INVITED REVIEW

Auxin: Regulation, Action, and Interaction

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● *Background* The phytohormone auxin is critical for plant growth and orchestrates many developmental processes.
● *Scope* This review considers the complex array of mechanisms plants use to control auxin levels, the movement of auxin through the plant, the emerging view of auxin-signalling mechanisms, and several interactions between auxin and other phytohormones. Though many natural and synthetic compounds exhibit auxin-like activity in bioassays, indole-3-acetic acid (IAA) is recognized as the key auxin in most plants. IAA is synthesized both from tryptophan (Trp) using Trp-dependent pathways and from an indolic Trp precursor via Trp-independent pathways; none of these pathways is fully elucidated. Plants can also obtain IAA by β-oxidation of indole-3-butyric acid (IBA), a second endogenous auxin, or by hydrolysing IAA conjugates, in which IAA is linked to amino acids, sugars or peptides. To permanently inactivate IAA, plants can employ conjugation and direct oxidation. Consistent with its definition as a hormone, IAA can be transported the length of the plant from the shoot to the root; this transport is necessary for normal development, and more localized transport is needed for tropic responses. Auxin signalling is mediated, at least in large part, by an SCF^{TIR1} E3 ubiquitin ligase complex that accelerates Aux/IAA repressor degradation in response to IAA, thereby altering gene expression. Two classes of auxin-induced genes encode negatively acting products (the Aux/IAA transcriptional repressors and GH3 family of IAA conjugating enzymes), suggesting that timely termination of the auxin signal is crucial. Auxin interaction with other hormone signals adds further challenges to understanding auxin response.
● *Conclusions* Nearly six decades after the structural elucidation of IAA, many aspects of auxin metabolism, transport and signalling are well established; however, more than a few fundamental questions and innumerable details remain unresolved.

Key words: Auxin, IAA, indole-3-acetic acid, 2,4-D, IBA, phytohormone, hormone signalling, proteasome, auxin biosynthesis, auxin conjugate, auxin transport, *Arabidopsis thaliana*.

INTRODUCTION

To fully understand auxin regulation, action, and interactions will be to understand many aspects of plant growth and development. As a critical plant hormone, auxin modulates such diverse processes as tropic responses to light and gravity, general root and shoot architecture, organ patterning, vascular development and growth in tissue culture (Davies, 1995). The importance of auxin for human sustenance is both vital and readily apparent: auxin is required for plant growth. Anthropogenic manipulation of auxin physiology has assisted plant propagation, and, through the blind pressure of artificial selection, the development of modern crop varieties (Multani *et al*., 2003; Salamini, 2003).

Auxin biology is among the oldest fields of experimental plant research. Charles Darwin performed early auxin experiments, observing the effects of a hypothetical substance modulating plant shoot elongation to allow tropic growth toward light (Darwin, 1880). Darwin's experiments expanded upon Theophil Ciesielski's research examining roots bending toward gravity (Ciesielski, 1872). The term auxin was coined by scientists examining plant growth-modulating substances in human urine named auxins A and B (Kögl and Haagen Smit, 1931). A structurally distinct compound with auxin activity isolated from fungi was called heteroauxin; auxins A and B were gradually abandoned for the reproducibly bioactive heteroauxin, which was later determined to be indole-3-acetic acid (IAA) (Thimann, 1977).

COMPOUNDS WITH AUXIN ACTIVITY

Because auxins influence virtually every aspect of plant growth and development, numerous bioassays for auxin response have been described. These assays have proven useful in the isolation of endogenous auxins, the identification of auxin precursors, and the development of synthetic auxin-like compounds (Thimann, 1977). One of the earliest noted auxin effects was in phototropism, the curvature of stems toward a light source (Darwin, 1880). Application of auxin to decapitated shoots can induce such bending in the absence of a light stimulus (Went, 1926), and several nonphototropic mutants are deficient in auxin signalling components (Harper *et al*., 2000; Tatematsu *et al*., 2004).

The pea curvature test also employs auxin-regulated differential growth: dark-grown (etiolated) *Pisum sativum* stems are decapitated, sliced along part of their length, and floated in solution containing compounds being tested (Wain and Wightman, 1954; Fawcett *et al*., 1960). In auxin solution, stem segments bend inward, while in water they curl outward (Went and Thimann, 1937). Other tests to establish whether a given compound exerts auxin-like effects include spraying tomato plants and application to

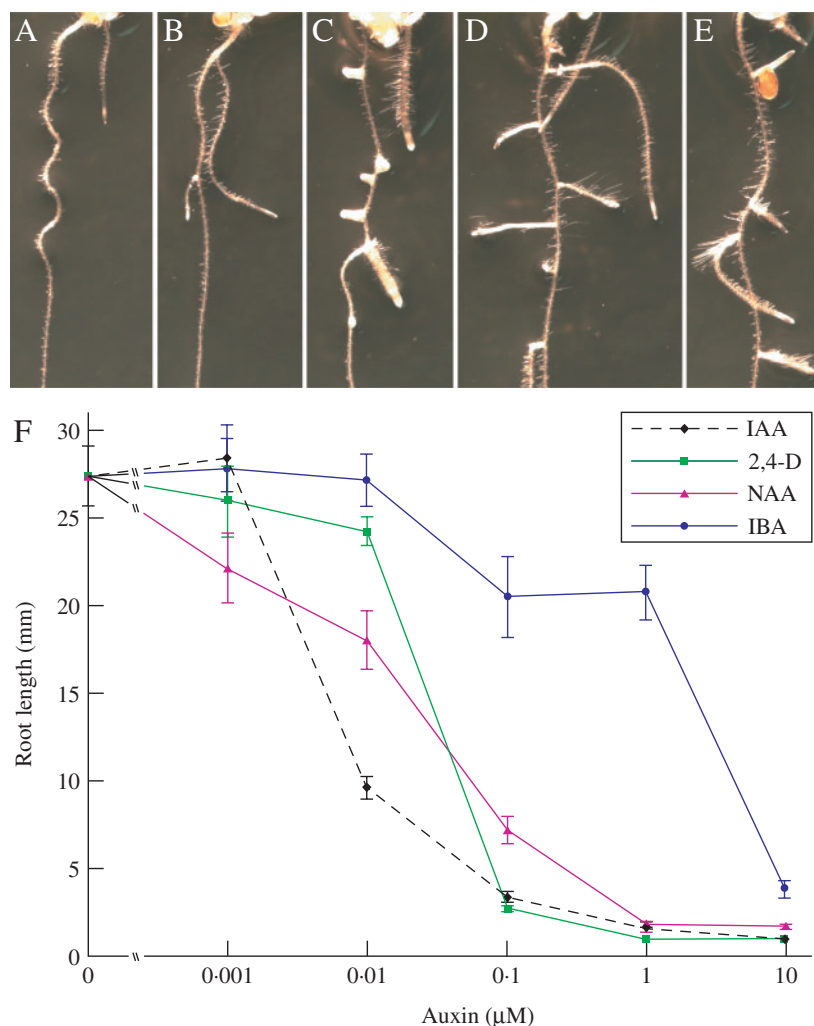* For correspondence. E-mail bartel@rice.edu

FIG. 1. Auxins promote lateral root formation and inhibit root elongation. *Arabidopsis thaliana* Col-0 ecotype plants were grown on unsupplemented medium (Haughn and Somerville, 1986) for 6 d, then transferred to unsupplemented medium (A) or medium supplemented with 10 nM IAA (B), 100 nM 2,4-D (C), 100 nM NAA (D) or 10 μM IBA (E) and grown for 6 additional days. (F) Plants were grown on various concentrations of natural and synthetic auxins for 8 d. Points represent means ± standard error, $n \geqslant 8$. All plants were grown at 22 °C under yellow light.

wheat coleoptiles, where auxin causes characteristic stem bending and elongation, respectively (Wain and Wightman, 1954; Fawcett *et al*., 1960).

Another early assay for auxin activity was in tissue culture, where auxins promote rooting from undifferentiated callus (Skoog and Miller, 1957). Along with the phytohormone cytokinin, which induces shoot formation, auxin allows regeneration of plants from cultured callus (Krikorian, 1995).

Current assays for auxin response in the model plant *Arabidopsis thaliana* often involve growth of seedlings on medium supplemented with the compound of interest. Auxins profoundly influence root morphology, inhibiting root elongation, increasing lateral root production (Fig. 1), and inducing adventitious roots (Zimmerman and Hitchcock, 1942). The relevance of these bioassays to normal plant physiology is supported by the observation that mutants that overproduce auxin tend to have abundant lateral and adventitious roots, along with long hypocotyls and

petioles, and epinastic leaves and cotyledons (Boerjan *et al*., 1995; King *et al*., 1995; Delarue *et al*., 1998; Zhao *et al*., 2001). Conversely, mutants deficient in auxin responses are often characterized by long primary roots, few lateral roots, and short hypocotyls when grown on unsupplemented medium in the light, in addition to reduced auxin responses in the bioassays described above (Estelle and Somerville, 1987; Hobbie and Estelle, 1995; Monroe-Augustus *et al*., 2003).

Many naturally occurring compounds that exert auxin-like effects have been revealed by these bioassays (Fig. 1). IAA, an extensively studied endogenous auxin, is active in all bioassays described above and is often potent at nanomolar concentrations (Fig. 1). A chlorinated form of IAA with high auxin activity, 4-Cl-IAA, is found in several plants (Slovin *et al*., 1999). In addition to the indolic auxins, phenylacetic acid (PAA) has been identified in plants and is an active auxin (Wightman, 1977; Ludwig-Müller and Cohen, 2002).

Certain IAA precursors, such as indole-3-acetonitrile and indole-3-pyruvic acid, are also active in bioassays, presumably because of conversion in the tissue to IAA (Thimann, 1977). Similarly, indole-3-butyric acid (IBA), identical to IAA except for two additional methylene groups in the side chain, is effective in bioassays. Like IAA, exogenous IBA inhibits arabidopsis root elongation (Zolman et al., 2000) and induces lateral (Zolman et al., 2000) and adventitious (King and Stimart, 1998) root formation. IBA, originally classified as a synthetic auxin, is in fact an endogenous plant compound (Epstein and Ludwig-Müller, 1993; Ludwig-Müller, 2000; Bartel et al., 2001). IBA is more effective than IAA at lateral root induction, perhaps because, unlike IAA, IBA efficiently induces lateral roots at concentrations that only minimally inhibit root elongation (Zolman et al., 2000); IBA is employed commercially for this purpose (Hartmann et al., 1990). Biochemical analyses in a variety of plants and genetic studies in arabidopsis indicate that IBA acts primarily through conversion to IAA in a process resembling peroxisomal fatty acid β-oxidation (Bartel et al., 2001), though roles for IBA independent of conversion to IAA have been proposed (Ludwig-Müller, 2000; Poupart and Waddell, 2000).

Two main types of synthetic plant growth regulators with auxin-like activity have been described: 1-naphthalacetic acid (NAA) and 2,4-D-related compounds. Both compounds exert auxin-like influences, including root elongation inhibition and lateral root promotion (Fig. 1). The NAA isomer 2-NAA has little activity in bioassays (Thimann, 1977) and provides a weak acid control for auxin experiments employing the active 1-NAA. 2,4-Dichlorophenoxybutyric acid (2,4-DB) is a 2,4-D derivative with two additional methylene groups in the side chain (analogous to the structural relationship between IBA and IAA) that elicits similar responses to those observed after 2,4-D treatment. In general, 2,4-dichlorophenoxyacetic acid (2,4-D) and IAA derivatives with even-numbered carbon side chains have more activity than derivatives with odd-numbered carbon side chains (Wain and Wightman, 1954; Fawcett et al., 1960). This result suggests that a process such as β-oxidation could remove two-carbon units from the side chains, arriving at the active acetate form if the substrate started with an even carbon number (Wain and Wightman, 1954; Fawcett et al., 1960). 2,4,5-Tricholorphenoxybutyric acid (2,4,5-TB) also exerts auxin-like activity; the infamous defoliant herbicide Agent Orange was a mixture of 2,4-D and 2,4,5-TB (Fallon et al., 1994). Agent Orange was particularly toxic because of dioxin produced as a by-product of 2,4,5-TB synthesis (Courtney et al., 1970; Schwetz et al., 1973). Today, 2,4-D alone is a widely used herbicide. In addition to NAA and 2,4-D, several alkylated and halogenated forms of IAA elicit auxin-like growth responses in various bioassays (Antolić et al., 1996; Nigović et al., 2000). Though IAA, 2,4-D, NAA, and other synthetic compounds can cause similar physiological responses in bioassays, the molecules cause distinct but overlapping changes in gene expression (Pufky et al., 2003), perhaps reflecting differences in metabolism, transport, or interaction with the signalling machinery.

IAA BIOSYNTHETIC PATHWAYS

Arabidopsis seedlings can synthesize IAA in leaves, cotyledons and roots; young leaves have the highest biosynthetic capacity (Ljung et al., 2001). Although it is widely accepted that plants use several pathways to synthesize IAA, none of the pathways is yet defined to the level of knowing each relevant gene, enzyme, and intermediate. Plant genes implicated in IAA biosynthesis are listed in Table 1, and the reactions catalysed by the encoded enzymes are illustrated in Fig. 2. Plants use both tryptophan (Trp)-dependent and Trp-independent routes to synthesize IAA; several Trp-dependent pathways have been suggested. Multiple IAA biosynthetic pathways may contribute to regulation of IAA production, but the paucity of informative loss-of-function mutations in IAA biosynthetic enzymes, coupled with functional redundancy, has limited analysis of pathway control and prevented definitive determination of the importance of each pathway. For example, arabidopsis seedlings grown at high temperature accumulate free IAA (Gray et al., 1998) and display high-auxin phenotypes (Gray et al., 1998; Rogg et al., 2001), but the source of the excess IAA is unknown.

Trp-dependent IAA biosynthesis

Several Trp-dependent pathways, which are generally named after an intermediate, have been proposed: the indole-3-pyruvic acid (IPA) pathway, the indole-3-acetamide (IAM) pathway, the tryptamine pathway, and the indole-3-acetaldoxime (IAOx) pathway. An arabidopsis enzymatic complex that converts Trp to IAA in vitro has been partially purified (Müller and Weiler, 2000b), and future biochemical and genetic dissection of the process is likely to reveal the relative importance of the pathways discussed below.

The IPA pathway [Trp → IPA → indole-3-acetaldehyde (IAAld) → IAA] is important in some IAA-synthesizing microorganisms (Koga, 1995) and may operate in plants as well (Cooney and Nonhebel, 1991). IPA is found in arabidopsis seedlings (Tam and Normanly, 1998), but genes encoding a Trp aminotransferase that oxidatively transaminates Trp to IPA or an IPA decarboxylase that converts IPA to IAAld have not been identified in plants. The final enzyme in the proposed IPA pathway is an IAAld-specific aldehyde oxidase protein (AAO1) that has increased activity in the IAA-overproducing superroot1 (sur1) mutant (Seo et al., 1998). The identification of arabidopsis AAO1 does not verify the existence of the IPA pathway, however, as IAAld may be an intermediate in other IAA biosynthetic pathways (see below).

