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Inputs to the Active Indole-3-Acetic Acid Pool: *De Novo* Synthesis, Conjugate Hydrolysis, and Indole-3-Butyric Acid β-Oxidation

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Abstract

The phytohormone auxin is important in virtually all aspects of plant growth and development, yet our understanding of auxin homeostasis is far from complete. Plants use several mechanisms to control levels of the active auxin, indole-3-acetic acid (IAA). Plants can synthesize IAA both from tryptophan (Trp-dependent pathways) and from a Trp precursor but bypassing Trp (Trp-independent pathways). Despite progress in identifying enzymes in Trp-dependent IAA biosynthesis, no single IAA biosynthetic pathway is yet defined to the level that all of the relevant genes, enzymes, and intermediates are identified. In addition to *de novo* synthesis, vascular plants can obtain IAA from the hydrolysis of IAA conjugates. IAA can be conjugated to amino acids, sugars, and peptides; endogenous conjugates that are active in bioassays and hydrolyzed in plants are likely to be important free IAA sources. Conjugation is also used to permanently inactivate excess IAA, and these conjugates may be distinct from the hydrolyzable conjugates. The peroxisomal β-oxidation of endogenous indole-3-butyric acid (IBA) also can supply plants with IAA, which may account for part of the auxin activity of exogenous IBA. Compartmentalization of enzymes and precursors may contribute to the regulation of auxin metabolism. IAA obtained through *de novo* synthesis, conjugate hydrolysis, or IBA β-oxidation may have different functions in plant development, and possible roles for the IAA derived from the various pathways are discussed.

Key words: Auxin biosynthesis; Auxin conjugate hydrolysis; Fatty acid β-oxidation; IAA; IBA; Indole; Glucosinolates; Phytohormone homeostasis; Plant peroxisome; Tryptophan

IAA Biosynthesis: Multiple Pathways

Many of the molecular components of *de novo* IAA biosynthesis, an essential aspect of IAA homeostasis (Figure 1), remain undefined despite decades of effort. Although several IAA biosynthetic pathways are now established in plant-associated microbes (Costacurta and Vanderleyden 1995; Patten and Glick 1996), no plant IAA biosynthetic pathway has been fully elucidated. The current state of our knowledge is illustrated in Figure 2 and genes implicated in IAA biosynthesis are listed in Table 1. Plants can produce IAA from both Trp-dependent and Trp-independent pathways (Normanly and others 1995; Bartel 1997; Normanly and Bartel 1999; Slovin and others 1999). Multiple routes to the same product may allow precise regulation of IAA biosyn-

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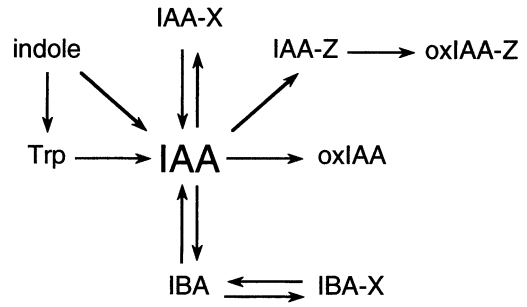

Figure 1. A simplified model of IAA homeostasis. IAA can be synthesized using at least two *de novo* pathways, from Trp and from a Trp precursor, perhaps indole. In addition, IBA β-oxidation and conjugate (IAA-X) hydrolysis can contribute to the free IAA pool. IAA can be inactivated by oxidation (OxIAA) or through formation of non-hydrolyzable conjugates (IAA-Z), which may also be oxidized (OxIAA-Z). In addition, as hydrolyzable IAA conjugates and IBA are likely to be derived from IAA, synthesis of these compounds may contribute to IAA inactivation. Formation and hydrolysis of IBA conjugates (IBA-X) may also be important for IAA homeostasis. See text for details.

thesis, but our understanding of this control is rudimentary. Two complementary approaches are contributing to breakthroughs in the study of IAA biosynthesis: the identification and quantification of trace amounts of indolic compounds from plants using stable isotope dilution and GC-MS, and the advance of genetic and genomic analyses in model plants to identify the specific enzymes involved.

Trp-Independent IAA Biosynthesis

A variety of plants use both Trp-dependent and Trp-independent IAA biosynthetic pathways (Normanly and others 1995; Slovin and others 1999). Although it was long assumed that plants would synthesize IAA from Trp, early suggestions of a Trp-independent pathway came from the demonstration that indole, but not Trp, has auxin activity in an *Avena* coleoptile bioassay (Winter 1966). Trp-independent IAA biosynthesis is important not only in angiosperms, but also in lower land plants, including liverworts, mosses, and ferns (Sztein and others 2000).

Feeding plants heavy isotope-labeled intermediates has been used to determine the importance of various biosynthetic pathways. These studies are based on the premise that, for a linear pathway, a precursor will contain higher isotopic enrichment than its derivative. Feeding studies with *Lemna gibba* indicate that Trp availability does not limit IAA biosynthesis in this system (Baldi and others 1991). In *Arabidopsis* seedlings, the Trp precursor [^{15}N]-anthranilate (Figure 2) labels IAA more completely than Trp, whereas [$^{2}H_5$]-Trp is not efficiently converted into IAA (Normanly and others 1993), supporting a Trp-independent IAA biosynthetic pathway.

Analyses of Trp biosynthetic mutants also demonstrate that IAA biosynthesis is not solely Trp-dependent. The maize *orange pericarp* (*orp*) mutant is a Trp auxotroph with defects in two Trp synthase β loci (Figure 2). Instead of having reduced IAA levels, as would be expected if all IAA is derived from Trp, the *orp* mutant contains more total (free plus conjugated) IAA than wild type (Wright and others 1991). In the mutant, D_2O feeding enriches the IAA pool more than the Trp pool, and [^{15}N]-anthranilate labels IAA but not Trp (Wright and others 1991).

The *Arabidopsis trp3-1* and *trp2-1* mutants, which are defective in Trp synthase α and β, respectively, (Last and others 1991; Radwanski and others 1996), have low soluble Trp levels (Müller and Weiler 2000b; Ouyang and others 2000) but accumulate amide- and ester-linked IAA conjugates (Normanly and others 1993; Ouyang and others 2000), suggesting that excess IAA is inactivated through conjugation. The *trp3-1* mutant also accumulates the Trp precursor indole-3-glycerol phosphate (Figure 2), which may be converted to IAA upon the *in vitro* base hydrolysis that is used to quantify IAA-conjugates (Müller and Weiler 2000b).

In contrast to *trp2* and *trp3,* blocks in earlier steps of the Trp pathway (Figure 2) do not result in IAA accumulation. The *Arabidopsis trp1-1* anthranilate phosphoribosyltransferase mutant (Last and Fink 1988) does not accumulate excess IAA (Normanly and others 1993). Similarly, *Arabidopsis* plants with decreased indole-3-glycerol phosphate synthase levels have reduced IAA accumulation (Ouyang and others 2000). These results imply that a Trp-independent pathway branches from indole-3-glycerol phosphate (Figure 2). In maize, Trp synthase α-like enzymes synthesize the indole destined for release as a volatile (Frey and others 2000) or used as a precursor for certain Trp-independent secondary metabolites (Frey and others 1997; Melanson and others 1997). It has not been reported whether other plants use Trp synthase α-like enzymes similarly, but it is intriguing that *Arabidopsis* contains a Trp synthaseα-like enzyme (GenBank accession number T01088) in addition to TSA1, the Trp synthase α defective in *trp3* (Radwanski and others 1996).

