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# Funneling auxin action: specificity in signal transduction

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Auxin regulates a broad spectrum of developmental processes, mediating transcriptional regulation via protein degradation. The molecular mechanisms of auxin action are partially understood whereas the molecular basis for developmental specificity in auxin responses is currently unclear. Recent biochemical and chemical-genetics studies have narrowed the search for regulators in auxin signaling that act upstream of auxin-dependent protein degradation. The auxin response requires the degradation of Aux/IAA inhibitors, which causes interacting ARF transcription factors to be released to regulate target genes. There are many ARFs and Aux/IAA proteins in *Arabidopsis*, and recent genetic studies suggest that their cell-specific combinations may determine the various auxin responses in development.

## Addresses

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## Abbreviations

<b>ABP1</b>	auxin binding protein 1
<b>ARF</b>	auxin response factor
<b>ASK1</b>	<i>Arabidopsis</i> SKP1-like 1
<b>Aux/IAA</b>	auxin/indole-3-acetic acid
<b>AXR</b>	auxin-resistant
<b>BDL</b>	bodenlos
<b>CAND1</b>	cullin-associated neddylation-dissociated 1
<b>COP9</b>	constitutively photomorphogenic 9
<b>CSN</b>	COP9 signalosome
<b>CUL1</b>	AtCULLIN1
<b>MP</b>	monopteros
<b>MSG2</b>	massugu 2
<b>NEDD8</b>	neural precursor cell expressed, developmentally downregulated 8
<b>NPH4</b>	non-phototropic hypocotyl 4
<b>PCIB</b>	p-chlorophenoxyisobutyric acid
<b>PPI-ase</b>	peptidyl-prolyl-isomerase
<b>RCE1</b>	rub-conjugating enzyme 1
<b>RUB1</b>	related to ubiquitin 1
<b>SCF</b>	SKP1–CULLIN/cdc53–F-box
<b>SHY2</b>	short hypocotyl 2
<b>SIR1</b>	sirtinol-resistant 1
<b>TIR1</b>	transport inhibitor resistant 1

## Introduction

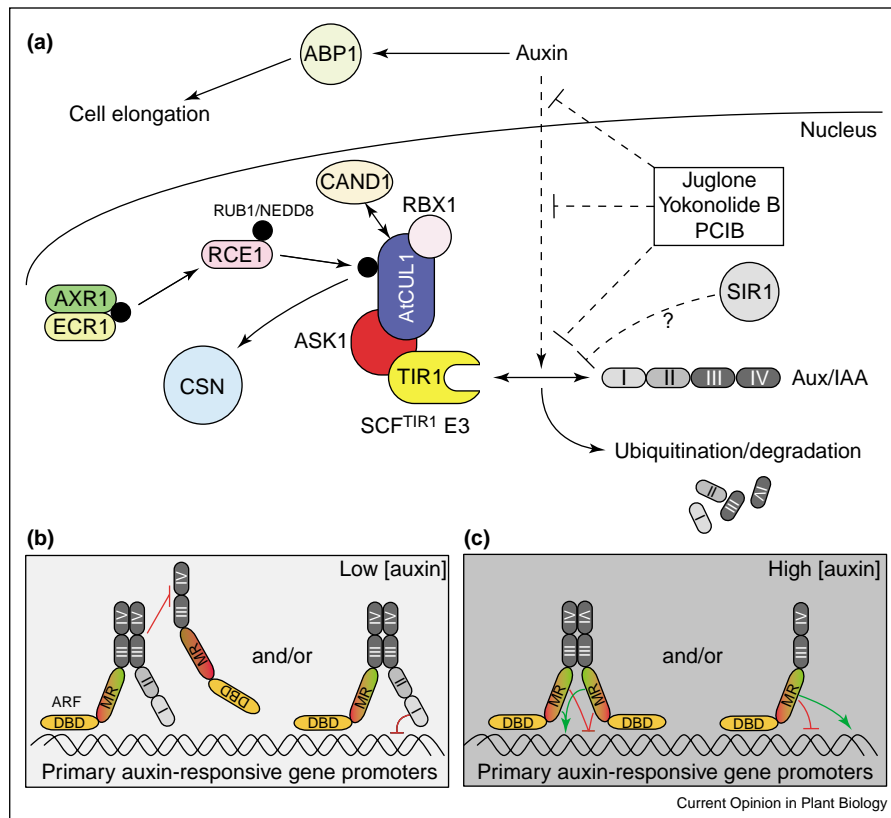
The plant signaling molecule auxin has been implicated in embryogenesis, root and shoot patterning, growth, branching and organogenesis, as well as in directional growth responses [1]. Hence, the acronym for auxin, IAA (indole-3-acetic acid) could also stand for ‘Influences Almost Anything’. Knowledge of the mechanisms of auxin signaling and action is steadily increasing, with recent leaps including the identification of a family of proteins required for the precise distribution of auxin within different plant tissues [1], and the appreciation of regulated protein degradation as a central process in auxin signal transduction [2]. Two major issues in auxin biology have, however, remained largely unresolved to date. Despite great efforts, no convincing candidate for an auxin receptor has been found. Another open question is how the generic auxin signal is converted into a very precise cellular response for each biological process. In the following sections, we will discuss recent advances in our understanding of auxin signal transduction pathways that lead to changes in gene expression. We also address the problem of how specific auxin responses could be generated.

