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Point of VIEW

Auxin Is Surfacing

Steffen Abel*

Department of Plant Sciences, University of California–Davis, One Shields Avenue, Davis, California 95616

ABSTRACT Indole-3-acetic acid (IAA or auxin) is essential throughout the life cycle of a plant. It controls diverse cellular processes, including gene expression. The hormone is perceived by a ubiquitin protein ligase (E3) and triggers the rapid destruction of repressors, called Aux/IAA proteins. The first structural model of a plant hormone receptor illustrates how auxin promotes Aux/IAA substrate recruitment by extending the hydrophobic protein-interaction surface. This work establishes a novel mechanism of E3 regulation by small molecules and promises a novel strategy for the treatment of human disorders associated with defective ubiquitin-dependent proteolysis.

*Corresponding author, sabel@ucdavis.edu.

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Plants are masters of resilience: once their seeds decide to sprout, they are destined to hold their ground and weather the elements, on occasion for centuries to come. As a consequence of sessility, plants respond to a local challenge or opportunity with directional growth, to evade or to explore, and with the synthesis of an arsenal of bioactive chemicals, to communicate or to self-defend. Several classes of hormones and their associated signaling networks govern plant development and adapt plant growth to its circumstance. The small tryptophan-related hormone indole-3-acetic acid (IAA), commonly known as auxin (a term derived from the Greek *aux-ein*, meaning "to expand or to grow"), has received much attention, partly because its discovery can be traced as far back as the late 19th century, when Charles Darwin (*1*) studied phototropism in canary grass and postulated the existence of a mobile substance promoting growth. More significantly, auxin is thought to impact nearly every facet during the life cycle of a plant, and a total failure to produce the hormone has not been reported for any living plant. Not surprisingly, the biology of auxin and the underlying mechanisms of its action and perception have captivated generations of researchers and even lay scientists. Whereas a molecular and biochemical strategy charted early auxin action and remained in hot pursuit of its tell-tale signs, it was the assertive cross fire delivered by forward genetics, thorough biochemistry, and structural biology that forced auxin to surface and emerge into full view. And what a sight it is! In a recent *Nature* issue, the laboratories directed by Zheng and Estelle (*2*) present several crystal structures of the transport inhibitor response 1 (TIR1)–auxin receptor complex, the first structural model of a plant hormone receptor, and reveal an unprecedented mechanism of small-ligand perception. Binding of auxin to TIR1, a subunit of a Skp1/Cullin/F-box protein (SCF)-type E3 ubiquitin protein ligase, directly promotes substrate protein recruitment by closing a polar gap in the hydrophobic contact surface on TIR1, which subsequently triggers ubiquitinylation and destruction of the target. These crystallographic studies represent a spectacular breakthrough in auxin biology and, more importantly, establish a novel mode for the sensing of small molecules, which likely will have far-reaching implications for general biology and medicine.

The execution of auxin response depends on hierarchical control of gene expression, which leads to profound changes in cellular fate and activity. A molecular approach to elucidating mechanisms of auxin action focused on primary response genes and uncovered an essential interplay of two transcription factor classes, known as Aux/IAA (-inducible) and auxin-response factor (ARF) proteins (*3*). Much has been learned from the study of the Aux/IAA family, which directly links auxin perception to the control of nuclear gene expression (Figure 1). Many *Aux/IAA* genes are rapidly induced by a variety of auxin compounds and encode extremely short-lived proteins of low abundance, the first hint of the importance of proteolysis in auxin signaling (*4, 5*). The promoters of *Aux/IAA* and other early auxin