None of the enzymes catalyzing Trp-independent IAA biosynthesis have been identified. However, maize seedling extracts efficiently convert indole to IAA without going through a Trp intermediate (Östin and others 1999), and further analysis of this

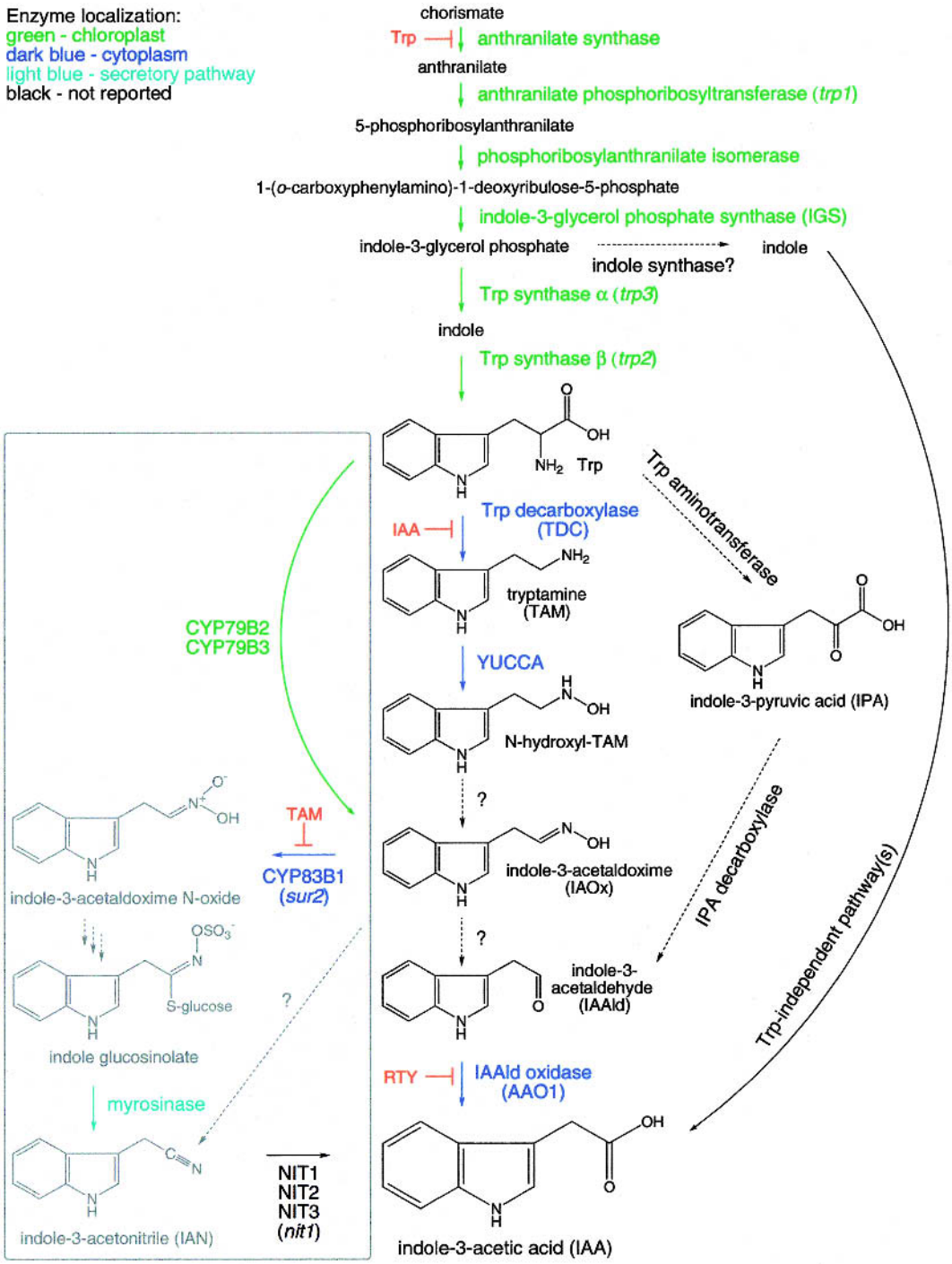

Figure 2. Proposed pathways of *de novo* IAA synthesis in plants. *De novo* IAA biosynthetic pathways initiate from Trp or Trp precursors. Trp biosynthesis and the P450-catalyzed conversion of Trp to IAOx are chloroplastic (shown in green), whereas many Trp-dependent IAA biosynthetic enzymes are apparently cytoplasmic (shown in blue). Suggested conversions for which plant genes have not been identified are dashed. Several points of negative regulation are shown by blunt arrows (red): anthranilate synthase is inhibited by Trp (Belser and others 1971), CYP83B1 is inhibited by tryptamine (Bak and others 2001), *TDC* transcription is repressed by IAA (Gooddijn and others 1992; Pasquali and others 1992), and AAO1 levels are elevated in the *rty* mutant (Sekimoto and others 1998; Seo and others 1998). The IAN pathway (boxed in gray) may be limited to families in the Capparales order that make indole glucosinolates (Bak and others 1998).

Table 1. Plant Genes Implicated in *de novo* IAA Biosynthesis

Gene	Species	Product	Putative Localization	Loss-of-Function (LOF) or Overexpression (OE) Phenotype	Reference
AAO1	*Arabidopsis*	IAA1d oxidase	Cytoplasm	OE in *rty* LOF not reported	(Sekimoto and others 1998; Seo and others 1998
CYP79B2	*Arabidopsis*	Cytochrome P450	Chloroplast	OE: resistant to Trp analogs OE: high indole glucosinolates OE: high IAN and IAA-X	(Hull and others 2000) (Mikkelsen and others 2000) (Celenza and Normanly, personal communication)
CYP79B3	*Arabidopsis*	Cytochrome P450	Chloroplast	not reported	(Hull and others 2000)
CYP83B1/ SUR2	*Arabidopsis*	Cytochrome P450	Cytoplasm	LOF: high IAA, IAA1d, and IAA-Asp; normal IAN; low indole glucosinolates OE: high indole glucosinolates	(Delarue and others 1998; Barlier and others 2000; Bak and others 2001) (Bak and others 2001)
NIT1	*Arabidopsis*	Nitrilase	Not reported	LOF: IAN resistant, normal IAA	(Normanly and others 1997)
NIT2	*Arabidopsis*	Nitrilase	Not reported	OE: increased sensitivity to IAN, normal IAA	(Normanly and others 1997)
SUR1/ RTY/ ALF1/ HLS3	*Arabidopsis*	Amino-transferase-like	Not reported	LOF: high IAA and IAA-X	(Boerjan and others 1995; Celenza and others 1995; King and others 1995; Golparaj and others 1996; Lehman and others 1996)
TDC	*C. roseus*	Trp decarboxylase	Cytoplasm	OE (canola): low indole glucosinolates OE (tobacco): low Trp	(Chavadej and others 1994) (Guillet and others 2000)
TRP2	*Arabidopsis*	Trp synthase β	Chloroplast	LOF: high IAA-X and IAN, normal free IAA, low Trp	(Normanly and others 1993; Ouyang and others 2000)
ORP	*Zea mays*	Trp synthase β	Chloroplast	LOF: high IAA-X, normal free IAA	(Wright and others 1991)
TRP3	*Arabidopsis*	Trp synthase α	Chloroplast	LOF: high IAA-X and IAN, normal free IAA, low Trp LOF: high IGP, IAN, and indole glucosinolates; normal IAA	(Normanly and others 1993; Ouyang and others 2000 (Müller and Weiler 2000b)
YUCCA, YUCCA2, YUCCA3	*Arabidopsis*	FMO-like	Cytoplasm	OE: high IAA	(Zhao and others 2001)

system may yield the relevant intermediates and enzymes.

Trp-Dependent IAA Biosynthesis: Indole-3-Pyruvate

In contrast to the Trp-independent pathway, the Trp-dependent pathway of IAA biosynthesis is rich with potential intermediates (Figure 2). Enzymes that convert Trp to indole-3-pyruvic acid (IPA), tryptamine, and indole-3-acetaldoxime (IAOx) have all been proposed to catalyze initial steps in Trp-dependent IAA biosynthesis. IPA has been identified in tomato shoots (Cooney and Nonhebel 1991), *Arabidopsis* seedlings (Tam and Normanly 1998), and pea root nodules (Badenoch-Jones and others 1984), and accumulates more label than IAA following D_2O feeding of tomato shoots (Cooney and Nonhebel 1991), consistent with an IAA precursor role.