## Beyond genetics – new advances in auxin signal transduction

The expression of many plant genes rapidly changes upon auxin treatment, with some genes responding within minutes. This implicates highly efficient downstream signaling that does not require *de novo* protein synthesis [3]. Over the past decade, the cloning of several genes that are required for normal auxin responses has elucidated that auxin promotes the degradation of a class of transcriptional repressors, the Aux/IAA proteins. These proteins mediate transcriptional responses to auxin through their inhibitory interactions with another class of transcriptional regulators, the auxin response factors (ARFs) (Figure 1). Auxin promotes the degradation of Aux/IAA proteins by stimulating their interaction with an SCF-type (SKP1–CULLIN/cdc53–F-box) E3 ubiquitin ligase protein complex. Ubiquitin ligases tag substrate proteins with a polyubiquitin polypeptide and thereby mark them for degradation by the 26S proteasome. The ubiquitin ligase involved in Aux/IAA degradation consists of AtCULLIN1 (CUL1), an *Arabidopsis* SKP1-like protein (ASK1) and transport inhibitor resistant 1 (TIR1), an F-box protein (Figure 1) [4–6].

How auxin promotes SCF<sup>TIR1</sup>–Aux/IAA interactions is presently unclear. It is conceivable that auxin signaling promotes the chemical modification of the SCF<sup>TIR1</sup> complex, of its Aux/IAA substrate or of yet another unknown

Figure 1



Model for auxin action on plant cells. **(a)** Auxin induces cell elongation, possibly through the activity of ABP1, and stimulates the interaction between nuclear Aux/IAA proteins and SCF<sup>TIR1</sup> (RBX1 is a RING-domain-containing protein that is associated with the SCF complex [21,41]). This interaction is followed by the ubiquitination and degradation of the Aux/IAA substrate by the 26S proteasome (not depicted). SCF activity requires AXR1/ECR1- and RCE1-dependent modification of AtCUL1. The CSN catalyzes the deconjugation of RUB1/NEDD8 and regulates SCF activity (whereas CAND1 binds to unmodified AtCUL1) and thereby influences SCF assembly. Auxin-dependent Aux/IAA degradation can be inhibited by Yokonolide B, PCIB and juglone. The sirtinol target SIR1 may act at the same step. **(b)** At low auxin concentrations, Aux/IAA proteins (I, II, III and IV represent conserved domains) interact with ARF transcription factors (through the DNA-binding domain [DBD] and the middle region [MR]), presumably at the target DNA site in primary auxin-responsive gene promoters, and counteract ARF activity either by competitive inhibition of homodimerization (left), or by directly influencing the activity of transcriptional complexes (right). **(c)** Upon Aux/IAA degradation stimulated by high auxin concentrations, ARFs either repress or activate target gene transcription as dimers (left) or as monomers (right). ECR1, E1 C-terminus related 1.

