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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

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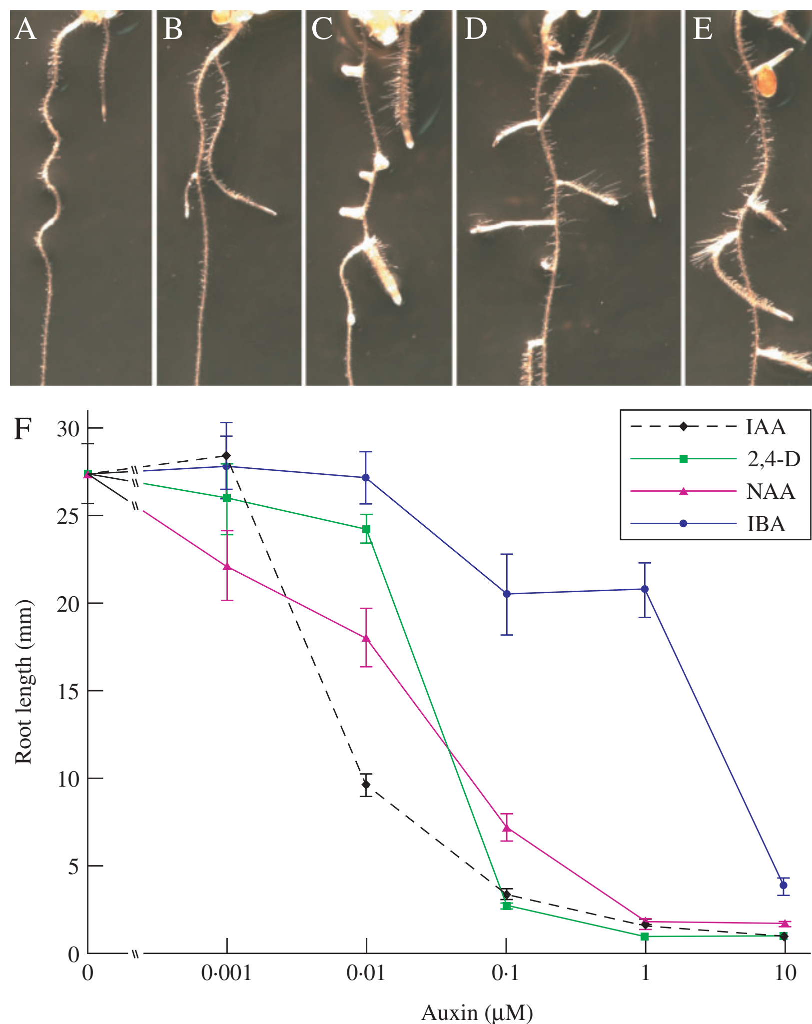

FIG. 1. Auxins promote lateral root formation and inhibit root elongation. *Arabidopsis thaliana* Col-0 ecotype plants were grown on unsupplemented medium (Haughn and Somerville, 1986) for 6 d, then transferred to unsupplemented medium (A) or medium supplemented with 10 nM IAA (B), 100 nM 2,4-D (C), 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, $n \geq 8$. 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).

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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, 2000*b*), 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

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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

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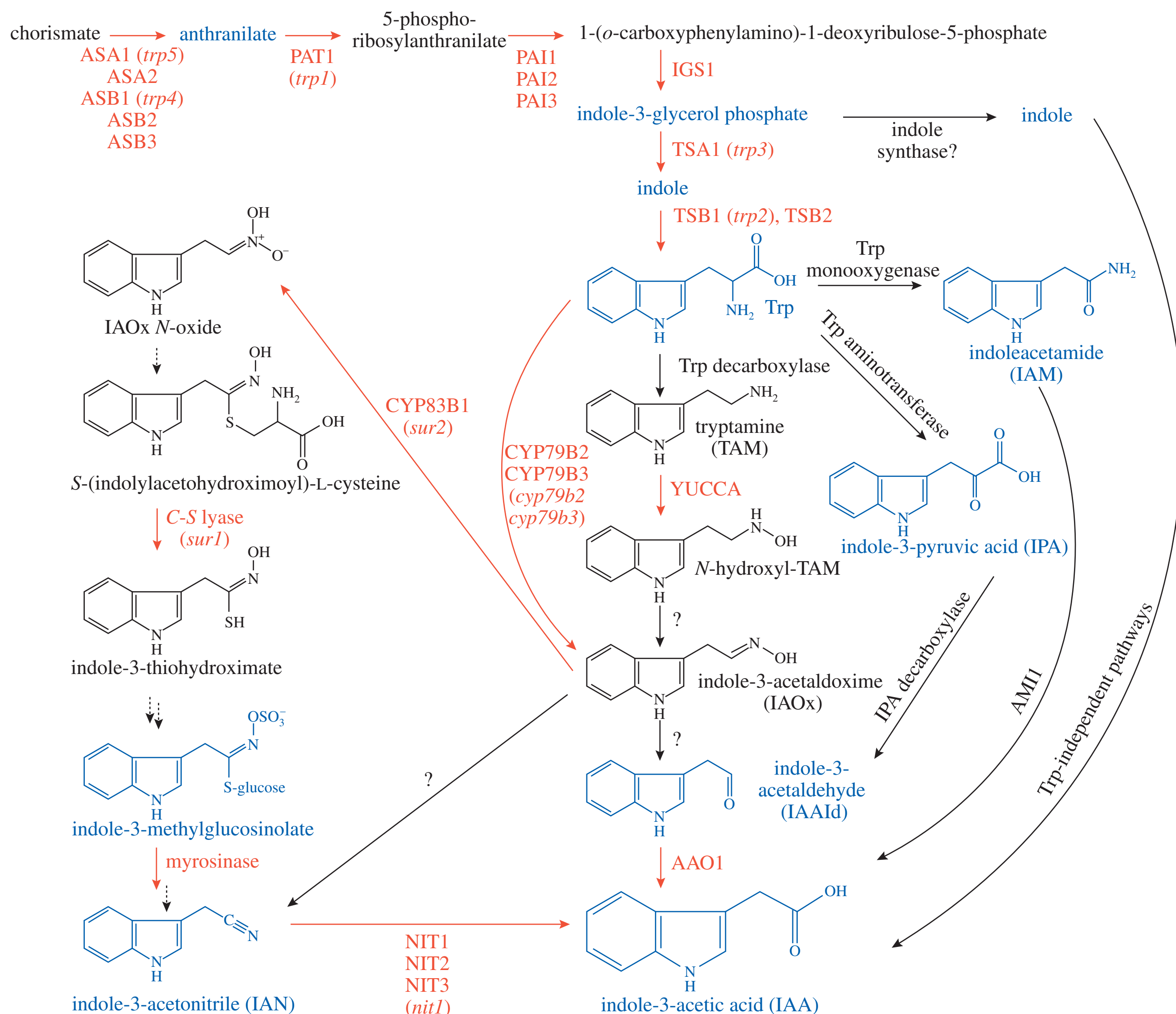

FIG. 2. Potential pathways of IAA biosynthesis in arabidopsis. *De novo* IAA biosynthetic pathways initiate from Trp or Trp precursors. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are identified are in red, and arabidopsis mutants are in lower-case italics. 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

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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

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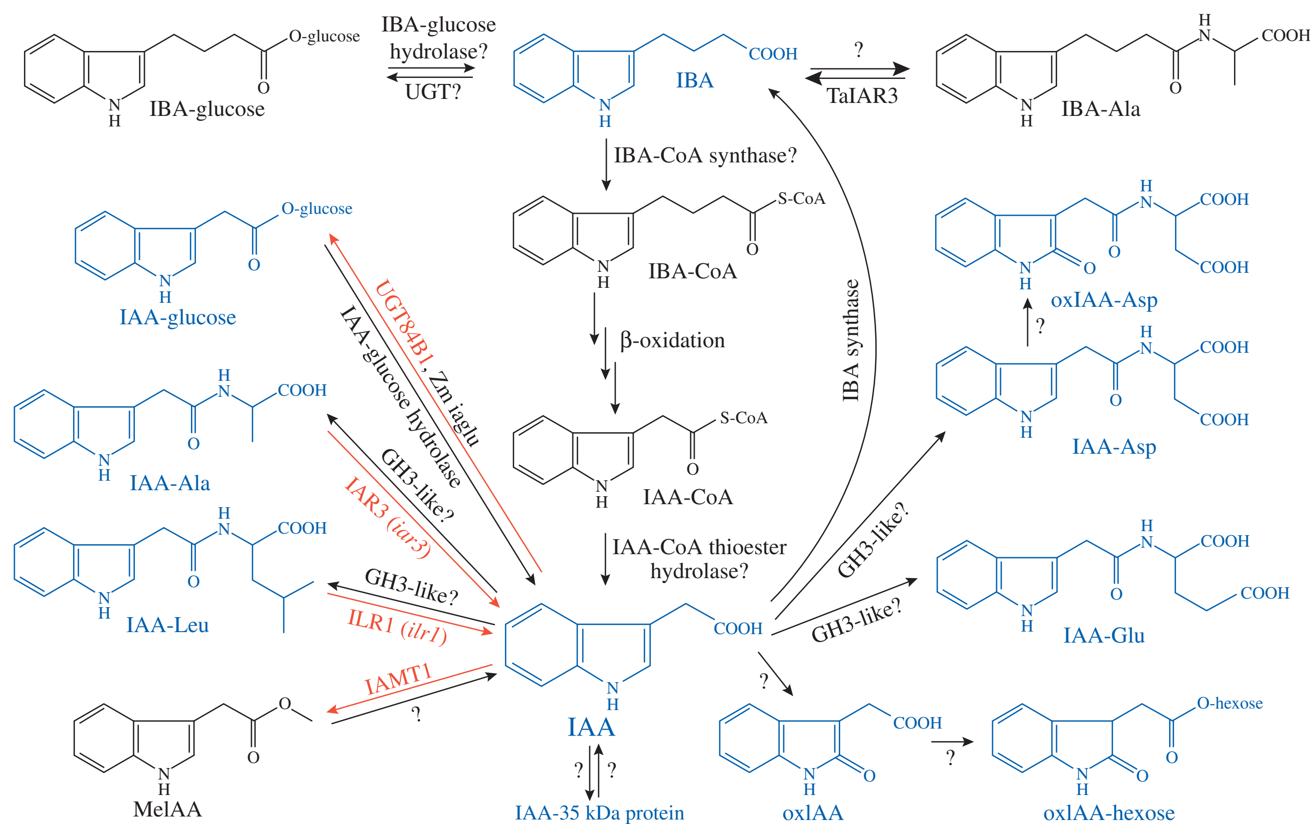

FIG. 3. Potential pathways of IAA metabolism. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are cloned are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which plant genes are not identified are indicated with question marks. 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 $[^2H_5]$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 $[^2H_5]$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

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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

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TABLE 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).