If IPA is an IAA precursor, as in certain IAA-synthesizing microorganisms (Koga 1995), a Trp aminotransferase could convert Trp to IPA and an IPA decarboxylase could convert IPA to indole-3-acetaldehyde (IAAld; Figure 2). However, genes for these enzymes have not been identified in plants. Homology inspection alone is inadequate to identify plant versions of the microbial enzymes. For example, *Arabidopsis* encodes four apparent decarboxylases similar to pyruvate decarboxylase, but it is not obvious whether any are IPA decarboxylases, as the microbial IPA decarboxylases are more similar to microbial pyruvate decarboxylases than to any plant protein.

Trp-Dependent IAA Biosynthesis: Renewed Focus on Tryptamine

Tryptamine was proposed to be an IAA precursor based on its auxin activity in *Avena* coleoptile elongation assays (Winter 1966). Tryptamine has been identified in tomato (Cooney and Nonhebel 1991), and tryptamine-derived alkaloids have been identified in a variety of plants. Tryptamine accumulates more label than IAA in D_2O-fed tomato shoots, consistent with a precursor role (Cooney and Nonhebel 1991).

Trp is converted to tryptamine by Trp decarboxylase (TDC), a well-studied enzyme necessary for the biosynthesis of pharmaceutically valuable monoterpenoid indole alkaloids in *Catharanthus roseus* (Facchini and others 2000). Tryptamine accumulation may be subject to feedback inhibition (Figure 2), as *C. roseus TDC* transcription is downregulated by exogenous auxin (Gooddijn and others 1992; Pasquali and others 1992).

The potential importance of the tryptamine pathway is highlighted by the identification of YUCCA, an *Arabidopsis* flavin monooxygenase (FMO)-like enzyme that apparently catalyzes the conversion of tryptamine to N-hydroxyl-tryptamine. Plants overexpressing *YUCCA* were identified by activation-tagging (Zhao and others 2001). These plants accumulate free IAA and display high auxin phenotypes, including long hypocotyls in the light, hookless development in the dark, epinastic cotyledons and leaves, long petioles, auxin-independent growth in tissue culture, and increased apical dominance (Zhao and others 2001). *YUCCA* overexpression also confers resistance to toxic Trp analogs, suggesting that the accumulating IAA is Trp-derived (Zhao and others 2001). Whereas *TDC*-overexpressing tobacco or canola accumulate tryptamine without high auxin phenotypes (Songstad and others 1990; Chavadej and others 1994; Guillet and others 2000), the finding that *YUCCA* overexpression in tobacco or *Arabidopsis* leads to high auxin phenotypes (Zhao and others 2001) suggests that YUCCA is rate-limiting in the tryptamine pathway. This hypothesis has not been definitively tested, as a family of YUCCA-like enzymes is present in *Arabidopsis,* and disrupting *YUCCA, YUCCA2,* or both does not confer any obvious phenotypes (Zhao and others 2001). Moreover, tryptamine has not yet been identified as an endogenous compound in *Arabidopsis.* The isolation of YUCCA accentuates the importance of non-biased genetic approaches in understanding IAA synthesis, as the tryptamine to N-hydroxyl-tryptamine conversion catalyzed by this enzyme was not uncovered in previous biochemical studies.

Trp-Dependent IAA Biosynthesis: Indole-3-Acetaldoxime and Branchpoint Control

A third potential tryptophan-derived IAA precursor, indole-3-acetaldoxime (IAOx), also serves as an indole glucosinolate precursor (Figure 2). IAOx has been identified in Chinese cabbage (Ludwig-Müller and Hilgenberg 1988), but is not detectable in tomato (Cooney and Nonhebel 1991).

Microsomal membranes from a variety of plants, including cabbage, maize, and pea, can convert Trp to IAOx (Ludwig-Müller and Hilgenberg 1988), and two cytochrome P450 monooxygenases, CYP79B2 and CYP79B3, catalyze this conversion *in vitro* (Hull and others 2000; Mikkelsen and others 2000). CYP79B2 was identified in a yeast screen for *Arabidopsis* proteins that confer resistance to 5-fluoroindole (Hull and others 2000). 5-Fl-indole is toxic because it is converted to 5-Fl-Trp, which inhibits

anthranilate synthase and is incorporated into proteins. *CYP79B2* expression in yeast presumably converts 5-Fl-Trp to 5-Fl-IAOx, which relieves the toxicity, and overexpression in *Arabidopsis* leads to resistance to toxic Trp analogs (Hull and others 2000) and increased indole glucosinolate (Mikkelsen and others 2000), indole-3-acetonitrile (IAN), and IAA conjugate levels (J. Celenza and J. Normanly, personal communication). Like the *trp2* and *trp3* mutants, *CYP79B2*-overexpressing plants have normal free IAA levels (J. Celenza and J. Normanly, personal communication), suggesting that the excess IAA is inactivated through conjugation.

A third cytochrome P450, CYP83B1, converts IAOx to its N-oxide, funneling carbon into the indole-glucosinolate pathway (Figure 2; Bak and others 2001). *superroot2* (*sur2*), a recessive mutant defective in this enzyme, was isolated based on its high auxin phenotypes (Delarue and others 1998) and independently in a reverse-genetic screen for cytochrome P450 mutants (Winkler and others 1998). The mutant accumulates free IAA (Delarue and others 1998; Barlier and others 2000), indole-3-acetaldehyde (IAAld), and IAA-Asp and IAA-Glu conjugates; but has normal IAN, IAA-Leu, and IAA-Ala levels (Barlier and others 2000). It is likely that IAOx accumulates in the *sur2* mutant and is converted to IAA, perhaps through IAAld (see below).

The *sur2* mutant phenotype can be rescued by growth at low pH or on low concentrations of IAA (Barlier and others 2000). Exogenous IAA might inhibit *de novo* IAA biosynthesis, thus reducing the IAA accumulation caused by the *sur2* block. It will be interesting to determine whether *yucca* also can be rescued by low pH or exogenous IAA, as the chloroplast targeting signals on CYP79B2/3 and probable cytoplasmic localization of YUCCA suggest that *yucca* and *sur2* might accumulate IAOx in different compartments (Figure 2; Table 1).

TDC overexpression in *Brassica napus* leads to tryptamine accumulation and dramatically reduces indole glucosinolate content (Chavadej and others 1994). The observation that tryptamine inhibits CYP83B1/SUR2 *in vitro* (Bak and others 2001) could explain this decrease. Regulation of branchpoint enzymes, such as CYP83B1, may be necessary to ensure adequate IAA production, as Trp-derived secondary metabolites, such as indole glucosinolates and indole alkaloids, compete with IAA for precursors.

Trp-Dependent IAA Biosynthesis: Pathways Converge at Indole-3-Acetaldehyde

Indole-3-acetaldehyde (IAAld) is a possible convergence point for the proposed Trp-dependent IAA biosynthetic pathways discussed above (Figure 2). The Trp → IPA → IAAld → IAA pathway has been thoroughly characterized in microorganisms (Patten and Glick 1996) and is considered a significant pathway in plants (Nonhebel and others 1993). IAAld could also be derived from IAOx (Rajagopal and Larsen 1972; Rajagopal and others 1991); a soluble protein activity from Chinese cabbage converts IAOx to IAA, apparently through an IAAld intermediate (Helmlinger and others 1987). Both *CYP79B2* overexpressors and *cyp83b1/sur2* mutants are expected to accumulate IAOx, although this has not been directly demonstrated. *sur2* accumulates IAAld (Barlier and others 2000), which is probably derived from IAOx. *YUCCA* overexpression probably increases conversion of tryptamine to N-hydroxyltryptamine (Zhao and others 2001), which could be dehydrogenated to IAOx or dehydrogenated and hydrolyzed to IAAld in a third pathway of IAAld production. However, enzymes that catalyze IAAld formation from N-hydroxyl-tryptamine or IAOx have not been identified.

