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Auxin Receptors and Plant Development: A New Signaling Paradigm

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hormone receptor, plant development, SCF, gene transcription

Abstract

The plant hormone auxin, in particular indole-3-acetic acid (IAA), is a key regulator of virtually every aspect of plant growth and development. Auxin regulates transcription by rapidly modulating levels of Aux/IAA proteins throughout development. Recent studies demonstrate that auxin perception occurs through a novel mechanism. Auxin binds to TIR1, the F-box subunit of the ubiquitin ligase complex SCF^{TIR1}, and stabilizes the interaction between TIR1 and Aux/IAA substrates. This interaction results in Aux/IAA ubiquitination and subsequent degradation. Regulation of the Aux/IAA protein family by TIR1 and TIR1-like auxin receptors (AFBs) links auxin action to transcriptional regulation and provides a model by which the vast array of auxin influences on development may be understood. Moreover, auxin receptor function is the first example of small-molecule regulation of an SCF ubiquitin ligase and may have important implications for studies of regulated protein degradation in other species, including animals.

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INTRODUCTION: AUXIN IS A MASTER REGULATOR OF GROWTH AND DEVELOPMENT

Two hundred and fifty years have passed since Henri-Louis Duhamel du Monceau reported that callus and roots formed above, but not below, the position of rings tightly clamped around young tree trunks (Duhamel 1758). He attributed this phenomenon to the accumulation of an organogenic sap that moved down from the leaves. The concept of plant hormones initially began to take shape in the nineteenth century as Julius von Sachs suggested that plant organ-forming substances move directionally within the plant, including a root-forming substance produced in leaves that moves downward (Sachs 1880). Charles Darwin likewise proposed that a mobile substance regulates the

bending of grass coleoptiles toward the sun (Darwin 1880). Subsequent attempts to purify and characterize substances responsible for each of these phenomena converged on a class of compounds called the auxins (Kögl & Smit 1931; Went 1926; Went & Thimann 1937, pp. 6–17), predominantly indole-3-acetic acid (IAA). These early observations, that auxin initiates processes as divergent as root formation and phototropic bending of stems, anticipated the breadth and complexity of the role(s) of auxin in plant development.

Much of plant growth depends on auxin-induced cell expansion and division, and numerous mutants deficient in auxin response exhibit overall dwarfism. Altered organ morphology is also commonly observed in auxin mutants owing to localized defects in growth.

The regulation of auxin distribution plays an essential role in many developmental processes. Recent excellent reviews describe the transport mechanisms that promote auxin movement from cell to cell during development and in response to environmental signals (Leyser 2006, Scheres & Xu 2006, Teale et al. 2006). Light regulates the orientation of shoot growth to optimize plant survival, a process termed phototropism, by promoting asymmetric auxin distribution in stems. This asymmetry produces a corresponding growth asymmetry resulting in organ bending. Originally proposed in the Cholodny-Went hypothesis, this mechanism explains Darwin's original observations of phototropism (Blancaflor & Masson 2003, Darwin 1880). In addition to light, other signals can influence auxin distribution. Recent studies indicate that brassinosteroid hormones influence development in part by affecting the distribution of auxin (Bao et al. 2004). Alterations in auxin localization can modify differential growth among cells within an organ, thus influencing organ shape, and can modify localized auxin-induced organogenesis, influencing overall plant architecture.

Auxin is required for the generation and maintenance of primary meristems as well as the formation of axillary meristems. The importance of auxin in both cell proliferation and meristem organization appears to contrast with its role in the expansion of already differentiated cells. Auxin is thought to be essential as early as the first zygotic division (Weijers & Jurgens 2005). During embryogenesis, auxin is necessary for specification of the initial cell of the root meristem and the vascular connectivity of all developing organs. As the plant matures, auxin reactivates differentiated cells to promote additional vascular tissue development and regulate lateral organ formation. Auxin also regulates organ polarity. Leaves and flowers that are severely auxin deficient or have reduced auxin response exhibit loss of asymmetry and develop as radial or funnel-shaped organs (Pekker et al. 2005).

Although a detailed understanding of the molecular basis for the complexity of auxin ac-

tivity has not yet emerged, progress during the past 15 years has identified critical components of auxin signaling and provided a framework for addressing how auxin regulates diverse developmental processes. Perhaps the most dramatic development is the recent discoveries that the *Arabidopsis* F-box protein TIR1 is an auxin receptor and that auxin functions by directly regulating the ubiquitin (Ub) protein ligase SCF^{TIR1}. Here we provide an overview of current research describing this auxin receptor, the Ub cycle in which it functions, and the transcriptional regulators it targets.

AUXIN RAPIDLY REGULATES TRANSCRIPTION

Three decades of studies have explored the rapid effects of auxin on gene expression. Genome-wide studies indicate that the transcriptional response to auxin is rapid and broad, influencing the expression of a large and diverse set of genes within minutes (Goda et al. 2004, Nemhauser et al. 2006, Overvoorde et al. 2005, Tian et al. 2002; K. Mockaitis & M. Estelle, unpublished). Early work identified the *Aux/IAA* genes on the basis of their rapid transcriptional activation by auxin, and auxin response factors (ARFs) on the basis of their ability to bind auxin-responsive promoters and regulate transcription (Abel & Theologis 1996, Guilfoyle & Hagen 2007). Subsequent studies demonstrated that the *Aux/IAA* proteins are unstable transcriptional repressors that can interact with ARFs (Abel et al. 1995, Ainley et al. 1988, Conner et al. 1990, Guilfoyle et al. 1993, Theologis et al. 1985, Yamamoto et al. 1992). In *Arabidopsis* the ARF and *Aux/IAA* proteins are encoded by families of 23 and 29 members, respectively, and together remain the focal point of studies of auxin response.

The structures of the *Aux/IAA* and ARF proteins have been reviewed extensively (Guilfoyle & Hagen 2007, Reed 2001). ARFs are defined by their conserved N-terminal DNA-binding domain and an adjacent domain [middle region (MR)] that determines their effects on transcription. For 5 of the ARFs, this MR is a

glutamine-rich domain that activates transcription either as part of an intact ARF protein or as an isolated domain. Up to 15 of the remaining ARF proteins may function as transcriptional repressors, whereas the activities of 3 more highly diverged ARFs remain unknown (Okushima et al. 2005, Tiwari et al. 2003, Ulmasov et al. 1999a).

Both ARF and Aux/IAA proteins contain conserved sequences near the C terminus termed domains III and IV. These domains mediate ARF-ARF, ARF-Aux/IAA, and Aux/IAA-Aux-IAA interactions in yeast two-hybrid tests. Recent quantitative assessments show that, for at least some ARF-Aux/IAA pairs, the heterodimer is more stable than either homodimer (Muto et al. 2006). Thus, ARF binding to Aux/IAA proteins may inhibit ARF-ARF dimer formation (Ulmasov et al. 1999b). Evidence exists, however, for an alternative or additional function of Aux/IAAs as active transcriptional repressors (Tiwari et al. 2001). A region in most Aux/IAA proteins, called domain I, contains a Leu-rich motif conserved among other families of transcriptional repressors. When fused to a DNA-binding domain, domain I effectively represses a heterologous transcriptional activator (VP16) and the activity of intact ARF proteins (Tiwari et al. 2004). Repression by domain I requires proximity to the activator on promoters, and domain I fails to act at a distance, consistent with the possibility that in some promoter complexes Aux/IAAs repress transcription through direct association with DNA-bound ARFs (Tiwari et al. 2004). Recently, Szemenyei et al. (2008) found that Aux/IAA proteins interact with a transcriptional corepressor called TOPLESS (TPL). During embryogenesis, TPL binds to domain I of the Aux/IAA protein BDL/IAA12 and represses MP/ARF5-dependent development. Thus, one function of Aux/IAAs appears to be the recruitment of corepressors.