The IAM pathway [Trp → IAM → IAA] is a second microbial pathway that also may act in plants. In Agrobacterium tumifaciens and Pseudomonas syringae, for example, Trp monooxygenase (IaaM) converts Trp to IAM, and an IAM hydrolase (IaaH) converts IAM to IAA (Patten and Glick, 1996). IAM lacks auxin activity in arabidopsis, which allows the iaaH gene to be used as a screenable marker that confers IAM sensitivity (Brusslan et al., 1993). Intriguingly, IAM is found in arabidopsis

Table 1. *Plant genes implicated in* de novo *IAA biosynthesis*

Gene	Product*	Putative localization	Loss-of-function (LOF) or overexpression (OE) phenotype	Reference
AAO1	IAAld oxidase	Cytoplasm	OE in *sur1*	Sekimoto *et al.* (1998); Seo *et al.* (1998)
AMI1	IAM hydrolase	Not reported	Not reported	Pollmann *et al.* (2003)
CYP79B2, *CYP79B3*	P450 monooxygenases	Chloroplast	LOF: *cyp79B2 cyp79B3*: low glucosinolates, IAN, and IAA OE: resistant to Trp analogues; high indolic glucosinolate, IAN, and IAA–X levels	Zhao *et al.* (2002); Hull *et al.* (2000); Mikkelsen *et al.* (2000);
CYP83B1/SUR2	P450 monooxygenase	Cytoplasm	LOF: high IAA, IAAld, and IAA–Asp levels; normal IAN levels; low indolic glucosinolate levels; altered Trp biosynthetic gene expression; defective photomorphogenesis in red light OE: high indolic glucosinolate levels, reduced apical dominance	Delarue *et al.* (1998); Barlier *et al.* (2000); Bak *et al.* (2001); Smolen and Bender (2002); Hoecker *et al.* (2004) Bak *et al.* (2001)
NIT1	Nitrilase	Not reported	LOF: IAN resistant, normal IAA levels	Normanly *et al.* (1997)
NIT2	Nitrilase	Not reported	OE: increased sensitivity to IAN, normal IAA levels	Normanly *et al.* (1997)
ZmNIT2	Nitrilase (maize)	Not reported	Not reported	Park *et al.* (2003)
SUR1/RTY/ALF1/HLS3	C-S lyase	Not reported	LOF: high IAA and IAA–X levels, low glucosinolates	Boerjan *et al.* (1995); Celenza *et al.* (1995); King *et al.* (1995); Golparaj *et al.* (1996); Lehman *et al.* (1996); Mikkelsen *et al.* (2004)
TDC	Trp decarboxylase (*C. roseus*)	Cytoplasm	OE: enhanced root curling	De Luca *et al.* (1989); Guillet *et al.* (2000)
TSA1/TRP3	Trp synthase α	Chloroplast	LOF: high IAA–X, IAN, indole-glycerol phosphate, and indolic glucosinolate levels; normal free IAA; low Trp; Trp auxotroph	Normanly *et al.* (1993); Müller and Weiler (2000*a*); Ouyang *et al.* (2000)
TSB1/TRP2	Trp synthase β	Chloroplast	LOF: high IAA–X and IAN levels, normal free IAA, low Trp, Trp auxotroph	Normanly *et al.* (1993); Ouyang *et al.* (2000)
ORP	Trp synthase β (maize)	Chloroplast	LOF: high IAA–X, normal free IAA, Trp auxotroph	Wright *et al.* (1991, 1992)
YUCCA, *YUCCA2*	FMO-like	Cytoplasm	LOF: no phenotype OE: high IAA levels	Zhao *et al.* (2001)
FLOOZY	FMO-like (petunia)	Not reported	LOF: defective leaf venation and apical dominance OE: high IAA levels	Tobeña-Santamaria *et al.* (2002)

*Listed genes are from arabidopsis unless otherwise noted.

seedlings at levels similar to free IAA (Pollmann *et al.*, 2002), and an arabidopsis amidohydrolase (AMI1) converts IAM to IAA *in vitro* (Pollmann *et al.*, 2003). It will be interesting to learn whether disruption of *AMI1* or *AAO1* decreases IAA levels.

YUCCA may catalyse a rate-limiting step in a tryptamine pathway

A tryptamine (TAM) pathway [Trp → TAM → N-hydroxyl-TAM → indole-3-acetaldoxime (IAOx) → IAAld → IAA] could also convert Trp to IAA (Fig. 2). Trp decarboxylase converts Trp to tryptamine in the first committed step in the biosynthesis of *Catharanthus roseus* monoterpenoid indole alkaloids (Facchini *et al.*, 2000). The arabidopsis genome contains potential Trp decarboxylase genes, but the encoded enzymes have not been characterized, and tryptamine has not been identified in arabidopsis.

The identification of *yucca*, an IAA-accumulating mutant with classic high-auxin phenotypes (Zhao *et al.*, 2001), suggests that a tryptamine IAA biosynthetic pathway may operate in some plants. *yucca* is resistant to toxic Trp analogues, suggesting that the accumulating IAA is Trp-derived (Zhao *et al.*, 2001). The *yucca* phenotype derives from overexpression of a flavin monooxygenase (FMO)-like enzyme that oxidizes tryptamine to N-hydroxyl-tryptamine *in vitro* (Zhao *et al.*, 2001). The homologous *Petunia × hybrida* enzyme FLOOZY is defective in a mutant deficient in leaf venation and apical dominance (Tobeña-Santamaria *et al.*, 2002). Although the loss-of-function *floozy* mutant has wild-type IAA levels, over-expressing *FLOOZY* results in increased IAA levels in shoot apices and young leaves (Tobeña-Santamaria *et al.*, 2002). YUCCA may be a rate-limiting enzyme in the tryptamine pathway, but a test of this hypothesis is hampered by genetic redundancy. Arabidopsis has a family of ten
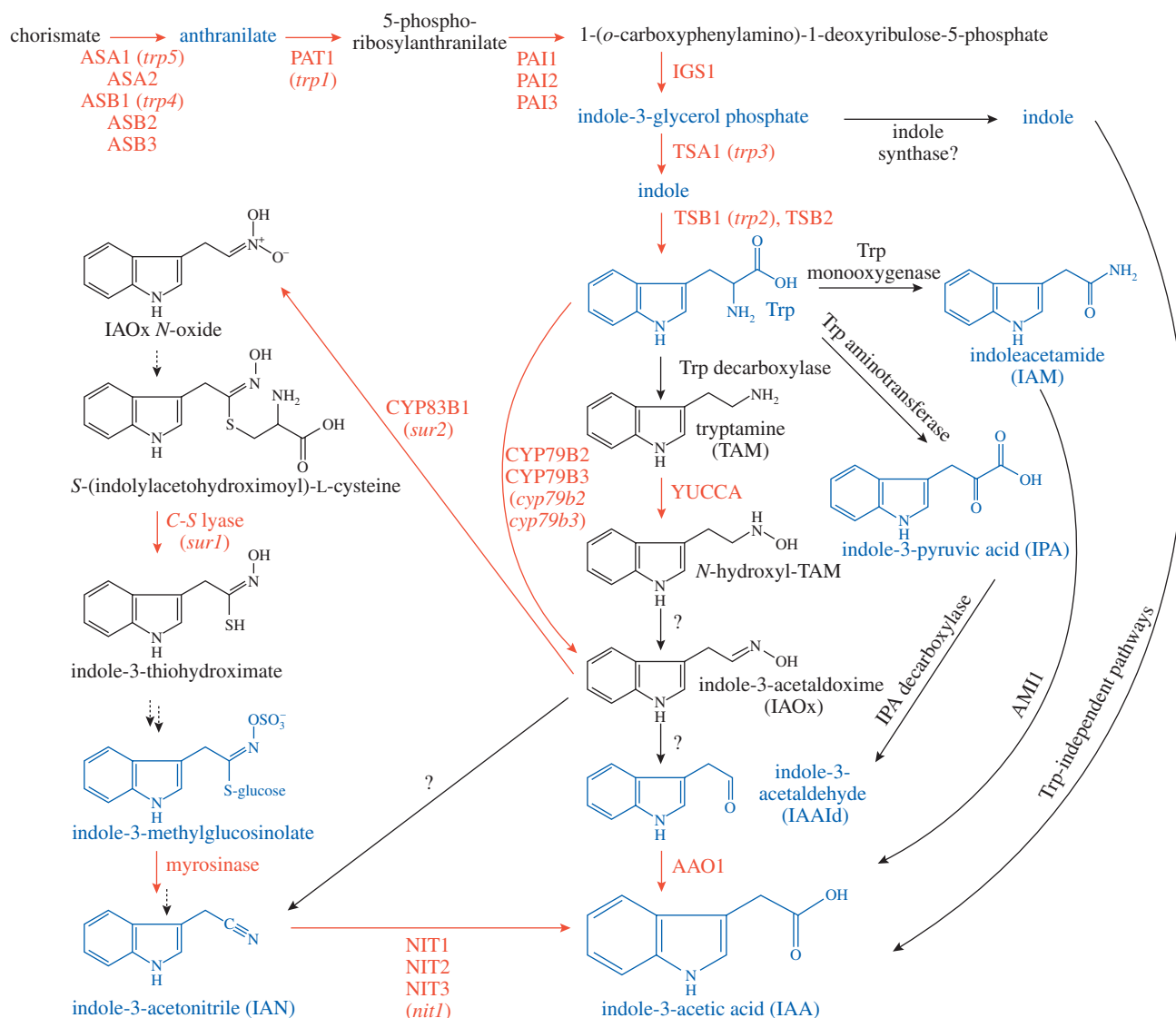
FIG. 2. Potential pathways of IAA biosynthesis in arabidopsis. *De novo* IAA biosynthetic pathways initiate from Trp or Trp precursors. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are identified are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which genes are not identified are indicated with question marks. Trp biosynthesis and the P450-catalysed conversion of Trp to IAOx are chloroplastic, whereas many Trp-dependent IAA biosynthetic enzymes are apparently cytoplasmic. See Table 1 for references.

YUCCA-like enzymes, and insertional mutations in *YUCCA* and *YUCCA2* confer no morphological phenotypes (Zhao *et al*., 2001). The *N*-hydroxyl-tryptamine produced by YUCCA could be dehydrogenated to IAOx or dehydrogenated and hydrolysed to IAAld (Fig. 2). Enzymes that catalyse these conversions have not been identified.

Indole-3-acetaldoxime is a precursor to indolic glucosinolates that can be converted to IAA

The IAOx pathway [Trp → IAOx → IAN or IAAld → IAA] is of particular interest in plants like arabidopsis that make indolic glucosinolate secondary metabolites (Fahey *et al*., 2001), because IAOx is the branch-point between indole-3-methylglucosinolate and IAA biosynthesis (Fig. 2). Two arabidopsis P450 monooxygenases,

CYP79B2 and CYP79B3, oxidize Trp to IAOx *in vitro* (Hull *et al*., 2000; Mikkelsen *et al*., 2000). *CYP79B2* overexpressors have increased IAA, IAN (Zhao *et al*., 2002) and indolic glucosinolate levels (Mikkelsen *et al*., 2000). Conversely, the *cyp79B2 cyp79B3* double mutant has morphological phenotypes suggestive of low auxin, reduced IAA in certain growth conditions, lowered IAN levels, and no detectable indolic glucosinolates (Zhao *et al*., 2002). Taken together, these results are consistent with IAOx serving as a precursor that can be shunted to either auxin or indolic glucosinolates.

A third P450 monooxygenase, CYP83B1, converts IAOx to its *N*-oxide, the first committed step in indole-3-methylglucosinolate biosynthesis (Fig. 2; Bak *et al*., 2001). Loss-of-function *cyp83b1* alleles were independently isolated in screens for high-auxin seedling

phenotypes (*superroot2* or *sur2*; Delarue *et al*., 1998), altered resistance to toxic Trp analogues (Smolen and Bender, 2002), defective photomorphogenesis in red light (Hoecker *et al*., 2004), and P450 monooxygenase insertional disruptions (Winkler *et al*., 1998). The *sur2/ cyp83B1* mutant accumulates free IAA (Delarue *et al*., 1998; Barlier *et al*., 2000) and the IAA precursor IAAld (Barlier *et al*., 2000). This phenotypic analysis, along with the nature of the defective gene, suggests that IAOx accumulates in the mutant and is converted to IAAld, which is oxidized to IAA (Fig. 2).

The *sur1* mutant (Boerjan *et al*., 1995), also isolated as *rooty* (King *et al*., 1995), *alf1* (Celenza *et al*., 1995) and *hookless3* (Lehman *et al*., 1996), provides another link between high auxin and defects in glucosinolate production. This mutant has high-auxin phenotypes resembling *sur2* and *yucca*, and accumulates free IAA and IAA conjugates (Boerjan *et al*., 1995; King *et al*., 1995; Lehman *et al*., 1996). *sur1* is defective in a *C-S* lyase that apparently cleaves *S*-(indolylacetohydroximoyl)-L-cysteine to indole-3-thiohydroximate, the third step in glucosinolate production from IAOx (Golparaj *et al*., 1996; Mikkelsen *et al*., 2004). Indeed, indolic glucosinolates are undetectable in *sur1* (Mikkelsen *et al*., 2004). Given the multiplicity of available pathways to modulate IAA levels, it is intriguing that arabidopsis plants cannot adequately compensate for the increased IAA precursor levels that result when indolic glucosinolate production is dampened.

Indole-3-acetonitrile and nitrilases in IAA biosynthesis

Nitrilases that can hydrolyse IAN to IAA are found in several plant families, including crucifers and grasses (Thimann and Mahadevan, 1964). These enzymes are encoded by the arabidopsis *NIT* genes (Bartling *et al*., 1992, 1994; Bartel and Fink, 1994) and *Zea mays* (maize) *ZmNIT2* (Park *et al*., 2003). NIT1 and NIT2 can hydrolyse IAN applied to plants (Schmidt *et al*., 1996; Normanly *et al*., 1997), and an enzymatic complex with nitrilase immunoreactivity converts Trp to IAA *in vitro* (Müller and Weiler, 2000*b*). IAN is present in arabidopsis (Normanly *et al*., 1993; Ilić *et al*., 1996) and maize (Park *et al*., 2003), suggesting that this conversion could contribute to IAA homeostasis. In the brassica, IAN is formed following myrosinase-catalysed indole-3-methylglucosinolate hydrolysis, and IAN levels tend to track with indolic glucosinolate levels in arabidopsis mutants (Normanly *et al*., 1993; Mikkelsen *et al*., 2000; Müller and Weiler, 2000*a*; Reintanz *et al*., 2001; Zhao *et al*., 2002), consistent with nitrilases acting downstream of glucosinolates in arabidopsis. However, it has also been suggested that IAN is an intermediate in IAOx to IAA conversion (Fig. 2), although enzymes catalysing the conversion of IAOx to IAN have not been isolated, and the source of IAN in maize, which lacks indolic glucosinolates, is unknown.

NIT1 is the most highly expressed of the four arabidopsis *NIT* genes (Bartel and Fink, 1994). *nit1* mutants are resistant to exogenous IAN (Normanly *et al*., 1997), but lack obvious low-auxin phenotypes, indicating that any role played by NIT1 in IAA biosynthesis is redundant. The *NIT2* gene is normally expressed at a low level, but is induced by a bacterial pathogen (Bartel and Fink, 1994), by *Plasmodiophora* (Grsic-Rausch *et al*., 2000), during arabidopsis leaf senescence (Quirino *et al*., 1999), and in response to IAN treatment (Grsic *et al*., 1998). *NIT2* induction correlates with decreased IAN levels and increased IAA levels during senescence (Quirino *et al*., 1999), increased IAA levels in *Plasmodiophora*-infected roots (Grsic-Rausch *et al*., 2000) and higher nitrilase immunoreactivity (Müller and Weiler, 2000*a*) in the IAN-accumulating *trp3* mutant (Normanly *et al*., 1993). *NIT3* expression is induced by sulfur starvation, and is correlated with reduced indolic glucosinolate levels and lateral root proliferation (Kutz *et al*., 2002). Expression of maize nitrilase *ZmNIT2* is elevated in embryonic tissue (Park *et al*., 2003). Upgrading these correlations between expression and IAA levels to causal relationships awaits the analysis of additional *nit* family mutants and would be aided by an arabidopsis *nit1 nit2 nit3* triple mutant.