The conversion of IAAld into IAA is catalyzed by IAAld oxidases, which have been characterized from several plants (Bower and others 1978; Sekimoto and others 1997; Seo and others 1998). An *Arabidopsis* aldehyde oxidase isozyme (AAO1) specific for IAAld accumulates in the *rooty* (*rty*) mutant (Seo and others 1998). The *rty* mutant (King and others 1995), also isolated as *sur1* (Boerjan and others 1995), *hookless3* (Lehman and others 1996), and *alf1* (Celenza and others1995), has high auxin phenotypes. This recessive mutant, defective in a tyrosine aminotransferase-like enzyme (Golparaj and others 1996), accumulates free IAA and IAA conjugates (Boerjan and others 1995; King and others 1995; Lehman and others 1996). By analogy to CYP83B1/SUR2, RTY might normally act to divert an IAA precursor to a secondary metabolite (Celenza 2001). It has not been reported if other indolic metabolites accumulate in *rooty* or whether the excess IAA is produced from Trp or independently of Trp. The observation that *AAO1* transcription and enzymatic activity are elevated in the *rty* mutant (Sekimoto and others 1998; Seo and others 1998) indicates that RTY acts genetically as a negative regulator of *AAO1* expression (Figure 2). It will be interesting to determine the level at which this regulation occurs, and whether *AAO1* disruption results in IAA deficiency or rescue of the *rty* high auxin phenotypes.

Trp-Dependent IAA Biosynthesis: A Limited Role for Indole-3-Acetonitrile

The importance and ubiquity of IAN in IAA biosynthesis remains uncertain. IAOx is converted to IAN

in Chinese cabbage and maize tissues (Helmlinger and others 1985), and IAN can be hydrolyzed to IAA by nitrilases encoded by the *Arabidopsis NIT* genes (Bartling and others 1992; Bartel and Fink 1994; Bartling and others 1994). Extracts from the Cruciferae, Graminae, and Musaceae families can hydrolyze IAN to IAA, suggesting the existence of a nitrilase pathway in these families (Thimann and Mahadevan 1964). *Arabidopsis* NIT1, NIT2, and NIT3 enzymes have apparent K_m values for IAN that are an order of magnitude greater than those for their preferred substrate, 3-phenyl-propionate (Vorwerk and others 2001), so IAN may not be the most relevant substrate. However, NIT1 and NIT2 can hydrolyze IAN *in vivo* (Schmidt and others 1996; Normanly and others 1997), and an *Arabidopsis* enzymatic complex with nitrilase immunoreactivity converts Trp to IAA *in vitro* (Müller and Weiler 2000a), making it possible that substrate channeling *in vivo* compensates for the high K_m.

Rather than hydrolyzing IAN made directly from IAOx, nitrilases may act on IAN formed following hydrolysis of indole glucosinolates by myrosinases (Figure 2). Plants with excess indole glucosinolates, including *CYP79B2* overexpressors (Mikkelsen and others 2000), the *trp3* mutant (Müller and Weiler 2000b), and the *bus* mutant (Reintanz and others 2001), also accumulate IAN (Celenza and Normanly, personal communication; Normanly and others 1993; Müller and Weiler 2000b; Reintanz and others 2001). The *trp3* mutant has higher nitrilase immunoreactivity than wild type (Müller and Weiler 2000b), suggesting that increased nitrilase mediated hydrolysis of indole glucosinolate-derived IAN might supply the extra IAA in the mutant. The *NIT2* gene is induced by bacterial pathogen infiltration of leaves (Bartel and Fink 1994) and by *Plasmodiophora* infection of roots (Grsic-Rausch and others 2000), situations in which myrosinases might hydrolyze glucosinolates. The observations that IAN levels are normal in the *sur2* mutant (Barlier and others 2000), and that the IAN-resistant *nit1* mutant (Normanly and others 1997) fails to rescue *sur2* defects (Bak and others 2001), also suggest that nitrilases act downstream of glucosinolates. Determination of the importance of nitrilases in *Arabidopsis* IAA biosynthesis awaits identification of an enzyme that converts IAOx to IAN and isolation of a triple *nit1 nit2 nit3* mutant.

Regulation of De Novo IAA Synthesis

Trp-dependent and Trp-independent pathways are developmentally regulated. Carrot cells switch from Trp-dependent to Trp-independent IAA biosynthesis when callus cultures are induced to form embryos (Michalczuk and others 1992). In zygotic carrot embryogenesis, an 80-fold surge in free IAA concentration is derived from Trp, but basal IAA biosynthesis is Trp-independent (Ribnicky and others 2001). During *Arabidopsis* leaf senescence, IAN levels decrease and IAA levels increase, which is correlated with a strong induction of *NIT2* expression, implying that the increased IAA might be IAN-derived (Quirino and others 1999). Scots pine seedlings begin synthesizing IAA from Trp approximately 3 days after sowing, and induce a Trp-independent pathway after 6 days (Ljung and others 2001). Developmental differences can be recapitulated *in vitro;* extracts from maize coleoptile tips (Koshiba and Matsuyama 1993; Koshiba and others 1995) or endosperm (Ilic' and others 1999; Glawischnig and others 2000) synthesize IAA from Trp, whereas seedling extracts use a Trp-independent pathway (Östin and others 1999). Tobacco protoplasts from vegetative shoot apices use both Trp-dependent and Trp-independent IAA biosynthetic pathways (Sitbon and others 2000), suggesting that both sources may be important later in development.

An emerging theme in IAA biosynthesis is that plants use Trp-independent pathways for IAA maintenance, but switch to Trp-dependent pathways when high IAA levels are required (Ribnicky and others 2001; Sztein and others 2001). Intact *Arabidopsis* seedlings convert more [^{15}N]-anthranilate into IAA than into Trp, and do not efficiently convert [$^{2}H_5$]-Trp into IAA (Normanly and others 1993), indicating that Trp-independent biosynthesis is important during normal growth. In contrast, *Arabidopsis* shoot or root explants efficiently convert [$^{2}H_5$]-Trp to IAA (Müller and others 1998b; Müller and Weiler 2000b), suggesting that a Trp-dependent pathway is wound-induced. Similarly, Trp-independent IAA biosynthesis predominates in 6-day-old bean seedlings, but a Trp-dependent pathway is induced by wounding (Sztein and others 2001).

IAA Conjugates: Storage and Inactivation

In addition to *de novo* synthesis, hydrolysis of IAA conjugates is an important source of free IAA in higher plants (Figure 1). Whereas most IAA is conjugated in vascular plants (Slovin and others 1999; Sztein and others 1999), conjugation and hydrolysis are apparently less important in nonvascular plants such as liverworts, which rely mostly on IAA biosynthesis and degradation to modulate IAA levels (Sztein and others 1995; Sztein and others 1999; Sztein and others 2000). IAA can be conjugated ei-

ther to sugars via ester linkages or to amino acids and peptides via amide linkages, and these conjugates may act in IAA storage or transport, protection of IAA against peroxidative degradation, and compartmentalization or detoxification of excess IAA (Cohen and Bandurski 1982). Despite the complexity of conjugate metabolism, which differs not only between species but also between developmental stages of the same species, patterns of conjugate functions are emerging. Conjugates that are biologically active, present endogenously, and hydrolyzed *in planta* are likely to be important IAA stores, whereas biologically inactive conjugates are probably precursors for IAA degradation.

Several approaches are elucidating conjugate roles in auxin metabolism. IAA conjugates present in unstressed plants and those formed in response to high exogenous auxin are being identified and quantified (Slovin and others 1999), the effects of conjugates in bioassays are being analyzed (Feung and others 1977; Hangarter and others 1980; Hangarter and Good 1981; Bialek and others 1983), and mutant screens are identifying loci important in conjugate perception and metabolism (Bartel and Fink 1995; Campanella and others 1996; Barratt and others 1999; Davies and others 1999; Lasswell and others 2000).

IAA Conjugates Identified in Plants

Conjugate synthesis pathways in higher plants appear complex and diverged. Many identified conjugates have only been found in a single plant species, which may result from incomplete analysis or biological differences. Because IAA-ester and IAA-amide conjugates are hydrolyzed under different conditions (Bialek and Cohen 1986; Baldi and others 1989), total ester- and amide-linked conjugates can be quantified. In general, monocots appear to accumulate ester conjugates, whereas dicots accumulate mostly amide conjugates (Cohen and Bandurski 1982; Slovin and others 1999).