protein. By systematically studying which factors interfere with the ability of recombinant AXR2/IAA7 or AXR3/IAA17 protein to pull-down TIR1 protein from auxin-treated plant extracts, Dharmasiri *et al.* [7••] have gained significant insight into the requirements for this particular auxin signaling process. Interestingly, the auxin-dependent SCF<sup>TIR1</sup>-Aux/IAA interaction requires neither integral membrane proteins nor the candidate auxin receptor, auxin binding protein 1 (ABP1). Neither removal of ATP nor inhibition of kinases or phosphatases interfered with the interaction. Furthermore, proline hydroxylation did not affect the SCF<sup>TIR1</sup>-Aux/IAA interaction. However, the interaction was sensitive to juglone, which inhibits peptidyl-prolyl-isomerases (PPI-ases) of the parvulin type. Although this effect is consistent with the fact that two conserved proline residues in domain II of Aux/IAAs are required

for auxin-dependent degradation, a direct involvement of parvulins remains to be demonstrated.

A novel signaling component, sirtinol-resistant 1 (SIR1), that may act on the same proline residues in Aux/IAA proteins was identified through a chemical genetics approach and subsequent mutagenesis [8••]. Sirtinol was identified from a chemical library as a compound with auxin-like activity. A mutation in the *SIR1* gene was found to render plants resistant to the growth-inhibitory effects of sirtinol. SIR1 contains a domain with distant homology to E1 ubiquitin-activating enzymes, as well as to a rhodanese domain that is also found in some PPI-ases. The biochemical activity of SIR1 as ubiquitin-activating enzyme or PPI-ase, however, has not yet been shown. Several auxin-resistant mutants (*axr1*, *axr2*, *axr3* and *axr6*) are also sirtinol resistant [9]. There is no evidence,

on the other hand, that *sir1* is auxin-resistant and this might therefore be a special mutation [8\*\*]. Further work, including the analysis of additional alleles of SIR1, will be required to determine the precise mode of action of SIR1 in auxin signaling upstream of auxin-dependent Aux/IAA degradation. In addition, further progress is to be expected from investigation into the molecular targets of other chemicals that also interfere with aspects of auxin-dependent Aux/IAA degradation. Two such compounds are p-chlorophenoxyisobutyric acid (PCIB) [10\*] and Yokonolide B [11\*]. Both were recently shown to interfere specifically with auxin signaling, acting upstream of Aux/IAA protein degradation.

### Specificity of gene expression responses

A central question in auxin biology is how specific auxin responses are generated throughout development. Conceivably, general components of auxin signaling may be modulated in a context-specific manner by signaling inputs that are currently unknown. However, a more likely explanation could lie in the fact that most of the proteins involved in the auxin response are members of protein families, different members of which might be differentially expressed and/or encode proteins with different inherent specificity or activity.

Given the model for auxin-dependent gene expression, specificity could be generated at several levels; for example by Aux/IAA protein degradation or by tissue-specific combinations of Aux/IAA and ARF proteins. Specific SCF complexes, recognizing only a given subset of substrate Aux/IAs could be assembled. Alternatively or additionally, given that the *Arabidopsis* genome encodes 23 ARF and 29 Aux/IAA proteins [12], each cell could have its own set of functionally distinct ARF and Aux/IAA proteins. The Aux/IAA proteins could be differentially susceptible to degradation after recognition by a common SCF complex.

### Do SCF complexes have specificity for Aux/IAA substrates?

There is only one cullin for SCF-type E3 ligases and 21 ASK proteins, but there are about 700 F-box proteins in *Arabidopsis* [13\*,14]. F-box proteins are receptors for degradation substrates, and should provide the SCF complex with specificity [2,5,14]. Importantly, complete loss of CUL1 activity leads to early zygotic arrest, a phenotype that has not yet been found in any auxin-related mutant [15]. Indeed, CUL1 is integral to SCF complexes that regulate jasmonic-acid responses [16] or flower development [17]. AXR1 and E1 C-terminus related 1 (ECR1) regulate SCF<sup>TIR1</sup> activity by promoting the modification of CUL1 by RUB1/NEDD8 [18\*]. This reaction is reversible, with the COP9 signalosome (CSN) promoting RUB1/NEDD8 deconjugation [14]. Additionally, AtCAND1 regulates SCF assembly by binding to unmodified AtCUL1 [9,19,20]. Mutations that affect ARX1,