Analyses of trp *mutants reveal Trp-independent IAA biosynthesis*

In addition to the proposed Trp-dependent IAA biosynthetic pathways (Fig. 2), analyses of Trp biosynthetic mutants demonstrate that plants also can synthesize IAA without using a Trp intermediate. The arabidopsis *trp3-1* and *trp2-1* mutants are defective in Trp synthase α and β, respectively (Last *et al*., 1991; Radwanski *et al*., 1996). These mutants accumulate amide- and ester-linked IAA conjugates (Normanly *et al*., 1993; Ouyang *et al*., 2000), despite having low soluble Trp levels (Müller and Weiler, 2000*a*; Ouyang *et al*., 2000). Similarly, the maize *orange pericarp* Trp synthase β mutant accumulates IAA conjugates (Wright *et al*., 1991, 1992). Unlike *trp2* and *trp3*, plants blocked earlier in the Trp pathway, such as *trp1* (Last and Fink, 1988) and antisense plants with decreased indole-3-glycerol phosphate synthase (IGS) levels, do not accumulate IAA conjugates (Normanly *et al*., 1993; Ouyang *et al*., 2000).

Analyses of the *trp* mutants imply that a Trp-independent IAA biosynthetic pathway branches from indole-3-glycerol phosphate or indole (Fig. 2). Trp synthase α and β normally channel indole-3-glycerol phosphate to Trp without indole release. In maize, however, Trp synthase α-like enzymes can act without β subunits to produce indole released as a volatile or converted into certain defense compounds (Frey *et al*., 1997, 2000; Melanson *et al*., 1997) or possibly IAA. Arabidopsis contains two apparent Trp synthase α genes: *TSA1*, the gene defective in the *trp3* mutant (Radwanski *et al*., 1996), and a second uncharacterized gene (*At4g02610*).

Because IAA conjugates are hydrolysed under alkaline conditions (Bialek and Cohen, 1986; Baldi *et al*., 1989), total (free plus conjugated) IAA is often inferred without knowledge of the conjugates present by quantifying free IAA after alkaline hydrolysis. The specificity of the alkaline hydrolysis evidence used to support the importance of the Trp-independent pathway has been questioned (Müller and Weiler, 2000*a*). Application of this technique requires
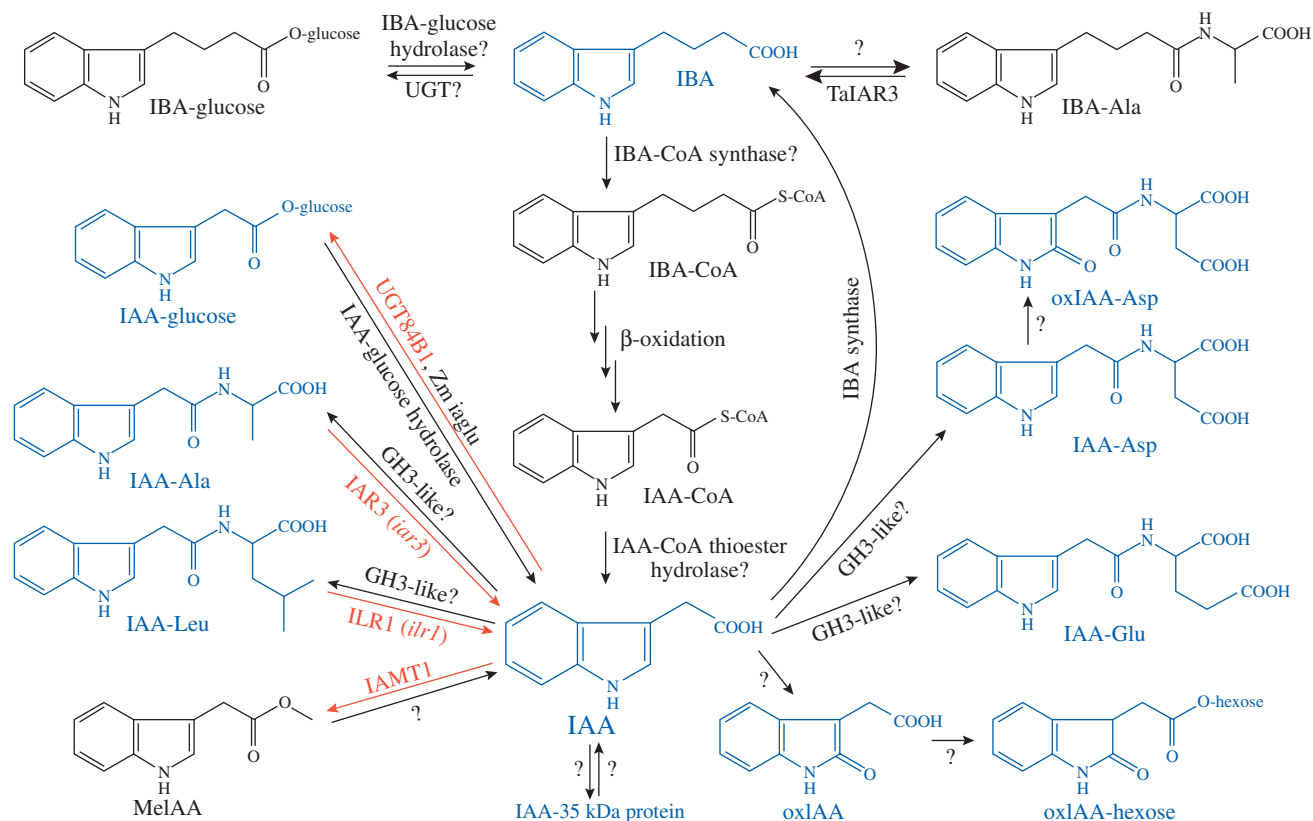
FIG. 3. Potential pathways of IAA metabolism. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are cloned are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which plant genes are not identified are indicated with question marks. A family of amidohydrolases that apparently resides in the ER lumen can release IAA from IAA conjugates. ILR1 has specificity for IAA–Leu (Bartel and Fink, 1995), whereas IAR3 prefers IAA–Ala (Davies *et al*., 1999). Maize (Zm) iaglu and arabidopsis UGT84B1 esterify IAA to glucose (Szerszen *et al*., 1994; Jackson *et al*., 2001); the enzymes that form and hydrolyse IAA–peptides have not been identified. IBA is likely to be converted to IAA–CoA in a peroxisomal process that parallels fatty acid β-oxidation to acetyl-CoA (Bartel *et al*., 2001). IAA can be inactivated by oxidation (oxIAA) or by formation of non-hydrolysable conjugates (IAA–Asp and IAA–Glu). IAA–amino acid conjugates can be formed by members of the GH3/JAR1 family (Staswick *et al*., 2002, 2005). OxIAA can be conjugated to hexose, and IAA–Asp can be further oxidized (Östin *et al*., 1998). IAMT1 can methylate IAA (Zubieta *et al*., 2003), but whether this activates or inactivates IAA is not known. IBA and hydrolysable IAA conjugates are presumably derived from IAA; biosynthesis of these compounds may contribute to IAA inactivation. Formation and hydrolysis of IBA conjugates may also contribute to IAA homeostasis; the wheat (Ta) enzyme TaIAR3 hydrolyses IBA–Ala (Campanella *et al*., 2004).

accommodation for the indolic biochemistry of the plant under study. For example, IAN, which is present in arabidopsis, is hydrolysed to IAA under alkaline conditions, so IAN must be separately quantified and subtracted from apparent total IAA values (Ilić *et al*., 1996). As the individual conjugates of arabidopsis are identified and quantified, it will be interesting to learn the precise conjugate profiles in the various *trp* mutants, and to reinvestigate alkaline-releasable IAA in mutant plants that lack indolic glucosinolates, for example.

An independent method to clarify biosynthetic pathways involves feeding plants isotopically labelled substrates, which, in a linear pathway, will result in isotopic enrichment of a precursor relative to its product. Intact arabidopsis seedlings do not efficiently convert [^{2}H$_5$]Trp into IAA, but the Trp precursor [^{15}N]anthranilate labels IAA more completely than Trp (Normanly *et al*., 1993), confirming the importance of Trp-independent IAA biosynthesis during normal growth. Arabidopsis shoot and root explants, however, do efficiently convert [^{2}H$_5$]Trp to IAA (Müller

et al., 1998*b*; Müller and Weiler, 2000*a*). Because the explant process may damage tissue, this result suggests that Trp-dependent IAA biosynthesis may be wound-induced in arabidopsis, as it is in bean (Sztein *et al*., 2002). Plants may switch from basal Trp-independent IAA biosynthesis to Trp-dependent pathways during stress, when more IAA may be needed (Ribnicky *et al*., 2002; Sztein *et al*., 2002). Studies examining metabolism of a recently synthesized, isotopically labelled indole may allow dissection of Trp-independent IAA biosynthesis (Ilić and Cohen, 2004).

IAA STORAGE: CONJUGATES AND INDOLE-3-BUTYRIC ACID

Higher plants can store IAA in the form of IAA conjugates and indole-3-butyric acid (IBA), which can provide free IAA upon hydrolysis or β-oxidation, respectively (Fig. 3). IAA can be ester-linked to sugars or amide-linked to amino

acids and peptides. Proposed functions for these conjugates include storage, transport, compartmentalization, excess IAA detoxification, and protection against peroxidative degradation (Cohen and Bandurski, 1982). Certain IAA conjugates are active in auxin bioassays, and several plants store IAA conjugates in seeds that are hydrolysed during germination to provide free IAA to developing seedlings. In contrast, biologically inactive conjugates present in plants are probably intermediates in IAA degradation. Analyses of arabidopsis mutants defective in various facets of IAA homeostasis are revealing the roles of the diverse IAA sources during plant growth and development.

IAA conjugate identification and functions

Different plant species have distinct IAA conjugate profiles (Cohen and Bandurski, 1982; Slovin *et al*., 1999). Experiments using alkaline hydrolysis to release free IAA from conjugates indicate that arabidopsis maintains approx. 90 % of IAA in amide linkages, with an additional approx. 10 % as ester-linked conjugates and approx. 1 % as free IAA (Normanly *et al*., 1993; Tam *et al*., 2000). Low levels of IAA–Ala, IAA–Asp, IAA–Glu and IAA–Leu are present in arabidopsis seeds (Rampey *et al*., 2004) and seedlings (Tam *et al*., 2000; Kowalczyk and Sandberg, 2001; Rampey *et al*., 2004). However, most of the amide-linked conjugates in arabidopsis seeds are solvent insoluble (Ljung *et al*., 2002), suggesting that single-amino acid conjugates constitute only part of the amide fraction in this tissue. A 35-kDa IAA–peptide is present in arabidopsis seeds; the large size of this conjugate may contribute to the solvent insolubility of amide conjugates (Ljung *et al*., 2002). Although genes encoding arabidopsis IAA–peptides have not been identified, an IAA-modified bean protein is similar to a soybean late seed maturation protein (Walz *et al*., 2002), suggesting that certain seed storage proteins may function in both amino acid and phytohormone storage. In addition to amide conjugates, the ester conjugate IAA–glucose has also been quantified in several dicotyledonous plants (including arabidopsis) and the monocot maize (Tam *et al*., 2000; Jakubowska and Kowalczyk, 2004).

Among divergent plant phyla, endogenous IAA, IAA–amide and IAA–ester levels are quite variable (Sztein *et al*., 1999). The lycophyte *Selaginella kraussiana* accumulates large quantities of conjugates, particularly IAA–amide compounds (Sztein *et al*., 1999). After feeding labelled IAA to the lycophyte *S. kraussiana*, the fern *Ceratopteris richardii* and various mosses and liverworts, varied species-specific conjugate profiles become apparent; the conjugates formed include both previously identified and unknown IAA conjugates (Sztein *et al*., 1999). These results suggest ancient roles for conjugates in plant biology.

IAA–amino acid conjugates found in plants can be classified into two groups based on bioassay activity and susceptibility to hydrolysis *in planta* or by plant enzymes. IAA–Ala and IAA–Leu efficiently inhibit arabidopsis root elongation and are substrates of arabidopsis amidohydrolases (Bartel and Fink, 1995; Davies *et al*., 1999; LeClere *et al*., 2002; Campanella *et al*., 2003; Rampey *et al*., 2004).

In arabidopsis, IAA–Ala is present at highest levels in shoots, whereas IAA–Leu accumulates in roots (Kowalczyk and Sandberg, 2001), but neither conjugate is formed at detectable levels following IAA application to seedlings or leaves (Östin *et al*., 1998; Barratt *et al*., 1999). These results suggest that IAA–Ala and IAA–Leu function to supply free IAA.

In contrast, although IAA–Asp and IAA–Glu also are present in arabidopsis (Tam *et al*., 2000; Kowalczyk and Sandberg, 2001), they are not appreciably hydrolysed by arabidopsis seedlings (Östin *et al*., 1998), and are inefficient inhibitors of root elongation (Campanella *et al*., 1996; LeClere *et al*., 2002). Tissues such as expanding leaves and roots that contain the highest free IAA levels also contain the highest levels of IAA–Asp and IAA–Glu (Kowalczyk and Sandberg, 2001). These results are consistent with an IAA catabolic role for IAA–Asp and IAA–Glu (see 'IAA inactivation' section).

Genetic analysis of IAA conjugate hydrolysis

Several mutant screens using different bioactive IAA–amino acid conjugates have been conducted. If conjugates with auxin activity function solely through free IAA release, then conjugate-resistant mutants that retain wild-type sensitivity to IAA may have defects in conjugate uptake or hydrolysis. If bioactive conjugates play additional roles, these also may be uncovered through mutant analyses. *ilr1* was isolated as an IAA–Leu resistant mutant with reduced sensitivity to root elongation inhibition caused by exogenous IAA–Leu. *ilr1* is defective in an amidohydrolase that cleaves IAA–Leu and IAA–Phe (Bartel and Fink, 1995). Similarly, *iar3* is IAA–Ala resistant and is defective in an amidohydrolase homologous to ILR1 that specifically hydrolyses IAA–Ala (Davies *et al*., 1999). The ILR1-like protein ILL2 is the most active IAA amidohydrolase *in vitro* (LeClere *et al*., 2002); however, no *ill2* alleles were isolated in genetic screens for conjugate-resistant root elongation. Though *ILR1* and *IAR3* are expressed in seedling roots, *ILL2* appears to be expressed predominantly in the shoot (Rampey *et al*., 2004). An *ill2* T-DNA allele is sensitive to IAA–Leu, IAA–Phe and IAA–Ala, but, when combined in double and triple mutants with *ilr1* and *iar3*, *ill2* contributes to IAA–Phe resistance in roots and hypocotyls and IAA–Ala resistance in hypocotyls (Rampey *et al*., 2004).