In maize, soybean, and bean seeds, the identified and quantified IAA conjugates account for most of the stored IAA. Maize kernels contain primarily ester-linked conjugates, including IAA-*myo*-inositol, IAA-*myo*-inositol glycosides, IAA-glucose, and a large cellulosic glucan conjugate (Cohen and Bandurski 1982). In soybean seeds, IAA-Asp and IAA-Glu are the predominant IAA conjugates (Cohen 1982; Epstein and others 1986). Bean seeds apparently lack amino acid conjugates, and the IAA is instead conjugated to several polypeptides ranging in size from 3– 60 kDa (Bialek and Cohen 1986; Walz and others 2001). One of these IAA-modified bean proteins, IAP1, is encoded by a homolog of a soybean late seed maturation protein (Walz and others 2001), suggesting a new role for seed storage proteins in phytohormone action.

In other species, quantification of identified conjugates does not account for all of the IAA released following base hydrolysis, so additional conjugates may remain to be identified. Oats contain an IAA-glycoprotein ester conjugate (Percival and Bandurski 1976), and rice contains IAA-*myo*-inositol (Hall 1980). *Arabidopsis* conjugates include IAA-Ala, IAA-Leu (Barlier and others 2000), IAA-Asp, IAA-Glu, IAA-glucose (Tam and others 2000), and an IAA-peptide (Walz and others 2001).

IAA conjugates that accumulate following exposure to exogenous IAA often differ from those apparently used for IAA storage (Figure 1). In response to high auxin levels from IAA application or *iaaM* transgene expression (Sitbon and others 1993), a distinct pathway is activated to detoxify the excess IAA. *Arabidopsis* inactivates low levels of IAA by oxidation (Östin and others 1998), and forms conjugates to Asp and Glu (Östin and others 1998) or Asp, Glu, and glucose (Barratt and others 1999) in response to high exogenous IAA. Similarly, IAA applied to the epicarp of orange fruit is primarily inactivated by oxidation and by conjugation to Asp (Chamarro and others 2001). This high-auxin conjugation pathway is evolutionarily conserved, as a variety of vascular plants challenged with IAA apparently accumulate IAA-Asp, IAA-Glu, or IAA-glucose (Sztein and others 1995). IAA-Asp and IAA-Glu can be further oxidized to oxIAA-conjugates (Östin and others 1992; Östin and others 1998; Chamarro and others 2001), which permanently inactivates the hormone. Additional catabolites, such as N-linked products of the indole ring, have also been identified (Östin and others 1995). Asp and Glu conjugates of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) are apparently sequestered in the vacuole (Davidonis and others 1982), supporting the hypothesis that these conjugates are catabolites.

Activity of IAA Conjugates

Certain endogenous IAA conjugates elicit auxin responses in bioassays, and conjugate activity often correlates with hydrolysis. For example, IAA-Ala is present in *Arabidopsis* (Barlier and others 2000) and *Picea abies* (Östin and others 1992) and has auxin activity in numerous bioassays (Hangarter and others 1980; Hangarter and Good 1981; Bialek and others 1983; Magnus and others 1992b; Davies and others 1999). Similarly, IAA-Leu is an endog-

enous *Arabidopsis* conjugate (Barlier and others 2000) and has activity in some bioassays (Feung and others 1977; Hangarter and others 1980; Bartel and Fink 1995). Bioactive conjugates often can be hydrolyzed in plants. IAA-Ala is hydrolyzed by bean stem sections (Bialek and others 1983), *Arabidopsis* extracts (LeClere and Bartel, unpublished), and the *Arabidopsis* IAR3 amidohydrolase (Davies and others 1999). IAA-Leu is hydrolyzed by the *Arabidopsis* ILR1 amidohydrolase (Bartel and Fink 1995), and IAA-Ala and IAA-Leu are hydrolyzed in Chinese cabbage extracts (Ludwig-Müller and others 1996).

Hydrolysis rates do not always fully account for conjugate bioactivity, however. For example, IAA-Ala has unique effects on tomato tissue cultures; pretreatment of cultures with IAA-Ala can inhibit IAA-induced shoot growth and root initiation, suggesting a possible competition between IAA and some conjugates for a common binding site(s) (Magnus and others 1992a).

Other conjugates found in plants do not consistently elicit auxin effects in bioassays. Although IAA-Asp promotes *Avena* coleoptile (Feung and others 1977) and soybean hypocotyl (Cohen and Baldi 1983) elongation, it has only low auxin activity in other systems, including bean stems (Bialek and others 1983) and *Arabidopsis* roots (Campanella and others 1996). IAA-Glu has auxin activity and is hydrolyzed in bean stem sections (Bialek and others 1983). However, neither IAA-Asp nor IAA-Glu are hydrolyzed by intact *Lemna gibba* plants (Slovin 1997), extracts from uninfected Chinese cabbage (Ludwig-Müller and others 1996), or *Arabidopsis* seedlings (Östin and others 1998).

Uptake and transport differences may contribute to the activities of IAA conjugates in bioassays. In *Lemna gibba,* IAA-Glu is taken up faster than IAA-Ala, whereas IAA-*myo*-inositol is taken up more slowly than either amide-linked conjugate (Slovin 1997). Conjugate uptake differences may reflect differences in influx carrier binding affinities, conjugate compartmentalization, or metabolism inside the cell. The *Arabidopsis aux1* auxin influx carrier mutant (Bennett and others 1996) is resistant to IAA-Ala and IAA-Leu, suggesting that at least some conjugates enter cells similarly to free IAA (our unpublished data). However, it is not known how conjugates exit cells. IAA-Ala and IAA-Gly are apparently not subject to polar auxin transport in pea stem sections, but rather disperse through diffusion (Hangarter and others 1980), suggesting that conjugates are not substrates of the auxin efflux carrier. In contrast, IAA-*myo*-inositol is transported 400 times faster than IAA from maize endosperm to shoots (Nowacki and Bandurski 1980). One possibility is that vascular transport contributes to the high rate of ester conjugate transport (Ludwig-Müller and others 1996). It is intriguing to speculate that sugar-linked conjugates may be directed to the vascular system for rapid movement through the plant, whereas amide-linked conjugates provide more localized responses. Analysis of different auxin transport mutants on various conjugates may allow further dissection of conjugate roles in IAA trafficking.

Developmental Regulation of Conjugation and Conjugate Hydrolysis

IAA conjugates stored in seeds can provide free IAA to seedlings. IAA-ester hydrolysis supplies free IAA to germinating maize seedlings (Epstein and others 1980), and amide conjugates are rapidly hydrolyzed after bean seed imbibition (Bialek and Cohen 1992). Conjugate hydrolysis in Scots pine peaks just prior to radicle emergence, and the disappearance of ester-linked conjugates correlates with an increase in free IAA (Ljung and others 2001). Interestingly, amide-linked IAA is low in these seeds, and IAA-Asp conjugation and IAA catabolism are initiated concurrently with *de novo* synthesis, which occurs after seed-stored ester conjugates are hydrolyzed (Ljung and others 2001).

The catabolic conjugation system is probably present during normal growth because IAA-Asp, IAA-Glu, and IAA-glucose are present at low levels in *Arabidopsis* seedlings (Tam and others 2000). In response to elevated IAA levels, storage conjugation pathways may be down-regulated as catabolic pathways are up-regulated. For example, the *sur2* mutant, which accumulates free IAA (see above), has a reduced ability to make IAA-Leu (Barlier and others 2000), a putative *Arabidopsis* IAA storage form (see above). However, *sur2* plants do accumulate IAA-Asp (Barlier and others 2000), an intermediate in permanent IAA inactivation (Normanly 1997; Slovin and others 1999). It appears that plants use different conjugates to detoxify excess IAA and to store IAA, suggesting that the conjugated moiety may dictate the fate of the attached IAA for storage, transport, or degradation (Cohen and Bandurski 1982).