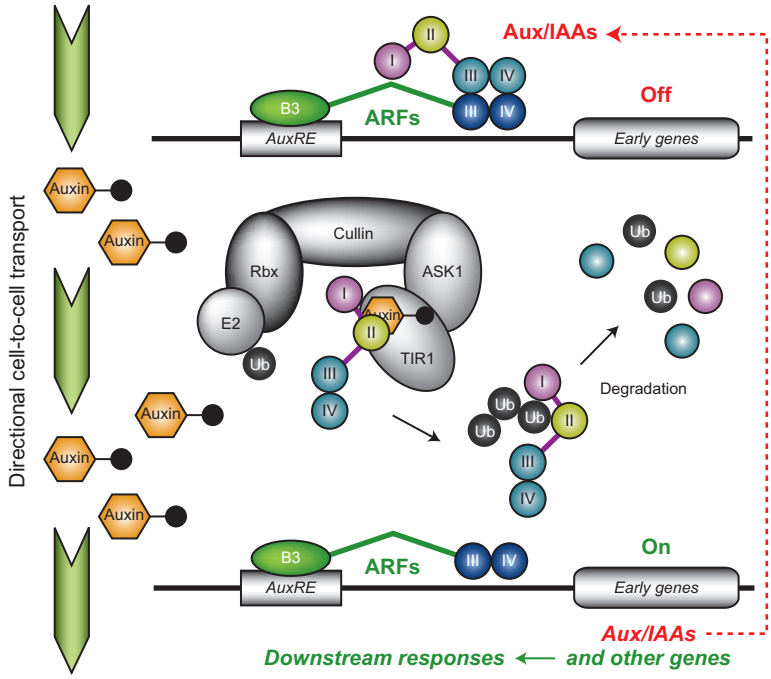

Figure 1. Control of auxin-responsive gene expression. The transcription of primary genes is induced by ARF activators, which bind to *AuxRE* promoter elements of early auxin genes *via* a B3-type, plant-specific, N-terminal DNA-binding domain. Members of the Aux/IAA family repress ARF function *via* heterodimerization of shared conserved domains (III and IV) on their C-termini and *via* the N-terminal repressor domain I. Aux/IAAs are labile proteins that contain a degron sequence in domain II. Auxin further stimulates Aux/IAA protein degradation by the ubiquitin/proteasome pathway, which leads to derepression of primary genes. Because *Aux/IAA* genes are a class of early genes, the interaction of both transcription factor families establishes negative feedback loops that often result in transient auxin responses. The SCF^{TIR1} E3 ubiquitin protein ligase is essential for auxin sensing in the regulation of gene expression. The complex consists of a Cullin–Rbx dimer and TIR1, an F-box LRR protein that is attached to the Cullin scaffold by the ASK1 adapter. Intracellular auxin, derived from polar cell-to-cell transport, directly mediates recruitment of Aux/IAA polypeptides to SCF^{TIR1} or related SCF^{AFB} complexes. Ubiquitin (Ub) is first activated by the E1 activating enzyme (not shown), then transferred to an E2 ubiquitin-conjugating enzyme, and finally transferred to TIR1-associated Aux/IAAs *via* Rbx activity. Subsequent polyubiquitinylation triggers Aux/IAA protein destruction by the 26S proteasome, which is thought to release ARF factors and promote transcription.

genes often share a common *auxin-responsive element* (*AuxRE*), which led to the identification of its associated ARF proteins (*6*). Most of the 29 Aux/IAA polypeptides encoded by the *Arabidopsis* genome are characterized by the presence of four conserved domains (I–IV). The two C-terminal domains (III and IV) mediate heterodimerization of Aux/IAA polypeptides, as well as the interaction between Aux/IAA and ARF proteins (*7, 8*). Almost all ARF proteins, encoded by 23 genes in *Arabidopsis*, have two similar domains at their C-termini and recognize *AuxRE*s *via* a conserved, plant-specific DNA binding domain on their N-terminal half (*7, 9*). Depending on the amino acid composition of their variable internal region, the largely constitutively expressed ARF proteins activate or repress gene transcription. Thus, given that the N-terminal domain I of Aux/IAA proteins acts as a repressor domain, the physical Aux/IAA:ARF association establishes a negative feedback loop in primary gene regulation, which often shapes a transient auxin response (*10, 11*). Conserved domain II confers instability to Aux/IAA proteins and comprises a transferable degron peptide with a characteristic GWPPV amino acid motif at its core. Remarkably, elevated auxin concentration rapidly (<2 min) accelerates proteasome-dependent Aux/IAA protein destruction, an indication that derepression of primary genes by auxin-stimulated proteolysis is an immediate–early and pivotal event in auxin transduction (*12–14*).