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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,

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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 1* (*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

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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 glucosyltransferase 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

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(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

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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*., 1999*a*; 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, 1997*b*). 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*., 1997*a*). ARF proteins can either activate or repress target gene transcription, depending on the nature of a central domain (Ulmasov *et al*., 1999*b*; 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*., 1997*a*, 1999*b*). 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 *hooklessl 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.

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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;

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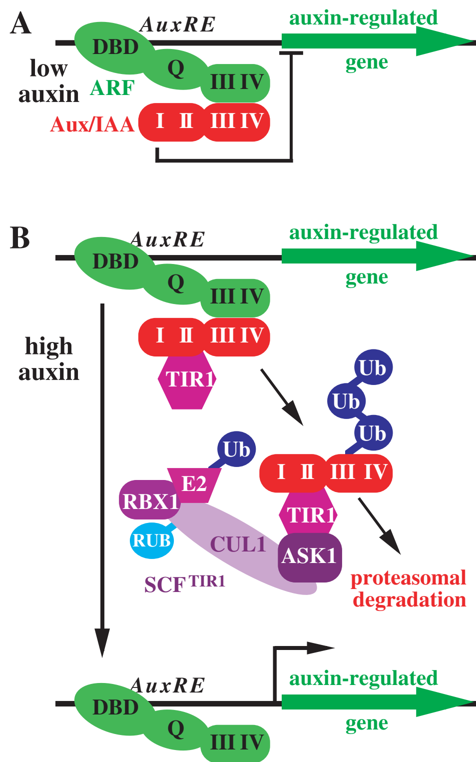

FIG. 4. The SCF^{TIR1} relieves Aux/IAA repression of activating ARFs. (A) An activating ARF protein (green) binds an *AuxRE* promoter element via an N-terminal DNA binding domain (DBD). Under low-auxin conditions, an Aux/IAA repressor (red) binds the activating ARF via heterodimerization between Aux/IAA 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

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TABLE 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).

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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

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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 overproducing 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

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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

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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.

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LITERATURE CITED

Abel S, Nguyen MD, Chow W, Theologis A. 1995*a*. *ACS4*, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. *Journal of Biological Chemistry* **270**: 19093–19099.

Abel S, Nguyen MD, Theologis A. 1995*b*. The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *Journal of Molecular Biology* **251**: 533–549.

Abel S, Oeller PW, Theologis A. 1994. Early auxin-induced genes encode short-lived nuclear proteins. *Proceedings of the National Academy of Science of the USA* **91**: 326–330.

Abel S, Theologis A. 1996. Early genes and auxin action. *Plant Physiology* **111**: 9–17.

Adham AR, Zolman BK, Millius A, Bartel B. 2005. Mutations in Arabidopsis acyl-CoA oxidase genes reveal distinct and overlapping roles in β-oxidation. *Plant Journal* **41**: 859–874.

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. 1997. Genes involved in organ separation in Arabidopsis: an analysis of the *cup-shaped cotyledon* mutant. *The Plant Cell* **9**: 841–857.

Antolić S, Kojić-Prodić B, Tomić S, Nigović B, Magnus V, Cohen JD. 1996. Structural studies on monofluorinated derivatives of the phytohormone indole-3-acetic acid (auxin). *Acta Crystallographica B* **52**: 651–661.

Avsian-Kretchmer O, Cheng JC, Chen L, Moctezuma E, Sung ZR. 2002. Indole acetic acid distribution coincides with vascular differentiation pattern during Arabidopsis leaf ontogeny. *Plant Physiology* **130**: 199–209.

Bak S, Tax FE, Feldmann KA, Galbraith DW, Feyereisen R. 2001. CYP83B1, a cytochrome P450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in Arabidopsis. *The Plant Cell* **13**: 101–111.

Baldi BG, Maher BR, Cohen JD. 1989. Hydrolysis of indole-3-acetic acid esters exposed to mild alkaline conditions. *Plant Physiology* **91**: 9–12.

Ballas N, Wong LM, Theologis A. 1993. Identification of the auxin-responsive element, *AuxRE*, in the primary indoleacetic acid-inducible gene, *PS-IAA4/5*, of pea (*Pisum sativum*). *Journal of Molecular Biology* **233**: 580–596.

Baraldi R, Bertazza G, Predieri S, Bregoli AM, Cohen JD. 1993. Uptake and metabolism of indole-3-butyric acid during the *in vitro* rooting phase in pear cultivars (*Pyrus communis*). *Acta Horticulturae* **329**: 289–291.

Barlier I, Kowalczyk M, Marchant A, Ljung K, Bhalerao R, Bennett M, Sandberg G, Bellini C. 2000. The *SUR2* gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. *Proceedings of the National Academy of Science of the USA* **97**: 14819–14824.

Barratt NM, Dong W, Gage DA, Magnus V, Town CD. 1999. Metabolism of exogenous auxin by *Arabidopsis thaliana*: identification of the conjugate N_{α}-(indol-3-ylacetyl)-glutamine and initiation of a mutant screen. *Physiologia Plantarum* **105**: 207–217.

Bartel B, Bartel DP. 2003. MicroRNAs: at the root of plant development? *Plant Physiology* **132**: 709–717.

Bartel B, Fink GR. 1994. Differential regulation of an auxin-producing nitrilase gene family in *Arabidopsis thaliana*. *Proceedings of the National Academy of Science of the USA* **91**: 6649–6653.

Bartel B, Fink GR. 1995. ILR1, an amidohydrolase that releases active indole-3-acetic acid from conjugates. *Science* **268**: 1745–1748.

Bartel B, LeClere S, Magidin M, Zolman BK. 2001. Inputs to the active indole-3-acetic acid pool: *de novo* synthesis, conjugate hydrolysis, and indole-3-butyric acid β-oxidation. *Journal of Plant Growth Regulation* **20**: 198–216.

Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281–297.

Bartling D, Seedorf M, Mithöfer A, Weiler EW. 1992. Cloning and expression of an *Arabidopsis* nitrilase which can convert indole-3-acetonitrile to the plant hormone, indole-3-acetic acid. *European Journal of Biochemistry* **205**: 417–424.

Bartling D, Seedorf M, Schmidt RC, Weiler EW. 1994. Molecular characterization of two cloned nitrilases from *Arabidopsis thaliana*: key enzymes in the biosynthesis of the plant hormone indole-3-acetic acid. *Proceedings of the National Academy of Science of the USA* **91**: 6021–6025.

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schultz B, Feldmann KA. 1996. *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. *Science* **273**: 948–950.

Bhalerao RP, Eklöf J, Ljung K, Marchant A, Bennett M, Sandberg G. 2002. Shoot-derived auxin is essential for early lateral root emergence in *Arabidopsis* seedlings. *Plant Journal* **29**: 325–332.

Bialek K, Cohen JD. 1986. Isolation and partial characterization of the major amide-linked conjugate of indole-3-acetic acid from *Phaseolus vulgaris* L. *Plant Physiology* **80**: 99–104.

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inzé D. 1995.