Interestingly, *ilr1 iar3 ill2* triple mutant seedlings display reductions in lateral root number, hypocotyl elongation in the light, sensitivity to exogenous IAA, and free IAA levels (Rampey *et al*., 2004). These results suggest that the endogenous IAA conjugate substrates of these hydrolases (IAA–Ala and IAA–Leu) are physiologically relevant sources of free IAA. The IAA–Leu insensitivity of the *ilr1 iar3 ill2* mutant implies that at least some IAA conjugates with auxin activity act solely via their hydrolysis to free IAA. However, the triple hydrolase mutant retains partial responsiveness to IAA–Ala (Rampey *et al*., 2004), suggesting that IAA–Ala has some hydrolysis-independent activity or that additional enzymes hydrolysing IAA–Ala remain to be discovered.

The *iar1* mutant is resistant to the known substrates of the ILR1 and IAR3 amidohydrolases and is defective

T ABLE 2. *Plant genes implicated in IAA conjugate metabolism*

Gene	Product*	Putative localization	Loss-of-function (LOF) or overexpression (OE) phenotype	Reference
ILR1	IAA–amino acid amidohydrolase	ER lumen	LOF: IAA–Leu resistant	Bartel and Fink (1995)
IAR3	IAA–amino acid amidohydrolase	ER lumen	LOF: IAA–Ala resistant	Davies *et al.* (1999)
TaIAR3	IBA–Ala amidohydrolase (wheat)	Not reported	Not reported	Campanella *et al.* (2004)
ILL1	IAA–amino acid amidohydrolase	ER lumen	Not reported	Bartel and Fink (1995)
ILL2	IAA–amino acid amidohydrolase	ER lumen	LOF: enhances IAA–amino acid conjugate resistance of *ilr1* and *iar3*	Bartel and Fink (1995); Rampey *et al.* (2004)
GH3.1	Putative IAA–amino acid synthase	Not reported	LOF: IAA hypersensitive	Staswick *et al.* (2005)
YDK1/GH3.2	IAA–amino acid synthase	Not reported	OE: dwarf, few lateral roots, de-etiolated LOF: IAA hypersensitive	Takase *et al.* (2004); Staswick *et al.* (2005)
GH3.3	IAA–amino acid synthase	Not reported	None reported	Staswick *et al.* (2005)
GH3.4	IAA–amino acid synthase	Not reported	None reported	Staswick *et al.* (2005)
GH3.5	IAA–amino acid synthase	Not reported	LOF: IAA hypersensitive	Staswick *et al.* (2005)
DFL1/GH3.6	IAA–amino acid synthase	Not reported	OE: IAA resistant, few lateral roots, dwarf, short hypocotyl in light, accumulates IAA–Asp	Nakazawa *et al.* (2001); Staswick *et al.* (2005)
GH3.17	IAA–amino acid synthase	Not reported	LOF: IAA hypersensitive	Staswick *et al.* (2005)
IAR1	Putative ZIP family transporter	Membrane	LOF: IAA–amino acid conjugate resistant	Lasswell *et al.* (2000)
ILR2	Novel protein	Not reported	LOF: IAA–Leu resistant	Magidin *et al.* (2003)
IAR4	Putative pyruvate dehydrogenase E1α subunit	Mitochondrion	LOF: IAA–Ala resistant	LeClere *et al.* (2004)
ICR1	Not reported	Not reported	IAA–Phe resistant	Campanella *et al.* (1996)
ICR2	Not reported	Not reported	IAA–Phe resistant	Campanella *et al.* (1996)
ILR3	Not reported	Not reported	IAA–Leu resistant	R. A. Rampey, M. Tierney, and B. Bartel (unpubl. res.)
UGT84B1	IAA glucosyl-transferase	Not reported	OE: IAA resistant, reduced apical dominance	Jackson *et al.* (2001, 2002)
iaglu	IAA glucosyl-transferase (maize)	Not reported	Not reported	Szerszen *et al.* (1994)
IAMT1	IAA–methyl transferase	Not reported	Not reported	Zubieta *et al.* (2003)

*Listed genes are from arabidopsis unless otherwise noted.

in a membrane protein (Lasswell *et al.*, 2000) that weakly resembles the ZIP family of metal transporters (Guerinot, 2000). Although the substrate and membrane localization of IAR1 are unknown, the fact that the amidohydrolases require divalent cations such as Mn^{2+}, Co^{2+} or Cu^{2+} for activity *in vitro* (Bartel and Fink, 1995; Davies *et al.*, 1999; LeClere *et al.*, 2002) suggests that metal homeostasis could impact conjugate hydrolysis by modulating amidohydrolase activity. Further supporting a role for metal homeostasis in IAA conjugate metabolism, the IAA–Leu and IAA–Phe resistant *ilr2* mutant is also resistant to exogenous Co^{2+} and Mn^{2+} (Magidin *et al.*, 2003). Because the novel ILR2 protein appears to influence metal transport and the *ilr2* mutant has a resistance profile similar to *ilr1*, ILR2 may indirectly affect IAA-conjugate metabolism by negatively regulating transport of metals that influence ILR1 activity (Magidin *et al.*, 2003).

The IAA–Ala resistant mutant *iar4* harbours a defective mitochondrial-type pyruvate dehydrogenase E1α (LeClere *et al.*, 2004). *iar4* is generally defective in root elongation, but is resistant to several IAA–amino acid conjugates. Although a direct role for pyruvate dehydrogenase in IAA-conjugate hydrolysis is difficult to envision, the slight resistance of *iar4* to the synthetic auxin 2,4-D implies that the mutant may be generally deficient in auxin metabolism or response. It is possible that pyruvic

acid itself, or an anabolic or catabolic product, influences IAA homeostasis. Alternatively, a complex including IAR4 may function directly in IAA biosynthesis, catalysing indole-3-pyruvic acid dehydrogenation to yield IAA–CoA, a hypothetical precursor of IAA or IAA conjugates (LeClere *et al.*, 2004).

The genes defective in the *icr1* (IAA-conjugate resistant), *icr2* (Campanella *et al.*, 1996), and *ilr3* (R. A. Rampey, M. Tierney and B. Bartel, unpubl. res.) mutants have not been reported. Genes currently implicated in IAA-conjugate responses are listed in Table 2. Because *ilr2*, *ilr3*, *iar4*, *icr1* and *icr2* are each represented by a single allele isolated in forward genetic screens, it is likely that conjugate resistance screens are not yet saturated. Sequence analysis suggests that the IAA–amino acid conjugate hydrolases reside in the ER (endoplasmic reticulum) lumen (Bartel and Fink, 1995; Davies *et al.*, 1999). Interestingly, the essential auxin binding protein ABP1 (Chen *et al.*, 2001) is also predominantly ER-localized (Jones, 1994), reinforcing the possibility of a role for this compartment in auxin biology. Analysis of additional mutants may reveal genes required for conjugate import into or IAA efflux from the ER, amidohydrolase transcript accumulation, or amidohydrolase localization, activity or stability. In theory, conjugate-resistant mutants that fail to import conjugates from the medium might be isolated as well (see 'Auxin transport' section).

TABLE 3. *Arabidopsis genes implicated in IBA metabolism*

Gene	Product	Putative localization	Mutant phenotypes*	Reference
ACX1	Long chain acyl-CoA oxidase	Peroxisome	IBA resistant, sucrose independent	Hooks *et al.* (1999); Adham *et al.* (2005)
ACX3	Medium chain acyl-CoA oxidase	Peroxisome	IBA resistant, 2,4-DB resistant, sucrose independent	Eastmond *et al.* (2000); Adham *et al.* (2005)
ACX4	Short chain acyl-CoA oxidase	Peroxisome	IBA resistant, 2,4-DB resistant, sucrose independent	Rylott *et al.* (2003); Adham *et al.* (2005)
AIM1	Multifunctional protein	Peroxisome	abnormal inflorescence meristems, IBA resistant, sucrose dependent	Richmond and Bleecker (1999); Zolman *et al.* (2000)
PED1	Thiolase	Peroxisome	IBA resistant, 2,4-DB resistant, sucrose dependent	Hayashi *et al.* (1998); Zolman *et al.* (2000)
PXA1/PED3/CMT	ABC transporter-like	Peroxisome	IBA resistant, 2,4-DB resistant, sucrose dependent, fewer lateral roots	Zolman *et al.* (2001*b*); Footitt *et al.* (2002); Hayashi *et al.* (2002)
PEX5	Receptor for peroxisomal matrix protein import	Peroxisome	IBA resistant, weak sucrose dependence	Brickner *et al.* (1998); Zolman *et al.* (2000)
PEX6	ATPase	Peroxisome	IBA resistant, sucrose dependent, fewer lateral roots	Zolman and Bartel (2004)
PEX7	Receptor for peroxisomal matrix protein import	Peroxisome	IBA resistant, sucrose independent; strong sucrose dependence and fewer lateral roots in *pex7-1 pex5-1* double mutant	Woodward and Bartel (2005)
PEX14/PED2	Docking protein for PEX5	Peroxisome	IBA resistant, sucrose dependent	Hayashi *et al.* (1998); Hayashi *et al.* (2000); Monroe-Augustus (2004)
IBR1	Not reported	Not reported	IBA resistant, sucrose independent	Zolman *et al.* (2000)
IBR3	Not reported	Not reported	IBA resistant, sucrose independent	Zolman *et al.* (2000)

*All mutants are presumed loss-of-function alleles.

The endogenous auxin IBA is converted to IAA in peroxisomes

IBA is a naturally occurring auxin in a variety of plants (Epstein and Ludwig-Müller, 1993; Ludwig-Müller, 2000; Bartel *et al.*, 2001). Arabidopsis seedlings contain somewhat less free IBA than IAA (Ludwig-Müller *et al.*, 1993), although detailed studies indicating whether this trend holds at all developmental stages have not been completed. Conditions that change IAA levels tend to similarly alter IBA levels (Ludwig-Müller *et al.*, 1993), suggesting that IAA and IBA metabolism are linked. Indeed, arabidopsis seedlings fed labelled IAA make labelled IBA, suggesting that IBA is synthesized from IAA (Ludwig-Müller and Epstein, 1994). Because IBA also acts as an IAA precursor (see below), IBA could function similarly to bioactive IAA conjugates in IAA homeostasis (Bartel *et al.*, 2001).

The auxin activity of IBA results, at least in part, from its conversion to IAA (Fig. 3). Isolation and characterization of arabidopsis mutants with IBA-resistant, IAA-sensitive root elongation are clarifying our understanding of IBA action (Poupart and Waddell, 2000; Zolman *et al.*, 2000, 2001*a, b*; Zolman and Bartel, 2004; Woodward and Bartel, 2005). Mutants with specific β-oxidation defects are IBA resistant, suggesting that IBA is converted to IAA in a process paralleling fatty acid β-oxidation. Because plants β-oxidize fatty acids solely in peroxisomes (Gerhardt, 1992; Kindl, 1993), and several IBA-response mutants also have peroxisomal defects, IBA to IAA conversion is likely peroxisomal.

Peroxisomal β-oxidation of seed storage lipids provides energy to germinating seedlings in oil-seed plants like arabidopsis. As a result, arabidopsis fatty acid utilization mutants require supplemental sucrose after germination to prevent developmental arrest (Hayashi *et al.*, 1998). Similarly, many IBA-response mutants are sucrose-dependent during seedling development, have reduced rates of seed storage lipid utilization, and are IBA resistant in both root elongation and lateral root initiation (Zolman *et al.*, 2000, 2001*a, b*; Zolman and Bartel, 2004; Woodward and Bartel, 2005). These phenotypes suggest defects in the peroxisomal β-oxidation of long-chain fatty acids and IBA. Other IBA-response mutants appear to metabolize long-chain fatty acids normally during germination (Zolman *et al.*, 2000; Adham *et al.*, 2005), but may still have defects in IBA β-oxidation, perhaps due to lesions in isozymes specific to short-chain substrates and IBA.

Cloning the genes defective in several IBA-response mutants (Table 3) has substantiated the essential role of peroxisomal β-oxidation in IBA activity. In addition to the proteins required directly in peroxisomal metabolism, more than 20 proteins are required for peroxisome biogenesis and import of peroxisomal matrix proteins from the cytoplasm (Olsen, 1998; Subramani, 1998; Tabak *et al.*, 1999; Mullen *et al.*, 2001). Mutations in *PEX5* or *PEX7*, receptors that bind and transport proteins into the peroxisomal matrix (Olsen, 1998; Subramani, 1998), confer IBA-response defects (Zolman *et al.*, 2000; Woodward and Bartel, 2005). *pex5* and *pex7* are likely to have defects importing β-oxidation enzymes from the cytoplasm,

slowing β-oxidation and causing IBA resistance. Another IBA-response mutant is defective in the peroxisome biogenesis gene *PEX6* and has abnormal peroxisome morphology (Zolman and Bartel, 2004). PXA1, a membrane protein that is approx. 30 % identical to human and yeast ATP-binding cassette transporters implicated in importing long-chain fatty acids into peroxisomes (Dubois-Dalcq *et al.*, 1999; Holland and Blight, 1999), is defective in another IBA-response mutant (Zolman *et al.*, 2001*b*). Because *pxa1* is resistant to IBA and is sucrose-dependent during seedling development, PXA1 is probably necessary for the import of both IBA and fatty acids (or the corresponding CoA esters) into peroxisomes (Zolman *et al.*, 2001*b*; Footitt *et al.*, 2002; Hayashi *et al.*, 2002).

Defects in β-oxidation enzymes can also lead to IBA resistance (Table 3). Several arabidopsis peroxisomal β-oxidation defective mutants have been isolated using resistance to the IBA analogue 2,4-dichlorophenoxybutyric acid (2,4-DB) (Hayashi *et al.*, 1998), which is converted to the active synthetic auxin 2,4-D similarly to IBA β-oxidation (Wain and Wightman, 1954). 2,4-DB-resistant mutants include *acx3* (Eastmond *et al.*, 2000), *acx4* (Rylott *et al.*, 2003), *aim1* (Richmond and Bleecker, 1999) and *ped1* (Hayashi *et al.*, 1998), which are also IBA resistant (Zolman *et al.*, 2000; Adham *et al.*, 2005). *acx* mutants have defects in acyl-CoA oxidases catalysing the second step of fatty acid β-oxidation, *aim1* (*abnormal inflorescence meristem*) is a mutant in a multifunctional protein acting in the third and fourth steps of fatty acid β-oxidation (Richmond and Bleecker, 1999), and *peroxisome defective l* (*ped1*) is defective in a thiolase catalysing the final step of β-oxidation (Hayashi *et al.*, 1998). Moreover, mutations in the gene encoding PEX14/PED2, which docks PEX5 at the peroxisome membrane, confer resistance to 2,4-DB (Hayashi *et al.*, 1998, 2000) and IBA (Monroe-Augustus, 2004).

Because arabidopsis mutants defective in fatty acid β-oxidation enzymes and peroxisome biogenesis proteins are IBA resistant, IBA is likely to be converted to IAA in peroxisomes. It remains to be determined whether enzymes that catalyse fatty acid β-oxidation also directly catalyse IBA β-oxidation, or whether there are peroxisomal enzymes dedicated to IBA β-oxidation. At least some fatty acid β-oxidation enzymes appear not to act on IBA, as evidenced by the normal IBA and 2,4-DB responses of the *lacs6 lacs7* double mutant, which is sucrose dependent due to defects in peroxisomal acyl-CoA synthetases catalysing the first step of fatty acid β-oxidation (Fulda *et al.*, 2004). If IBA to IAA conversion requires dedicated enzymes, one would expect to recover IBA-response mutants defective in these isozymes that retain normal fatty acid β-oxidation. Moreover, the inferred peroxisomal localization of IBA to IAA conversion implies the existence of a hydrolase that releases IAA from the CoA ester (Fig. 3), unless this thioester is efficiently hydrolysed nonenzymatically, and a transporter that effluxes IAA or IAA–CoA out of the peroxisome. Indeed, several sucrose-independent IBA-response mutants, including *ibr1* and *ibr3*, are candidates for having defects in such functions (Zolman *et al.*, 2000).