RCE1, CSN or AtCAND1 not only impair auxin response but also affect several other SCF-dependent processes [21,22]. Despite AtCUL1 and its regulators being involved in auxin-independent pathways, weak mutations in *axr6* alleles of CUL1 [23\*] and a double mutant of *axr1* and *ree1* [18\*] cause auxin-related embryo phenotypes. This could indicate that auxin response depends critically on SCF complex activity, and might be the first process to break down if SCF activity is compromised.

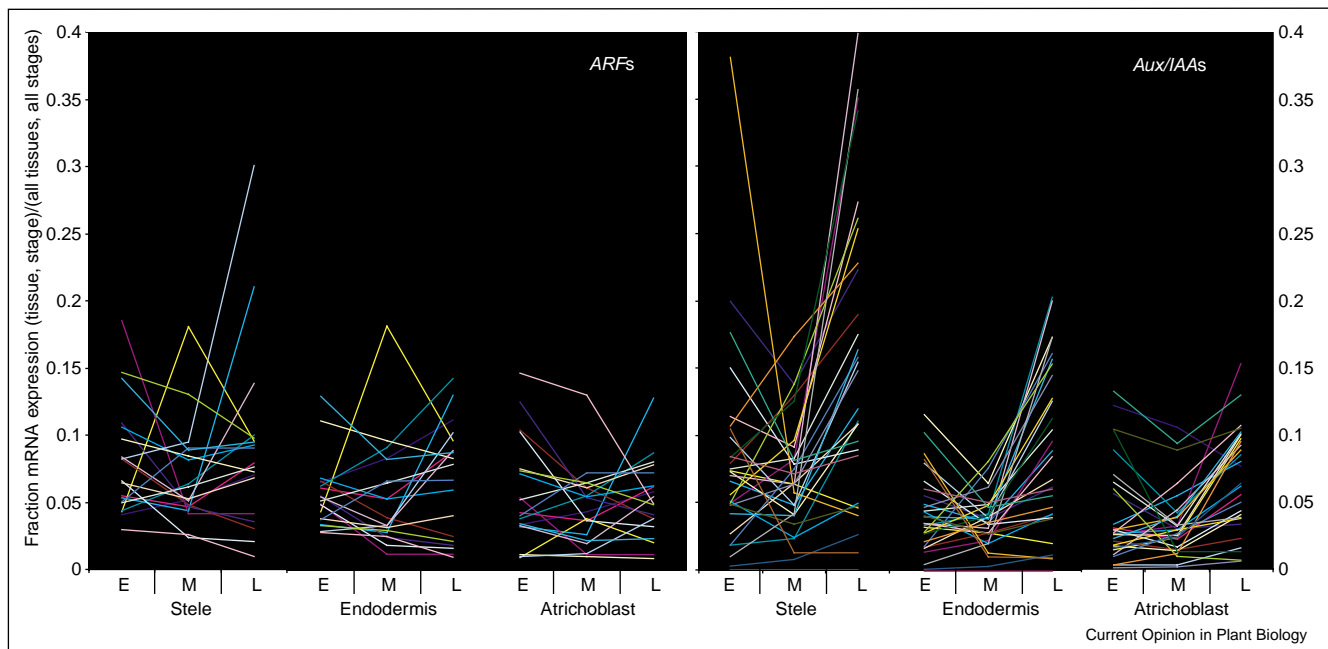
A mutation in the ASK1 component of the SCF complex leads to weak auxin resistance [4], but this subunit probably makes only a limited contribution to complex specificity because various different pairwise combinations of ASK1 with other F-box proteins were demonstrated in yeast two-hybrid assays [13\*] and *in vivo* [4,5,17]. The *tir1* mutant shows only auxin-related defects, so TIR1 is probably specifically involved in the degradation of Aux/IAA proteins. However, most mutations that stabilize Aux/IAs have phenotypes that are stronger than that of *tir1*, suggesting functional overlap between TIR1 and other F-box proteins. The F-box protein family in *Arabidopsis* is very large, and only a subset of these proteins have a domain composition similar to that of TIR1. Among the TIR1-related F-box proteins, several relatively close homologs have completely different biological functions [2], and therefore their predicted substrates are different. Thus, functional overlap could be restricted to the small subfamily comprised of closest TIR1 homologues, which consists of four more members [4]. If this assumption proves correct, given the size of the Aux/IAA protein family, each TIR1-related F-box protein must recognize several Aux/IAA proteins.