Earlier studies showed that ARF activity varies with promoter context (Tiwari et al. 2003). Recent studies either have suggested (Nemhauser et al. 2004) or have demonstrated (Shin et al. 2007) that unrelated transcrip-

tion factors act within or near ARF complexes. Whether Aux/IAAs function within such complexes or interact with ARFs prior to complex formation remains to be determined. Substantial sequence divergence in regions outside the conserved domains suggests that members of the Aux/IAA family may have diverse binding partners, perhaps explaining some of the complexity of auxin-mediated development. The identification of protein complexes that act on auxin-responsive promoters and the influence of Aux/IAAs on the assembly and/or activities of these complexes are important areas of ongoing investigation.

What is clear is that transcriptional and developmental responses to auxin are sensitive to the levels of Aux/IAA proteins (Dreher et al. 2006; Knox et al. 2003; Ouellet et al. 2001; Ramos et al. 2001; Tian et al. 2002; Timpte et al. 1994; Worley et al. 2000; Zenser et al. 2001, 2003). Multiple lines of evidence show that a small, conserved structure in the Aux/IAA proteins, called domain II, is essential for the degradation of these repressors and links their concentration directly to auxin levels. Developmental genetic screens conducted by several groups recovered dominant or semidominant gain-of-function mutations in a number of *Aux/IAA* genes (Table 1). In each line the mutation occurred within the conserved domain II sequence (GWPPV/I) (Reed 2001). Subsequent demonstration that domain II functions as an auxin-dependent degron suggested that the gain-of-function mutations stabilize the affected protein (Gray et al. 2001, Ramos et al. 2001). This was later confirmed for several of the *aux/iaa* mutants (Ouellet et al. 2001, Tian et al. 2003).

Plants harboring stabilized mutant forms of Aux/IAA proteins exhibit an array of developmental phenotypes, including aberrant embryonic patterning, seedling and mature organ development, tropic growth, maturation, and fertility (Liscum & Reed 2002). As expected, Aux/IAA stabilization in plants correlates with reduced auxin-regulated transcription. Studies of more recently available loss-of-function mutants in the *ARF* genes are beginning to

Table 1 Aux/IAA proteins in *Arabidopsis thaliana* and evidence for their roles in auxin-mediated development

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
<i>LAA1</i>	IAA1	Auxin decreases protein half-life; <i>axr5-1</i> gain-of-function mutation is in degron	<i>axr5-1</i> degron mutation reduces multiple auxin responses	Abel et al. 1995; Park et al. 2002; Yang et al. 2004; Zenser et al. 2001, 2003
<i>LAA2</i>	IAA2	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
<i>LAA3</i>	SUPPRESSOR OF <u>HY2</u> or <u>SHORT HYPOCOTYL 2</u> (SHY2/IAA3)	<i>shy2-1, -2, -3, -6</i> mutations are in degron	<i>shy2-1, -2, -3, -6</i> degron mutations reduce multiple auxin responses	Abel et al. 1995, Kim et al. 1996, Reed 2001, Reed et al. 1998, Soh et al. 1999, Tian & Reed 1999, 2003
<i>LAA4</i>	IAA4	Pea ortholog shows rapid turnover in vivo	Phylogenetic relationship	Abel et al. 1994, 1995; Liscum & Reed 2002
<i>LAA5</i>	IAA5	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
<i>LAA6</i>	SUPPRESSOR OF <u>HY1</u> (SHY1/IAA6)	Pea ortholog shows rapid turnover in vivo; <i>shy1-1</i> mutation is in degron	<i>shy1-1</i> -stabilizing mutation reduces multiple auxin responses	Abel et al. 1994, 1995; Kim et al. 1996; Ramos et al. 2001; Reed 2001
<i>LAA7</i>	<u>AUXIN RESISTANT 2</u> (AXR2/IAA7)	Auxin decreases protein half-life; protein can interact with TIR1; <i>axr2-1</i> mutation is in degron; <i>axr2-1</i> mutation abolishes protein interaction with TIR1 and increases protein half-life	<i>axr2-1</i> -stabilizing mutations reduce multiple auxin responses	Abel et al. 1995, N. Dharmasiri et al. 2003, Gray et al. 2001, Nagpal et al. 2000, Timpte et al. 1994
<i>LAA8</i>	IAA8	Protein shows rapid turnover in vivo; contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Dreher et al. 2006, Liscum & Reed 2002
<i>LAA9</i>	IAA9	Protein shows rapid turnover in vivo; contains domain II degron	RNAi-reduced levels in tomato increase sensitivity to auxin in multiple developmental processes	Abel et al. 1995, Dreher et al. 2006, Liscum & Reed 2002, Wang et al. 2005
<i>LAA10</i>	IAA10	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995
<i>LAA11</i>	IAA11	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
<i>LAA12</i>	BODENLOS (BDL/IAA12)	<i>bdl</i> mutation is in degron	<i>bdl</i> degron mutation reduces multiple auxin responses	Abel et al. 1995; Hamann et al. 1999, 2002; Liscum & Reed 2002
<i>LAA13</i>	IAA13	Contains domain II degron	Degrone mutant transgene impairs auxin-related development	Abel et al. 1995, Weijers et al. 2005
<i>LAA14</i>	<u>SOLITARY ROOT</u> (SLR/IAA14)	<i>slr-1</i> mutation is in degron	<i>slr-1</i> degron mutation reduces multiple auxin responses	Abel et al. 1995; Fukaki et al. 2002, 2005; Vanneste et al. 2005
<i>LAA15</i>	IAA15	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
<i>LAA16</i>	IAA16	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002

(Continued)

Table 1 (Continued)

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
<i>IAA17</i>	AUXIN RESISTANT 3 (AXR3/IAA17)	Auxin decreases protein half-life; protein can interact with TIR1; <i>axr3</i> mutations are in degron and increase protein half-life	<i>axr3-1</i> and <i>-3</i> degron mutations reduce multiple auxin responses	N. Dharmasiri et al. 2003, Gray et al. 2001, Leyser et al. 1996, Ouellet et al. 2001, Overvoorde et al. 2005, Rouse et al. 1998
<i>IAA18</i>	IAA18	<i>iaa18-1</i> mutation is in degron	<i>iaa18-1</i> degron mutation reduces multiple auxin responses	Reed 2001
<i>IAA19</i>	MASSUGU 2 (MSG2/IAA19)	<i>msg2-1</i> to <i>-4</i> mutations are in degron	<i>msg2-1</i> to <i>-4</i> degron mutations reduce multiple auxin responses	Liscum & Reed 2002, Tatematsu et al. 2004
<i>IAA26</i>	Phytochrome interacting protein 1 (PAP1/IAA26)	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
<i>IAA27</i>	Phytochrome interacting protein 2 (PAP2/IAA27)	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
<i>IAA28</i>	IAA28	Auxin decreases protein half-life; <i>iaa28-1</i> mutation is in degron	<i>iaa28-1</i> degron mutations reduce multiple auxin responses	Dreher et al. 2006, Rogg et al. 2001
<i>IAA29</i>	IAA29	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
<i>IAA31</i>	IAA31	Auxin decreases protein half-life; imperfect conservation of domain II correlates with a half-life longer than that of other Aux/IAAs in vivo	Phylogenetic relationship	Dreher et al. 2006, Liscum & Reed 2002

contribute to our understanding of auxin-regulated transcription in development. Selected examples are described below. Similarities in the phenotypes conferred by loss-of-function *arf* mutations and stabilizing *aux/iaa* domain II mutations have led to recent functional pairing of some Aux/IAA and ARF proteins (Fukaki et al. 2006; Tatematsu et al. 2004; Weijers et al. 2005, 2006). In each case, yeast two-hybrid or other interaction assays have confirmed the potential for direct protein-protein interactions.