Several peroxisome defective IBA-response mutants have reduced lateral root initiation not only following IBA exposure (Zolman *et al.*, 2000), but also in the absence of exogenous auxin (Zolman *et al.*, 2001*b*; Zolman and Bartel, 2004; Woodward and Bartel, 2005). Similarly, certain *Pyrus communis* (pear) plants with adventitious root formation defects apparently do not convert IBA to IAA (Baraldi *et al.*, 1993). These defects imply that the IAA formed from endogenous IBA β-oxidation during seedling development is important for lateral root initiation. The lateral rooting defects in the peroxisome defective IBA-response mutants (Zolman *et al.*, 2001*b*; Zolman and Bartel, 2004; Woodward and Bartel, 2005) are more severe than those of the conjugate hydrolase triple mutant (Rampey *et al.*, 2004), suggesting that conjugate hydrolysis does not fully compensate for a lack of IBA β-oxidation, and vice versa.

A few IBA-response mutants with apparently normal fatty acid β-oxidation are less sensitive than wild type to the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and auxin transport inhibitors (Zolman *et al.*, 2000). The *rib1* (resistant to IBA) mutant is in this class (Poupart and Waddell, 2000). Moreover, the *Lateral rootless* (*Lrt1*) *Oryza sativa* (rice) mutant is resistant to IAA, IBA and 2,4-D in terms of root elongation, but only IBA can restore lateral root initiation to the mutant (Chhun *et al.*, 2003). Identifying the genes defective in these IBA-response mutants may reveal IAA-independent roles for IBA or unique features of IBA biology, such as factors differentially mediating IBA and IAA transport (Rashotte *et al.*, 2003).

Like IAA, much of the IBA in plants is conjugated to other moieties through amide- and ester-linkages (Epstein and Ludwig-Müller, 1993; Ludwig-Müller, 2000). A wheat homologue of the arabidopsis IAR3 IAA–Ala hydrolase is inactive on IAA conjugates, but rather hydrolyses amino acid conjugates of IBA including IBA–Ala, which is present in wheat extracts (Campanella *et al.*, 2004). It will be interesting to learn whether the other members of the monocot amidohydrolase family have specificity for IAA– or IBA–amino acid conjugates. In arabidopsis, IBA is largely ester linked (Ludwig-Müller *et al.*, 1993), suggesting that a different family of enzymes will catalyse IBA release. Although the complete IAA and IBA conjugate profiles have not been reported for any plant, it is likely that these profiles will be complex and reflect the diversity and specificities of the corresponding conjugate hydrolases and synthases.

IAA INACTIVATION

Pathways that inactivate IAA (Fig. 3) counteract the inputs to the IAA pool. As discussed above, IAA conjugates that accumulate following exposure of arabidopsis to IAA apparently differ from those used for IAA storage, consistent with the conjugated moiety dictating the fate of the attached IAA (Cohen and Bandurski, 1982). Arabidopsis permanently inactivates applied IAA by ring oxidation to oxIAA (Fig. 3), which can then be conjugated to hexose (Östin *et al.*, 1998). In addition, IAA is conjugated to Asp and Glu after applying 5 μM IAA (Östin *et al.*, 1998), and to Asp, Glu, Gln and glucose in response to 500 μM IAA (Barratt *et al.*, 1999). Arabidopsis seedlings do not

appreciably hydrolyse IAA–Asp and IAA–Glu, and IAA–Asp can be further oxidized to oxIAA–Asp (Östin *et al.*, 1998), reinforcing the catabolic nature of Asp conjugation. The catabolic conjugation system is probably present during normal growth, because IAA–Asp and IAA–Glu are present at low levels in arabidopsis seedlings (Tam *et al.*, 2000; Kowalczyk and Sandberg, 2001; Rampey *et al.*, 2004).

In response to elevated IAA levels, catabolic conjugation pathways may be up-regulated and storage conjugation pathways down-regulated. For example, the *sur2* mutant accumulates free IAA (see above) and IAA–Asp (Barlier *et al.*, 2000), an intermediate in permanent IAA inactivation (Normanly, 1997; Slovin *et al.*, 1999). However, *sur2* plants inefficiently form the putative arabidopsis IAA storage compound IAA–Leu (Barlier *et al.*, 2000). The high-auxin phenotype in the *yucca* mutant is suppressed by expressing *iaaL* (Zhao *et al.*, 2001), a microbial IAA-conjugating enzyme (Glass and Kosuge, 1986), suggesting that IAA-conjugating activities are insufficient in *yucca*. In contrast, the *trp2* and *trp3* mutants apparently accumulate IAA conjugates but not free IAA (Normanly *et al.*, 1993), implying that the conjugation pathways are sufficient to accommodate the accumulating IAA in this case.

A screen for mutants accumulating new conjugates following exposure to prolonged high-IAA challenge revealed that the photorespiration mutant *gluS* accumulates IAA–Gln at the expense of IAA–Asp following IAA treatment (Barratt *et al.*, 1999). Because the *gluS* mutant, which is defective in chloroplastic glutamate synthase, has increased soluble Gln levels (Somerville and Ogren, 1980), this altered conjugate profile implies that conjugates formed following IAA challenge are, in part, dependent on amino acid pool sizes.

Remarkably, the enzymes that conjugate IAA to amino acids are encoded by members of the *GH3* family of auxin-induced genes (see 'Auxin-induced transcripts' section). These enzymes are in the luciferase superfamily (Staswick *et al.*, 2002) and are related to the JAR1 enzyme that conjugates the hormone jasmonic acid to amino acids (Staswick and Tiryaki, 2004). Two members of the arabidopsis *GH3* family have been uncovered as genes overexpressed in dwarf mutants with reduced apical dominance, *dfl1-D* (Nakazawa *et al.*, 2001) and *ydk1-D* (Takase *et al.*, 2004). *dfl1-D* is resistant to applied IAA, and both *dfl1-D* and *ydk1-D* have reduced lateral rooting and hypocotyl elongation (Nakazawa *et al.*, 2001; Takase *et al.*, 2004). These phenotypes are consistent with decreased free auxin levels, which would be expected when overexpressing an IAA-conjugating enzyme; indeed, IAA–Asp levels are elevated in *dfl1-D* (Staswick *et al.*, 2005). Further, disruption of certain *GH3* genes confers hypersensitivity to specific forms of auxin conjugated by the encoded GH3 (Staswick *et al.*, 2005). The characterized GH3-like enzymes apparently prefer to synthesize inactivating (IAA–Asp and –Glu) over hydrolysable (IAA–Ala and -Leu) conjugates *in vitro* (Staswick *et al.*, 2005); it will be interesting to learn which enzymes are responsible for synthesizing hydrolysable conjugates *in vivo*.

In addition to IAA–amino acid conjugates, the esterified conjugate IAA–glucose is found in plants (Chisnell and Bandurski, 1988; Tam *et al.*, 2000; Jakubowska and Kowalczyk, 2004) and plant proteins with IAA glucosyl-transferase activity have been identified (Leznicki and Bandurski, 1988; Szerszen *et al.*, 1994; Jackson *et al.*, 2001; Jakubowska and Kowalczyk, 2004). Maize *iaglu* (Szerszen *et al.*, 1994) and arabidopsis *UGT84B1A* (Jackson *et al.*, 2001) encode glucosyl transferases that conjugate IAA to glucose. Overexpressing *UGT84B1* renders plants resistant to exogenous IAA and disrupts gravitropism (Jackson *et al.*, 2002), consistent with a role in IAA inactivation. Paradoxically, free IAA levels are actually increased in *UGT84B1*-overexpressing lines (Jackson *et al.*, 2002). Because UGT84B1 also has considerable activity on IBA (Jackson *et al.*, 2001), it or a related glucosyl transferase may be responsible for the IBA–glucose formed from labelled IBA fed to arabidopsis seedlings (Ludwig-Müller and Epstein, 1993).

An enzyme that methylates the carboxyl side chain of IAA has recently been described (Zubieta *et al.*, 2003). This enzyme, IAMT1, is a member of a family of carboxyl methyltransferases; other members methylate plant hormones such as jasmonic acid (JA) and salicylate (Zubieta *et al.*, 2003). Methylation will increase the volatility of IAA, but it is not clear whether this modification activates or inactivates the hormone. In the case of JA, overexpressing the gene encoding jasmonate methyltransferase JMT increases resistance to a fungal pathogen (Seo *et al.*, 2001), implying that methyl jasmonate is an active signalling molecule. Understanding of the *in vivo* roles of methylated IAA awaits analysis of plants with increased and decreased accumulation of this derivative.

Finally, as arabidopsis can convert IAA to IBA (Ludwig-Müller and Epstein, 1994), IBA synthase might contribute to IAA inactivation (Fig. 3). An IBA synthase regulated by a variety of biotic and abiotic stresses (Ludwig-Müller, 2000) has been partially purified from maize (Ludwig-Müller and Hilgenberg, 1995), but the specific components or its effects on the free IAA pool have not been determined.

AUXIN TRANSPORT

Although many tissues can synthesize auxin (Ljung *et al.*, 2001), auxin transport is complex and highly regulated, involving many identified proteins. Chemical and genetic studies have revealed that transport of auxin to distant sites is clearly required for normal development. For example, IAA transport is necessary for proper lateral root development (R.C. Reed *et al.*, 1998; Bhalerao *et al.*, 2002), vascular development (Mattsson *et al.*, 1999), phyllotaxis (Reinhardt *et al.*, 2003), embryonic axis development (Friml *et al.*, 2003) and tropisms (Friml *et al.*, 2002).

Auxin is produced largely in shoot apical regions, historically identified as the shoot apical meristem. However, application of auxin transport inhibitors blocks IAA accumulation in the shoot apex, suggesting that apical auxin is transported from other regions, probably young leaves and developing leaf primordia (Ljung *et al.*, 2001; Avsian-Kretchmer *et al.*, 2002). IAA is transported basipetally in shoots (Lomax *et al.*, 1995) and suppresses lateral shoot growth (Thimann and Skoog, 1934). Both acropetal

(Scott and Wilkins, 1968) and basipetal (Davies and Mitchell, 1972) transport occurs in roots. Opposing directions of auxin transport in roots is achieved by spatial separation, with acropetal transport in the central cylinder and basipetal transport in the epidermis (Tsurumi and Ohwaki, 1978).

Several arabidopsis mutants are defective in proteins mediating polar auxin transport. Among the earliest genes cloned that were defective in auxin-resistant mutants was *AUX1*, which encodes a transmembrane protein similar to amino acid permeases (Bennett *et al*., 1996). AUX1, and possibly other closely related proteins, mediates influx of IAA into cells (Marchant *et al*., 1999). AUX1 is localized asymmetrically in the plasma membrane of certain cell files, facilitating directional auxin transport (Swarup *et al*., 2001, 2004). Interestingly, IAA and the synthetic auxin 2,4-D appear to be AUX1 substrates, but *aux1* remains sensitive to the synthetic compound NAA; indeed, NAA can restore *aux1* gravitropism (Yamamoto and Yamamoto, 1998).

Once IAA has entered a cell via AUX1, several factors regulate efflux. The *pin-formed* (*pin1*) arabidopsis mutant is characterized by shoot meristem defects causing inflorescences to terminate in pin-shaped points generally lacking lateral organs (Okada *et al*., 1991). *PIN1* is a member of a multigene family (including *EIR1/AGR1/PIN2*, another gene implicated in polar auxin transport) that encodes transmembrane auxin efflux facilitator proteins with homology to bacterial efflux carriers (Chen *et al*., 1998; Gälweiler *et al*., 1998; Luschnig *et al*., 1998; Müller *et al*., 1998*a*; Utsuno *et al*., 1998). Like AUX1, PIN1 is asymmetrically localized in the cell, consistent with a role in polar auxin efflux (Gälweiler *et al*., 1998).

The polar localization of PIN proteins is complex and dynamic. After a change in the gravity vector, PIN3 quickly moves from all lateral sides of columella cells specifically to the lateral side newly oriented toward gravity (Friml *et al*., 2002). Both PIN1 (Geldner *et al*., 2001) and PIN3 (Friml *et al*., 2002) rapidly cycle between the plasma membrane and unidentified endosomal compartments. PIN1 cycling is inhibited by the auxin transport inhibitor TIBA; movement from endosomal compartments to the plasma membrane is likewise inhibited by the vesicular trafficing inhibitor BFA (Geldner *et al*., 2001). PIN localization responds to cues from the PINOID serine-threonine kinase; *PINOID* overexpression or disruption alters the polar localization of PIN proteins in the cell (Friml *et al*., 2004). PIN cycling is actin-dependent (Geldner *et al*., 2001), and links between actin, polar auxin transport and gravitropism have been reported in several plants (Hou *et al*., 2003, 2004; Sun *et al*., 2004).

Flavonoids, compounds that accumulate in specific locations in light-grown arabidopsis (Buer and Muday, 2004), negatively regulate auxin transport (Brown *et al*., 2001) and PIN1 cycling (Peer *et al*., 2004). PIN protein expression and localization are altered in flavonoid mutants (Peer *et al*., 2004) and the arabidopsis *transparent testa4* (*tt4*) mutant lacks flavonoids and has increased root basipetal and acropetal IAA transport and delayed gravitropism (Buer and Muday, 2004).

In addition to PIN proteins, certain MULTIDRUG RESISTANCE-like (MDR) proteins similar to mammalian MDR proteins are necessary for polar auxin transport in arabidopsis (Noh *et al*., 2001), maize and *Sorghum bicolor* (Multani *et al*., 2003). The arabidopsis MDR proteins MDR1 and P-GLYCOPROTEIN 1 (PGP1) bind the auxin transport inhibitor NPA (Noh *et al*., 2001). Basal PIN1 localization is disrupted in the *mdr1 pgp1* double mutant (Noh *et al*., 2003). PXA1, the peroxisomal membrane transporter necessary for IBA β-oxidation (Zolman *et al*., 2001*b*), is also an MDR-like protein.

The arabidopsis mutant *transport inhibitor response 3* (*tir3*, allelic to *doc1*, *big* and *umb1*; see 'Auxin interactions with other hormones' section) has reduced auxin transport (Ruegger *et al*., 1997). The mutant, resistant to root elongation inhibition by NPA, has reduced NPA binding to microsomal fractions (Ruegger *et al*., 1997). Another mutant with pleiotropic phenotypes, *roots curl in NPA* (*rcn1*, allelic to *eer1*; see 'Auxin interactions with other hormones' section), is defective in a protein phosphatase 2A subunit (Deruère *et al*., 1999) and actually has increased basipetal auxin transport (Rashotte *et al*., 2001).

Differential transport of auxin precursors and storage forms offers a potential point of auxin regulation. Little is known about the transport of IAA conjugates. The *aux1* auxin influx carrier mutant (Bennett *et al*., 1996) is resistant to IAA–Ala and IAA–Leu (B. Bartel, unpubl. res.), as well as to IAA (Pickett *et al*., 1990), so it is possible that at least these conjugates enter cells similarly to free IAA. Alternatively, the conjugate resistance of *aux1* could reflect failure to take up IAA freed by conjugate hydrolysis.