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superroot, a recessive mutation in Arabidopsis, confers auxin overproduction. *The Plant Cell* **7**: 1405–1419.
Bostick M, Lochhead SR, Honda A, Palmer S, Callis J. 2004. Related to ubiquitin 1 and 2 are redundant and essential and regulate vegetative growth, auxin signaling, and ethylene production in Arabidopsis. *The Plant Cell* **16**: 2418–2432.
Brady SM, Sarkar SF, Bonetta D, McCourt P. 2003. The *ABSCISIC ACID INSENSITIVE 3 (ABI3)* gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. *Plant Journal* **34**: 67–75.
Brickner DG, Brickner JH, Olsen LJ. 1998. Sequence analysis of a cDNA encoding Pex5p, a peroxisomal targeting signal type 1 receptor from Arabidopsis. *Plant Physiology* **118**: 330.
Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, Taiz L, Muday GK. 2001. Flavonoids act as negative regulators of auxin transport *in vivo* in Arabidopsis. *Plant Physiology* **126**: 524–535.
Brusslan JA, Karlin-Neumann GA, Huang L, Tobin EM. 1993. An Arabidopsis mutant with a reduced level of *cab140* RNA is a result of cosuppression. *The Plant Cell* **5**: 667–677.
Buer CS, Muday GK. 2004. The *transparent testa4* mutation prevents flavonoid synthesis and alters auxin transport and the response of Arabidopsis roots to gravity and light. *The Plant Cell* **16**: 1191–1205.
Burg SP, Burg EA. 1966. The interaction between auxin and ethylene and its role in plant growth. *Proceedings of the National Academy of Science of the USA* **55**: 262–269.
Campanella JJ, Ludwig-Mueller J, Town CD. 1996. Isolation and characterization of mutants of *Arabidopsis thaliana* with increased resistance to growth inhibition by indoleacetic acid-amino acid conjugates. *Plant Physiology* **112**: 735–745.
Campanella JJ, Ludwig-Mueller J, Bakllamaja V, Sharma V, Cartier A. 2003. ILR1 and sILR1 amidohydrolase homologs differ in expression pattern and substrate specificity. *Plant Growth Regulation* **41**: 215–223.
Campanella JJ, Olajide AF, Magnus V, Ludwig-Müller J. 2004. A novel auxin conjugate hydrolase from wheat with substrate specificity for longer side-chain auxin amide conjugates. *Plant Physiology* **135**: 2230–2240.
Celenza JL, Grisafi PL, Fink GR. 1995. A pathway for lateral root formation in *Arabidopsis thaliana*. *Genes and Development* **9**: 2131–2142.
Chen J-G, Ullah H, Young JC, Sussman MR, Jones AM. 2001. ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis. *Genes and Development* **15**: 902–911.
Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH. 1998. The *Arabidopsis thaliana AGRAVITROPIC 1* gene encodes a component of the polar-auxin-transport efflux carrier. *Proceedings of the National Academy of Science of the USA* **95**: 15112–15117.
Cheng Y, Dai X, Zhao Y. 2004. AtCAND1, a HEAT-repeat protein that participates in auxin signaling in Arabidopsis. *Plant Physiology* **135**: 1020–1026.
Chhun T, Taketa S, Tsurumi S, Ichii M. 2003. Interaction between two auxin-resistant mutants and their effects on lateral root formation in rice (*Oryza sativa* L.). *Journal of Experimental Botany* **54**: 2701–2708.
Chisnell JR, Bandurski RS. 1988. Translocation of radiolabeled indole-3-acetic acid and indole-3-acetyl-*myo*-inositol from kernel to shoot of *Zea mays* L. *Plant Physiology* **86**: 79–84.
Chuang HW, Zhang W, Gray WM. 2004. Arabidopsis *ETA2*, an apparent ortholog of the human cullin-interacting protein CAND1, is required for auxin responses mediated by the SCF^{TIR1} ubiquitin ligase. *The Plant Cell* **16**: 1883–1897.
Ciesielski T. 1872. Untersuchungen über die Abwärtskrümmung der Wurzel. *Beitrage zur Biologie der Pflanzen* **1**: 1–30.
Cluis CP, Mouchel CF, Hardtke CS. 2004. The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. *Plant Journal* **38**: 332–347.
Cohen JD, Bandurski RS. 1982. Chemistry and physiology of the bound auxins. *Annual Review of Plant Physiology* **33**: 403–430.
Cooney TP, Nonhebel HM. 1991. Biosynthesis of indole-3-acetic acid in tomato shoots: Measurement, mass-spectral identification and incorporation of ^2H from ^2H_2O into indole-3-acetic acid, D- and L-tryptophan, indole-3-pyruvate and tryptamine. *Planta* **184**: 368–376.
Courtney KD, Gaylor DW, Hogan MD, Falk HL, Bates RR, Mitchell I. 1970. Teratogenic evaluation of 2,4,5-T. *Science* **168**: 864–866.
Darwin C. 1880. *The power of movement in plants*. London: John Murray.
Davies PJ. 1995. *Plant hormones*. Dordrecht: Kluwer Academic Publishers.
Davies PJ, Mitchell EK. 1972. Transport of indoleacetic acid in intact roots of *Phaseolus coccineus*. *Planta* **105**: 139–154.
Davies RT, Goetz DH, Lasswell J, Anderson MN, Bartel B. 1999. *IAR3* encodes an auxin conjugate hydrolase from Arabidopsis. *The Plant Cell* **11**: 365–376.
De Luca V, Marineau C, Brisson N. 1989. Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase: comparison with animal dopa decarboxylases. *Proceedings of the National Academy of Science of the USA* **86**: 2582–2586.
del Pozo JC, Estelle M. 1999. The *Arabidopsis* cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proceedings of the National Academy of Science of the USA* **96**: 15342–15347.
del Pozo JC, Dharmasiri S, Hellmann H, Walker L, Gray WM, Estelle M. 2002. AXR1-ECR1-dependent conjugation of RUB1 to the Arabidopsis Cullin AtCUL1 is required for auxin response. *The Plant Cell* **14**: 421–433.
del Pozo JC, Timpte C, Tan S, Callis J, Estelle M. 1998. The ubiquitin-related protein RUB1 and auxin response in *Arabidopsis*. *Science* **280**: 1760–1763.
Delarue M, Prinsen E, Van Onckelen H, Caboche M, Bellini C. 1998. *Sur2* mutations of *Arabidopsis thaliana* define a new locus involved in the control of auxin homeostasis. *Plant Journal* **14**: 603–611.
Deruère J, Jackson K, Garbers C, Söll D, DeLong A. 1999. The *RCN1*-encoded A subunit of protein phosphatase 2A increases phosphatase activity *in vivo*. *Plant Journal* **20**: 389–399.
Deshaies RJ. 1999. SCF and Cullin/RING H2-based ubiquitin ligases. *Annual Review of Cell and Development Biology* **15**: 435–467.
De Smet I, Signora L, Beeckman T, Inzé D, Foyer CH, Zhang H. 2003. An abscisic acid-sensitive checkpoint in lateral root development of *Arabidopsis*. *Plant Journal* **33**: 543–555.
Dharmasiri N, Dharmasiri S, Jones AM, Estelle M. 2003. Auxin action in a cell-free system. *Current Biology* **13**: 1418–1422.
Dharmasiri S, Dharmasiri N, Hellmann H, Estelle M. 2003. The RUB/Nedd8 conjugation pathway is required for early development in *Arabidopsis*. *EMBO Journal* **22**: 1762–1770.
Dharmasiri S, Estelle M. 2002. The role of regulated protein degradation in auxin response. *Plant Molecular Biology* **49**: 401–409.
Dill A, Thomas SG, Hu J, Steber CM, Sun TP. 2004. The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *The Plant Cell* **16**: 1392–1405.
Dubois-Dalcq M, Feigenbaum V, Aubourg P. 1999. The neurobiology of X-linked adrenoleukodystrophy, a demyelinating peroxisomal disorder. *Trends in Neuroscience* **22**: 4–12.
Dunlap JR, Robacker KM. 1990. Abscisic acid alters the metabolism of indole-3-acetic acid in senescing flowers of *Cucumis melo* L. *Plant Physiology* **94**: 870–874.
Eastmond PJ, Hooks MA, Williams D, Lange P, Bechtold N, Sarrobert C, Nussaume L, Graham IA. 2000. Promoter trapping of a novel medium-chain acyl-CoA oxidase, which is induced transcriptionally during *Arabidopsis* seed germination. *Journal of Biological Chemistry* **275**: 34375–34381.
Eklöf S, Åstot C, Sitbon F, Moritz T, Olsson O, Sandberg G. 2000. Transgenic tobacco plants co-expressing *Agrobacterium iaa* and *ipt* genes have wild-type hormone levels but display both auxin- and cytokinin-overproducing phenotypes. *Plant Journal* **23**: 279–284.
Epstein E, Ludwig-Müller J. 1993. Indole-3-butyric acid in plants: occurrence, synthesis, metabolism, and transport. *Physiologia Plantarum* **88**: 382–389.
Estelle MA, Somerville C. 1987. Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology. *Molecular and General Genetics* **206**: 200–206.
Facchini PJ, Huber-Allanach KL, Tari LW. 2000. Plant aromatic L-amino acid decarboxylases: Evolution, biochemistry, regulation, and metabolic engineering applications. *Phytochemistry* **54**: 121–138.
Fahey JW, Zalcmann AT, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocynanates among plants. *Phytochemistry* **56**: 5–51.
Fallon H, Tollerud D, Breslow N, Berlin J, Bolla K, Colditz G, Goetz C, Kaminski N, Kriebel D, Mottet NK, *et al.* 1994. The U.S. military and the herbicide program in Vietnam. In *Veterans and agent orange:*

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health effects of herbicides used in Vietnam Washington, DC: National Academy Press, 74–110.

Farrás R, Ferrando A, Jásik J, Kleinow T, Ökrész L, Tiburcio A, Salchert K, del Pozo JC, Schell J, Koncz C. 2001. SKP1-SnRK protein kinase interactions mediate proteasomal binding of a plant SCF ubiquitin ligase. *EMBO Journal* **20**: 2742–2756.

Fawcett CH, Wain RL, Wightman F. 1960. The metabolism of 3-indolylalkanecarboxylic acids, and their amides, nitriles and methyl esters in plant tissues. *Proceedings of the Royal Society of London. Series B. Biological Science* **152**: 231–254.

Feng S, Shen Y, Sullivan JA, Rubio V, Xiong Y, Sun TP, Deng XW. 2004. Arabidopsis CAND1, an unmodified CUL1-interacting protein, is involved in multiple developmental pathways controlled by ubiquitin/proteasome-mediated protein degradation. *The Plant Cell* **16**: 1870–1882.

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

Frey M, Chomet P, Glawischnig E, Stettner C, Grün S, Winklmair A, Eisenreich W, Bacher A, Meeley RB, Briggs SP, Simcox K, Gierl A. 1997. Analysis of a chemical plant defense mechanism in grasses. *Science* **277**: 696–699.

Frey M, Stettner C, Paré PW, Schmelz EA, Tumlinson JH, Gierl A. 2000. An herbivore elicitor activates the gene for indole emission in maize. *Proceedings of the National Academy of Science of the USA* **97**: 14801–14806.

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* **426**: 147–153.

Friml J, Wísniewska J, Benková E, Mendgen K, Palme K. 2002. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* **415**: 806–809.

Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PBF, Ljung K, Sandberg G, Hooykaas PJ, Palme K, Offringa R. 2004. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**: 862–865.

Fu X, Harberd NP. 2003. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. *Nature* **421**:d40–743.

Fukaki H, Tameda S, Masuda H, Tasaka M. 2002. Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. *Plant Journal* **29**: 153–168.

