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Auxin signaling

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Abstract

Auxin regulates a host of plant developmental and physiological processes, including embryogenesis, vascular differentiation, organogenesis, tropic growth, and root and shoot architecture. Genetic and biochemical studies carried out over the past decade have revealed that much of this regulation involves the $SCF^{TIR1/AFB}$-mediated proteolysis of the Aux/IAA family of transcriptional regulators. With the recent finding that the TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN SIGNALING F-BOX (AFB) proteins also function as auxin receptors, a potentially complete, and surprisingly simple, signaling pathway from perception to transcriptional response is now before us. However, understanding how this seemingly simple pathway controls the myriad of specific auxin responses remains a daunting challenge, and compelling evidence exists for $SCF^{TIR1/AFB}$-independent auxin signaling pathways.

Introduction

In the plant life cycle, few developmental processes occur without the involvement of the phytohormone auxin. Initially described by Charles and Francis Darwin as a mobile substance that mediates phototropism in grass coleoptiles, auxin was chemically identified in the 1930s as indole-3-acetic acid [1]. Subsequent experiments involving the application of auxin to plant tissues implicated this hormone in a plethora of physiological and developmental processes. In the 1980s, it was recognized that auxin regulates the transcription of many genes, thereby linking the auxin signal to changes in gene expression [2•]. However, the nature of the molecular mechanisms of auxin perception and signal transduction underlying this regulation remained a mystery. In this review, we focus on these central questions and the impressive progress that has been made toward illuminating this fundamental area of plant biology.

Auxin-responsive gene expression

Early molecular approaches to elucidate auxin action identified several genes that exhibit a rapid and specific increase in expression in response to exogenous auxin. Among the most thoroughly characterized are three gene families (*SAURs [Small Auxin-Up RNAs]*, *GH3s* and *Aux/IAAs*), which are induced within minutes of auxin application. The promoter regions of several auxin-responsive genes contain one or more auxin-responsive elements (*AuxRE*), which are capable of conferring auxin-regulated gene expression to reporter constructs [3]. The identification of the *AuxRE* sequence led to the isolation of the *Arabidopsis AUXIN RESPONSE FACTOR 1 (ARF1)* gene [4], and subsequent genetic, genomic, and molecular studies have identified 23 *ARF* genes in *Arabidopsis* [5]. In addition to a conserved amino-terminal (N-terminal) domain that mediates *AuxRE* binding [6], most ARF transcription factors also contain carboxyl-terminal (C-terminal) dimerization elements (domains III and IV). In between is a variable middle region (MR) that generally is either glutamine (Q)-rich or serine (S)-rich. This difference is apparently a major determinant of ARF function, with S-rich ARFs

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acting as transcriptional repressors and Q-rich ARFs as transcriptional activators in protoplast transfection assays [6].

Genetic studies have implicated various *ARFs* in distinct developmental processes, including embryogenesis (*ARF5/MONOPTEROS [MP]* [7] and *ARF17* [8•]), root development (*ARF7, ARF19* [9•], *ARF10* and *ARF16* [8•,10•]), flower development (*ARF2* [11], *ARF3/ETTIN [ETT]* [12], *ARF6* and *ARF8* [13,14]), and senescence (*ARF1* and *ARF2* [13]). Owing to some functional redundancy, several *arf* mutants lack obvious phenotypes, but exacerbated developmental defects are seen in several double mutant combinations [9•,14].

Although the transcription of most *ARF* genes is not auxin-regulated, hormonal control of at least the Q-rich ARFs is achieved through their interactions with Aux/IAA proteins (Figure 1a). The 29 members of the *Arabidopsis Aux/IAA* family of auxin-inducible genes encode nuclear proteins, most of which share four conserved domains [5], including the C-terminal domains III and IV that are found in most ARF proteins. These domains mediate Aux/IAA-ARF heterodimerization [15], which has been shown to repress transcriptional activation by Q-rich ARFs in protoplast assays [6]. Aux/IAA domain I has recently been shown to mediate this transcriptional repression, although the mechanistic details remain to be elucidated [16].