Genetic Analysis of IAA Conjugate Functions

The isolation and characterization of IAA-conjugate resistant mutants that remain sensitive to IAA provide an additional tool to examine conjugate functions. A number of genes are implicated in IAA conjugate responses (Table 2), and several *Arabidopsis*

Table 2. Plant Genes Implicated in IAA Conjugate Metabolism

Gene	Species	Product	Putative Localization	Mutant Phenotype	Reference
ILR1	*Arabidopsis*	IAA-amino acid amidohydrolase	ER lumen	IAA-Leu resistant	(Bartel and Fink 1995)
IAR3	*Arabidopsis*	IAA-amino acid amidohydrolase	ER lumen	IAA-Ala resistant	(Davies and others 1999)
ILL1	*Arabidopsis*	IAA-amino acid amidohydrolase	ER lumen	Not reported	(Bartel and Fink 1995)
ILL2	*Arabidopsis*	IAA-amino acid amidohydrolase	ER lumen	Not reported	(Bartel and Fink 1995)
IAR1	*Arabidopsis*	Transporter?	Membrane	IAA-amino acid conjugate resistant	(Lasswell and others 2000)
ILR2	*Arabidopsis*	Not reported	Not reported	IAA-Leu resistant	(Magidin and Bartel, unpublished)
IAR4	*Arabidopsis*	Not reported	Not reported	IAA-Ala resistant	(LeClere and Bartel, unpublished)
ICR1	*Arabidopsis*	Not reported	Not reported	IAA-Phe resistant	(Campanella and others 1996)
ICR2	*Arabidopsis*	Not reported	Not reported	IAA-Phe resistant	(Campanella and others 1996)
iaglu	*Zea mays*	IAA glucosyl-transferase	Not reported	Not reported	(Szerszen and others 1994)
UGT84B1	*Arabidopsis*	IAA glucosyl-transferase	Not reported	Not reported	(Jackson and others 2001)
IAP1	*Phaseolus vulgaris*	Seed protein modified by IAA	Not reported	Not reported	(Walz and others 2001)

IAA-amino acid conjugate resistant mutants have been identified: *ilr1* (Bartel and Fink 1995) and *ilr2* (Magidin and Bartel, unpublished) are IAA-Leu and IAA-Phe resistant; *iar3* (Davies and others 1999) and *iar4* (LeClere and Bartel, unpublished) are IAA-Ala resistant; *icr1* and *icr2* (IAA conjugate resistant) are resistant to IAA-Ala, IAA-Phe, and IAA-Gly (Campanella and others 1996); and *iar1* is resistant to several IAA conjugates, including IAA-Ala, IAA-Leu, and IAA-Phe (Lasswell and others 2000).

If conjugates are IAA precursors, then conjugate-resistant mutants may be defective in conjugate hydrolysis or uptake. If conjugates have additional roles, however, it may be possible to genetically separate conjugate functions from those of IAA. ILR1 and IAR3 are amidohydrolases that cleave IAA-Leu and IAA-Phe (Bartel and Fink 1995) or IAA-Ala (Davies and others 1999), respectively. IAR1 (Lasswell and others 2000) is a multi-pass transmembrane protein with weak similarity to the ZIP family of metal transporters (Guerinot 2000). The biochemical activity of IAR1 is unknown, but it may transport a co-factor to facilitate amidohydrolase activity (Lasswell and others 2000). To date, the analysis of IAA conjugate-resistant mutants suggests that IAA conjugates with auxin activity act via their hydrolysis to free IAA. Although the genes defective in *icr1, icr2, ilr2,* and *iar4* have not been reported, it will be interesting to learn whether these mutants are also defective in some aspect of conjugate hydrolysis or if they reveal hydrolysis-independent conjugate functions.

Indole-3-ButyricAcid: Another IAA Storage Form

Indole-3-butyric acid (IBA) was long regarded as a synthetic auxin, but recent evidence demonstrates that IBA is present in numerous plant species. IBA is used widely in horticulture because of its efficacy in inducing lateral and adventitious roots on cuttings. Most IBA research has focused on identifying optimal conditions for secondary root development for the vegetative propagation of commercially important trees and ornamental plants.

Examination of the molecular mechanisms of IBA action indicates that IBA β-oxidation provides a source of free IAA in addition to *de novo* biosynthesis and conjugate hydrolysis (Figure 1). Some evidence suggests that IBA acts as an auxin on its own, independently of IAA. However, biochemical studies in numerous plants and genetic studies of *Arabidopsis* IBA-response mutants indicate that IBA acts primar-

ily via its conversion to IAA, which occurs in a mechanism similar to peroxisomal fatty acid β-oxidation.

IBA is an Endogenous Auxin

IBA is found in a variety of plants and tissues, including *Arabidopsis* seedlings (Ludwig-Müller and Epstein 1993; Ludwig-Müller and others 1993); carrot roots (Epstein and others 1991); tobacco leaves (Sutter and Cohen 1992); maize kernels, leaves, and roots (Epstein and others 1989; Ludwig-Müller and Epstein 1991); and pea roots, epicotyls, and shoots (Schneider and others 1985; Nordström and others 1991). A few studies have quantified IAA and IBA levels in plant tissues. For example, tobacco leaves contain 9 ng/g free IBA compared to 26 ng/g free IAA (Sutter and Cohen 1992) and there is slightly less total IBA than IAA in *Arabidopsis* (Ludwig-Müller and others 1993). However, these comparisons are complicated by variations in auxin concentrations under different growth conditions and between species (Ludwig-Müller and Epstein 1993). For instance, potato peelings contain elevated IBA levels at the start of sprouting (Blommaert 1954), and tumor-prone tobacco genotypes have higher IBA levels than other lines (Bayer 1969). In addition, auxin concentration varies during development. For example, free IAA peaks in 3-day-old *Arabidopsis* seedlings, whereas free IBA peaks between 5 and 9 days (Ludwig-Müller and Epstein 1993; Ludwig-Müller and others 1993).

IBA has auxin effects in many bioassays and initiates rooting significantly better than IAA in numerous plant species (Hartmann and others 1990), including adventitious root formation in pea (Nordström and others 1991), mung bean (Wiesman and others 1988, 1989), and apple cuttings (Alvarez and others 1989; van der Krieken and others 1992; van der Krieken and others 1993). In addition, exogenous IBA stimulates stem elongation similarly to IAA in intact pea plants (Yang and Davies 1999). IBA also has auxin effects on *Arabidopsis* seedlings: IBA inhibits root elongation (Zolman and others 2000) and induces lateral (Zolman and others 2000) and adventitious (King and Stimart 1998) root formation. Whereas IBA and IAA responses are qualitatively similar, effective IBA concentrations are generally higher than those of IAA.

The increased ability of IBA versus IAA to initiate lateral and adventitious roots may result from differences in receptor binding, compartmentalization, stability, tissue sensitivity, uptake, transport, or conjugation between the two auxins (Epstein and Ludwig-Müller 1993; de Klerk and others 1999; Ludwig-Müller 2000). IBA is more stable than IAA, both *in vivo* and in solution (Robbins and others 1988; Nissen and Sutter 1990; Nordström and others 1991). In addition, plants form inactive oxidation products following exposure to exogenous IAA (Normanly 1997; Slovin and others 1999); after this irreversible oxidation, IAA levels can drop below the optimal concentration for lateral root initiation at the time when auxin is required. IBA may be better at initiating lateral roots because it is not readily oxidized (Epstein and Ludwig-Müller 1993; de Klerk and others 1997; de Klerk and others 1999).

IBA is taken up and transported more slowly than IAA in a variety of systems, perhaps leaving more hormone at the plant base where it can affect root initiation (Epstein and Ludwig-Müller 1993; Ludwig-Müller 2000). The *aux1* mutant, defective in an auxin influx carrier (Bennett and others 1996), is resistant to the inhibitory effects of IBA (Zolman and others 2000), suggesting that IBA enters cells similar to IAA and IAA-conjugates (see above). In contrast, the *eir1/agr1/pin2* auxin efflux carrier mutant (Chen and others 1998; Luschnig and others 1998; Müller and others 1998a; Utsuno and others 1998) responds differently to IBA and IAA, suggesting that IBA is not an EIR1 substrate (Poupart and Waddell 2000; Zolman and others 2000).