Fulda M, Schnurr J, Abbadi A, Heinz E, Browse J. 2004. Peroxisomal acyl-CoA synthetase activity is essential for seedling development in *Arabidopsis thaliana*. *The Plant Cell* **16**: 394–405.

Furutani M, Vernoux T, Traas J, Kato T, Tasaka M, Aida M. 2004. *PIN-FORMED1* and *PINOID* regulate boundary formation and cotyledon development in *Arabidopsis* embryogenesis. *Development* **131**: 5021–5030.

Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, Yanagisawa S, Vierstra RD. 2004. *Arabidopsis* EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. *Proceedings of the National Academy of Science of the USA* **101**: 6803–6808.

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* **282**: 2226–2230.

Garbers C, DeLong A, Deruére J, Bernasconi P, Söll D. 1996. A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in *Arabidopsis*. *EMBO Journal* **15**: 2115–2124.

Geldner N, Friml J, Stierhof Y-D, Jürgens G, Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**: 425–428.

Gerhardt B. 1992. Fatty acid degradation in plants. *Progress in Lipid Research* **31**: 417–446.

Gil P, Dewey E, Friml J, Zhao Y, Snowden KC, Putterill J, Palme K, Estelle M, Chory J. 2001. BIG: a calossin-like protein required for polar auxin transport in *Arabidopsis*. *Genes and Development* **15**: 1985–1997.

Gil P, Liu Y, Orbović V, Verkamp E, Poff KL, Green PJ. 1994. Characterization of the auxin-inducible *SAUR-AC1* gene for use as a molecular genetic tool in *Arabidopsis*. *Plant Physiology* **104**: 777–784.

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM. 1992. Isolation of the Arabidopsis *ABI3* gene by positional cloning. *The Plant Cell* **4**: 1251–1261.

Glass NL, Kosuge T. 1986. Cloning of the gene for indoleacetic acid-lysine synthetase from *Pseudomonas syringae* subsp. *savastanoi*. *Journal of Bacteriology* **166**: 598–603.

Goda H, Sawa S, Asami T, Fujioka S, Shimada Y, Yoshida S. 2004. Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. *Plant Physiology* **134**: 1555–1573.

Golparaj M, Tseng T-S, Olszewski N. 1996. The *Rooty* gene of *Arabidopsis* encodes a protein with highest similarity to aminotransferases. *Plant Physiology* **111** (suppl.): 114.

Gray WM, del Pozo JC, Walker L, Hobbie L, Risseeuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M. 1999. Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes and Development* **13**: 1678–1691.

Gray WM, Hellmann H, Dharmasiri S, Estelle M. 2002. Role of the Arabidopsis RING-H2 protein RBX1 in RUB modification and SCF function. *The Plant Cell* **14**: 2137–2144.

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M. 2001. Auxin regulates SCF^{TIR1}-dependent degradation of Aux/IAA proteins. *Nature* **414**: 271–276.

Gray WM, Muskett PR, Chuang HW, Parker JE. 2003. Arabidopsis SGT1b is required for SCF^{TIR1}-mediated auxin response. *The Plant Cell* **15**: 1310–1319.

Gray WM, Östin A, Sandberg G, Romano CP, Estelle M. 1998. High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*. *Proceedings of the National Academy of Science of the USA* **95**: 7197–7202.

Grsic S, Sauerteig S, Neuhaus K, Albrecht M, Rossiter J, Ludwig-Müller J. 1998. Physiological analysis of transgenic *Arabidopsis thaliana* plants expressing one nitrilase isoform in sense or antisense direction. *Journal of Plant Physiology* **153**: 446–456.

Grsic-Rausch S, Kobelt P, Siemens JM, Bischoff M, Ludwig-Müller J. 2000. Expression and localization of nitrilase during symptom development of the clubroot disease in Arabidopsis. *Plant Physiology* **122**: 369–378.

Guerinot ML. 2000. The ZIP family of metal transporters. *Biochimica et Biophysica Acta* **1465**: 190–198.

Guillet G, Poupart J, Basurco J, De Luca V. 2000. Expression of tryptophan decarboxylase and tyrosine decarboxylase genes in tobacco results in altered biochemical and physiological phenotypes. *Plant Physiology* **122**: 933–943.

Guo H, Ecker JR. 2003. Plant responses to ethylene gas are mediated by $SCF^{EBF1/EBF2}$-dependent proteolysis of EIN3 transcription factor. *Cell* **115**: 667–677.

Hagen G, Kleinschmidt A, Guilfoyle T. 1984. Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections. *Planta* **162**: 147–153.

Hager A. 2003. Role of the plasma membrane H^+-ATPase in auxin-induced elongation growth: historical and new aspects. *Journal of Plant Research* **116**: 483–505.

Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G. 2002. The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes and Development* **16**: 1610–1615.

Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO Journal* **17**: 1405–1411.

Hardtke CS, Ckurshumova W, Vidaurre DP, Singh SA, Stamatiou G, Tiwari SB, Hagen G, Guilfoyle TJ, Berleth T. 2004. Overlapping and non-redundant functions of the *Arabidopsis* auxin response factors *MONOPTEROS* and *NONPHOTOTROPIC HYPOCOTYL 4*. *Development* **131**: 1089–1100.

Harper RM, Stowe-Evans EL, Luesse DR, Muto H, Tatematsu K, Watahiki MK, Yamamoto K, Liscum E. 2000. The *NPH4* locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. *The Plant Cell* **12**: 757–770.

Hartmann HT, Kester DE, Davies FT. 1990. Anatomical and physiological basis of propagation by cuttings. In *Plant propagation: principles and practices* Englewood Cliffs, NJ: Prentice Hall, 199–245.

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Haughn GW, Somerville C. 1986. Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. *Molecular and General Genetics* **204**: 430–434.

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

Hayashi M, Nito K, Toriyama-Kato K, Kondo M, Yamaya T, Nishimura M. 2000. *At*Pex14p maintains peroxisomal functions by determining protein targeting to three kinds of plant peroxisomes. *EMBO Journal* **19**: 5701–5710.

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

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, del Pozo C, Reinhardt D, Estelle M. 2003. *Arabidopsis AXR6* encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. *EMBO Journal* **22**: 3314–3325.

Himanen K, Vuylsteke M, Vanneste S, Vercruysse S, Boucheron E, Alard P, Chriqui D, Van Montagu M, Inzé D, Beeckman T. 2004. Transcript profiling of early lateral root initiation. *Proceedings of the National Academy of Science of the USA* **101**: 5146–5151.

Hobbie L, Estelle M. 1995. The *axr4* auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. *Plant Journal* **7**: 211–220.

Hoecker U, Toledo-Ortiz G, Bender J, Quail PH. 2004. The photomorphogenesis-related mutant *red1* is defective in *CYP83B1*, a red light-induced gene encoding a cytochrome P450 required for normal auxin homeostasis. *Planta* **219**: 195–200.

Holland IB, Blight MA. 1999. ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. *Journal of Molecular Biology* **293**: 381–399.

Hooks MA, Kellas F, Graham IA. 1999. Long-chain acyl-CoA oxidases of *Arabidopsis*. *Plant Journal* **20**: 1–13.

Hou G, Kramer VL, Wang YS, Chen R, Perbal G, Gilroy S, Blancaflor EB. 2004. The promotion of gravitropism in *Arabidopsis* roots upon actin disruption is coupled with the extended alkalinization of the columella cytoplasm and a persistent lateral auxin gradient. *Plant Journal* **39**: 113–125.

Hou G, Mohamalawari DR, Blancaflor EB. 2003. Enhanced gravitropism of roots with a disrupted cap actin cytoskeleton. *Plant Physiology* **131**: 1360–1373.

Hull AK, Vij R, Celenza JL. 2000. *Arabidopsis* cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proceedings of the National Academy of Science of the USA* **97**: 2379–2384.

Ilić N, Cohen JD. 2004. Synthesis of [^{13}C]-isotopomers of indole and tryptophan for use in the analysis of indole-3-acetic acid biosynthesis. *Journal of Labelled Compounds and Radiopharmaceuticals* **47**: 635–646.

Ilić N, Normanly J, Cohen JD. 1996. Quantification of free plus conjugated indoleacetic acid in Arabidopsis requires correction for the nonenzymatic conversion of indolic nitriles. *Plant Physiology* **111**: 781–788.

Itoh H, Matsuoka M, Steber CM. 2003. A role for the ubiquitin-26S-proteasome pathway in gibberellin signaling. *Trends in Plant Science* **8**: 492–497.

Jackson RG, Kowalczyk M, Li Y, Higgins G, Ross J, Sandberg G, Bowles DJ. 2002. Over-expression of an *Arabidopsis* gene encoding a glucosyltransferase of indole-3-acetic acid: phenotypic characterization of transgenic lines. *Plant Journal* **32**: 573–583.

Jackson RG, Lim E-K, Li Y, Kowalczyk M, Sandberg G, Hoggett J, Ashford DA, Bowles DJ. 2001. Identification and biochemical characterization of an *Arabidopsis* indole-3-acetic acid glucosyltransferase. *Journal of Biological Chemistry* **276**: 4350–4356.

Jakubowska A, Kowalczyk S. 2004. The auxin conjugate 1-*O*-indole-3-acetyl-β-D-glucose is synthesized in immature legume seeds by IAGlc synthase and may be used for modification of some high molecular weight compounds. *Journal of Experimental Botany* **55**: 791–801.

Johnson MA, Pérez-Amador MA, Lidder P, Green PJ. 2000. Mutants of *Arabidopsis* defective in a sequence-specific mRNA degradation pathway. *Proceedings of the National Academy of Science of the USA* **97**: 13991–13996.

Jones AM. 1994. Auxin-binding proteins. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**: 393–420.

Jones-Rhoades MW, Bartel DP. 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Molecular Cell* **14**: 787–799.

Kanyuka K, Praekelt U, Franklin KA, Billingham OE, Hooley R, Whitelam GC, Halliday KJ. 2003. Mutations in the huge *Arabidopsis* gene *BIG* affect a range of hormone and light responses. *Plant Journal* **35**: 57–70.