AUX/IAA AND ARF PROTEINS MEDIATE AUXIN ACTION IN VASCULAR DEVELOPMENT AND ORGANOGENESIS

Auxin plays a pivotal role in vascular tissue and organ initiation. Studies of these processes pro-

vide the most detailed descriptions to date of the role of auxin in development. During embryogenesis auxin is required for normal organ formation, as evidenced by early developmental arrest in several auxin response mutants. Loss of ARF5 function in the *Arabidopsis* mutant *monopteros* (*mp*) completely prevents root formation (Berleth & Jurgens 1993, Hardtke & Berleth 1998, Weijers & Jurgens 2005). Identical effects are seen in the *bodenlos* (*bdl*) mutant, in which the Aux/IAA protein IAA12 is stabilized by a mutation in domain II (Hamann et al. 1999, 2002; Weijers & Jurgens 2005). Lack of root development in *mp* and *bdl* is due to impaired development of a single cell called the hypophysis, the founder cell of the basal meristem. In the apical portion of *mp* (and *bdl*) embryos, vascular tissue development is severely reduced, reflecting a general loss of normal

cell proliferation. Smaller meristems, reduced cotyledon/leaf emergence, and defects in floral meristem formation in *mp* plants suggest that ARF5 acts early in promoting cell division prior to differentiation (Przemeck et al. 1996, Vidaurre et al. 2007).

The analysis of *arf5* hypomorphic lines indicates that this transcription factor also functions during postembryonic development to positively regulate vascular tissue formation (Hardtke et al. 2004). In leaves, as in embryos, expression of ARF5 precedes vascular development (Scarpella et al. 2006, Weijers et al. 2006, Wenzel et al. 2007). ARF5 expression in these contexts colocalizes with and precedes the expression of *PIN1*, which encodes a membrane protein that facilitates the movement of auxin from cell to cell and helps to establish gradients of auxin within developing organs. Extensive analyses show that PIN1 and related auxin carriers are asymmetrically localized within many cell types and that this distribution contributes to polar movement of auxin within tissues and organs. Asymmetric distribution of PIN1 is first observed at the embryonic 32-cell stage in provascular cells adjacent to the hypophysis (Friml et al. 2003) and in preprocambial cells of developing leaves (Scarpella et al. 2006). During formation of the embryonic root, expression of *ARF5* and *IAA12* is limited to embryonic cells directly adjacent to the hypophysis. Auxin movement into the hypophysis and provascular tissue of other organs appears to be essential for determining these cells' fates, implying that other auxin signaling proteins act in these cells to initiate auxin-regulated differentiation (Weijers et al. 2006). These findings add new validation to the hypothesis that auxin establishes and enhances its own transport during organogenesis, the time-honored developmental model termed canalization. Auxin-induced derepression of ARF5 transcriptional activity in one cell leads to the expression of PIN1, which promotes the movement of auxin into the adjacent cell, inducing additional transcriptional responses. Details of PIN-mediated mechanisms that establish cellular polarity and influence auxin allocation are described in an-

other review in this volume (Kleine-Vehn & Friml 2008).

During the formation of lateral roots, auxin influences the reactivation of differentiated vascular cells in the primary root. The first anticlinal divisions during lateral root formation occur in pericycle cells adjacent to the xylem. As in embryogenesis, the division and differentiation of these cells require auxin (Casimiro et al. 2001, Himanen et al. 2002, Malamy & Benfey 1997). Mutations in several auxin response components cause defects in auxin-induced lateral root formation and reduce cell cycle activity in the xylem pole pericycle (Fukaki et al. 2006, Gray et al. 1999). One of these, a mutation in the degron motif of *SLR/IAA14*, stabilizes the IAA14 protein and completely blocks the early development of lateral roots (Fukaki et al. 2002). Targeted expression of the stabilized form of IAA14 indicates that the protein normally functions in xylem pole pericycle cells (Fukaki et al. 2005). Further studies suggest that IAA14-regulated transcription contributes to both cell cycle reactivation and subsequent regulation of cellular identity (Vanneste et al. 2005). Expression of *ARF7* and *ARF19* coincides with that of *SLR/IAA14* in lateral root initials, and the *arf7arf19* double mutant lacks lateral roots in the presence of auxin (Okushima et al. 2007), similar to *shr-1*. Results from two additional studies show that ARF7 and ARF19 can substitute for one another in activating some promoters (Li et al. 2006, Okushima et al. 2005).

AUXIN SIGNALING REQUIRES THE UBIQUITIN-PROTEASOME PATHWAY

As mentioned above, most of the *Aux/IAA* genes are transcriptionally regulated by auxin in an ARF- and Aux/IAA-dependent manner (Abel et al. 1995, Tatematsu et al. 2004, Tian et al. 2002). Auxin-regulated expression of these repressors serves as a rapid negative feedback mechanism in auxin signaling. The activity of the auxin receptor, described below, completes this regulatory loop by destabilizing

the Aux/IAA proteins. As noted above, severe developmental consequences result when this loop is disrupted and the Aux/IAA repressors accumulate.

The Ub-proteasome pathway is responsible for the regulated degradation of diverse proteins in eukaryotes. Ub is attached to substrate proteins through a series of highly conserved enzymatic reactions, described below. Plants devote a remarkably large fraction of their genomes to this pathway, suggesting that protein degradation is particularly important for cellular regulation in plants (Smalle & Vierstra 2004).

The Ubiquitin-Conjugation Cycle

The Ub-protein conjugation pathway is initiated by an enzyme called the Ub-activating enzyme, or E1 (Hershko & Ciechanover 1998). This enzyme catalyzes an ATP-dependent reaction that activates a Ub monomer and transfers it to the second enzyme in the pathway, the Ub-conjugating enzyme (E2) (Hershko & Ciechanover 1998). A third protein or protein complex called the Ub-protein ligase (E3) interacts with both the Ub-E2 and specific substrate proteins, thus promoting the transfer of Ub to the substrate (Hershko & Ciechanover 1998, Pickart 2001). The SCF (Skp1-Cul1-F-box) protein complexes make up a major class of E3s in all eukaryotes and appear to be the most abundant type of E3 in plants (Moon et al. 2004, Smalle & Vierstra 2004). Each SCF contains a highly conserved central scaffold protein, called a cullin, that is associated with the adaptor protein Skp1 (a member of the ASK family in plants) (Petroski & Deshaies 2005, Smalle & Vierstra 2004). This adaptor protein provides the binding site for the F-box protein, which functions as the substrate-binding component of the SCF. The fourth subunit, variously called RBX1, ROC, or Hrt1, binds the Ub-E2 and promotes transfer of Ub to the F-box-protein-bound substrate. Ub-E2 docking to RBX1 is thought to allosterically promote the transfer of Ub, indicating that the purpose of SCF-complex architecture is to position

the protein substrate to receive the transferred Ub effectively (Petroski & Deshaies 2005). Indeed, the structure of the CUL1 subunit establishes a critical distance between the substrate protein and Ub-E2. Upon transfer by the E2, an isopeptide bond is formed between a lysyl ϵ -amino group on the substrate protein and the C-terminal glycyl residue of Ub (Petroski & Deshaies 2005).

Substrate marking for recognition by the 26S proteasome requires the addition of a chain of polymerized Ub (Petroski & Deshaies 2005). It is not clear if polyubiquitination occurs through the successive addition of single Ub molecules to SCF-bound substrate or by the attachment of a preformed Ub chain (Hochstrasser 2006). Members of a family of deubiquitinating enzymes (DUBs) assist in Ub recycling upon breakdown of polyubiquitinated substrates (Amerik & Hochstrasser 2004). Some DUBs may have additional roles in selectively reversing the ubiquitination of substrates, thereby preventing substrate degradation. Diversity among subunits of the proteasome may also contribute complexity to proteasome-substrate interactions (Brukhin et al. 2005, Demartino & Gillette 2007, Smalle et al. 2002, Ueda et al. 2004).