Similarly, the *aux1* mutant (Bennett *et al*., 1996) is less sensitive than wild type to root elongation inhibition by exogenous IBA (Zolman *et al*., 2000) and IAA competes effectively for labelled IBA uptake into arabidopsis seedlings (Ludwig-Müller *et al*., 1995), consistent with a shared importer. However, experiments with labelled IBA suggest that it is transported more efficiently than IAA in roots, and labelled IBA transport is unaffected in the *aux1* mutant (Rashotte *et al*., 2003). Thus, the IBA-resistance of *aux1* root elongation may reflect resistance to IAA derived from IBA.

IBA appears not to be a good substrate of the IAA efflux carrier. Unlike wild type, roots of the *eir1/agr1/pin2* auxin transport mutant (Chen *et al*., 1998; Luschnig *et al*., 1998; Müller *et al*., 1998*a*; Utsuno *et al*., 1998) bend and enter vertically oriented medium containing substrates of the efflux carrier, such as IAA or NAA (Utsuno *et al*., 1998). However, *eir1* roots fail to bend and enter media containing 2,4-D (Utsuno *et al*., 1998) or IBA (Poupart and Waddell, 2000; Zolman *et al*., 2000), suggesting that IBA, like 2,4-D, does not use the IAA efflux carrier. In support of this observation, labelled IBA transport is unaffected in *eir1* roots and is NPA-insensitive in wild-type roots (Rashotte *et al*., 2003). Proteins specifically mediating IBA transport remain to be identified.

AUXIN SIGNALLING

Auxin-induced transcripts

IAA biosynthesis, metabolism, and transport together ensure that appropriate auxin levels are in place to

orchestrate plant development. How the signalling between auxin and downstream effectors occurs is the subject of much research. Auxin rapidly and transiently induces accumulation of at least three families of transcripts: *SMALL AUXIN-UP RNAs* (*SAURs*), *GH3*-related transcripts and *AUXIN/INDOLE-3-ACETIC ACID* (*Aux/IAA*) family members.

SAUR transcripts accumulate rapidly after auxin exposure in soybean (Walker and Key, 1982) and many other species, including arabidopsis (Gil *et al.*, 1994). Maize ZmSAUR2 is a small nuclear protein that, like the encoding transcript, is rapidly degraded (Knauss *et al.*, 2003). The short half-lives of *SAUR* mRNAs appear to be conferred by downstream elements (DSTs) in the 3′ untranslated region of the messages (Sullivan and Green, 1996). Arabidopsis mutants that stabilize DST-containing RNAs, and thus stabilize *SAUR* transcripts, have no reported morphological phenotype (Johnson *et al.*, 2000), and the function of these small RNAs remains unknown.

GH3 transcript accumulation is also induced by auxin (Hagen *et al.*, 1984) and numerous plants have auxin-responsive *GH3*-like genes. At least some IAA-induced *GH3* genes encode IAA–amino acid conjugating enzymes (Staswick *et al.*, 2005), whereas several GH3-related proteins that are not auxin regulated function to adenylate or conjugate amino acids to molecules other than IAA, including jasmonic acid (Staswick *et al.*, 2002; Staswick and Tiryaki, 2004). Thus, the auxin induction of *GH3* genes likely serves to dampen the auxin signal by inactivating IAA via conjugation (see 'IAA inactivation' section).

Like the *GH3* family, *Aux/IAA* transcripts accumulate following auxin exposure, and the encoded proteins also apparently serve to dampen auxin signalling. Induction of some *Aux/IAA* genes occurs within minutes of auxin application and does not require new protein synthesis (Abel *et al.*, 1994; Abel and Theologis, 1996). The Aux/IAA family includes 28 proteins in arabidopsis (Liscum and Reed, 2002; Remington *et al.*, 2004), and homologous genes, some of which are also auxin-induced, are present in other plants. The encoded proteins share extensive sequence identity in four conserved domains. Domain I is a transcriptional repressor (Tiwari *et al.*, 2004). Domain II is critical for Aux/IAA instability; several mutations in this domain have been isolated as gain-of-function alleles that stabilize the proteins and confer auxin-resistant phenotypes (Kim *et al.*, 1996; Rouse *et al.*, 1998; Soh *et al.*, 1999; Tian and Reed, 1999; Nagpal *et al.*, 2000; Rogg *et al.*, 2001; Fukaki *et al.*, 2002; Hamann *et al.*, 2002; Tatematsu *et al.*, 2004; Yang *et al.*, 2004). In fact, a 13-amino acid fragment of domain II from IAA7/AXR2 is sufficient to confer auxin-mediated degradation to a fusion protein (Ramos *et al.*, 2001). Domains III and IV are involved in homodimerization and heterodimerization with other Aux/IAA proteins and with AUXIN REPONSE FACTORs (ARFs) (Kim *et al.*, 1997; Ulmasov *et al.*, 1999a; Hardtke *et al.*, 2004).

Many genes with auxin-induced expression, including most *SAUR*, *GH3* and *Aux/IAA* genes, share a common sequence in their upstream regulatory regions, TGTCTC or variants, first identified from the promoter region of the pea *PS-IAA4/5* gene (Ballas *et al.*, 1993). Regions including this sequence, known as the *Auxin-Responsive Element*, or *AuxRE*, confer auxin-induced gene expression in synthetic constructs (Ulmasov *et al.*, 1995, 1997b). More recently, genome-wide profiling experiments have revealed a wealth of auxin-induced genes (Sawa *et al.*, 2002; Pufky *et al.*, 2003; Cluis *et al.*, 2004; Himanen *et al.*, 2004), many of which contain *AuxRE*s in putative regulatory regions (Pufky *et al.*, 2003; Nemhauser *et al.*, 2004).

Auxin response factors mediate auxin-induced changes in gene expression

Identification of the *AuxRE* led to isolation of ARF1, the founding member of the *AuxRE*-binding protein family, using a yeast one-hybrid screen (Ulmasov *et al.*, 1997a). ARF proteins can either activate or repress target gene transcription, depending on the nature of a central domain (Ulmasov *et al.*, 1999b; Tiwari *et al.*, 2003). ARFs can bind tandem repeat *AuxRE* sequences as homodimers, dimers with other ARFs, or dimers with repressive Aux/IAA proteins (Ulmasov *et al.*, 1997a, 1999b). Auxin responsiveness depends on ARF motifs similar to Aux/IAA protein domains III and IV and is mediated through dimerization with Aux/IAA proteins (Tiwari *et al.*, 2003). Because the dimerization domains are dispensable for ARF activation of reporter genes in *Daucus carota* protoplast assays, it is likely that ARFs can function as monomers (Tiwari *et al.*, 2003).

Mutations in several arabidopsis *ARF* genes confer gene-specific developmental defects. Mutations in *ETTIN/ARF3* (*ETT*) lead to floral abnormalities (Sessions and Zambryski, 1995; Sessions *et al.*, 1997) that can be phenocopied by NPA application to flowers (Nemhauser *et al.*, 2000). Genetic interaction between *ETT* and the *SEUSS* gene is necessary for proper floral development (Pfluger and Zambryski, 2004). Further, *seuss* mutants are defective not only in flower morphology, but are generally defective in auxin responses (Pfluger and Zambryski, 2004).

A screen for suppressors of a *hookless1* (*hls1*) mutant, which fails to form an ethylene-mediated apical hook in darkness (Lehman *et al.*, 1996), identified *hookless1 suppressor 1/arf2* (Li *et al.*, 2004). Ethylene acts through HLS1 to negatively regulate ARF2 levels and achieve an apical hook; *ARF2* overexpression, like *HLS1* deficiency, inhibits apical hook formation (Lehman *et al.*, 1996; Li *et al.*, 2004). Loss of *arf2* function has no observable effect on apical hook formation when not in combination with *hls1*, but does cause various defects in adult morphology (Li *et al.*, 2004).

Defects in *MONOPTEROS/ARF5* (*MP*), a transcriptionally activating ARF, result in aberrant seedling morphology, often with a single cotyledon and a loss of basal structures (Hardtke and Berleth, 1998). Mutations in a second activating ARF, *NON-PHOTOTROPIC HYPOCOTYL4* (*NPH4/TIR5/MSG1/ARF7*), confer deficient shoot phototropism, an auxin-mediated process (Harper *et al.*, 2000). While the phenotypes of *mp/arf5* and *nph4/arf7* initially appeared unrelated, combining a weak allele of *mp* with *nph4* in a double mutant enhances the *mp* fused cotyledon phenotype (Hardtke *et al.*, 2004), suggesting some functional overlap between the two ARFs.

Thus, mutation of several *ARF* genes confers developmental phenotypes, and abundant evidence from studies employing reporter constructs in protoplast assays implicates ARFs in auxin responsive transcription. Studies in intact plants are beginning to directly link ARFs with auxin-responsive transcription at native promoters. For example, *nph4/arf7* mutant seedlings display dramatically reduced levels of several auxin-induced transcripts both before and after auxin application (Stowe-Evans *et al*., 1998) and expression of several *Aux/IAA* genes responds to MP/ARF5 levels (Mattsson *et al*., 2003).

The diversity of *arf* mutant phenotypes makes it clear that the rules governing the interactions between ARFs and *AuxRE*s will be complex; the fact that only a few *arf* mutants have been reported indicates that much of this complexity remains to be uncovered. *ARF8* disruption leads to mild but significant high-auxin phenotypes such as stronger apical dominance, increased lateral root number, and a longer hypocotyl in the light; conversely, *ARF8* overexpression results in opposite phenotypes, suggesting reduced auxin response (Tian *et al*., 2004). These observations are initially counterintuitive, because ARF8 is an activating ARF (Ulmasov *et al*., 1999*b*). However, these results can be neatly explained by the observation that ARF8 induces expression of several *GH3* genes (Tian *et al*., 2004) involved in auxin inactivation (Staswick *et al*., 2005). Indeed, free IAA levels are reduced in hypocotyls of *ARF8* overexpression lines (Tian *et al*., 2004), demonstrating the intimate connection between auxin responses and auxin levels.

Aux/IAA proteins repress ARF function

Aux/IAA proteins interact with ARF proteins via C-terminal domains III and IV conserved between the Aux/IAA family and most ARF proteins (Ulmasov *et al*., 1999*a*). At least some Aux/IAA proteins can directly repress transcription (Tiwari *et al*., 2004), and the interaction of Aux/IAA proteins with activating ARF proteins can prevent transcriptional activation in protoplast assays (Tiwari *et al*., 2003).

It may seem counterintuitive that expression of some *Aux/IAA* genes, which function to repress auxin signalling, is auxin-induced. However, Aux/IAA protein levels plummet immediately following auxin exposure (see below). Increased transcription of *Aux/IAA* genes after an auxin stimulus is likely to be mediated by ARF proteins via *AuxRE*s in *Aux/IAA* promoter regions. Thus, Aux/IAA accumulation is subject to negative feedback; Aux/IAA protein levels decline after auxin exposure, allowing increased transcription of *Aux/IAA* genes and thereby ensuring a transient auxin response.

Gain-of-function *Aux/IAA* mutations generally reduce auxin sensitivity in root elongation assays and confer dramatic auxin-related developmental defects, including altered gravitropism and apical dominance in *axr2/iaa7* (Wilson *et al*., 1990; Nagpal *et al*., 2000), *axr3/iaa17* (Rouse *et al*., 1998) and *axr5/iaa1* (Yang *et al*., 2004), severe lateral root defects in *iaa28* (Rogg *et al*., 2001) and *slr/iaa14* (Fukaki *et al*., 2002), photomorphogenic defects in *shy2/iaa3* (Soh *et al*., 1999; Tian and Reed, 1999), hypocotyl tropism defects in *msg2/iaa19*

(Tatematsu *et al*., 2004), and embryonic patterning defects in *bdl/iaa12* (Hamann *et al*., 2002). Remarkably, these dominant missense mutations all map to a small region of domain II and several have been shown to stabilize the encoded Aux/IAA proteins (Worley *et al*., 2000; Gray *et al*., 2001; Ramos *et al*., 2001; Tiwari *et al*., 2001; Zenser *et al*., 2001), underscoring the importance of this region *in vivo*. In contrast to the dramatic defects conferred by stabilizing Aux/IAA proteins, the few reported loss-of-function *Aux/IAA* alleles confer only subtle phenotypes. Most were isolated as suppressors of gain-of-function alleles mutated in the same gene (Rouse *et al*., 1998; Tian and Reed, 1999; Nagpal *et al*., 2000). Loss-of-function *shy2/iaa3* mutants have large cotyledons and short hypocotyls (Tian and Reed, 1999), and null *axr2/iaa7* mutants have slightly longer hypocotyls than wild type (Nagpal *et al*., 2000). The dramatic phenotypes of the gain-of-function *Aux/IAA* mutants coupled with the subtle effects of losing individual *Aux/IAA* genes suggests that these genes play important but largely overlapping roles in wild-type plants.

Aux/IAA degradation is mediated by the SCF^{TIR1} ubiquitin ligase

The Aux/IAA proteins, which inhibit auxin responses, are unstable even in the absence of a stimulus (Abel *et al*., 1994). Auxin application further destabilizes Aux/IAA proteins (Gray *et al*., 2001; Zenser *et al*., 2001), which is presumed to free activating ARF proteins from repression and thereby allow auxin-induced gene expression (Fig. 4). The transient nature of auxin-induced transcription is likely to result in part from many of the *Aux/IAA* genes themselves being transcriptionally induced by auxin (Abel *et al*., 1995*b*).

Aux/IAA proteins are unstable because they are targets of ubiquitin-mediated degradation. Ubiquitin is covalently attached to substrate proteins in a three-step process that begins with activation of the ubiquitin C-terminus by an E1 enzyme, followed by ubiquitin transfer from the E1 to an E2 intermediary protein. Finally, the E2–ubiquitin complex is brought into proximity to the target protein via a specificity-providing E3 protein or protein complex, which mediates the ubiquitination of target proteins. Once targets are multiply ubiquitinated, they are substrates for degradation by the 26S proteasome. One type of E3 complex is the Skp1-Cullin-F-box (SCF) class, named for the defining components. SCF complexes are comprised of mostly general subunits, while one of numerous F-box proteins confers target specificity. The F-box protein associates with a cullin (CUL1) via a SKP adapter protein named ASK (from arabidopsis SKP1-like). CUL1 also binds to the ubiquitin E2-interacting protein RBX1 (also known as HRT1 or ROC1). Thus, the SCF complex recruits an E2 protein bearing ubiquitin to a specific protein target to facilitate ubiquitination of the target protein.