Kasschau KD, Xie Z, Allen E, Llave C, Chapman EJ, Krizan KA, Carrington JC. 2003. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with *Arabidopsis* development and miRNA function. *Developmental Cell* **4**: 205–217.

Kepinski S, Leyser O. 2004. Auxin-induced SCF^{TIR1}–Aux/IAA interaction involves stable modification of the SCF^{TIR1} complex. *Proceedings of the National Academy of Science of the USA* **101**: 12381–12386.

Kim BC, Soh MS, Kang BJ, Furuya M, Nam HG. 1996. Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. *Plant Journal* **9**: 441–456.

Kim J, Harter K, Theologis A. 1997. Protein-protein interactions among the Aux/IAA proteins. *Proceedings of the National Academy of Science of the USA* **94**: 11786–11791.

Kim SK, Chang SC, Lee EJ, Chung WS, Kim YS, Hwang S, Lee JS. 2000. Involvement of brassinosteroids in the gravitropic response of primary root of maize. *Plant Physiology* **123**: 997**–**1004**.**

Kindl H. 1993. Fatty acid degradation in plant peroxisomes: function and biosynthesis of the enzymes involved. *Biochimie* **75**: 225–230.

King JJ, Stimart DP. 1998. Genetic analysis of variation for auxin-induced adventitious root formation among eighteen ecotypes of *Arabidopsis thaliana* L. Heynh. *Journal of Heredity* **89**: 481–487.

King JJ, Stimart DP, Fisher RH, Bleecker AB. 1995. A mutation altering auxin homeostasis and plant morphology in Arabidopsis. *The Plant Cell* **7**: 2023–2037.

Kitagawa K, Skowyra D, Elledge SJ, Harper JW, Hieter P. 1999. *SGT1* encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Molecular Cell* **4**: 21–33.

Knauss S, Rohrmeier T, Lehle L. 2003. The auxin-induced maize gene *ZmSAUR2* encodes a short-lived nuclear protein expressed in elongating tissues. *Journal of Biological Chemistry* **278**: 23936–23943.

Koga J. 1995. Structure and function of indolepyruvate decarboxylase, a key enzyme in indole-3-acetic acid biosynthesis. *Biochimica et Biophysica Acta* **1249**: 1–13.

Kögl F, Haagen Smit AJ. 1931. Über die Chemie des Wuchsstoffs. *K. Akad. Wetenschap. Amsterdam Proceedings. Section Science* **34**: 1411–1416.

Koornneef M, Rolff E, Spruit CJP. 1980. Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. *Zeitschrift fur Pflanzenphysiology. International Journal of Plant Physiology* **100S**: 147–160.

Kovtun Y, Chiu WL, Tena G, Sheen J. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proceedings of the National Academy of Science of the USA* **97**: 2940–2945.

Kovtun Y, Chiu WL, Zeng W, Sheen J. 1998. Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature* **395**: 716–720.

Kowalczyk M, Sandberg G. 2001. Quantitative analysis of indole-3-acetic acid metabolites in Arabidopsis. *Plant Physiology* **127**: 1845–1853.

Krikorian AD. 1995. Hormones in tissue culture and micropropagation. In: Davies PJ, ed. *Plant hormones*. Dordrecht: Kluwer, 774–796.

Kutz A, Müller A, Hennig P, Kaiser WM, Piotrowski M, Weiler EW. 2002. A role for nitrilase 3 in the regulation of root morphology in sulphur-starving *Arabidopsis thaliana*. *Plant Journal* **30**: 95–106.

Kwak JM, Moon JH, Murata Y, Kuchitsu K, Leonhardt N, DeLong A, Schroeder JI. 2002. Disruption of a guard cell-expressed protein phosphatase 2A regulatory subunit, *RCN1*, confers abscisic acid insensitivity in Arabidopsis. *The Plant Cell* **14**: 2849–2861.

Larsen PB, Cancel JD. 2003. Enhanced ethylene responsiveness in the *Arabidopsis eer1* mutant results from a loss-of-function mutation in the protein phosphatase 2A A regulatory subunit, *RCN1*. *Plant Journal* **34**: 709–718.

Larsen PB, Cancel JD. 2004. A recessive mutation in the RUB1-conjugating enzyme, *RCE1*, reveals a requirement for RUB modification for

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control of ethylene biosynthesis and proper induction of *basic chitinase* and *PDF1.2* in *Arabidopsis*. *Plant Journal* **38**: 626–638.
Larsen PB, Chang C. 2001. The Arabidopsis *eer1* mutant has enhanced ethylene responses in the hypocotyl and stem. *Plant Physiology* **125**: 1061–1073.
Lasswell J, Rogg LE, Nelson DC, Rongey C, Bartel B. 2000. Cloning and characterization of *IAR1*, a gene required for auxin conjugate sensitivity in Arabidopsis. *The Plant Cell* **12**: 2395–2408.
Last RL, Bissinger PH, Mahoney DJ, Radwanski ER, Fink GR. 1991. Tryptophan mutants in *Arabidopsis*: The consequences of duplicated tryptophan synthase β genes. *The Plant Cell* **3**: 345–358.
Last RL, Fink GR. 1988. Tryptophan-requiring mutants of the plant *Arabidopsis thaliana*. *Science* **240**: 305–310.
Laufs P, Peaucelle A, Morin H, Traas J. 2004. MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. *Development* **131**: 4311–4322.
LeClere S, Rampey RA, Bartel B. 2004. *IAR4*, a gene required for auxin conjugate sensitivity in Arabidopsis, encodes a pyruvate dehydrogenase E1α homolog. *Plant Physiology* **135**: 989–999.
LeClere S, Tellez R, Rampey RA, Matsuda SPT, Bartel B. 2002. Characterization of a family of IAA-amino acid conjugate hydrolases from *Arabidopsis*. *Journal of Biological Chemistry* **277**: 20446–20452.
Lehman A, Black R, Ecker JR. 1996. *HOOKLESS1*, an ethylene response gene, is required for differential cell elongation in the Arabidopsis hypocotyl. *Cell* **85**: 183–194.
Leyser HMO, Lincoln CA, Timpte C, Lammer D, Turner J, Estelle M. 1993. *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. *Nature* **364**: 161–164.
Leznicki AJ, Bandurski RS. 1988. Enzymic synthesis of indole-3-acetyl-1-*O*-β-D-glucose. I. Partial purification and characterization of the enzyme from *Zea mays*. *Plant Physiology* **88**: 1474–1480.
Li H, Johnson P, Stepanova A, Alonso JM, Ecker JR. 2004. Convergence of signaling pathways in the control of differential cell growth in *Arabidopsis*. *Developmental Cell* **7**: 193–204.
Li H-M, Altschmied L, Chory J. 1994. *Arabidopsis* mutants define downstream branches in the phototransduction pathway. *Genes and Development* **8**: 339–349.
Li L, Deng XW. 2003. The COP9 signalosome: an alternative lid for the 26S proteasome? *Trends in Cell Biology* **13**: 507–509.
Lincoln C, Britton JH, Estelle M. 1990. Growth and development of the *axr1* mutants of *Arabidopsis*. *The Plant Cell* **2**: 1071–1080.
Liscum E, Reed JW. 2002. Genetics of Aux/IAA and ARF action in plant growth and development. *Plant Molecular Biology* **49**: 387–400.
Liu J, Furukawa M, Matsumoto T, Xiong Y. 2002. NEDD8 modification of CUL1 dissociates $p120^{CAND1}$, an inhibitor of CUL1-SKP1 binding and SCF ligases. *Molecular Cell* **10**: 1511–1518.
Ljung K, Bhalerao RP, Sandberg G. 2001. Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *Plant Journal* **28**: 465–474.
Ljung K, Hull AK, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G. 2002. Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. *Plant Molecular Biology* **50**: 309–332.
Lomax TL, Muday GK, Rubery PH. 1995. Auxin transport. In: Davies PJ, ed. *Plant hormones*. Dordrecht: Kluwer, 509–530.
Ludwig-Müller J, Epstein E. 1993. Indole-3-butyric acid in *Arabidopsis thaliana*. II. *In vivo* metabolism. *Plant Growth Regulation* **13**: 189–195.
Ludwig-Müller J. 2000. Indole-3-butyric acid in plant growth and development. *Plant Growth Regulation* **32**: 219–230.
Ludwig-Müller J, Cohen JD. 2002. Identification and quantification of three active auxins in different tissues of *Tropaeolum majus*. *Physiologia Plantarum* **115**: 320–329.
Ludwig-Müller J, Epstein E. 1994. Indole-3-butyric acid in *Arabidopsis thaliana*. III. *In vivo* biosynthesis. *Plant Growth Regulation* **14**: 7–14.
Ludwig-Müller J, Hilgenberg W. 1995. Characterization and partial purification of indole-3-butyric acid synthetase from maize (*Zea mays*). *Physiologia Plantarum* **94**: 651–660.
Ludwig-Müller J, Raisig A, Hilgenberg W. 1995. Uptake and transport of indole-3-butyric acid in *Arabidopsis thaliana*: comparison with other natural and synthetic auxins. *Journal of Plant Physiology* **147**: 351–354.
Ludwig-Müller J, Sass S, Sutter EG, Wodner M, Epstein E. 1993. Indole-3-butyric acid in *Arabidopsis thaliana*. I. Identification and quantification. *Plant Growth Regulation* **13**: 179–187.
Luschnig C, Gaxiola RA, Grisafi P, Fink GR. 1998. EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes and Development* **12**: 2175–2187.
McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM. 2003. The Arabidopsis *SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *The Plant Cell* **15**: 1120–1130.
Magidin M, Pittman JK, Hirschi KD, Bartel B. 2003. *ILR2*, a novel gene regulating IAA conjugate sensitivity and metal transport in *Arabidopsis thaliana*. *Plant Journal* **35**: 523–534.
Mallory AC, Dugas DV, Bartel DP, Bartel B. 2004. MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Current Biology* **14**: 1035–1046.
Marchant A, Kargul J, May ST, Muller P, Delbarre A, Perrot-Rechenmann C, Bennett MJ. 1999. AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. *EMBO Journal* **18**: 2066–2073.
Mattsson J, Ckurshumova W, Berleth T. 2003. Auxin signaling in Arabidopsis leaf vascular development. *Plant Physiology* **131**: 1327–1339.
Mattsson J, Sung ZR, Berleth T. 1999. Responses of plant vascular systems to auxin transport inhibition. *Development* **126**: 2979–2991.
Melanson D, Chilton M-D, Masters-Moore D, Chilton WS. 1997. A deletion in an indole synthase gene is responsible for the DIMBOA-deficient phenotype of *bxbx* maize. *Proceedings of the National Academy of Science of the USA* **94**: 13345–13350.
Mikkelsen MD, Hansen CH, Wittstock U, Halkier BA. 2000. Cytochrome P450 CYP79B2 from *Arabidopsis* catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. *Journal of Biological Chemistry* **275**: 33712–33717.
Mikkelsen MD, Naur P, Halkier BA. 2004. *Arabidopsis* mutants in the *C-S* lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. *Plant Journal* **37**: 770–777.
Mockaitis K, Howell SH. 2000. Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of Arabidopsis seedlings. *Plant Journal* **24**: 785–796.
Monroe-Augustus M. 2004. Genetic approaches to elucidating the mechanisms of indole-3-acetic acid and indole-3-butyric acid function in *Arabidopsis thaliana*. PhD Thesis, Department of Biochemistry and Cell Biology, Rice University, Houston, TX, 202 pp.
Monroe-Augustus M, Zolman BK, Bartel B. 2003. IBR5, a dual-specificity phosphatase-like protein modulating auxin and abscisic acid responsiveness in Arabidopsis. *The Plant Cell* **15**: 2979–2991.
Morgan PW, Hall WC. 1962. Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. *Physiologia Plantarum* **15**: 420–427.
Mullen RT, Flynn CR, Trelease RN. 2001. How are peroxisomes formed? The role of the endoplasmic reticulum and peroxins. *Trends in Plant Science* **6**: 256–261.
Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K. 1998*a*. *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. *EMBO Journal* **17**: 6903–6911.
Müller A, Hillebrand H, Weiler EW. 1998*b*. Indole-3-acetic acid is synthesized from L-tryptophan in roots of *Arabidopsis thaliana*. *Planta* **206**: 362–369.
Müller A, Weiler EW. 2000*a*. Indolic constituents and indole-3-acetic acid biosynthesis in the wild-type and a tryptophan auxotroph mutant of *Arabidopsis thaliana*. *Planta* **211**: 855–863.
Müller A, Weiler EW. 2000*b*. IAA-synthase, an enzyme complex from *Arabidopsis thaliana* catalyzing the formation of indole-3-acetic acid from (*S*)-tryptophan. *Biology and Chemistry* **381**: 679–686.
Multani DS, Briggs SP, Chamberlin MA, Blakeslee JJ, Murphy AS, Johal GS. 2003. Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants. *Science* **302**: 81–84.
Nagpal P, Walker LM, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW. 2000. *AXR2* encodes a member of the Aux/IAA protein family. *Plant Physiology* **123**: 563–573.