Like IAA (see above), much of the IBA in plants is conjugated to other moieties through amide- and ester-linkages (Epstein and Ludwig-Müller 1993; Ludwig-Müller 2000). Although the IBA and IAA conjugate levels are similar in tobacco leaves (Sutter and Cohen 1992), differences in metabolism may contribute to the increased efficacy of IBA in lateral root induction (Wiesman and others 1988; Epstein and Ludwig-Müller 1993; van der Krieken and others 1997; Ludwig-Müller 2000). Unlike IAA, which is predominantly amide linked in dicots, IBA is largely ester linked (Ludwig-Müller and others 1993), and IBA conjugates may be more easily hydrolyzed (Epstein and Ludwig-Müller 1993) or differentially transported than IAA conjugates (see above). In addition, it has been reported that certain IBA conjugates, including IBA-Asp, are themselves active in secondary root initiation (Wiesman and others 1989).

IBA is Converted to IAA

One explanation for the auxin activity of IBA is that IBA is a "slow-release" form of IAA (van der Krieken and others 1997), similar to certain IAA conjugates (see above). IBA may supply plants with a continuous IAA source, providing auxin when it is required for root initiation. Even-chain length derivatives of

the synthetic auxin 2,4-D, including 2,4-dichlorophenoxybutyric acid (2,4-DB), have auxin activity in wheat cylinder tests, pea curvature assays, and tomato leaf epinasty tests (Wain and Wightman 1954). Similarly, even chain-length derivatives of IAA, including IBA, are active in auxin bioassays (Fawcett and others 1960). Wheat and pea extracts shorten these derivatives two carbons at a time (Fawcett and others 1960), suggesting that IBA and 2,4-DB are converted to IAA and 2,4-D similarly to fatty acid β-oxidation, which shortens fatty acids in two carbon increments.

Radiolabeled IBA is converted to IAA by a variety of plants, including apple (van der Krieken and others 1992), olive (Epstein and Lavee 1984), grape (Epstein and Lavee 1984), pear (Baraldi and others 1993), pea (Nordström and others 1991), bean (Wiesman and others 1988), and maize (Ludwig-Müller and Epstein 1991). In pea shoots, IBA treatment causes a transient tenfold increase in IAA levels during the first day of exposure, but IAA levels return to normal by day 4 (Nordström and others 1991). After apple shoots are incubated with [^{3}H]-IBA for 2 days, 4% of the label is in free IBA and 1% is in free IAA, which is a higher proportion of labeled free IAA than after treatment with [^{3}H]-IAA (van der Krieken and others 1992; van der Krieken and others 1993), consistent with a "slow-release" function for IBA. However, when shoots are treated with IBA or IAA concentrations that produce similar free IAA levels, IBA induces more secondary roots, suggesting that IBA also may act on its own or synergistically with IAA (van der Krieken and others 1992; van der Krieken and others 1993).

IBA to IAA Conversion is Peroxisomal

Our understanding of IBA action is being clarified by the isolation and characterization of *Arabidopsis* mutants with IBA-resistant root elongation that respond normally to IAA (Poupart and Waddell 2000; Zolman and others 2000; Zolman and others 2001a; Zolman and others 2001b). As discussed below, defects in β-oxidation can lead to IBA resistance, supporting the hypothesis that IBA is converted to IAA in a mechanism that parallels fatty acid β-oxidation (Figure 3). In plants, β-oxidation is exclusively peroxisomal (Gerhardt 1992; Kindl 1993). Peroxisomes are small organelles that contain no DNA; proteins acting in the peroxisomal matrix are imported from the cytoplasm post-translationally. At least 20 proteins are required for peroxisomal import, biogenesis, and function (Olsen 1998; Subramani 1998; Tabak and others 1999). It is likely that most of these proteins will be required for IBA to IAA conversion.

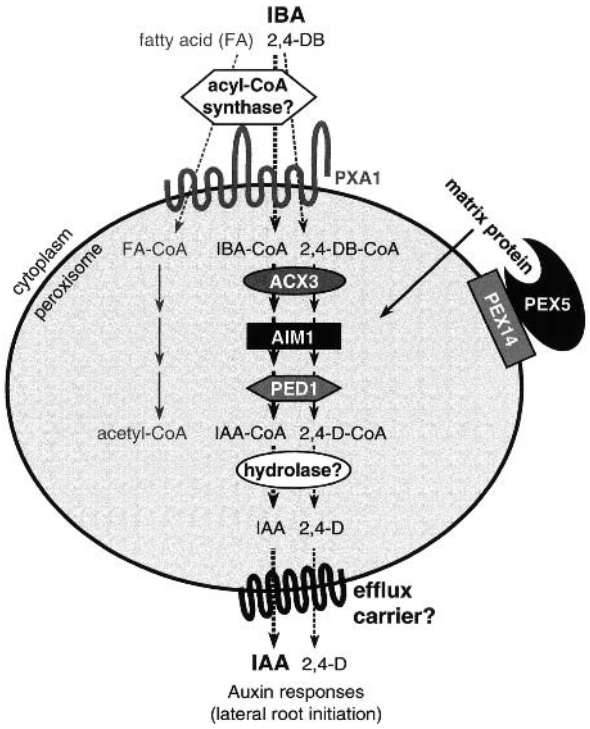

Figure 3. The conversion of IBA to IAA. IBA is likely to be converted to IAA-CoA in peroxisomes in a process that parallels fatty acid β-oxidation to acetyl-CoA. The IBA analog 2,4-DB is probably converted to the synthetic auxin 2,4-D by the same enzymes. Mutations in genes encoding the ABC-transporter-like protein PXA1 (Zolman and others 2001b), the medium chain acyl-CoA oxidase ACX3 (Eastmond and others 2000), the multifunctional protein AIM1 (Richmond and Bleecker 1999), and the thiolase PED1 (Hayashi and others 1998) each confer reduced sensitivity to IBA and 2,4-DB (Zolman and others 2000; Zolman and others 2001b). Moreover, mutations in *PEX5* and *PEX14,* which are necessary for the import of most matrix enzymes, including AIM1, also confer IBA and/or 2,4-DB resistance (Hayashi and others 2000; Zolman and others 2000). The acyl-CoA synthase that esterifies IBA to IBA-CoA, the hydrolase that releases the free acid, and the transporter that effluxes IAA from the peroxisome remain to be identified. The acyl-CoA hydrolase is shown in the peroxisome, but alternatively could be cytoplasmic.

Long-chain fatty acids stored in seeds undergo peroxisomal β-oxidation to provide energy during *Arabidopsis* germination. Mutants defective in fatty acid utilization therefore do not develop after germination unless provided with sucrose (Hayashi and others 1998). Analysis of sucrose-dependence and auxin-related phenotypes, including lateral root initiation and responses to synthetic auxins and auxin transport inhibitors, allows classification of IBA-response mutants into four groups (Zolman and oth-

ers 2000). Some mutants are IBA resistant in both root elongation and lateral root initiation, and are sucrose-dependent during early seedling development, indicating possible defects in enzymes required for the peroxisomal β-oxidation of IBA and long-chain fatty acids. A second subset of mutants are resistant to IBA in root elongation and have slight peroxisomal defects, but induce lateral roots normally in response to IBA, perhaps reflecting defects in tissue-specific β-oxidation isozymes. Other mutants are IBA resistant in root elongation and lateral root initiation, but lack obvious peroxisomal defects; these may be defective in isozymes specific to short-chain substrates and IBA. Finally, additional IBA-response mutants, including *rib1* (resistant to IBA; Poupart and Waddell 2000), respond normally to IAA but have altered responses to synthetic auxins and auxin transport inhibitors, and thus may function in an aspect of IBA action, such as transport, that is independent of its conversion to IAA.

Additional *Arabidopsis* peroxisomal β-oxidation mutants have been isolated using resistance to the IBA analog 2,4-DB. 2,4-DB is converted to 2,4-D similarly to IBA β-oxidation (Wain and Wightman 1954; Hayashi and others 1998). 2,4-DB resistant mutants that also have defects in growth without sucrose include *ped1, ped2, ped3* (Hayashi and others 1998), *acx3* (Eastmond and others 2000), *aim1* (Richmond and Bleecker 1999), and *dbr5* (Lange and Graham 2000). *acx3, aim1,* and *ped1* mutants are also IBA resistant (Zolman and others 2000; Zolman and Bartel, unpublished data), suggesting that 2,4-DB resistant mutants will be IBA resistant as well, and that the defects in these mutants will affect the conversion of IBA to IAA in addition to the conversion of 2,4-DB to 2,4-D.