A number of loss-of-function mutations in *Aux/IAA* genes have been characterized but they confer no apparent mutant phenotypes, suggesting genetic redundancy within this large gene family [17•]. Most of the insight into the biological function of Aux/IAA proteins gathered to date comes from the characterization of dominant, gain-of-function mutations [2•] in several *Aux/IAA* genes that confer dramatic phenotypes characteristic of auxin signaling defects. Each of these mutations alters the highly conserved core of domain II. Most Aux/IAAs are extremely short-lived proteins, whose half-lives decrease further in the presence of auxin [18]. The 13-amino-acid domain II sequence functions as a degron that targets the Aux/IAA for ubiquitin-mediated proteolysis [19,20], and these dominant mutations disrupt this regulation, thereby stabilizing the affected protein. Recent findings suggest that sequences outside of domain II also contribute to Aux/IAA proteolysis, and it is interesting to note that degradation rates vary considerably among different Aux/IAA family members [21•].

Although genetic studies suggest considerable functional redundancy within the *ARF* and *Aux/IAA* gene families [9•,17•], the fact that several *arf* mutants exhibit specific auxin-related defects clearly indicates some functional specialization. Likewise, the phenotypes of the different gain-of-function *Aux/IAA* mutants that have been characterized vary considerably. Recent results suggest that auxin signals are converted into specific responses by matching pairs of co-expressed ARF and Aux/IAA proteins. For example, gain-of-function mutations in the *Aux/IAA* gene *IAA12/BODENLOS [BDL]* confer a dramatic embryo- or seedling-lethal phenotype similar to those associated with loss-of-function mutations in *arf5/mp* [22]. These findings suggest an antagonistic relationship between *IAA12/BDL* and *ARF5/MP*, and indeed, IAA12 was found to interact with ARF5 and to negatively regulate its activity [23]. Although yeast two-hybrid studies have not revealed substantial specificity in the interactions between individual ARF and Aux/IAA proteins, the above findings suggest that such specificity might exist *in planta*. The spatiotemporal transcriptional control of *Aux/IAA* gene expression is also a key determinant in the regulation of specific ARFs: promoter-swap experiments have suggested that IAA12/BDL and IAA3/SHORT HYPOCOTYL 2 [SHY2] are largely functionally interchangeable [24••]. The precise control of expression pattern might explain why several ARF mRNAs are under the additional control of microRNA (miRNA)- and *trans*-acting small interfering RNAs (tasiRNA)-mediated degradation [8•,10•,25].

Proteolysis and auxin signaling

The genetic identification of *Arabidopsis* auxin-resistant mutants has provided crucial insight into the molecular mechanisms underlying auxin signaling. Molecular and biochemical studies of the gene products that are affected by these mutations have positioned the SCF^{TIR1} ubiquitin-ligase complex as a central regulator of auxin signaling. E3 ubiquitin ligases catalyze the conjugation of ubiquitin to substrate proteins. Once ubiquitinylated, these substrates are targeted to the 26S proteasome for degradation. SCF complexes are the largest family of ubiquitin ligases in plants. They are composed of Skp1, cullin, an F-box protein, and the small RING protein Rbx1 (for RING-BOX 1). The cullin subunit acts as a scaffold, binding Skp1 at its N-terminus and Rbx1 at its C-terminus. Skp1 binds the F-box domain of the F-box protein, which functions as a substrate-specific adaptor, recruiting specific proteins to the complex for ubiquitinylation [26,27].