Regulation of SCF Assembly and Function

A number of factors that regulate SCF assembly and/or activity have been identified. These include the Ub-like protein RUB (or Nedd8 in animals) (Kerscher et al. 2006, Parry & Estelle 2004). RUB is conjugated to the cullin subunit of the SCF through a separate pathway consisting of dedicated E1 and E2 enzymes. The RUB molecule is removed from the cullin by the COP9 signalosome (CSN) complex (Kerscher et al. 2006, Wei & Deng 2003). RUB modification may be important for the recruitment of the Ub-E2 to the SCF (Kerscher et al. 2006). In addition, RUB appears to block binding of CUL by CAND1, a 120-kDa protein that sterically hinders the CUL-Skp1/ASK interaction. Several recent reviews have discussed

in detail the role of these proteins in SCF assembly/disassembly (Petroski & Deshaies 2005, Wu et al. 2006).

Regulation of SCF-Substrate Recognition

The regulation of SCF-substrate recognition has now been characterized for many animal and fungal SCFs (Petroski & Deshaies 2005). In general the SCF-substrate interaction is regulated by posttranslational modification of the substrate. In almost all known cases, phosphorylation marks the substrate for SCF recognition. For example, the mammalian SCF^{Fbw7} promotes the degradation of a number of growth regulators, including cyclin E, c-Myc, c-Jun, Notch, Presenilin, and sterol regulatory element-binding proteins (SREBP) (Koepp et al. 2001, Minella & Clurman 2005, Nateri et al. 2004, Orlicky et al. 2003, Ye et al. 2004). Each of these proteins contains conserved Cdc4 phospho-degron (CPD) motifs. When a threonyl residue within the CPD is phosphorylated, the protein is recognized by SCF^{Fbw7}, ubiquitinated, and rapidly degraded. Phosphorylation also promotes degradation of the mammalian Cdk inhibitor p27^{Kip1} by SCF^{Skp2} (Nakayama & Nakayama 2005). In this case, however, an additional adaptor protein termed Cks1 is required for substrate recognition (Ganoth et al. 2001, Spruck et al. 2001, Xu et al. 2007). When p27^{Kip1} is phosphorylated at T187 by Cdk2–cyclin A, the phosphothreonyl motif interacts with Cks1 associated in the complex, whereas the F-box protein Skp2 interacts with another portion of the substrate to promote binding.

GENETIC STUDIES IN *ARABIDOPSIS* DEMONSTRATE THAT THE UBIQUITIN-PROTEASOME PATHWAY IS REQUIRED FOR AUXIN SIGNALING

The connection between auxin and the Ub pathway was established through a very simple screen for *Arabidopsis* mutants with altered

auxin response (Walker & Estelle 1998). The roots of *Arabidopsis* seedlings are inhibited by low levels of auxin in the growth medium, making it relatively straightforward to isolate large numbers of auxin-resistant mutants. The first auxin-resistant mutant to be characterized in detail was called *axr1* (Leyser et al. 1993, Lincoln et al. 1990). The *AXR1* gene encodes a subunit of the heterodimeric RUB-E1 enzyme, the first enzyme in the RUB-conjugation pathway (del Pozo et al. 1998). Because RUB modification of CUL1 is important for SCF function, these results suggested that auxin response depends on the action of a cullin-containing E3 ligase. This idea was supported by later studies of another auxin-resistant mutant called *tir1* (Ruegger et al. 1998). The *TIR1* gene encodes a leucine-rich-repeat (LRR)-containing F-box protein that interacts with CUL1, ASK1 or ASK2, and RBX1 to form SCF^{TIR1} (Gray et al. 1999, Ruegger et al. 1998). Typically the biggest challenge in characterizing a newly discovered E3 ligase is identifying its substrates. In the case of SCF^{TIR1}, there were some obvious candidates because the Aux/IAA proteins were known to be unstable repressors of auxin-regulated transcription. Both genetic and biochemical studies confirmed that the Aux/IAs are substrates of SCF^{TIR1} (Gray et al. 1999). Several members of the Aux/IAA family are stabilized in the *axr1* and *tir1* mutants, and both mutants exhibit reduced auxin-regulated gene expression (Gray et al. 1999; K. Mockaitis & M. Estelle, unpublished). Furthermore, in vitro studies demonstrated that Aux/IAA proteins interact with TIR1 and that auxin stimulates this interaction (Gray et al. 1999). Taken together, these results indicate that auxin acts by promoting the degradation of the Aux/IAs through the action of SCF^{TIR1}, a model that is summarized in **Figure 1**.

Support for the model was provided by analyses of mutants that affect other proteins in the Ub pathway, including CUL1, ASK1, CAND1, and subunits of the CSN (Chuang et al. 2004; Gray et al. 2001, 2003; Hellmann et al. 2003; Moon et al. 2007; Quint et al. 2005; Schwechheimer et al. 2001) (**Table 2**).

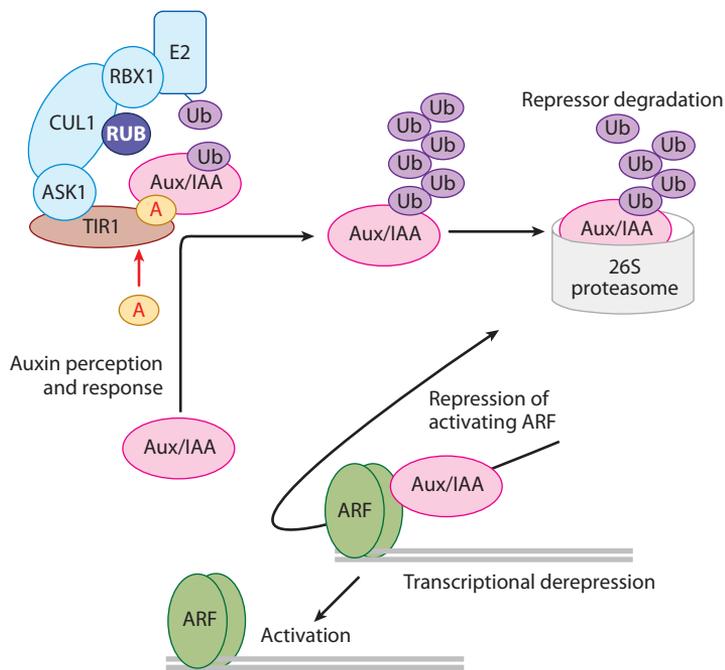


Figure 1

Auxin regulates transcription by promoting ubiquitin (Ub)-mediated degradation of Aux/IAA repressors. Auxin (A) binds to the F-box protein TIR1 in SCF^{TIR1} and stabilizes the interaction between TIR1 and an Aux/IAA substrate. The repressor is polyubiquitinated and degraded by the 26S proteasome. Loss of the Aux/IAA permits auxin response factor (ARF)-dependent transcription of auxin-regulated genes. E2, Ub-conjugating enzyme.

Additionally, investigators have isolated mutants that affect an AXR1-like enzyme (AXL), the partner of AXR1 required for RUB-E1 activity (ECR1), the RUB-E2 (RCE1), and other SCF regulators (Chuang et al. 2004, S. Dharmasiri et al. 2003, Dharmasiri et al. 2007, Walsh et al. 2006, Woodward et al. 2007). Like *axr1* and *tir1*, these mutants are deficient in auxin response and show a variety of auxin-related growth defects. For example, some *cul1* alleles (also called *axr6*) have a seedling lethal phenotype that is similar to the phenotypes of *mpl/arf5* and *bll/iaa12*, contributing to evidence that SCF-mediated degradation of IAA12 is required for formation of the embryonic root (Hellmann et al. 2003, Hobbie et al. 2000). Taken together, characterization of these mutants demonstrates that the regulation of SCF

function and the resulting Ub modification of Aux/IAA proteins are critical to auxin-regulated plant development.