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Nakazawa M, Yabe N, Ichikawa T, Yamamoto YY, Yoshizumi T, Hasunuma K, Matsui M. 2001. *DFL1*, an auxin-responsive *GH3* gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. *Plant Journal* **25**: 213–221.

Nemhauser JL, Feldman LJ, Zambryski PC. 2000. Auxin and *ETTIN* in *Arabidopsis* gynoecium morphogenesis. *Development* **127**: 3877–3888.

Nemhauser JL, Mockler TC, Chory J. 2004. Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biology* **2**: 1460–1471.

Nigović B, Antolić S, Kojić-Prodić B, Kiralj R, Magnus V, Salopek-Sondi B. 2000. Correlation of structural and physico-chemical parameters with the bioactivity of alkylated derivatives of indole-3-acetic acid, a phytohormone (auxin). *Acta Crystallographica B* **56**: 94–111.

Noh B, Bandyopadhyay A, Peer WA, Spalding EP, Murphy AS. 2003. Enhanced gravi- and phototropism in plant *mdr* mutants mislocalizing the auxin efflux protein PIN1. *Nature* **423**: 999–1002.

Noh B, Murphy AS, Spalding EP. 2001. *Multidrug Resistance*-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *The Plant Cell* **13**: 2441–2454.

Nordström A, Tarkowski P, Tarkowska D, Norbaek R, Åstot C, Dolezal K, Sandberg G. 2004. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin-regulated development. *Proceedings of the National Academy of Science of the USA* **101**: 8039–8044.

Normanly J. 1997. Auxin metabolism. *Physiologia Plantarum* **100**: 431–442.

Normanly J, Cohen JD, Fink GR. 1993. *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. *Proceedings of the National Academy of Science of the USA* **90**: 10355–10359.

Normanly J, Grisafi P, Fink GR, Bartel B. 1997. Arabidopsis mutants resistant to the auxin effects of indole-3-acetonitrile are defective in the nitrilase encoded by the *NIT1* gene. *The Plant Cell* **9**: 1781–1790.

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *The Plant Cell* **3**: 677–684.

Olsen LJ. 1998. The surprising complexity of peroxisome biogenesis. *Plant Molecular Biology* **38**: 163–189.

Oshikawa K, Matsumoto M, Yada M, Kamura T, Hatakeyama S, Nakayama KI. 2003. Preferential interaction of TIP120A with Cul1 that is not modified by NEDD8 and not associated with Skp1. *Biochemical and Biophysical Research Communications* **303**: 1209–1216.

Östin A, Kowalyczk M, Bhalerao RP, Sandberg G. 1998. Metabolism of indole-3-acetic acid in Arabidopsis. *Plant Physiology* **118**: 285–296.

Ouellet F, Overvoorde PJ, Theologis A. 2001. IAA17/AXR3: biochemical insight into an auxin mutant phenotype. *The Plant Cell* **13**: 829–841.

Ouyang J, Shao X, Li J. 2000. Indole-3-glycerol phosphate, a branchpoint of indole-3-acetic acid biosynthesis from the tryptophan biosynthetic pathway in *Arabidopsis thaliana*. *Plant Journal* **24**: 327–333.

Park WJ, Kriechbaumer V, Müller A, Piotrowski M, Meeley RB, Gierl A, Glawischnig E. 2003. The nitrilase ZmNIT2 converts indole-3-acetonitrile to indole-3-acetic acid. *Plant Physiology* **133**: 794–802.

Patten CL, Glick BR. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology* **42**: 207–220.

Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SN, Chen RJ, Masson PH, Murphy AS. 2004. Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in *Arabidopsis thaliana*. *The Plant Cell* **16**: 1898–1911.

Peng Z, Shen Y, Feng S, Wang X, Chitteti BN, Vierstra RD, Deng XW. 2003. Evidence for a physical association of the COP9 signalosome, the proteasome, and specific SCF E3 ligases *in vivo*. *Current Biology* **13**: R504–R505.

Pfluger J, Zambryski P. 2004. The role of *SEUSS* in auxin response and floral organ patterning. *Development* **131**: 4697–4707.

Pickett FB, Wilson AK, Estelle M. 1990. The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. *Plant Physiology* **94**: 1462–1466.

Pollmann S, Müller A, Piotrowski M, Weiler EW. 2002. Occurrence and formation of indole-3-acetamide in *Arabidopsis thaliana*. *Planta* **216**: 155–161.

Pollmann S, Neu D, Weiler EW. 2003. Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid. *Phytochemistry* **62**: 293–300.

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P. 2003. EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. *Cell* **115**: 679–689.

Poupart J, Waddell CS. 2000. The *rib1* mutant is resistant to indole-3-butyric acid, an endogenous auxin in Arabidopsis. *Plant Physiology* **124**: 1739–1751.

Pufky J, Qiu Y, Rao MV, Hurban P, Jones AM. 2003. The auxin-induced transcriptome for etiolated *Arabidopsis* seedlings using a structure/function approach. *Functional & Integrative Genomics* **3**: 135–143.

Quirino BF, Normanly J, Amasino RM. 1999. Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. *Plant Molecular Biology* **40**: 267–278.

Radwanski ER, Barczak AJ, Last RL. 1996. Characterization of tryptophan synthase alpha subunit mutants of *Arabidopsis thaliana*. *Molecularand General Genetics* **253**: 353–361.

Radwanski ER, Last RL. 1995. Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. *The Plant Cell* **7**: 921–934.

Ramos JA, Zenser N, Leyser O, Callis J. 2001. Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent. *The Plant Cell* **13**: 2349–2360.

Rampey RA, LeClere S, Kowalczyk M, Ljung K, Sandberg G, Bartel B. 2004. A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during Arabidopsis germination. *Plant Physiology* **135**: 978–988.

Rashotte AM, DeLong A, Muday GK. 2001. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. *The Plant Cell* **13**: 1683–1697.

Rashotte AM, Poupart J, Waddell CS, Muday GK. 2003. Transport of the two natural auxins, indole-3-butyric acid and indole-3-acetic acid, in Arabidopsis. *Plant Physiology* **133**: 761–772.

Reed JW, Elumalai RP, Chory J. 1998. Suppressors of an *Arabidopsis thaliana phyB* mutation identify genes that control light signaling and hypocotyl elongation. *Genetics* **148**: 1295–1310.

Reed RC, Brady SR, Muday GK. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiology* **118**: 1369–1378.

Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C. 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* **426**: 255–260.