A genetic approach, taken in parallel, validated the importance of the Aux/IAA–ARF circuit for auxin-regulated gene expression and directly guided the way into the realm of auxin perception. One group of auxin-resistant mutants in *Arabidopsis* provided overwhelming support for the critical role of Aux/IAA protein abundance. Gain-of-function mutations in a number of *Aux/IAA* genes were identified that change a conserved amino acid residue within the degron peptide of domain II (*3, 15*). As a consequence, the altered Aux/IAA proteins are stabilized and repress ARF function, and this often results in dramatic developmental defects because of decreased auxin sensitivity. It is thought that specific responses to auxin are mediated by pairs of interacting Aux/IAA and ARF proteins that are co-expressed *in planta*. For such established combinations, recessive *arf* mutations confer similar phenotypes as dominant *aux/iaa* mutations, which underscore the biological significance of negative feedback regulation in auxin-responsive gene expression (*16*). A second group of mutations conferring resistance to auxin or to inhibitors of its polar cell-to-cell transport stabilizes Aux/IAA proteins by disabling components of the SCF^{TIR1} complex or its associated regulatory

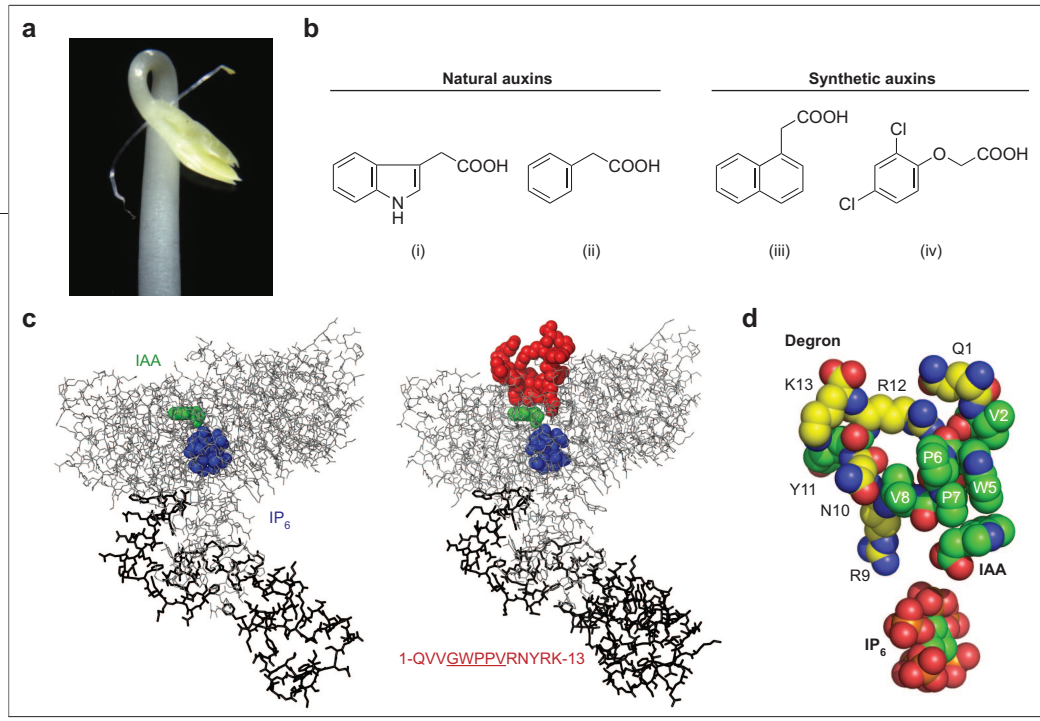

Figure 2. Auxin chemistry and biology. a) The classic biological systems for studying auxin response, pea seedlings (*Pisum sativum*) grown in the dark and small *Arabidopsis thaliana* sprouts. b) The chemical structures for some naturally occurring and synthetic auxin compounds: (i) IAA, (ii) phenylacetic acid (PAA), (iii) NAA, and (iv) 2,4-D. Tan *et al.* (*2*) used IAA, NAA, and 2,4-D to determine the 3D structure of the TIR1–ASK1 auxin receptor complex in association with a 13-amino-acid degron peptide of IAA7, an Aux/IAA protein from *Arabidopsis*. c) The TIR1–ASK1–IAA complex in the absence (on the left) or presence (on the right) of the substrate degron peptide. The TIR1 subunit is depicted in gray, and the ASK1 chain is shown as a black stick model. The auxin compound (IAA), shown in green space-filling representation, occupies a pocket on the top surface of the TIR1-LRR domain. An IP_6 cofactor (blue spheres), positioned in the center of the solenoid fold, interacts with several structural elements of TIR1 that are functionally important for auxin and substrate binding. The coiled degron peptide (red space-filling representation) covers the pocket and places its conserved GWPPV fold on top of the auxin indole ring, which extends the hydrophobic interaction surface of TIR1. d) A close-up view of the spatial arrangement of the three TIR1 ligands, which illustrates the hydrophobic stacking between the indole ring of IAA and the GWPPV motif of the degron. The C-atoms of hydrophobic residues of the degron peptide are shown in green and those of polar or charged residues in yellow. Blue spheres depict N-atoms and red spheres O-atoms. This figure was generated with PyMOL (http://pymol.sourceforge.net) with coordinates from Protein Data Bank entries 2P1P and 2P1Q.