Degradation of proteins other than the Aux/IAAs depends on many of the same Ub pathway regulators, and several newly described F-box proteins function in response to other plant hormones and environmental signals (Chini et al. 2007, Lechner et al. 2006, Moon et al. 2004, Thines et al. 2007). The observation that developmental defects in mutants of the Ub pathway, the CSN, and proteasome regulators often appear at least partially auxin related, mimicking phenotypes of Aux/IAA and ARF mutants, emphasizes the very early influences of auxin in plant developmental programs (Dohmann et al. 2005, Hellmann et al. 2003, Schwechheimer et al. 2001).

Auxin Perception through the SCF

The discovery that SCF^{TIR1} promotes the degradation of the Aux/IAA proteins was a major breakthrough in plant hormone signaling. However, there were many unresolved questions, including the identity of the auxin receptor and how TIR1-Aux/IAA recognition is regulated. On the basis of studies of SCFs in animals and fungi, it was assumed that Aux/IAA recognition would require a posttranslational modification, probably phosphorylation (Petroski & Deshaies 2005). However, in vitro studies strongly suggested that Aux/IAA modification was not required for recognition (N. Dharmasiri et al. 2003, Kepinski & Leyser 2004). For example, inhibitors of protein kinases and phosphatases do not affect the TIR1-Aux/IAA interaction (N. Dharmasiri et al. 2003, Kepinski & Leyser 2004). In addition, auxin promotes the interaction in membrane-depleted extracts, suggesting that the receptor and any intermediary components are not associated with cellular membranes (N. Dharmasiri et al. 2003).

Two important papers confirmed that auxin regulates SCF^{TIR1} through a novel mechanism (Dharmasiri et al. 2005a, Kepinski &

Table 2 Proteins involved in SCF assembly in *Arabidopsis thaliana* and evidence for their roles in auxin-mediated development

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
<i>ASK1</i>	<i>Arabidopsis</i> SKP1	Orthologous to yeast Skp1; links F-box protein to CUL1	<i>ask1</i> loss-of-function mutations reduce multiple auxin responses	Gray et al. 1999
<i>CUL1</i>	CULLIN1/AUXIN RESISTANT 6 (AXR6/CUL1)	Scaffold protein of SCF complex	Several <i>cul1</i> loss-of-function mutations reduce multiple auxin responses	Hellmann et al. 2003, Moon et al. 2007, Shen et al. 2002
<i>RBX1</i>	RING-H2 finger protein	Orthologous to RBX/ROC/Hrt, SCF docking subunit for Ub-E2	Decreased expression or overexpression reduces multiple auxin responses	Gray et al. 2002
<i>RUB1</i> and -2	RELATED TO UBIQUITIN 1 and 2	Orthologous to Nedd8	Reduced levels reduce multiple auxin responses; together <i>RUB1</i> and -2 are essential in development	Bostick et al. 2004
<i>AXR1</i>	AUXIN RESISTANT1	Subunit in RUB/Nedd8 activating enzyme	Loss-of-function mutations reduce multiple auxin responses and reduce RUB1 modification of CUL1	del Pozo & Estelle 1999, del Pozo et al. 2002, Leyser et al. 1993
<i>AXL</i>	AUXIN RESISTANT1-LIKE	Subunit in RUB/Nedd8 activating enzyme	Loss of function synergistically with <i>axr1</i> reduces multiple auxin responses	Dharmasiri et al. 2007
<i>ECR1</i>	RUB E1 enzyme subunit	With AXR1/AXL, activates RUB for transfer to RCE1	Loss-of-function mutation reduces multiple auxin responses	del Pozo & Estelle 1999, del Pozo et al. 2002, Woodward et al. 2007
<i>RCE1</i>	E2 enzyme for RUB conjugation	Interacts with RBX1 and SCF ^{TIR1}	Loss-of-function mutations reduce multiple auxin responses	del Pozo & Estelle 1999, S. Dharmasiri et al. 2003
<i>CSN5</i>	Component of COP9 signalosome	Interacts with SCF; removes RUB from CUL1; regulates SCF assembly	Loss-of-function mutations reduce multiple auxin responses	Dohmann et al. 2005, Peng et al. 2003, Schwecheimer et al. 2001
<i>CAND1</i>	Cullin-associated and neddylation-dissociated1	Orthologous to animal Cand1; interacts with CUL1; regulates SCF assembly	Loss-of-function mutations reduce multiple auxin responses	Alonso-Peral et al. 2006, Cheng et al. 2004, Chuang et al. 2004, Feng et al. 2004
<i>SGT1b</i>	Suppressor of G2 allele of <i>skp1</i> / Enhancer of TIR1 auxin resistance ETA3	Yeast ortholog interacts with Skp1	Loss-of-function mutations reduce multiple auxin responses	Gray et al. 2003, Walsh et al. 2006

Leyser 2005). In these studies, partially purified SCF^{TIR1} bound an Aux/IAA protein in an auxin-dependent manner, indicating that the receptor copurified with SCF^{TIR1}. Furthermore, labeled auxin (³H-IAA) bound specifi-

cally to SCF^{TIR1}, or a closely associated protein, signifying that an auxin receptor was present in the complex. One explanation for these results, something that previously seemed very unlikely, is that TIR1 is the auxin receptor.

That this is the case was strongly suggested by the discovery that TIR1 synthesized in insect cells or *Xenopus* embryos also binds recombinant Aux/IAA protein in the presence of auxin (Dharmasiri et al. 2005a, Kepinski & Leyser 2005). Because the only plant proteins in this assay are TIR1 and Aux/IAA, each synthesized in heterologous systems, the logical conclusion was that one of these two proteins binds auxin. Assays in vitro showed that Aux/IAA proteins do not bind auxin, leaving TIR1 as the best candidate.

AUXIN RECEPTOR ACTIVITY IS REVEALED

To address the mechanism of auxin perception, the structure of TIR1 in complex with ASK1 was determined in the presence of IAA and a peptide that includes the degron motif of IAA7/AXR2 (Tan et al. 2007). TIR1 is composed of the highly conserved F-box domain and 18 LRRs. The crystal structure reveals that the ASK1-TIR1 complex forms a mushroom-shaped structure, with ASK1 and the F-box domain forming the stem (Figure 2a). The cap