Several auxin-resistant mutants stabilize Aux/IAA proteins by disrupting components of the SCF^{TIR1} ubiquitin ligase or proteins that regulate the SCF (Table 4). *tir1* mutants are resistant to auxins, auxin transport inhibitors and the auxin mimic sirtinol (Ruegger *et al*., 1998;
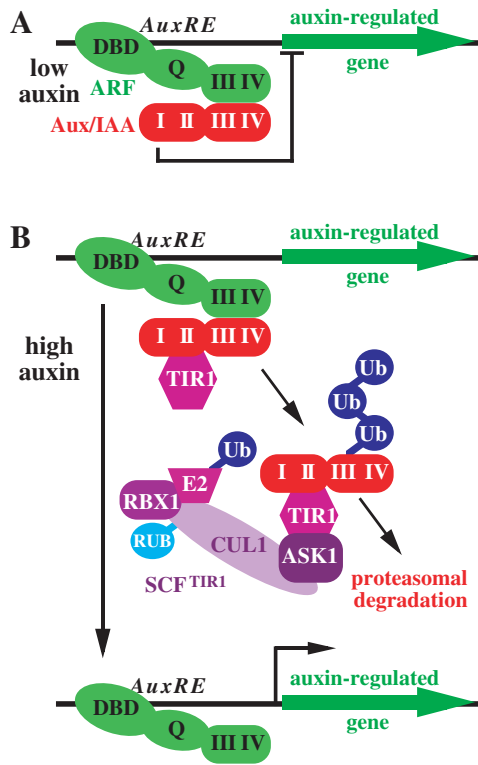
FIG. 4. The SCF^{TIR1} relieves Aux/IAA repression of activating ARFs. (A) An activating ARF protein (green) binds an *AuxRE* promoter element via an N-terminal DNA binding domain (DBD). Under low-auxin conditions, an Aux/IAA repressor (red) binds the activating ARF via heterodimerization between Aux/IAA and ARF domains III and IV. (B) Auxin promotes Aux/IAA domain II-TIR1 association, bringing the Aux/IAA protein to the SCF^{TIR1} complex (purple) for ubiquitination (Ub) and subsequent destruction by the 26S proteasome. The activating ARF, with a Gln-rich (Q) middle domain, is then freed to promote auxin-induced gene expression.

Zhao *et al*., 2003*b*). *TIR1* encodes a leucine-rich repeat-containing F-box protein (Ruegger *et al*., 1998) that lends Aux/IAA ubiquitination specificity to the SCF^{TIR1} complex. The auxin-resistant *axr6* mutant is defective in CUL1 (Hellmann *et al*., 2003), and misexpressing RBX1 results in auxin resistance (Gray *et al*., 2002). Also, ASK1 is necessary for proper auxin response, and ASK1 and ASK2, members of a 19-member family (Farrás *et al*., 2001), interact with the TIR1 F-box (Gray *et al*., 1999).

SCF^{TIR1} regulation is complex, and auxin resistance mutant screens have revealed regulatory components as well. *AXR1* encodes one subunit of a ubiquitin E1-like enzyme complex (Leyser *et al*., 1993) with the partner protein E1-LIKE CONJUGATING ENZYME-RELATED 1 (ECR1) (del Pozo *et al*., 1998, 2002). The AXR1-ECR1 dimer activates and transfers RELATED TO UBIQUITIN 1 (RUB1, known as NEDD8 in animals) to the RUB1-CONJUGATING ENZYME RCE1 (S. Dharmasiri *et al*., 2003). RUB is subsequently transferred to a specific lysine on CUL1 (del Pozo and Estelle, 1999), apparently using RBX1 as the RUB E3 ligase to facilitate RUB transfer from RCE1 to CUL1 (Gray *et al*., 2002). Although mutants defective in *ECR1* have not been reported, overexpression of ECR1 with the

active site cysteine mutated confers developmental defects in the shoot (del Pozo *et al*., 2002). Defects in AXR1 (Estelle and Somerville, 1987; Lincoln *et al*., 1990), RUB (Bostick *et al*., 2004) or RCE1 (S. Dharmasiri *et al*., 2003; Larsen and Cancel, 2004) confer auxin resistance, and *axr1 rce1* double mutants have embryonic defects similar to *mp/arf5* (S. Dharmasiri *et al*., 2003), prompting the hypothesis that RUB modification positively regulates SCF^{TIR1} activity.

Recent results suggest a more complicated situation. CONSTITUTIVE PHOTOMORPHOGENESIS 9 (COP9) is a component of the COP9 signalosome complex, which resembles the 26S proteasome lid (Li and Deng, 2003). COP9 signalosome activity is necessary both for RUB removal from CUL1 and for proper auxin response (Schwechheimer *et al*., 2001). In addition, overexpressing *RBX1* results in increased RUB-CUL1 levels and 2,4-D resistance (Gray *et al*., 2002). Thus, it appears that RUB addition to, and removal from, CUL1 are both required for SCF^{TIR1} function, implying a necessary RUB modification cycle. However, RUB deconjugation is separable from at least some developmental roles of the COP9 signalosome (Wang *et al*., 2002). Evidence that the COP9 signalosome interacts with proteasome components indicates that the COP9 signalosome may be a *bona fide* proteasome lid (Schwechheimer and Deng, 2001; Li and Deng, 2003; Peng *et al*., 2003) that could function directly in both RUB removal and ubiquitin-mediated substrate degradation.

A screen for mutations that enhance the 2,4-D resistance of *tir1* uncovered several previously identified SCF^{TIR1} components as well as *enhancer of tir1-1 auxin resistance 3* (*eta3*) (Gray *et al*., 2003). The *eta3* single mutant phenotype is similar to *tir1*, and when combined in *tir1 eta3*, enhances the *tir1* lateral root deficit, short hypocotyl and 2,4-D resistance phenotypes (Gray *et al*., 2003). *eta3* is deficient in SGT1b (Gray *et al*., 2003), a homologue of yeast SGT1, which interacts with SCF complexes (Kitagawa *et al*., 1999). While the role of SGT1b remains obscure, *eta3* is not deficient in SCF^{TIR1} assembly or auxin-responsive interaction of TIR1 with an Aux/IAA protein *in vitro* (Gray *et al*., 2003).

Further modulation of SCF is achieved by a plant orthologue of the human Cullin-Associated and Neddylation-Dissociated (CAND) protein, which is predicted to regulate SCF assembly (Liu *et al*., 2002; Zheng *et al*., 2002; Oshikawa *et al*., 2003). Defects in arabidopsis CAND1/ETA2 result in auxin resistance (Cheng *et al*., 2004; Chuang *et al*., 2004; Feng *et al*., 2004). Though CUL1 and RUB-CUL1 levels appear wild type in *cand1* mutants (Chuang *et al*., 2004; Feng *et al*., 2004), pulldowns with the SCF^{TIR1}-interacting domain of AXR2/IAA7 reveal increased CUL1, but decreased ASK1, association (Chuang *et al*., 2004). Further experiments examining SCF component and substrate interactions in *cand1* are needed to understand these results, but it is clear that CAND1 is essential for proper SCF^{TIR1} function.

Auxin induction of Aux/IAA–TIR1 interaction

Auxin application to plants (Gray *et al*., 2001) or addition to plant cell lysate (N. Dharmasiri *et al*., 2003) can quickly

T ABLE 4. *Auxin-related SCF components and SCF-regulatory genes from arabidopsis*

Gene	Function	Loss-of-function phenotype*	Assay
TIR1	Auxin F-box	Auxin resistant[1]	Root elongation inhibition
		NPA, CPD resistant[1]	Root elongation inhibition
		Reduced lateral root number[1]	
		Hypocotyl elongation defect[1]	Growth at elevated temperature
		Enhances *axr1-12* dwarfism[2]	
		Enhances *cand1* dwarfism[2]	
CUL1/AXR6	SCF scaffold	Auxin resistant[3]	Root elongation inhibition
		Embryo lethal (null)[4]	
ASK	CUL1/F-box adapter	Auxin resistant (*ask1*)[5]	Root elongation inhibition
		Reduced lateral root number (*ask1*)[5]	
		Dwarf (*ask1*)[5]	
		Floral abnormalities (*ask1* and *ask11*)[6]	
		Embryo lethal (*ask1 ask2*)[6]	
RBX1	CUL1/E2 adapter	Auxin resistant[7]	Root elongation inhibition
		Reduced lateral root number[7]	
		Dwarfism[7]	
		MeJA resistant[7]	Root elongation inhibition
		Delayed cold-induced gene expression[7]	Northern blot
RUB1 RUB2	Ubiquitin-like modifier	Auxin resistant[8]	Root elongation inhibition
		Reduced lateral root number[8]	
		Dwarfism[8]	
		Ethylene overproduction[8]	Hypocotyl elongation in darkness, GC
		Embryo lethal (null)[8]	
AXR1	RUB activating enzyme component	Auxin resistant[9]	Root elongation inhibition
		Reduced gravitropism[10]	Root reorientation
		Dwarfism (severe alleles)[9]	
		MeJA resistant[11]	Root elongation inhibition
		ACC resistant[12,13]	Hypocotyl elongation inhibition in darkness
		Enhances *cop10-4* deetiolation[7]	Growth in darkness
		Floral abnormalities[14]	
		Delayed cold-induced gene expression[7]	Northern blot
ECR1	RUB activating enzyme component	Reduced auxin-induced gene expression[14]	Northern blot of wild-type plants transformed with a mutant version of ECR1
		Dwarfism[14]	
		Floral abnormalities[14]	
RCE1	RUB E2 enzyme	Auxin resistance[10]	Root elongation inhibition
		Reduced lateral root proliferation[10]	Lateral root induction by auxin
		Reduced gravitropism[10]	Root reorientation
		Dwarfism[10]	
		MeJA resistance[10]	Root elongation inhibition
		Ethylene overproduction[15]	Gas chromatography
		Reduced hypocotyl elongation in darkness[15]	
CSN5	COP9 signalosome component	Auxin resistance[7]	Root elongation inhibition
		Reduced lateral root number[7]	
		Dwarfism[7]	
		MeJA resistance[7]	Root elongation inhibition
		Delayed cold-induced gene expression[7]	Northern blot
CAND1/ETA2	SCF regulator	Auxin resistance[2,13,17]	Root elongation inhibition
		Reduced lateral root number[2]	
		Dwarfism[2]	
		MeJA resistance[17]	Root elongation inhibition
		ACC resistance[2]	Hypocotyl elongation inhibition in darkness
		Reduced apical hook[13]	Growth in darkness
		Floral abnormalities[17]	
		ABA resistance[13]	Root elongation inhibition
		Enhanced red light response[13]	Hypocotyl elongation inhibition
		Late flowering[17]	
SGT1b	SCF regulator	Auxin resistance[16]	Root elongation inhibition
		Reduced lateral root number[16]	
		MeJA resistance[16]	Root elongation inhibition

*Red, auxin-related phenotypes; blue, ethylene-related phenotypes; purple, jasmonate-related phenotypes; green, floral development phenotypes.
[1]Ruegger *et al.* (1998); [2]Chuang *et al.* (2004); [3]Hellmann *et al.* (2003); [4]Shen *et al.* (2002); [5]Gray *et al.* (1999); [6]Zhao *et al.* (2003*a*); [7]Schwechheimer *et al.* (2002); [8]Bostick *et al.* (2004); [9]Lincoln *et al.* (1990); [10]Dharmasiri *et al.* (2003*b*); [11]Tiryaki and Staswick (2002); [12]Xu *et al.* (2002); [13]Cheng *et al.* (2004); [14]del Pozo *et al.* (2002); [15]Larsen and Cancel (2004); [16]Gray *et al.* (2003); [17]Feng *et al.* (2004).

induce Aux/IAA domain II interaction with TIR1. Thus, auxin could destabilize Aux/IAA proteins *in planta* by promoting association with the SCF^{TIR1} E3 ubiquitin ligase complex. The *in vitro* dissection of this TIR1–Aux/IAA interaction holds promise to reveal at least one of the long-sought auxin receptors.

Many substrates of SCF complexes require modification prior to ubiquitination (Deshaies, 1999). Several observations led to the early hypothesis that alterations to proline residues in domain II could provide a mechanism for auxin promotion of TIR1–Aux/IAA domain II interaction. First, several dominant auxin-resistant mutants harbour mutations altering one of two adjacent prolines within domain II (Rouse *et al.*, 1998; Soh *et al.*, 1999; Tian and Reed, 1999; Nagpal *et al.*, 2000; Rogg *et al.*, 2001; Fukaki *et al.*, 2002; Hamann *et al.*, 2002; Tatematsu *et al.*, 2004; Yang *et al.*, 2004); many of these mutations are known to stabilize the mutant Aux/IAA protein (Worley *et al.*, 2000; Ouellet *et al.*, 2001). In addition, a mutant resistant to the auxin-like effects of sirtinol is defective in SIR1, a protein containing a domain present in certain Peptidyl Prolyl *cis-trans* Isomerases (PPIases; Zhao *et al.*, 2003*b*), and the parvulin-class PPIase inhibitor juglone inhibits Aux/IAA–TIR1 interaction (N. Dharmasiri *et al.*, 2003; Tian *et al.*, 2003).

However, although proline modification in Aux/IAA domain II may be relevant to TIR1 interaction, it is not the change induced by auxin. Mass spectroscopic analysis of AXR3/IAA17 domain II reveals possible proline hydroxylation, but this state is not affected by auxin addition (Kepinski and Leyser, 2004). Indeed, auxin does not appear to cause any covalent modification of domain II (Kepinski and Leyser, 2004). In addition, sirtinol does not promote Aux/IAA–TIR1 interaction (Kepinski and Leyser, 2004); therefore, sirtinol (and SIR1) influence Aux/IAA turnover in some other way, perhaps by regulating SCF^{TIR1} or proteasome activity. SIR1 is in fact more closely related to E1 ligases than to PPIases, suggesting yet another ubiquitin-like modification (in addition to the known roles of ubiquitin and RUB) important for auxin responsiveness. Finally, juglone affects TIR1 or an associated protein, not Aux/IAA domain II, to repress Aux/IAA–TIR1 association (Kepinski and Leyser, 2004).

The signal by which auxin promotes Aux/IAA–TIR1 interaction remains unclear. Tantalizing results show that *in vitro* treatment of extracts with NAA induces modification of TIR1 or a TIR1-associated protein rather than the Aux/IAA proteins (Kepinski and Leyser, 2004). Thus the most direct hypothesis, that TIR1 is itself an auxin receptor and that association of auxin with TIR1 promotes its binding and ubiquitination of substrate Aux/IAA proteins, will soon be testable.

AUXIN INTERACTIONS WITH OTHER HORMONES

Auxin modulates hormone levels

Physiological studies suggest many links between phytohormones. Several other hormones modulate or are modulated by auxin levels and responses. One of the most-told stories in plant biology is the relationship between auxin and cytokinin, which can be employed *in vitro* to induce root and shoot development, respectively (Skoog and Miller, 1957). Auxin and cytokinin levels are inversely correlated *in vivo* (Eklöf *et al.*, 2000) and auxin treatment can rapidly inhibit cytokinin biosynthesis (Nordström *et al.*, 2004).

Auxin and the gaseous hormone ethylene are also intimately linked. Exogenous auxin exposure stimulates ethylene production (Morgan and Hall, 1962) through induction of a gene encoding the rate-limiting enzyme in ethylene biosynthesis (Abel *et al.*, 1995*a*). Conversely, ethylene inhibits lateral (Burg and Burg, 1966) and basipetal (Suttle, 1988) auxin transport.

As with ethylene, auxin elicits increased gibberellic acid (GA) production (Ross *et al.*, 2000), and basipetally transported auxin is necessary for the production of the active gibberellins GA$_1$ and GA$_3$ in barley (Wolbang *et al.*, 2004). GAs act, at least in part, through promoting degradation of DELLA repressors (Silverstone *et al.*, 2001); disrupting auxin transport precludes GA-mediated DELLA protein degradation (Fu and Harberd, 2003).

Auxin response is also connected to brassinosteroids (BRs), which act in concert with auxin to promote root gravitropic curvature in maize (Kim *et al.*, 2000). BR and auxin treatments induce accumulation of many of the same transcripts (Goda *et al.*, 2004; Nemhauser *et al.*, 2004). Exposure to the hormone abscisic acid (ABA) decreases free IAA levels while increasing esterified IAA conjugates in muskmelon ovaries (Dunlap and Robacker, 1990). Antagonistic to auxin, exogenous ABA inhibits lateral root formation (De Smet *et al.*, 2003).