Mutations in the *TRANSPORT INHIBITOR RESPONSE1 (TIR1)* gene confer reduced auxin response [28]. *TIR1* encodes a nuclear protein belonging to the F-box protein family of *Arabidopsis*, which has approximately 700 members. TIR1 interacts with the core SCF subunits [29,30], and additional support for SCF-mediated regulation of auxin signaling was rapidly obtained through genetic studies of *CULLIN 1 (CUL1)*, *ARABIDOPSIS SKP1-LIKE (ASK1)* and *RBX1* [29-32]. These findings established the SCF^{TIR1} complex as a positive regulator of auxin response and suggested a model invoking the SCF^{TIR1}-mediated ubiquitinylation of a repressor of auxin signaling. Given the short-lived nature of Aux/IAA proteins, together with the results of molecular and genetic studies implicating these proteins as negative regulators of auxin response, Aux/IAA proteins are attractive candidates for substrates of the SCF^{TIR1} complex. Indeed, Aux/IAA stability is increased in mutants that have defects in SCF^{TIR1} activity, and biochemical studies have confirmed that Aux/IAA proteins interact with TIR1 [20]. Furthermore, this interaction requires the Aux/IAA domain II degron and is rapidly promoted by auxin in a concentration-dependent manner.

The study of several additional auxin-resistant mutants has provided insight into the regulation of SCF^{TIR1} activity. Proper SCF^{TIR1} function requires the covalent modification of the CUL1 subunit by conjugation of the RELATED TO UBIQUITIN protein (RUB, known as NEDD8 in mammals). RUB conjugation requires RUB-specific E1 (AUXIN RESISTANT 1 [AXR1]-E1 C-TERMINAL RELATED1 [ECR1]) and E2 enzymes (RUB1 CONJUGATING ENZYME1 [RCE1]), as well as RBX1, which appears to function as a RUB E3 ligase. Defects in any of these enzymes severely impair SCF^{TIR1} activity, conferring a dramatic reduction in auxin response [20,30,33,34].

RUB modification of CUL1 is a dynamic process. The SCF interacts with the COP9 SIGNALOSOME (CSN) [35], which possesses a RUB-isopeptidase activity that cleaves the RUB modifier off of CUL1 [26]. Surprisingly, plants that have impaired CSN function exhibit diminished auxin response and increased Aux/IAA stability. Thus, like RUB conjugation, RUB cleavage off of CUL1 is also required for optimal SCF^{TIR1} activity [35]. While the molecular function of RUB modification remains unclear, an interesting avenue has emerged with the identification of CAND1 (CULLIN-ASSOCIATED and NEDD8-DISSOCIATED1). CAND1 specifically interacts with unmodified cullin, and can be dissociated by the RUB modification of CUL1 *in vitro* [26]. Furthermore, CAND1 and SKP1 binding to CUL1 are mutually exclusive, suggesting a model whereby CAND1 sequesters a fraction of the CUL1 pool and thus negatively regulates ubiquitin-ligase activity by preventing SCF assembly [26]. Genetic studies of *Arabidopsis cand1* mutants, however, suggest a more complicated scenario. Rather than exhibiting increased SCF^{TIR1} activity as might be expected, *cand1* mutants are defective in Aux/IAA proteolysis and display diminished auxin-response phenotypes similar to those of SCF mutants [36-38]. To reconcile these observations, it has been suggested that CAND1 is

required to sustain SCF activity *in vivo* by promoting cycles of SCF assembly and disassembly ([26]; Figure 1b). Such a cycle might be essential in a cellular context in which many F-box proteins compete for access to the common core SCF subunits. The cyclical conjugation and cleavage of the RUB modifier has been proposed to drive this cycle. The finding that auxin response in *cand1* mutants is still highly dependent on the RUB conjugation pathway is, however, difficult to reconcile with this model, and at least suggests that this is not the primary means by which RUB regulates SCF function [37].

Auxin receptors

Amid an ever-clearer picture of how auxin triggers changes in gene expression, the auxin receptor remained a conspicuous omission from models of auxin action. Early biochemical efforts to isolate auxin receptors identified AUXIN-BINDING PROTEIN1 (ABP1) [39]. The majority of ABP1 protein localizes to the endoplasmic reticulum, where the pH is too high for auxin binding. However, some ABP1 is also found on the plasma membrane, and ABP1 antibody experiments have implicated this pool in auxin-mediated cell expansion. Overexpression studies also support a role for ABP1 in cell expansion but no evidence has been obtained to link ABP1 to auxin-regulated transcriptional changes, which are clearly responsible for many auxin responses.