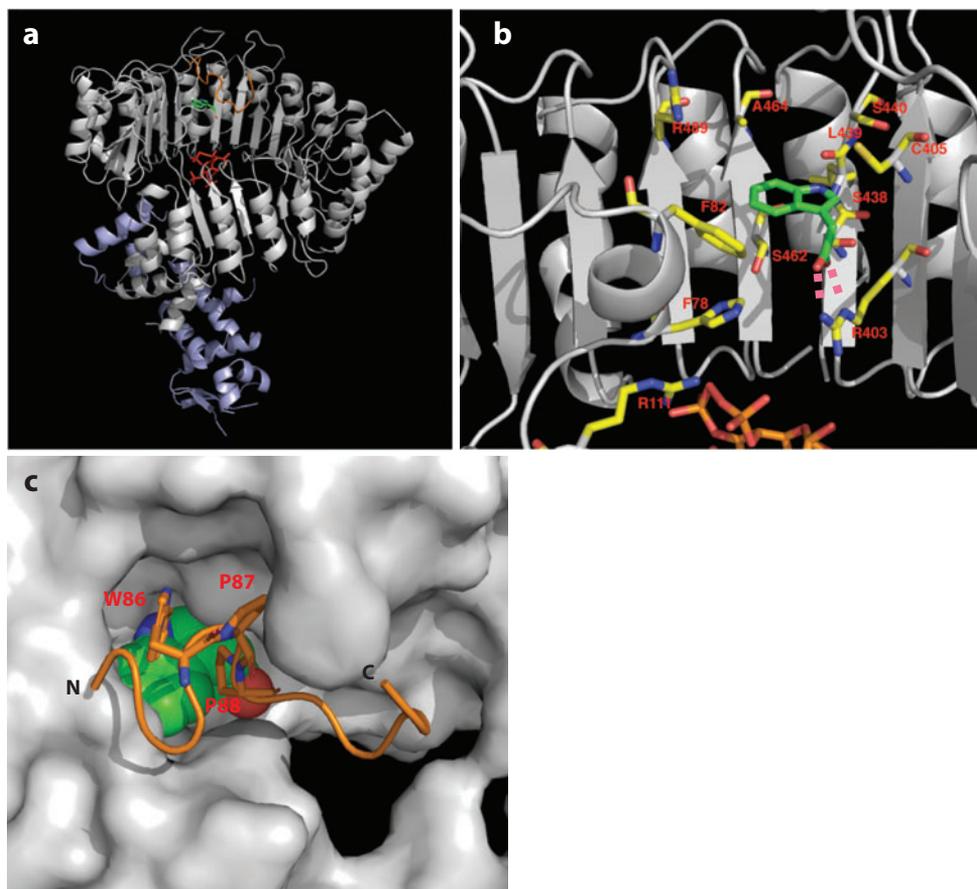


Figure 2

Auxin perception by the F-box protein TIR1. (a) Structure of TIR1 (gray) in complex with ASK1 (dark blue), indole-3-acetic acid (IAA) (green), Aux/IAA domain II peptide (orange), and inositol hexakisphosphate (red). (b) Close-up of the auxin-binding pocket occupied by IAA (green). Surrounding TIR1 residues are shown in yellow. Dashed pink lines indicate hydrogen bonds between the carboxyl group of IAA and conserved R403. (c) Surface view of TIR1 in complex with IAA (green) and domain II peptide (orange).

of the mushroom consists of the relatively long LRR domain that adopts a fold unique among known LRR domains (Tan et al. 2007). The TIR1 LRR domain forms a slightly twisted, incomplete ring-like structure of alternating solvent-facing alpha helices and core-lining beta strands. Auxin is bound within a mostly hydrophobic cavity (see below). Surprisingly, auxin binding does not induce major conformational changes in the receptor, indicating that the hormone does not act as an allosteric regulator. Instead, the auxin molecule fills the deepest portion of the cavity and provides an additional binding surface for the Aux/IAA degron. The Aux/IAA proteins appear to bind TIR1 in the absence of auxin, but with low affinity (Dharmasiri et al. 2005b, Kepinski & Leyser 2005, Tan et al. 2007). The auxin molecule acts as “molecular glue” between TIR1 and its substrate, binding both proteins and facilitating hydrophobic packing between TIR1 and its substrate. Once the TIR1-auxin-Aux/IAA complex is formed, the hormone is trapped within the auxin cavity, presumably until the ubiquitinated protein is released. Further biochemical studies are required to establish the precise requirements for auxin binding, but on the basis of the structure it is likely that high-affinity auxin binding requires both TIR1 and the Aux/IAA protein. If this is true, this may have important implications for the biological function of the auxin receptor (see below).

Importantly, a long-standing mystery regarding auxin activity is explained by the TIR1 structure. Over the years, a large number of synthetic auxins have been developed, mostly for use as herbicides. These compounds all have a planar ring and a free carboxyl group (Napier 2004, Napier et al. 2002, Sterling & Hall 2007). However, their ring structures vary greatly in size and substitution, leading to questions about how a single receptor can accommodate such different compounds. An examination of the auxin-binding pocket illustrates how this is achieved. The planar ring of the auxin molecule, indole in the case of IAA, fits into the generally hydrophobic cavity while the carboxyl

group forms a salt bridge with a key arginine (R403) (**Figure 2b**). Additional structural determinations were also performed with two different synthetic auxins, 1-NAA and 2,4-D. These compounds have a larger ring and smaller ring, respectively, than does IAA, and binding studies indicate that IAA has the highest affinity among these three compounds, followed by 1-NAA and 2,4-D (Dharmasiri et al. 2005a, Kepinski & Leyser 2005, Tan et al. 2007). Although the relatively spacious hydrophobic cavity accommodates the ring structure of all three molecules, the smaller size of the 2,4-D ring does not fill the cavity as well, and 2,4-D hence has the lowest affinity of the three compounds. IAA appears to bind more tightly than NAA because of electrostatic interactions involving the N on the indole ring (Dharmasiri et al. 2005a, Kepinski & Leyser 2005, Tan et al. 2007).

A recent theoretical analysis of auxin chemistry concluded that auxin activity may depend on the electron density of the ring, rather than its precise atomic composition (Ferro et al. 2006). This idea seems generally consistent with the similar shape-fitted binding of IAA, NAA, and 2,4-D into the TIR1 cavity.

The Auxin Receptor Structure Includes InsP₆

Another surprising feature of the TIR1 structure is the presence of a single inositol hexakisphosphate (InsP₆) molecule in the floor of the LRR domain (**Figure 2**). The position of InsP₆ appears to be critical for stabilizing the structure of the auxin-binding pocket. InsP₆ is found in all eukaryotes, and its putative role in a wide variety of cellular processes is beginning to be explored (Macbeth et al. 2005, Mulugu et al. 2007, Odom et al. 2000, Seeds & York 2007). In plants, InsP₆ (also called phytate) is an extremely abundant compound that has so far attracted attention primarily because of its animal antinutrient properties (Raboy 2007). Recent genetic studies suggest that it may have an important role in phosphate storage and signaling (Stevenson-Paulik et al. 2005). Currently it is not clear whether InsP₆ binding to TIR1 has

a regulatory function or a structural function, although the abundance of InsP₆ in plant cells suggests the latter. Further studies are required to establish a role of InsP₆ in TIR1 function and auxin signaling.

A FAMILY OF AUXIN RECEPTORS IN PLANTS

The *Arabidopsis* genome encodes five F-box proteins exhibiting 50–70% sequence identity with TIR1. These proteins have been named auxin signaling F-box protein 1 to 5 (AFB1–AFB5) (Table 3). Genetic and biochemical studies have implicated these proteins in auxin signaling (Dharmasiri et al. 2005b, Walsh et al. 2006; A. Santner, S. Mooney & M. Estelle, unpublished). Moreover, binding of radiolabeled IAA is diminished in extracts from mutants lacking TIR1 and AFB1–3, confirming that these proteins are very likely to function as auxin receptors. As for the *tir1* mutants, single *afb* loss-of-function mutations do not cause dramatic developmental defects.

However, combining *tir1* and *afb1–3* mutations leads to a severely reduced auxin response and a variety of auxin-related developmental defects (Dharmasiri et al. 2005b). The most severely affected *tir1afb2afb3* and *tir1afb1afb2afb3* seedlings arrest as young seedlings, with a phenotype strikingly similar to the phenotypes of the *mp1arf5* and *bdl/iaa12* mutants described above. This similarity is explained by the fact that IAA12 accumulates in *tir1afb2afb3* seedlings, presumably because these plants are deficient in auxin-dependent degradation of IAA12 early in embryogenesis (Dharmasiri et al. 2005b). As with *bdl*, only a fraction of the triple and quadruple *afb* mutant seedlings fail to form roots. Compensatory mechanisms within an auxin feedback loop or other aspects of the developmental network may overcome the embryonic arrest in the fraction of seedlings that develop roots. These, however, continue to display a broad array of auxin response defects as they mature.