Reintanz B, Lehnen M, Reichelt M, Gershenzon J, Kowalczyk M, Sandberg G, Godde M, Uhl R, Palme K. 2001. *bus*, a bushy Arabidopsis *CYP79F1* knockout mutant with abolished synthesis of short-chain aliphatic glucosinolates. *The Plant Cell* **13**: 351–367.

Remington DL, Vision TJ, Guilfoyle TJ, Reed JW. 2004. Contrasting modes of diversification in the *Aux/IAA* and *ARF* gene families. *Plant Physiology* **135**: 1738–1752.

Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP. 2002. Prediction of plant microRNA targets. *Cell* **110**: 513–520.

Ribnicky DM, Cohen JD, Hu W-S, Cooke TJ. 2002. An auxin surge following fertilization in carrots: a mechanism for regulating plant totipotency. *Planta* **214**: 505–509.

Richmond TA, Bleecker AB. 1999. A defect in β-oxidation causes abnormal inflorescence development in Arabidopsis. *The Plant Cell* **11**: 1911–1923.

Rogg LE, Bartel B. 2001. Auxin signaling: derepression through regulated proteolysis. *Developmental Cell* **1**: 595–604.

Rogg LE, Lasswell J, Bartel B. 2001. A gain-of-function mutation in *IAA28* suppresses lateral root development. *The Plant Cell* **13**: 465–480.

Ross JJ, O'Neill DP, Smith JJ, Kerckhoffs LHJ, Elliott RC. 2000. Evidence that auxin promotes gibberellin A_1 biosynthesis in pea. *Plant Journal* **21**: 547–552.

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O. 1998. Changes in auxin response from mutations in an *AUX/IAA* gene. *Science* **279**: 1371–1373.

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Ruegger M, Dewey E, Gray WM, Hobbie L, Turner J, Estelle M. 1998. The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast Grr1p. *Genes and Development* **12**: 198–207.

Ruegger M, Dewey E, Hobbie L, Brown D, Bernasconi P, Turner J, Muday G, Estelle M. 1997. Reduced naphthylphthalamic acid binding in the *tir3* mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. *The Plant Cell* **9**: 745–757.

Rylott EL, Rogers CA, Gilday AD, Edgell T, Larson TR, Graham IA. 2003. *Arabidopsis* mutants in short- and medium-chain acyl-CoA oxidase activities accumulate acyl-CoAs and reveal that fatty acid β-oxidation is essential for embryo development. *Journal of Biological Chemistry* **278**: 21370–21377.

Salamini F. 2003. Hormones and the green revolution. *Science* **302**: 71–72.

Samach A, Klenz JE, Kohalmi SE, Risseeuw E, Haughn GW, Crosby WL. 1999. The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. *Plant Journal* **20**: 433–445.

Sawa S, Ohgishi M, Goda H, Higuchi K, Shimada Y, Yoshida S, Koshiba T. 2002. The *HAT2* gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in *Arabidopsis*. *Plant Journal* **32**: 1011–1022.

Schmidt R-C, Müller A, Hain R, Bartling D, Weiler EW. 1996. Transgenic tobacco plants expressing the *Arabidopsis thaliana* nitrilase II enzyme. *Plant Journal* **9**: 683–691.

Schwechheimer C, Deng XW. 2001. COP9 signalosome revisited: a novel mediator of protein degradation. *Trends in Cell Biology* **11**: 420–426.

Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M, Deng X-W. 2001. Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} in mediating auxin response. *Science* **292**: 1379–1382.

Schwechheimer C, Serino G, Deng XW. 2002. Multiple ubiquitin ligase-mediated processes require COP9 signalosome and AXR1 function. *The Plant Cell* **14**: 2553–2563.

Schwetz BA, Norris JM, Sparschu GL, Rowe VK, Gehring PJ, Emerson JL, Gerbig CG. 1973. Toxicology of chlorinated dibenzo-*p*-dioxins. *Environmental Health Perspectives* **5**: 87–99.

Scott TK, Wilkins MB. 1968. Auxin transport in roots. II. Polar flux of IAA in *Zea* roots. *Planta* **83**: 323–334.

Sekimoto H, Seo M, Kawakami N, Komano T, Desloire S, Liotenberg S, Marion-Poll A, Caboche M, Kamiya Y, Koshiba T. 1998. Molecular cloning and characterization of aldehyde oxidases in *Arabidopsis thaliana*. *Plant Cell Physiology* **39**: 433–442.

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD. 2001. Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Science of the USA* **98**: 4788–4793.

Seo M, Akaba S, Oritani T, Delarue M, Bellini C, Caboche M, Koshiba T. 1998. Higher activity of an aldehyde oxidase in the auxin-overproducing *superroot1* mutant of *Arabidopsis thaliana*. *Plant Physiology* **116**: 687–693.

Sessions A, Nemhauser JL, McCall A, Roe JL, Feldmann KA, Zambryski PC. 1997. *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. *Development* **124**: 4481–4491.

Sessions RA, Zambryski PC. 1995. *Arabidopsis* gynoecium structure in the wild type and in *ettin* mutants. *Development* **121**: 1519–1532.

Shen WH, Parmentier Y, Hellmann H, Lechner E, Dong A, Masson J, Granier F, Lepiniec L, Estelle M, Genschik P. 2002. Null mutation of *AtCUL1* causes arrest in early embryogenesis in *Arabidopsis*. *Molecular Biology of the Cell* **13**: 1916–1928.

Silverstone AL, Jung HS, Dill A, Kawaide H, Kamiya Y, Sun TP. 2001. Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *The Plant Cell* **13**: 1555–1565.

Skoog F, Miller CO. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symposia of the Society for Experimental Biology* **11**: 118–131.

Slovin JP, Bandurski RS, Cohen JD. 1999. Auxin. In: Hooykaas PJJ, Hall MA, Libbenga KR, eds. *Biochemistry and molecular biology of plant hormones*. Amsterdam: Elsevier, 115–140.

Smolen G, Bender J. 2002. Arabidopsis cytochrome P450 *cyp83B1* mutations activate the tryptophan biosynthetic pathway. *Genetics* **160**: 323–332.

Soh MS, Hong SH, Kim BC, Vizir I, Park DH, Choi G, Hong MY, Chung Y-Y, Furuya M, Nam HG. 1999. Regulation of both light- and auxin-mediated development by the *Arabidopsis IAA3/SHY2* gene. *Journal of Plant Biology* **42**: 239–246.

Somerville CR, Ogren WL. 1980. Inhibition of photosynthesis in *Arabidopsis* mutants lacking leaf glutamate synthase activity. *Nature* **286**: 257–259.

Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W. 2005. Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-acetic acid. *The Plant Cell* **17**: 616–627.

Staswick PE, Tiryaki I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *The Plant Cell* **16**: 2117–2127.

Staswick PE, Tiryaki I, Rowe ML. 2002. Jasmonate response locus *JAR1* and several related Arabidopsis genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. *The Plant Cell* **14**: 1405–1415.

Stowe-Evans EL, Harper RM, Motchoulski AV, Liscum E. 1998. NPH4, a conditional modulator of auxin-dependent differential growth responses in Arabidopsis. *Plant Physiology* **118**: 1265–1275.

Strader LC, Ritchie S, Soule JD, McGinnis KM, Steber CM. 2004. Recessive-interfering mutations in the gibberellin signaling gene *SLEEPY1* are rescued by overexpression of its homologue, *SNEEZY*. *Proceedings of the National Academy of Science of the USA* **101**: 12771–12776.

Subramani S. 1998. Components involved in peroxisome import, biogenesis, proliferation, turnover, and movement. *Physiological Reviews* **78**: 171–188.

Sullivan ML, Green PJ. 1996. Mutational analysis of the DST element in tobacco cells and transgenic plants: identification of residues critical for mRNA instability. *RNA* **2**: 308–315.

Sun H, Basu S, Brady SR, Luciano RL, Muday GK. 2004. Interactions between auxin transport and the actin cytoskeleton in developmental polarity of *Fucus distichus* embryos in response to light and gravity. *Plant Physiology* **135**: 266–278.

Sunkar R, Zhu JK. 2004. Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. *The Plant Cell* **16**: 2001–2019.

Surpin M, Raikhel N. 2004. Traffic jams affect plant development and signal transduction. *Nature Reviews. Molecular Cell Biology* **5**: 100–109.

Suttle JC. 1988. Effect of ethylene treatment on polar IAA transport, net IAA uptake and specific binding of *N*-1-naphthylphthalamic acid in tissues and microsomes isolated from etiolated pea epicotyls. *Plant Physiology* **88**: 795–799.

Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M. 2001. Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the *Arabidopsis* root apex. *Genes and Development* **15**: 2648–2653.

Swarup R, Kargul J, Marchant A, Zadik D, Rahman A, Mills R, Yemm A, May S, Williams L, Millner P, *et al*. 2004. Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. *The Plant Cell* **16**: 3069–3083.

Szerszen JB, Szczyglowski K, Bandurski RS. 1994. *iaglu*, a gene from *Zea mays* involved in conjugation of growth hormone indole-3-acetic acid. *Science* **265**: 1699–1701.

Sztein AE, Cohen JD, de la Fuente IG, Cooke TJ. 1999. Auxin metabolism in mosses and liverworts. *American Journal of Botany* **86**: 1544–1555.

Sztein AE, Ilic N, Cohen JD, Cooke TJ. 2002. Indole-3-acetic acid biosynthesis in isolated axes from germinating bean seeds: the effect of wounding on the biosynthetic pathway. *Plant Growth Regulation* **36**: 201–207.

Tabak HF, Braakman I, Distel B. 1999. Peroxisomes: simple in function but complex in maintenance. *Trends in Cell Biology* **9**: 447–453.

Takase T, Nakazawa M, Ishikawa A, Kawashima M, Ichikawa T, Takahashi N, Shimada H, Manabe K, Matsui M. 2004. *ydk1-D*, an auxin-responsive *GH3* mutant that is involved in hypocotyl and root elongation. *Plant Journal* **37**: 471–483.