proteins (*3, 17*). SCF complexes are the largest class of E3 ubiquitin protein ligases in plants and catalyze the ubiquitinylation of substrate proteins as a prelude to their degradation by the 26S proteasome (*18*). Target proteins are recruited to the SCF complex *via* a specificity-lending F-box protein subunit that is tethered to its scaffold by an adaptor protein. Mutations in the *TIR1* gene, which encodes a leucine-rich-repeat (LRR)-containing F-box protein, confer reduced auxin response. TIR1 is localized to the cell nucleus and interacts with core SCF subunits, thus establishing the SCF^{TIR1} complex as a central regulator of auxin signaling (*19, 20*).

Identification of TIR1 as an F-box protein suggested recruitment of Aux/IAA polypeptides to the SCF^{TIR1} complex in an auxin-dependent manner. Indeed, biochemical studies demonstrated that SCF^{TIR1} physically interacts with Aux/IAA proteins *via* their degron peptide (*12, 21*). Auxin stimulates this interaction, even in a cell- and membrane-free extract, an indication that auxin response is mediated by a soluble receptor (*22*). The pressing question then arose: how does auxin regulate substrate recruitment and SCF^{TIR1} activity? Regulated ubiquitin/proteasome-mediated protein destruction is a highly conserved signal transduction mechanism in eukaryotic cells and requires signal-induced substrate modification, typically phosphorylation, for recognition by SCF-type E3 ubiquitin ligases. A series of meticulous biochemical experiments systematically ruled out post-translational Aux/IAA protein modification or the requirement of additional protein factors (*21, 22*). Thus, none of the established mechanisms controlling SCF-type E3 activity seemed to apply to the SCF^{TIR1} complex. Instead, to much surprise, auxin alone promotes TIR1:Aux/IAA association by binding directly to TIR1 (*23, 24*). This unexpected result strongly suggests that the nuclear TIR1 receptor and its Aux/IAA substrates are sufficient for auxin sensing by the SCF^{TIR1} pathway that activates early genes by derepression. Thus, auxin signaling is simple and direct, as suspected from the rapid kinetics of primary gene activation (*25, 26*).

But how does auxin enhance TIR1 affinity for its targets? This question is best addressed by structural biology. Tan and colleagues (*2*) have now determined the crystal structures of the free complex formed by *Arabidopsis* TIR1 and its SCF adapter (ASK1), as well as of the TIR1–ASK1 complex in association with an Aux/IAA degron peptide together with one of three different auxin compounds, including the natural IAA and two synthetic auxins, 1-naphthalene-acetic acid (NAA) and the herbicide 2,4-dichlorophenoxy-acetic acid (2,4-D) (see Figure 2, panel b). The F-box motif of TIR1 interacts with ASK1 to form a stem-like structure that is capped by the TIR1-LRR domain, which adopts a highly curved solenoid fold with the overall shape of a closed horseshoe. A single pocket on the top surface of the LRR domain binds to both the auxin compound and the core GWPPV motif of the degron peptide. Each auxin examined "surfaces" the bottom of the pocket such that its carboxyl group protrudes into the floor where it is anchored by