Although the available evidence suggests that TIR1 and AFB1–3 have similar functions

Table 3 Auxin receptors in *Arabidopsis thaliana*

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
<i>TIR1</i>	Transport inhibitor response 1, TIR1 F-box protein	Interacts with ASK1; interacts with Aux/IAAs; auxin increases Aux/IAA affinity; crystal structure shows TIR1-auxin-Aux/IAA complex	Loss-of-function mutations reduce multiple auxin responses	N. Dharmasiri et al. 2003, Dharmasiri et al. 2005b, Gray et al. 1999, Kepinski & Leyser 2005, Ruegger et al. 1998, Tan et al. 2007
<i>AFB1</i>	Auxin F-box protein 1 (AFB1)	Member of TIR1/AFB family; auxin increases Aux/IAA affinity	Loss of function with <i>tir1</i> , <i>afb2</i> , <i>afb3</i> dramatically impairs development	Dharmasiri et al. 2005b
<i>AFB2</i>	Auxin F-box protein 2 (AFB2)	Member of TIR1/AFB family; auxin increases Aux/IAA affinity	Loss of function with <i>tir1</i> reduces multiple auxin responses	Dharmasiri et al. 2005b
<i>AFB3</i>	Auxin F-box protein 3 (AFB3)	Member of TIR1/AFB; auxin increases Aux/IAA affinity	Loss of function with <i>tir1</i> and <i>afb2</i> dramatically impairs development	Dharmasiri et al. 2005b
<i>AFB4</i>	Auxin F-box protein 4 (AFB4)	Member of TIR1/AFB family		Dharmasiri et al. 2005b
<i>AFB5</i>	Auxin F-box protein 5 (AFB5)	Member of TIR1/AFB family	Loss-of-function mutation confers resistance to auxin analogs	Dharmasiri et al. 2005b, Walsh et al. 2006

in development, it is likely that more detailed studies will reveal specific roles for one or more of these proteins. Such specific functions have already been demonstrated for AFB5. Genetic studies showed that loss of AFB5 results in resistance to the synthetic auxin picloram (Walsh et al. 2006). Because *afb5* plants are only slightly resistant to IAA and other auxins, AFB5 appears to have important chemical selectivity. It will be interesting to learn how the biological functions of these proteins relate to differences in the auxin-binding cavities or other elements of AFB4 and AFB5 structures.

Auxin Receptor Specificities

Most members of the Aux/IAA protein family contain the auxin degron, implying that these proteins are all substrates of SCF^{TIR1/AFB}. However, several studies demonstrate substantial differences in the rates of degradation among family members (Dreher et al. 2006, Gray et al. 2001, Hayashi et al. 2003, Ouellet et al. 2001). Because the structure of a complete Aux/IAA protein in complex with TIR1 has not been determined, it is not clear if there are contacts between the two proteins outside the degron. Interestingly, Dreher et al. (2006) showed that substitution of a conserved basic residue pair distant from the known degron motif increases the basal half-life of an IAA17-luciferase fusion protein in plants without affecting auxin-stimulated degradation. This site may participate in an electrostatic interaction between the LRR surface and the Aux/IAA that is needed for low-affinity substrate binding when auxin is not present. It is not known if SCF^{TIR1/AFB} promotes Aux/IAA degradation in the absence of auxin.

A related question concerns the specificity of the TIR1/AFB-Aux/IAA interaction. In vitro experiments suggest that isolated TIR1 and AFB1–3 can interact with multiple members of the Aux/IAA family (Dharmasiri et al. 2005b, Yang et al. 2004). Further studies are required to determine the biological relevance of these interactions and in vivo functional specificities in auxin-mediated development.

JASMONIC ACID MAY BE PERCEIVED BY THE RELATED F-BOX PROTEIN COI1

TIR1 and AFB1–5 are part of a group of seven related F-box proteins. The last member of this group, a protein called COI1, is required for response to the hormone jasmonic acid (JA). Recent results demonstrate remarkable similarities between auxin and JA signaling. Like TIR1, COI1 promotes the degradation of transcriptional repressors in response to a hormone, in this case jasmonyl isoleucine (JA-Ile) (Chini et al. 2007, Thines et al. 2007). Stabilized versions of the repressors, the JAZ proteins, inhibit JA responses. On the basis of primary structure, the LRRs of COI1 should assume the same novel fold as does TIR1. The auxin-binding cavity is not present in COI1, but the InsP₆-binding pocket is conserved between the two proteins. On the basis of these similarities, it is tempting to speculate that COI1 functions as a JA-Ile receptor and that hormone binding stabilizes the interaction between COI1 and the JAZ proteins.

On the basis of the structural data, it is likely that both TIR1/AFB and the Aux/IAA protein are required for high-affinity binding of auxin. The mechanism of auxin binding offers an explanation for the failure to identify TIR1 in experiments designed to recover auxin-binding proteins (Napier 2004). Because the Aux/IAA proteins are rapidly ubiquitinated and degraded upon binding to TIR1, the TIR1-auxin-Aux/IAA complex is transient and present at low levels.

Auxin-Regulated Processes Are Sensitive to Levels of Both Auxin and Receptor

The abundance of auxin receptors may quantitatively modulate transcriptional responses to auxin and resulting developmental effects. The *tir1-1* mutation is semidominant (Ruegger et al. 1998), indicating that auxin response is sensitive to TIR1 levels, and loss of additional auxin receptor family members further impairs auxin-related development (Dharmasiri et al. 2005b).

Because the auxin signal does not appear to be amplified between hormone perception and gene regulation, transcription and its

developmental consequences are expected to be acutely sensitive to the localized concentration of auxin in the cell. Indeed, numerous studies demonstrate that the transient regulation of auxin intercellular transport and intracellular homeostasis critically influences development (Leyser 2006, Tanaka 2006). The *tir1* phenotype is greatly exacerbated in the context of reduced cellular auxin levels. Dramatic hypermorphic phenotypes are apparent when the *tir1-1* mutation is combined with mutations in the *TAA1/TIR2* gene that reduce auxin levels (M. Yamada, P. Jensen & M. Estelle, unpublished). *TAA1/TIR2* is an aminotransferase involved in auxin biosynthesis (Stepanova et al. 2008, Tao et al. 2008; M. Yamada, P. Jensen & M. Estelle, unpublished). Whereas *tir1* and *taa1/tir2* develop fully and show relatively mild auxin-related defects, root meristems of double-mutant seedlings are highly disorganized and underdeveloped (M. Yamada, P. Jensen & M. Estelle, unpublished). Because auxin levels are lower in *taa1/tir2* seedlings, they are sensitized to changes in the level of SCF^{TIR1}.

Consistent with these observations, mutants that are deficient in components of auxin response differ markedly from wild-type plants in their sensitivities to chemical or genetic inhibition of auxin transport. When the auxin transport inhibitor *N*-(1-naphthyl)phthalamic acid (NPA) is applied to floral meristems, auxin is depleted in that region to a level that minimally affects wild-type floral development (Nemhauser et al. 2000). In weak mutant alleles of *ett/arf3*, however, application of NPA leads to dramatic deformation of floral organs in a manner mimicking development in the strongest *ett/arf3* alleles (Nemhauser et al. 2000). In contrasting experiments, increasing localized auxin concentrations appears to compensate for a reduction in auxin receptor abundance. When NPA or other transport inhibitors are applied to seedlings, auxin accumulates in the root tip and stimulates excessive cell proliferation. The roots of *tir1* and other auxin response mutants are less sensitive to auxin transport inhibitors than are wild-type seedlings, and indeed this

difference is the basis for the screen in which the *tir1* mutant was identified (Ruegger et al. 1997).