An important breakthrough in the auxin receptor hunt came when auxin action was demonstrated in a cell-free system [40]. Specifically, the addition of auxin to crude plant extracts was shown to promote the interaction between Aux/IAA proteins and the SCF^{TIR1} complex, indicating the presence of a soluble auxin receptor. SCF-substrate interactions frequently require the post-translational modification of the substrate [26]. This did not appear to be the case in this instance, however, because Aux/IAA domain II, which was previously shown to be sufficient for auxin-induced TIR1 binding, is not modified when incubated with auxin-supplemented extracts [41•].

Finally, two recent reports revealed the remarkable simplicity of the TIR1 pathway by showing that radiolabeled auxin that was added to crude extracts co-purified with the SCF^{TIR1}-Aux/IAA complex [42••,43••]. This binding activity is saturable, specific for active auxins, and possesses the affinity expected of an auxin receptor. Although this evidence alone suggests only that a receptor is associated with SCF^{TIR1}, the finding that TIR1 that is expressed in animal cells also interacts with Aux/IAAs (or a domain II peptide) in an auxin-dependent manner strongly suggests that TIR1 is an auxin receptor. To date, it has not been possible to separate the auxin binding and Aux/IAA binding activities of TIR1, raising the possibility that auxin might coordinate the complex, with both TIR1 and domain II contacting the hormone.

The fact that *tir1* null mutants exhibit a relatively weak auxin response defect suggested that TIR1 might not be the only auxin receptor. Indeed, three additional AUXIN SIGNALING F-BOX proteins, AFB1-AFB3, are highly related to TIR1 and were recently shown to exhibit auxin-dependent binding to Aux/IAA proteins [44••]. Correspondingly, the introduction of *afb* mutations into *tir1* plants results in a progressive reduction in auxin response, culminating with *tir1 afb1 afb2 afb3* quadruple mutants exhibiting a seedling lethal phenotype similar to that of *mp* and *bdl* mutants. Curiously, a fraction of these quadruple mutants do survive and are able to complete development, suggesting that auxin signaling is not completely abolished in these mutants.

Conclusions

The discovery that the TIR1/AFB F-box proteins function as auxin receptors is a surprising development that fills in a crucial piece of the auxin puzzle and might well serve as a new paradigm for the regulation of SCF-mediated ubiquitinylation by small ligands. Many details

remain to be established, including a definition of the auxin-binding site and a comparison of the binding affinities of the receptors, but we can now follow the auxin signal from perception through to changes in gene expression (Figures 1a,c). But can this apparently simple signaling pathway account for the diversity of specific auxin responses? The presence of a small receptor family suggests the potential for functional specialization. However, the relatively ubiquitous expression patterns of the *TIR1/AFB* genes, their biochemical interactions with common Aux/IAA proteins and the results of genetic studies all suggest that the receptors act in a largely redundant fashion [44••]. This would again suggest that the control of specific auxin responses might largely occur downstream in the web of potential Aux/IAA-ARF interactions. The recent finding that Aux/IAA degradation kinetics vary considerably among different family members is an exciting development [21•], particularly if future structural studies reveal that Aux/IAA proteins contribute to auxin binding. If so, one intriguing possibility is that these stability differences reflect varying affinities for IAA, which could facilitate Aux/IAA proteins serving as sensors of auxin concentration.

The $SCF^{TIR1/AFB}$-mediated proteolysis of Aux/IAA proteins is clearly responsible for many of the effects of auxin, but the resulting changes in gene expression occur too slowly to account for the most rapid auxin responses, including ion fluxes across the plasma membrane [45], mitogen-activated protein (MAP) kinase activation [46], and vesicle trafficking [47••]. The success in elucidating the $SCF^{TIR1/AFB}$ pathway has resulted in other potential signaling mechanisms receiving significantly less attention than they probably deserve. There is evidence that implicates a cell-surface auxin receptor in auxin-mediated cell expansion [48]. This receptor might very likely be ABP1 [49], but the mechanism of ABP1 action and the identities of other components of this pathway await discovery. Furthermore, several factors, including the repressing ARFs, SAUR proteins, MAP kinase pathway components, and the INDOLE-3-BUTYRIC ACID-RESPONSE5 (IBR5) [50] dual-specificity phosphatase, have been implicated in auxin signaling but are presently without a home in current models. Clearly, much remains to be learned about the mechanics of auxin signaling.