Tam YY, Epstein E, Normanly J. 2000. Characterization of auxin conjugates in Arabidopsis. Low steady-state levels of indole-3-acetyl-aspartate, indole-3-acetyl-glutamate, and indole-3-acetyl-glucose. *Plant Physiology* **123**: 589–595.

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Tam YY, Normanly J. 1998. Determination of indole-3-pyruvic acid levels in *Arabidopsis thaliana* by gas chromatography-selected ion monitoring-mass spectrometry. *Journal of Chromatography A* **800**: 101–108.

Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto KT. 2004. *MASSUGU2* encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*. *The Plant Cell* **16**: 379–393.

Thimann KV. 1977. *Hormone action in the whole life of plants*. Amherst: University of Massachusetts Press.

Thimann KV, Mahadevan S. 1964. Nitrilase. I. Occurrence, preparation, and general properties of the enzyme. *Archives of Biochemistry and Biophysics* **105**: 133–141.

Thimann KV, Skoog, F. 1934. On the inhibition of bud development and other functions of growth substance in *Vicia faba. Proceedings of the Royal Society of London Series B. Biological Science* **114**: 317–339.

Tian CE, Muto H, Higuchi K, Matamura T, Tatematsu K, Koshiba T, Yamamoto KT. 2004. Disruption and overexpression of *auxin response factor 8* gene of *Arabidopsis* affect hypocotyl elongation and root growth habit, indicating its possible involvement in auxin homeostasis in light condition. *Plant Journal* **40**: 333–343.

Tian Q, Nagpal P, Reed JW. 2003. Regulation of *Arabidopsis* SHY2/IAA3 protein turnover. *Plant Journal* **36**: 643–651.

Tian Q, Reed JW. 1999. Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. *Development* **126**: 711–721.

Timpte C, Lincoln C, Pickett FB, Turner J, Estelle M. 1995. The *AXR1* and *AUX1* genes of *Arabidopsis* function in separate auxin-response pathways. *Plant Journal* **8**: 561–569.

Tiryaki I, Staswick PE. 2002. An Arabidopsis mutant defective in jasmonate response is allelic to the auxin-signaling mutant *axr1*. *Plant Physiology* **130**: 887–894.

Tiwari SB, Hagen G, Guilfoyle T. 2003. The roles of auxin response factor domains in auxin-responsive transcription. *The Plant Cell* **15**: 533–543.

Tiwari SB, Hagen G, Guilfoyle TJ. 2004. Aux/IAA proteins contain a potent transcriptional repression domain. *The Plant Cell* **16**: 533–543.

Tiwari SB, Wang X-J, Hagen G, Guilfoyle TJ. 2001. Aux/IAA proteins are active repressors, and their stability and activity are modulated by auxin. *The Plant Cell* **13**: 2809–2822.

Tobeña-Santamaria R, Bliek M, Ljung K, Sandberg G, Mol JNM, Souer E, Koes R. 2002. FLOOZY of petunia is a flavin mono-oxygenase-like protein required for the specification of leaf and flower architecture. *Genes and Development* **16**: 753–763.

Tsurumi S, Ohwaki Y. 1978. Transport of ^{14}C-labeled indoleacetic acid in *Vicia* root segments. *The Plant Cell Physiology* **19**: 1195–1206.

Ulmasov T, Hagen G, Guilfoyle TJ. 1997*a*. ARF1, a transcription factor that binds auxin response elements. *Science* **276**: 1865–1868.

Ulmasov T, Hagen G, Guilfoyle TJ. 1999*a*. Dimerization and DNA binding of auxin response factors. *Plant Journal* **19**: 309–319.

Ulmasov T, Hagen G, Guilfoyle TJ. 1999*b*. Activation and repression of transcription by auxin-response factors. *Proceedings of the National Academy of Science of the USA* **96**: 5844–5849.

Ulmasov T, Liu Z-B, Hagen G, Guilfoyle TJ. 1995. Composite structure of auxin response elements. *The Plant Cell* **7**: 1611–1623.

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ. 1997*b*. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *The Plant Cell* **9**: 1963–1971.

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

Wain RL, Wightman F. 1954. The growth-regulating activity of certain ω-substituted alkyl carboxylic acids in relation to their β-oxidation within the plant. *Proceedings of the National Academy of Science of the USA* **142**: 525–536.

Walker JC, Key JL. 1982. Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$ RNAs of elongating soybean hypocotyl. *Proceedings of the National Academy of Science of the USA* **79**: 7185–7189.

Walz A, Park S, Slovin JP, Ludwig-Müller J, Momonoki YS, Cohen JD. 2002. A gene encoding a protein modified by the phytohormone indoleacetic acid. *Proceedings of the National Academy of Science of the USA* **99**: 1718–1723.

Wang X, Kang D, Feng S, Serino G, Schwechheimer C, Wei N. 2002. CSN1 N-terminal-dependent activity is required for Arabidopsis development but not for Rub1/Nedd8 deconjugation of cullins: a structure-function study of CSN1 subunit of COP9 signalosome. *Molecular Biology of the Cell* **13**: 646–655.

Went FW. 1926. On growth-accelerating substances in the coleoptile of *Avena sativa*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen. Series C. Biological and Medical Sciences* **30**: 10–19.

Went FW, Thimann KV. 1937. *Phytohormones*. New York: The Macmillan Company.

Wightman F. 1977. *Plant growth regulation*. Berlin: Springer-Verlag.

Wilson AK, Pickett FB, Turner JC, Estelle M. 1990. A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene, and abscisic acid. *Molecular and General Genetics* **222**: 377–383.

Winkler RG, Frank MR, Galbraith DW, Feyereisen R, Feldmann KA. 1998. Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis: isolation of mutations in the cytochrome P450 gene superfamily. *Plant Physiology* **118**: 743–750.

Wolbang CM, Chandler PM, Smith JJ, Ross JJ. 2004. Auxin from the developing inflorescence is required for the biosynthesis of active gibberellins in barley stems. *Plant Physiology* **134**: 769–776.

Woodward AW, Bartel B. 2005. The *Arabidopsis* peroxisomal targeting signal type 2 receptor PEX7 is necessary for peroxisome function and dependent on PEX5. *Molecular Biology of the Cell* **16**: 573–583.

Worley CK, Zenser N, Ramos J, Rouse D, Leyser O, Theologis A, Callis J. 2000. Degradation of Aux/IAA proteins is essential for normal auxin signalling. *Plant Journal* **21**: 553–562.

Wright AD, Moehlenkamp CA, Perrot GH, Neuffer MG, Cone KC. 1992. The maize auxotrophic mutant *orange pericarp* is defective in duplicate genes for tryptophan synthase β. *The Plant Cell* **4**: 711–719.

Wright AD, Sampson MB, Neuffer MG, Michalczuk L, Slovin JP, Cohen JD. 1991. Indole-3-acetic acid biosynthesis in the mutant maize *orange pericarp*, a tryptophan auxotroph. *Science* **254**: 998–1000.

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG. 1998. *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* **280**: 1091–1094.

Xie Q, Frugis G, Colgan D, Chua N-H. 2000. *Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes and Development* **14**: 3024–3036.

Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D. 2002. The SCF$^{\mathrm{CoI1}}$ ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. *The Plant Cell* **14**: 1919–1935.

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

Yang X, Lee S, So JH, Dharmasiri S, Dharmasiri N, Ge L, Jensen C, Hangarter R, Hobbie L, Estelle M. 2004. The IAA1 protein is encoded by *AXR5* and is a substrate of SCF$^{\mathrm{TIR1}}$. *Plant Journal* **40**: 772–782.

Zenser N, Ellsmore A, Leasure C, Callis J. 2001. Auxin modulates the degradation rate of Aux/IAA proteins. *Proceedings of the National Academy of Science of the USA* **98**: 11795–11800.

Zhao D, Ni W, Feng B, Han T, Petrasek MG, Ma H. 2003*a*. Members of the *Arabidopsis-SKP1-like* gene family exhibit a variety of expression patterns and may play diverse roles in Arabidopsis. *Plant Physiology* **133**: 203–217.

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J. 2001. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **291**: 306–309.

Zhao Y, Dai X, Blackwell HE, Schreiber SL, Chory J. 2003*b*. SIR1, an upstream component in auxin signaling identified by chemical genetics. *Science* **301**: 1107–1110.

Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JR, Normanly J, Chory J, Celenza JL. 2002. Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes and Development* **16**: 3100–3112.

Zheng J, Yang X, Harrell JM, Ryzhikov S, Shim EH, Lykke-Andersen K, Wei N, Sun H, Kobayashi R, Zhang H. 2002. CAND1 binds to

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unneddylated CUL1 and regulates the formation of SCF ubiquitin E3 ligase complex. *Molecular Cell* **10**: 1519–1526.

Zimmerman PW, Hitchcock AE. 1942. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. *Contributions of the Boyce Thompson Institute* **12**.

Zolman BK, Bartel B. 2004. An *Arabidopsis* indole-3-butyric acid-response mutant defective in PEROXIN6, an apparent ATPase implicated in peroxisomal function. *Proceedings of the National Academy of Science of the USA* **101**: 1786–1791.

Zolman BK, Monroe-Augustus M, Thompson B, Hawes JW, Krukenberg KA, Matsuda SPT, Bartel B. 2001*a*. *chy1*, an *Arabidopsis* mutant with impaired β-oxidation, is defective in a peroxisomal β-hydroxyisobutyryl-CoA hydrolase. *Journal of Biological Chemistry* **276**: 31037–31046.

Zolman BK, Silva ID, Bartel B. 2001*b*. The Arabidopsis *pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid β-oxidation. *Plant Physiology* **127**: 1266–1278.

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

Zubieta C, Ross JR, Koscheski P, Yang Y, Pichersky E, Noel JP. 2003. Structural basis for substrate recognition in the salicylic acid carboxyl methyltransferase family. *The Plant Cell* **15**: 1704–1716.

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