The SCF links auxin signalling with other stimuli

Several mutants with defects in auxin response are also defective in responses to other phytohormones, suggesting communication or cross-talk between phytohormone response pathways. For example, mutations in the general components comprising or regulating the SCF cause deficient responses to multiple hormonal and environmental stimuli (Schwechheimer *et al.*, 2002). Though the full phenotypic complexity of mutants compromised in multiple pathways is likely to be revealed in years to come, pleiotropic defects have already been reported for many mutants with compromised auxin response (Table 4).

axr1 mutants fail to efficiently modify CUL1 with RUB (del Pozo *et al.*, 2002) and are resistant to the root elongation inhibition caused by auxins (Estelle and Somerville, 1987), cytokinin, ethylene (Timpte *et al.*, 1995), methyl jasmonate (Schwechheimer *et al.*, 2002) and epi-brassinolide (Tiryaki and Staswick, 2002). These data suggest that RUB modification is necessary for active SCF complexes and hormone responses in addition to SCF^{TIR1} and auxin.

Indeed, SCF^{COI1} is necessary for jasmonate responses (Xie *et al.*, 1998), and the RUB E2 enzyme-defective mutant *rce1* is auxin and methyl-jasmonate resistant (S. Dharmasiri *et al.*, 2003) and overproduces ethylene (Larsen and Cancel, 2004). In addition, reduced expression of the CSN5 subunit of the COP9 signalosome that removes

RUB from CUL1, or of the SCF component RBX1 that adds RUB to CUL1, or defects in the putative CUL1-regulatory protein SGT1b, each cause deficient auxin and methyl-jasmonate responses (Schwechheimer *et al.*, 2001; Gray *et al.*, 2003).

axr1 and *cand1* are also resistant to hypocotyl elongation inhibition by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in darkness (Cheng *et al.*, 2004). Apical hook formation in darkness, an auxin-mediated differential growth response promoted by ethylene, is reduced in *cand1* (Chuang *et al.*, 2004). However, the RUB1 E2 conjugating enzyme mutant *rce1*, though defective in auxin response (S. Dharmasiri *et al.*, 2003), overproduces ethylene and has an exaggerated triple response (Larsen and Cancel, 2004). Likewise, reducing RUB levels causes ethylene overproduction and enhanced triple response (Bostick *et al.*, 2004). Indeed, SCF complexes with the F-box proteins EBF1 and EBF2 repress ethylene response by targeting the ethylene response transcriptional activator EIN3 for degradation; *ebf1 ebf2* mutants show constitutive ethylene responses (Guo and Ecker, 2003; Potuschak *et al.*, 2003; Gagne *et al.*, 2004).

Gibberellins promote degradation of repressor DELLA proteins mediated by SCF^SLY1 (McGinnis *et al.*, 2003; Dill *et al.*, 2004) and the functionally redundant SCF^SNE (Strader *et al.*, 2004). Though no altered morphological response to GA has been reported in general SCF mutants, a DELLA protein–GFP fusion is more stable in *axr1-12* than wild type following GA application (Fu and Harberd, 2003).

In addition to hormone responses, SCF complexes can modulate development and environmental responses. For example, the F-box protein UFO is necessary for floral development (Samach *et al.*, 1999). *axr1* plants and transgenic plants overexpressing an inactive ECR1 both bear abnormal flowers; introducing the inactive *ECR1* transgene into *axr1* enhances the severity of *axr1* floral defects (del Pozo *et al.*, 2002). Also, though a role for an F-box complex in cold response has not been demonstrated, *axr1*, *csn5* and *rbx1* each display delayed cold-regulated gene induction (Schwechheimer *et al.*, 2002).

The full impact of many response pathways feeding through a single type of protein degradation apparatus, the SCF, remains to be determined. However, it is tempting to imagine competition for shared SCF components among hundreds of specificity-determining F-box proteins. Additionally, it is possible that regulatory mechanisms such as RUB modification may differentially regulate specific SCF complexes. Further, different phytohormones modulate SCF activity in opposing ways: repressive proteins are degraded in response to auxin (Rogg and Bartel, 2001), gibberellin (Itoh *et al.*, 2003) and presumably jasmonate (Xu *et al.*, 2002), whereas ethylene impedes destruction of the transcriptional activator EIN3 by SCF^EBF1/EBF2 (Guo and Ecker, 2003; Potuschak *et al.*, 2003; Gagne *et al.*, 2004).

Other genes bridge auxin response with other stimuli

In addition to factors affecting SCF function, defects in several proteins with less-defined roles in auxin response display altered response to other hormones and environmental conditions. For example, several lines of evidence suggest that protein phosphorylation influences response to various stimuli including auxin exposure. First, auxin, salicylic acid, wounding and salt treatments induce MAPK (mitogen activated protein kinase) activity in arabidopsis roots, whereas kinase inhibitors block auxin inducibility of a reporter gene (Mockaitis and Howell, 2000).

Second, expression of constitutively active forms of the *ANP* family members of arabidopsis mitogen-activated protein kinase kinase kinases (MAPKKKs) dampens responsiveness of an auxin-inducible reporter gene construct in protoplasts (Kovtun *et al.*, 2000). The same kinase family positively regulates responses to oxidative stress, and, like constitutively active ANPs, H_2O_2 exposure inhibits auxin-responsive reporter gene induction (Kovtun *et al.*, 2000). Likewise, a constitutively active form of the related tobacco MAPKKK NPK1 dampens auxin responsiveness of a reporter gene (Kovtun *et al.*, 1998) and confers resistance to freezing, heat and salt stresses (Kovtun *et al.*, 2000). Based on these results and auxin activation of kinase activity, there is both a positive and negative modulation of auxin responsiveness by kinases.

Third, a mutant isolated because of altered response to an auxin transport inhibitor, *roots curl in NPA 1* (*rcn1*) is deficient in a protein phosphatase 2A subunit (Garbers *et al.*, 1996; Deruère *et al.*, 1999). In addition to having increased IAA transport (Rashotte *et al.*, 2001) and over-producing ethylene (Larsen and Chang, 2001; Larsen and Cancel, 2003), *rcn1* is defective in NPA, gravity (Rashotte *et al.*, 2001) and ABA (Kwak *et al.*, 2002) responses. Likewise, protein phosphatase inhibitors phenocopy *rcn1* gravitropism and IAA transport defects when applied to wild-type arabidopsis (Rashotte *et al.*, 2001).

Finally, the *indole-3-butyric acid-response* mutant *ibr5* is deficient in an apparent dual-specificity phosphatase (Monroe-Augustus *et al.*, 2003). *ibr5* is resistant to auxins and ABA (Monroe-Augustus *et al.*, 2003). In addition, *ibr5* makes fewer lateral roots, a longer primary root, and a shorter hypocotyl than wild type, phenotypes consistent with generally deficient auxin response (Monroe-Augustus *et al.*, 2003).

In addition to phosphorylation-related genes, genes classically involved in auxin signalling are involved in response to other environmental cues. For example, Aux/IAA and ARF proteins are involved in light responses. Stabilizing gain-of-function mutations in *shy2/iaa3*, *axr2/iaa7* and *axr3/iaa17* cause de-etiolation in darkness (Kim *et al.*, 1996; J.W. Reed *et al.*, 1998; Nagpal *et al.*, 2000). Moreover, the constitutive photomorphogenic mutant *long hypocotyl 5* (*hy5*) (Koornneef *et al.*, 1980) can be partially rescued by overexpressing *AXR2/IAA7* (Cluis *et al.*, 2004). Further, *hookless1* (*hls1*) fails to make an apical hook in darkness (Lehman *et al.*, 1996), and this phenotype is suppressed by mutation of the auxin response factor *ARF2* (Li *et al.*, 2004).

The arabidopsis mutant *abscisic acid insensitive 3* (*abi3*) was isolated because of ABA-insensitive germination and is deficient in a transcription factor (Giraudat *et al.*, 1992). In addition to ABA resistance, *abi3* is resistant to lateral root

proliferation induced by auxin and lateral root repression by the auxin transport inhibitor NPA, and *ABI3:GUS* reporter accumulation is induced by auxin exposure (Brady *et al.*, 2003).

Additionally, certain mutants defective in auxin transport exhibit altered responses to other stimuli. The auxin influx mediator *aux1* is resistant to specific auxins and to ethylene (Pickett *et al.*, 1990). *rcn1* has increased auxin transport and numerous developmental and phytohormone phenotypes (see above). Further, mutations in the auxin transport facilitator *TRANSPORT INHIBITOR RESPONSE 3* (*TIR3*) (Ruegger *et al.*, 1997) cause not only low-auxin phenotypes, but also altered responses to light, ethylene, cytokinin and gibberellin (Kanyuka *et al.*, 2003). *tir3* alleles have been isolated as *dark overexpression of CAB1* (*doc1*; Li *et al.*, 1994), the cytokinin-resistant mutant *umbrella1*, and *attenuated shade avoidance1* (Kanyuka *et al.*, 2003); the gene has been renamed *BIG* (Gil *et al.*, 2001).

CONCLUSIONS AND FUTURE PROSPECTS

Auxin is a critical phytohormone. Complex and redundant regulation of IAA abundance, transport, and response allow an intricate system of auxin utilization that achieves a variety of purposes in plant development. As a result, the study of auxin biology is making an impact on our understanding of an astounding variety of processes, from regulated protein degradation to signal transduction cascades, from organelle biogenesis to plant morphogenesis. Despite prodigious historical and ongoing auxin research, many of the most fundamental original questions remain incompletely answered.

Redundancy is a key theme in auxin metabolism. IAA is produced both from the amino acid tryptophan and from an independent pathway utilizing a Trp precursor (Normanly *et al.*, 1993). IAA can be stored in certain conjugated forms, which can be hydrolysed (Bartel and Fink, 1995), or as IBA, which can be β-oxidized (Zolman *et al.*, 2000) to regenerate free IAA. Further, the isolation of a protein bearing covalently attached IAA (Walz *et al.*, 2002) suggests an exciting new area for auxin storage research. A variety of auxin degradation products are found in plants (Östin *et al.*, 1998). Future work will establish the relative contributions to auxin homeostasis from each of these redundant mechanisms.

In addition, tantalizing clues are emerging about the subcellular compartmentalization of auxin metabolism and response. Differential compartmentalization and controlled entry and exit from organelles may provide regulatory points for auxin and its precursors, although it is unknown where free IAA and its storage forms accumulate within the cell. Trp biosynthetic enzymes are plastidic (Radwanski and Last, 1995), whereas several potential downstream Trp-dependent IAA biosynthetic enzymes, including YUCCA and AAO1, are apparently cytoplasmic, and the subcellular location of Trp-independent IAA biosynthesis remains a mystery. Interestingly, CYP79B2 and CYP79B3 have chloroplast targeting signals (Hull *et al.*, 2000); perhaps

compartmentalization aids channelling common intermediates to either IAA or indolic glucosinolates. Directly comparing IAA and Trp precursor and metabolite levels in *yucca*, *sur1* and *sur2* mutants may reveal the importance of this potential compartmentalization. The subcellular location of IAA-conjugate biosynthesis and IAA degradation are also unknown. IAA-conjugate hydrolase sequence are consistent with ER retention (Bartel and Fink, 1995; Davies *et al.*, 1999). Also, β-oxidation of IBA to yield IAA apparently takes place in peroxisomes (Zolman *et al.*, 2000). Finally, it is unclear how many different sites in the cell can perceive auxin, though at least one auxin signalling apparatus is apparently entirely soluble (N. Dharmasiri *et al.*, 2003).

The effectors through which auxin signalling influences growth and development are beginning to be elucidated. These will include the direct targets of ARFs and downstream effectors of these genes, combined with any non-transcriptional auxin responses. Transcript changes associated with lateral root production in response to auxin are beginning to be identified (Himanen *et al.*, 2004); some of these are likely to be ARF targets. For example, lateral root proliferation in response to auxin is mediated by the transcription factor encoded by the auxin-induced *NAC1* gene, which acts downstream of TIR1 (Xie *et al.*, 2000).

In addition to auxin effectors downstream of TIR1, some processes appear to be mediated by auxin independent of the TIR1 signalling pathway. For example, auxin leads to rapid acidification of the extracellular space by enhancing plasma membrane ATPase activity, allowing cell wall loosening and thereby enabling cell growth (Hager, 2003). This process may be regulated by auxin, at least in part, via a signal transduction pathway acting in parallel to the SCF^{TIR1} pathway (Hager, 2003).

Auxin is also proving useful in the study of various cellular processes of general interest. For example, IBA and 2,4-DB response mutants are revealing key steps in peroxisome biogenesis and function, and the search for genes influencing IAA–amino acid conjugate responses has revealed new players in metal homeostasis (Lasswell *et al.*, 2000; Magidin *et al.*, 2003). Further, studies of disrupted auxin transport reveal that the actin cytoskeleton, unidentified endosomal compartments, and a rapid vesicular trafficking are all involved (Surpin and Raikhel, 2004). Finally, the study of auxin-response mutants has provided multiple new insights into the roles of SCF complexes in ubiquitin-mediated proteolysis (Dharmasiri and Estelle, 2002).

A tremendous area of future research will be the characterization and understanding of interactions between different phytohormones and environmental stimuli. The channelling of several hormone signalling pathways through specific SCF complexes with shared components is one possible arena for cross-talk. In addition, many mutants with altered response to auxin and other stimuli remain to be fully characterized or understood.

The recent identification of microRNAs and their targets in plants (Bartel and Bartel, 2003) has revealed intriguing links to auxin signalling. Several of these tiny riboregulators, which direct negative regulation of complementary

mRNAs (Bartel, 2004), target messages implicated in auxin responsiveness. For example, miR167 targets *ARF6* and *ARF8* mRNAs (Rhoades *et al.*, 2002; Kasschau *et al.*, 2003), which encode activating ARFs (Ulmasov *et al.*, 1999b), and miR160 targets *ARF10*, *ARF16*, and *ARF17* mRNAs (Rhoades *et al.*, 2002; Kasschau *et al.*, 2003), which have not been functionally characterized but resemble repressing ARFs. Moreover, miR393 targets mRNAs encoding TIR1 and the three most closely related F-box proteins (Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004). In addition to microRNAs influencing auxin signalling, microRNAs are likely to aid in signal interpretation. For example, miR164 targets *CUC1* and *CUC2* (Laufs *et al.*, 2004; Mallory *et al.*, 2004), which establish organ boundaries in embryos and flowers (Aida *et al.*, 1997) and are negatively regulated by PIN1 and PINOID (Furutani *et al.*, 2004). Thus microRNAs, like ubiquitin-mediated protein degradation, are likely to be post-transcriptional regulators defining the extent of auxin signalling and response during development and in response to environmental cues.

The single most pressing question of auxin biology remains the identity of the auxin receptors. Tremendous progress has been made in this area, starting with the identification of SCF^{TIR1} as a critical conduit of auxin response (Leyser *et al.*, 1993; Gray *et al.*, 1999). Recent results suggest that auxin causes modification of the TIR1 F-box, or a tightly associated protein, to increase TIR1 interaction with Aux/IAA repressors, bringing them to the SCF to be targeted for destruction (Kepinski and Leyser, 2004). However, the nature of the modification, as well as the proximity to the auxin receptor, remain undetermined. While it is likely that SCF^{TIR1} mediates many transcriptional responses to auxin, it remains to be seen whether there are SCF^{TIR1}-independent transcriptional auxin responses, or whether this signalling complex also mediates rapid responses to auxin that are not transcriptional.

ACKNOWLEDGEMENTS

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