Regulation of AFB and ARF Levels

A variety of developmental and environmental cues may modulate development by regulating the local abundance of auxin receptors and other auxin response components. Experiments with promoter::GUS lines indicate that the *TIR1* and *AFB1-3* promoters are active throughout the life cycle of the plant, with particularly high activity in growing tissues. However, recent findings that the *TIR1*, *AFB2*, and *AFB3* transcripts are targets of the microRNA (miRNA) *miR393* add complexity to our understanding of the dynamics of auxin receptor action (Navarro et al. 2006). Studies indicate that *miR393* regulates growth and development during the plant response to bacterial pathogens. When *Arabidopsis* seedlings are treated with the pathogen *Pseudomonas syringae* or the bacterial elicitor flagellin, levels of *miR393* become elevated, leading to a reduction in *TIR1* transcript and TIR1 protein levels. Concomitant stabilization of AXR3/IAA17 and downregulation of auxin-activated transcription demonstrate that changes in TIR1 levels can very rapidly alter auxin response. Suppression of auxin signaling by *miR393* correlates with reduced growth of the pathogen, suggesting that regulation of auxin receptor abundance may serve as a disease resistance strategy (Navarro et al. 2006).

Levels and localization of auxin response components downstream of the receptors are also regulated by transcriptional and posttranscriptional mechanisms. A number of recent studies demonstrate that expression of ARF family members is subject to regulation by silencing RNAs. Transcripts of several ARFs are targeted by known miRNAs or *trans*-acting small interfering RNAs (siRNAs). Inhibiting miRNA-mediated cleavage of *ARF17* (Mallory et al. 2005) or *ARF10* (Liu et al. 2007) transcripts increases ARF accumulation and causes defects in development throughout the plant (Mallory et al. 2005). Blocking siRNA regulation of *ARF3* and *ARF4* drastically impairs

normal development of leaves and flowers, in part owing to defects in cell differentiation and developmental timing (Fahlgren et al. 2006, Garcia et al. 2006, Hunter et al. 2006). In addition, regulation of translation initiation by upstream open reading frames (uORFs) modulates ARF3 and ARF5 levels during floral development, and a similar mechanism is predicted to influence nine additional *ARF* genes (Nishimura et al. 2005). Signals other than auxin promote proteasome-dependent degradation of ARF (Li et al. 2004) and ARF1 (Salmon et al. 2008) by as-yet-unknown mechanisms. Continued efforts to identify mechanisms by which the expression, localization, and association of auxin receptors and response components are regulated will add important insights to our understanding of how auxin signaling is integrated with the developmental network.

OTHER CELLULAR RESPONSES TO AUXIN MAY OCCUR INDEPENDENTLY OF THE TIR1/AFB RECEPTORS

Apart from the well-characterized transcriptional response, auxin affects cellular responses, such as ion transport through the plasma membrane, that are probably too rapid to be directly related to changes in transcription (Badescu & Napier 2006, Napier 2004, Yamagami et al. 2004). A variety of studies suggest that these responses may be mediated by a membrane-associated protein called AUXIN-BINDING PROTEIN1 (ABP1). This protein was isolated on the basis of its affinity for auxin compounds and is therefore considered a candidate auxin receptor (Napier 2004, Napier et al. 2002). Interestingly, loss of ABP1 function causes early embryo arrest as in *tir1afb2afb3*, *mp*, and *bdl* (above), but with a very different terminal phenotype (Chen et al. 2001). The *abp1* mutants exhibit pronounced defects in cell division, expansion, and arrest at the globular stage. ABP1 and the TIR1/AFBs are clearly not related, leaving open the possibility that ABP1 may serve an auxin perception role in cellular processes that are not yet well understood (David et al. 2007).

In addition, auxin regulates its own transport in part by inhibiting endocytosis of the auxin transporter protein PIN1 (Paciorek et al. 2005). This response does not appear to require transcription or the TIR1 auxin receptor, suggesting that it involves an independent signaling pathway. Whether this pathway includes ABP1 remains to be determined.

OTHER NATURAL AUXINS?

The structure of the auxin receptor explains for the first time how compounds with a variety of chemical structures can act as auxins. In addition, emerging evidence suggests that different members of the TIR1/AFB family may have distinct auxin specificities, at least with respect to synthetic auxins. These observations invite us to consider the possibility that natural auxins are also chemically diverse. IAA is generally thought to be the primary natural auxin, but very little is known regarding the possible existence of other auxins in plants. Compounds such as 4-chloroindole-3-acetic acid (4-Cl-IAA), indole-3-butyric acid (IBA), indole-3-pyruvic acid (IPA), and indole-3-acetonitrile (IAN), as well as phenylacetic acid (PPA) have been identified in plants and shown to have auxin-related effects (Ozga et al. 2002, Van Huizen et al. 1997, Woodward & Bartel 2005, Zolman et al. 2000). In some cases, such as IBA, the compound may be converted to IAA, whereas others may interact directly with the receptors. Because it is now possible to test this directly in the case of the TIR1/AFB proteins, we should have an answer to this question soon. If there are additional auxins present in the plant, it will be very interesting to explore their functions with regard to specific developmental processes (Campanoni & Nick 2005, Walsh et al. 2006).

DEVELOPMENTAL COMPLEXITY

As this review shows, auxin-dependent growth and development are sensitive to changes in the levels of auxin-regulated transcription complexes, auxin receptors, and local concentrations of auxin. Little is known regarding

protein-protein interactions that contribute additional complexity to auxin response pathways. Plant responses to other intrinsic and environmental signals interact with auxin responses through a variety of mechanisms that continue to be uncovered. For example, the possibility that the photoreceptor phytochrome may directly regulate selected Aux/IAAs suggests that transcriptional outputs of auxin signaling vary with light conditions (Colon-Carmona et al. 2000). Indeed, some of the Aux/IAA proteins were originally identified in genetic screens assessing light responsiveness (Tian & Reed 1999). Interesting recent findings in the signaling of pathogens and other hormones have begun to broaden our view of how other factors modify auxin signaling. Because auxin response

is dependent on the Ub-proteasome pathway, components of this pathway that function in developmental processes unrelated to auxin signaling may represent additional points of signal integration.

There are many outstanding questions regarding the cellular role of auxin in plant growth and development. Auxin receptor specificities are likely to explain important aspects of auxin-mediated development not yet understood. In addition, detailed knowledge of individual auxin target genes and the spatial and temporal controls governing the ARFs, Aux/IAAs, and other transcriptional regulators is required. These studies will reveal how such a small chemical signal has such a large impact on the progression of plant life.

SUMMARY POINTS

1. Plant development depends on auxin perception by newly identified receptors whose functions are integral to the ubiquitin-proteasome pathway of protein degradation. Auxin perception leads to the degradation of Aux/IAA transcriptional repressors and thereby rapidly alters gene expression.
2. The first auxin receptor structure reveals a novel mechanism of hormone-receptor and SCF-substrate interactions. Auxin is bound in a hydrophobic pocket within the F-box protein of the SCF. Auxin acts as a molecular glue to promote high-affinity binding of an Aux/IAA protein through its degron structure, allowing the substrate to be ubiquitinated without additional modification.
3. In *Arabidopsis* six auxin receptor F-box proteins appear to act similarly, and developmental specificities among these are being explored. Auxin receptor abundance is regulated through a variety of mechanisms, including RNA silencing. Localized availability of auxin to receptors is tightly regulated throughout development by the transcriptional output of auxin signaling as well as other signaling pathways.

FUTURE ISSUES

1. The selectivity of auxin receptors with substrate Aux/IAAs and the influence of different auxins on these interactions need to be determined. Such findings will contribute to our knowledge of auxin receptor specificities and/or biochemical redundancies.
2. Elucidating the role of phytate in auxin receptor structures may identify important physiological requirements for auxin signaling.
3. The identification of natural auxin-responsive promoters and higher-order ARF complexes will elaborate Aux/IAA functions and will better explain the role of the auxin receptor in regulating transcription.

4. Further elaborating mechanisms by which auxin receptors and their transcriptional effectors are spatially and temporally regulated will explain much of the complexity of the developmental progression and responses of plants to their environments.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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