The identification of the genes defective in several IBA-response mutants has confirmed the importance of peroxisomal β-oxidation in IBA action (Figure 3). One mutant is defective in PEX5 (Zolman and others 2000), a receptor that binds and transports proteins from the cytoplasm into the peroxisomal matrix (Olsen 1998; Subramani 1998). Similarly, the *ped2* (*pe*roxisome *d*efective) mutant is defective in PEX14, a peroxisomal membrane protein essential for the import of peroxisomal matrix proteins (Hayashi and others 2000). It is likely that *pex5* and *ped2* have defects in importing peroxisomal matrix proteins required for β-oxidation, which slow β-oxidation and cause IBA-resistant, sucrose-dependent phenotypes. A third IBA response mutant is defective in *PXA1* (Zolman and others 2001b). PXA1 is approximately 30% identical to human and yeast ATP-binding cassette transporters implicated in importing or activating long-chain fatty acids for β-oxidation (Dubois-Dalcq and others 1999; Holland and Blight 1999). Because *pxa1* is resistant to IBA and 2,4-DB and displays sucrose-dependent seedling development, it is likely that PXA1 imports IBA, 2,4-DB, and fatty acids into peroxisomes.

In contrast to peroxisome biogenesis defects, other IBA-response mutants have defects in β-oxidation enzymes (Figure 3). One IBA-response mutant is an *acx3* allele (Zolman and Bartel, unpublished). *acx3* is defective in a gene encoding an acyl-CoA oxidase catalyzing the second step of β-oxidation (Eastmond and others 2000). The weak sucrose-dependence of this mutant may reflect the specificity of ACX3 for medium-chain fatty acids (Eastmond and others 2000). The *aim1* (abnormal inflorescence meristem) mutant contains a mutation in a multifunctional protein acting in fatty acid β-oxidation (Richmond and Bleecker 1999), and *ped1* is defective in a thiolase that acts in the final step of β-oxidation (Hayashi and others 1998). Because *Arabidopsis* IBA-response mutants are defective in fatty acid β-oxidation enzymes and peroxisome biogenesis proteins, it is likely that IBA acts as an IAA precursor, and that IBA is converted to IAA in a peroxisomal process that uses at least a subset of fatty acid β-oxidation enzymes (Figure 3). The identification of genes defective in IBA-response mutants with apparently normal IBA to IAA conversion (Poupart and Waddell 2000; Zolman and others 2000) may elucidate IAA-independent roles for IBA.

Compartmentalization and Inputs to the IAA Pool

It is becoming clear that the subcellular localizations of the intermediates and enzymes controlling IAA homeostasis are potential control points. The enzymes in the Trp biosynthetic pathway are apparently chloroplastic. In contrast, most of the downstream proteins implicated in Trp-dependent IAA biosynthesis, including Trp decarboxylase, YUCCA, AAO1, and CYP83B1, appear cytoplasmic (Figure 2; Table 1), and a partially purified *Arabidopsis* IAA-synthase that converts Trp to IAA is soluble (Müller and Weiler 2000a). Only CYP79B2 and CYP79B3, the P450s that convert Trp to IAOx, possess apparent chloroplast-targeting signals (Hull and others 2000). It is possible that the IAOx made by these chloroplastic P450s is channeled to glucosinolate production, whereas the IAOx made via the YUCCA pathway is destined for IAA biosynthesis (Celenza 2001). Direct comparisons of the IAA and Trp metabolites that accumulate in *yucca* and *sur2* mutants

might reveal whether this subcellular distribution contributes to IAA homeostasis. It also will be interesting to learn where the Trp-independent IAA biosynthetic enzymes are localized.

Whereas Trp-dependent IAA biosynthesis is likely to be largely cytoplasmic, IBA to IAA conversion is almost certainly peroxisomal (Zolman and others 2000), and sequence analysis suggests that the IAA-amino acid conjugate hydrolases reside in the ER lumen (Bartel and Fink 1995; Davies and others 1999). It is intriguing that ABP1, an essential auxin binding protein (Chen and others 2001), is also predominantly localized in the ER lumen (Jones 1994), suggesting a role for auxin in this compartment. It will be informative to identify the membrane in which IAR1 resides, as the *iar1* mutant is resistant to the known substrates of the putative ER-resident hydrolases (Lasswell and others 2000). Furthermore, it is important to learn where the enzymes that catalyze IAA-amino acid conjugate formation are localized, as conjugates may need to move into the ER for hydrolysis. Although dramatic progress in identifying intercellular IAA transporters has been made in recent years (Estelle 1998; Palme and Gälweiler 1999), the apparent compartmentalization of auxin metabolism implies that intracellular transporters that move IAA out of the ER and the peroxisome (Figure 3) remain to be identified.

Future Directions

Recent advances in genetic technologies and analytical chemistry have advanced our understanding of auxin homeostasis, and are likely to contribute to future breakthroughs. Activation tagging (Weigel and others 2000) and related technologies (Wilson and others 1996; LeClere and Bartel 2001) may circumvent the redundancy (Zhao and others 2001) that has plagued analysis of auxin metabolism to date (Normanly and Bartel 1999). In addition, systematic reverse genetic screens for loss-of-function mutations in genes of interest are now routine (Krysan and others 1999), and classical genetic analysis has been accelerated by the *Arabidopsis* genome sequence completion (The Arabidopsis Genome Initiative 2000). Furthermore, screens in heterologous hosts based on enzyme activity (Corey and others 1993) or resistance to toxic compounds (Hull and others 2000) expanded to additional steps in auxin metabolism may complement traditional biochemical approaches.

As the various inputs to the IAA pool are elucidated, it will also be important to identify the enzymes that inactivate IAA (Figure 1) and determine how they are regulated. The high auxin phenotype in the *yucca* mutant is suppressed by expression of *iaaL* (Zhao and others 2001), a microbial IAA conjugating enzyme (Glass and Kosuge 1986), suggesting that IAA-inactivating activities are not sufficient in *yucca*. In contrast, endogenous conjugation pathways apparently inactivate the IAA that accumulates in *trp2* and *trp3* mutants (Normanly and others 1993) and in *CYP79B2* overexpressors (Celenza and Normanly, personal communication). Although genes encoding enzymes responsible for IAA-glucose synthesis have been cloned from maize (Szerszen and others 1994) and *Arabidopsis* (Jackson and others 2001), plant enzymes responsible for IAA- and IBA-amino acid conjugate synthesis remain to be identified. In addition, as IAA can be converted to IBA in plant extracts (Ludwig-Müller 2000), IBA synthase might contribute to IAA inactivation (Figure 1). A maize IBA synthase that is regulated by a variety of biotic and abiotic stresses (Ludwig-Müller 2000) has been partially purified (Ludwig-Müller and Hilgenberg 1995). It will be interesting to determine whether IBA synthesis impacts the free IAA pool.

A future challenge will be to determine whether *de novo* synthesis, conjugate hydrolysis, and IBA β-oxidation (Figure 1) are redundant IAA sources or instead supply specific auxin needs during development. For example, free IAA accumulates in *Arabidopsis* seedlings grown at high temperature (Gray and others 1998), resulting in high auxin phenotypes (Gray and others 1998; Rogg and others 2001), but the source of this IAA has not been identified. Mutants defective in various facets of IAA homeostasis may aid in assigning functions to individual IAA sources. Several peroxisome defective IBA-response mutants have reduced lateral root initiation, not only following exposure to IBA (Zolman and others 2000; Zolman and others 2001a), but also in the absence of exogenous auxin (Zolman and others 2001b). This defect implies that the IAA formed from endogenous IBA β-oxidation during seedling development is important for lateral root initiation. In support of this hypothesis, IBA apparently is not converted to IAA in a subset of pear plants with defects in lateral root formation (Baraldi and others 1993). In contrast, the *ilr1* and *iar3* amidohydrolase mutants have normal numbers of lateral roots (Magidin and Bartel, unpublished); the IAA released from conjugates may be less important for this process. Plants may use different IAA sources for different auxin requirements during development, and it will be interesting to learn which functions are specific to particular precursors and which can be supplied through any of several pathways.

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