highly selective polar residues. These are held in place by an unexpected inositol hexakisphosphate (IP_6) cofactor positioned in the interior of the solenoid fold. The hydrophobic planar ring system of each auxin, sited in a less selective cavity of fixed shape, faces to the open ceiling and extends the hydrophobic surface of the degron-binding pocket. The coiled Aux/IAA degron peptide seals the TIR1 pocket by packing the conserved GWPPV motif against the hormone, which is believed to remain trapped until Aux/IAA destruction ensues. Auxin binding does not induce an allosteric switch or a significant conformational change; instead, it fills a polar gap to form a continuous hydrophobic protein-interaction surface, much like an adhesive, or "molecular glue", as the authors described it. This regulatory mechanism is consistent with the weak affinity of SCF^{TIR1} to its Aux/IAA substrates observed in the absence of auxin and with their short basal half-lives. It also explains why several natural and synthetic compounds that share only a planar unsaturated ring structure and a side chain with a carboxyl group display "auxin activity" in many bioassays (*3*). As long as these diverse compounds can be anchored to the bottom of the TIR1-LRR pocket, are small enough to be accommodated by the auxin-binding cavity, and provide sufficient hydrophobic contact surface for GWPPV adhesion, Aux/IAA proteins will be marked for degradation.

Although Aux/IAA substrates do not have any detectable affinity to auxin, an optimal hormone binding site is cooperatively formed upon their docking to TIR1 (*2*). It is interesting that basal and auxin-stimulated degradation rates differ considerably among Aux/IAA family members (*27*); this points to a more active role of Aux/IAA polypeptides in auxin sensing. The mechanism of auxin-dependent substrate recruitment to TIR1 suggests complex kinetics for the cyclical assembly and disassembly of the TIR1–IAA–Aux/IAA holo-receptor complex. This may permit continual reassessment of cellular auxin status with each catalytic cycle of Aux/IAA removal. Thus, the mode of hormone perception by SCF^{TIR1} may allow for instantaneous and proportional readout of fluctuating intracellular auxin levels, which are governed by dynamic and directional cell-to-cell transport to create asymmetric local distribution patterns. Such auxin gradients are essential for the regulation of tropic growth and a wide spectrum of developmental processes (*28*). A genetic analysis of TIR1 and its three most closely related auxin-signaling F-box proteins (AFBs) demonstrate that these four auxin receptors collectively mediate auxin-responsive gene expression within the entire plant and during all stages of development (*29*). The protein-interaction networks of TIR1/AFB receptors and Aux/IAA–ARF transcription factors are thought to provide sufficient combinatorial flexibility for executing the myriad of highly specific downstream responses to the hormone. The future will tell whether this elegant perception system accounts for all auxin responses that require reprogramming of gene expression.

The structural model of the TIR1–auxin receptor complex is a milestone achievement in the long quest to understand an entire auxin signaling pathway, from the mechanism of auxin perception to the regulation of gene expression. As with any scientific breakthrough, questions are answered in surprising ways that have implications beyond their own field. For example, the presence of an inositol polyphosphate cofactor, IP_6, with a structural role in the TIR1–auxin receptor complex is an unexpected and intriguing observation because it may point to an additional layer of control for recruiting protein substrates. Inositol polyphosphates are known to regulate a variety of cellular functions, including nuclear activities such as chromatin remodeling or transcription (*30*). It will be interesting to see whether the IP_6 cofactor is unique to TIR1/AFB receptors and whether other plant signaling pathways that require IP_6 metabolism intersect with auxin response. However, this is the most tantalizing question: how widely are F-box proteins used as sensors of small molecules to trigger ubiquitin-dependent destruction of regulatory proteins? *Arabidopsis* has ~700 F-box proteins (*18*), and plants synthesize a plethora of low-molecular-weight chemicals with biological activity, an indication that just the tip of a new iceberg has been sighted in the plant kingdom. And of course, much anticipation exists in regard to treatment of disease by targeting F-box sensors of small molecules, because an increasing number of human disorders have been linked to a malfunctioning ubiquitin/proteasome system (*31*). Beyond its promise for human health, the path from the isolation of auxin response genes and mutants to the discovery of the TIR1 receptor and its structural mode of auxin perception is a beautiful illustration of the power of scientific reasoning and human intuition.

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