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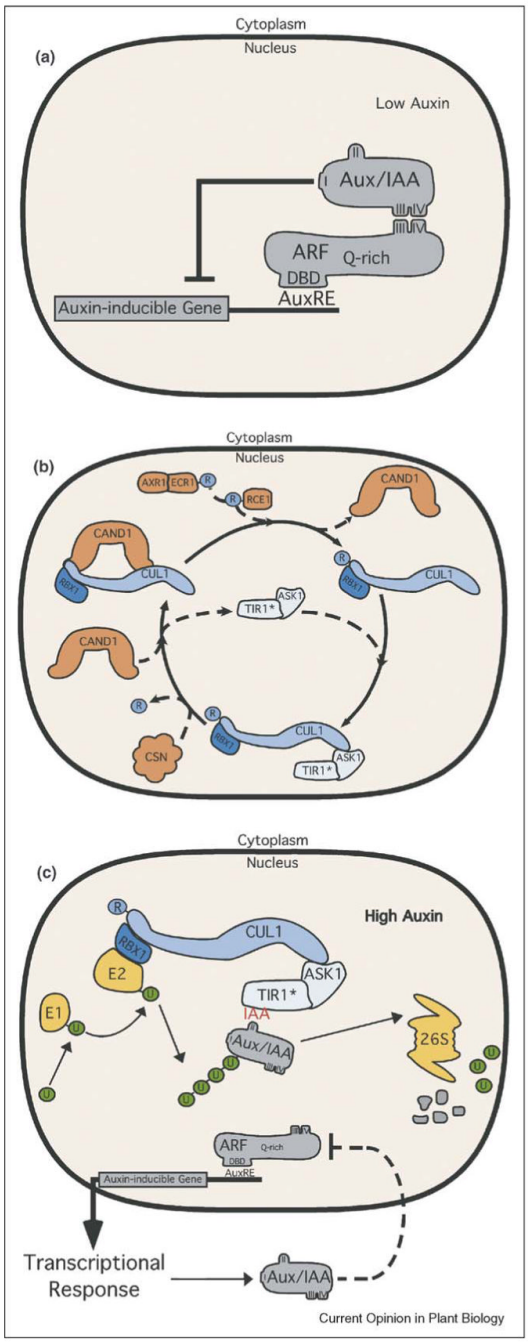

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Auxin regulation of gene expression. **(a)** Under sub-threshold auxin concentrations the Aux/IAA proteins heterodimerize with the ARF transcription factors, thereby repressing auxin-inducible gene expression. **(b)** The active SCF^{TIR1} complex, containing RUB-modified CUL1, is shown at the bottom. The CSN complex can cleave the RUB modifier from CUL1, thus facilitating CAND1 binding to CUL1 and SCF disassembly. Conjugation of RUB to CUL1 by the AXR1-ECR1 and RCE1 enzymes might free CUL1 from CAND1, promoting re-assembly of the active complex. Genetic studies have shown that all of the components depicted in this figure are required for optimal SCF^{TIR1} activity *in vivo*. **(c)** Auxin binding to the TIR1/AFB receptors promotes the recruitment of Aux/IAA proteins to the SCF complex. Subsequent Aux/

IAA ubiquitinylation and proteasome-mediated degradation results in a decline in Aux/IAA protein levels, thus de-repressing auxin-inducible gene expression. In addition to genes responsible for specific auxin responses, the *Aux/IAA* genes themselves are auxin-inducible. This might represent a negative feedback loop that ensures a transient response, with the nascent Aux/IAA proteins attenuating the signaling pathway as auxin levels fall by restoring repression of the ARF transcription factors. DBD, DNA-binding domain; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; U, ubiquitin; R, RUB. *,AFB1, AFB2, or AFB3.