### Are specific Aux/IAA-ARF combinations involved in developmental auxin responses?

One can easily envision how different combinations of ARFs and Aux/IAs could account for the entire spectrum of auxin responses. Preferential interactions between Aux/IAs and ARFs seem to exist according to yeast two-hybrid assays [24,25,26\*]. However, we currently know surprisingly little about biologically relevant homo and hetero interactions between ARFs and Aux/IAs.

The issue of ARF and Aux/IAA specificity can be broken down into two central questions. First, what is the expression pattern of all *ARF* and *Aux/IAA* genes? Naturally, if Aux/IAs and ARFs are qualitatively different, the cellular ensemble of regulators could determine a precise auxin response. Limited *ARF* gene expression data at cellular resolution are available [27,28], but a survey of global expression analysis in *Arabidopsis* roots [29\*\*] shows extensive specialization at the transcriptional level (Figure 2). More information is available on *Aux/IAA* gene expression. The promoters of genes including *IAA3*,

Figure 2



Expression of *ARF* and *Aux/IAA* mRNAs in *Arabidopsis* root tips. Graphical representation of the relative mRNA abundance of most *Arabidopsis* *ARF* (left) and *Aux/IAA* (right) genes in distal meristematic (E), elongating (M) and differentiating (L) regions of three tissue types (stele, endodermis and atrichoblasts) in *Arabidopsis* seedling root tips (data reproduced from [29\*\*]). mRNA expression in each given situation (stage, tissue) is plotted as a fraction of the total expression in all three stages and all five tissues, as reported in [29\*\*]. Each colour-coded line represents a single *ARF* or *Aux/IAA* gene. Note that *ARF* genes are highly specialized as judged from the many unique transcriptional profiles. In E and M stages, the expression levels of *Aux/IAA* genes are relatively divergent, whereas most are upregulated during differentiation (M to L stages).

*IAA12*, *IAA14* and *IAA19* define different subsets of cells [30,31,32\*] and the differential expression of many other *Aux/IAAs* is apparent from global expression analysis (Figure 2) [29\*\*]. Thus, different cells do seem to contain specific sets of *ARF* and *Aux/IAA* mRNAs. Second, are *ARFs* and *Aux/IAAs* functionally distinct? This issue has been addressed in separate reports for *ARFs* and *Aux/IAAs*. Overexpression of the closely related *MP/ARF5* and *NPH4/ARF7* proteins leads to qualitatively different phenotypes. Additionally, *NPH4/ARF7* cannot replace *MP/ARF5* function during embryogenesis [26\*]. However, when overexpressed, *MP/ARF5* can suppress defects in the *nph4* mutant. Taken together, these findings suggest that *MP/ARF5* and *NPH4/ARF7* are not functionally equivalent, and the capacity of *MP/ARF5* seems to be broader than that of *NPH4/ARF7*.

To date, gain-of-function mutations in ten *Aux/IAA* proteins have been described [2]. Their phenotypes are similar in some cases, but different or even opposite in others. Because of the distinct expression patterns of the mutated genes, it has not been possible to assess qualitative differences in their phenotypes unequivocally. A recent study indicated that *Aux/IAA* proteins can be functionally different [33\*]. *SHY2/IAA3* and *AXR3/IAA17* are both expressed in roots, and the corresponding

gain-of-function mutants affect root-hair development in different ways. Inducible overexpression of *shy2* or *axr3* genes under the same promoter recapitulated gene-specific effects on root-hair development. Interestingly, a novel phenotype was found after the induction of *axr3* expression in the *shy2* mutant, but not after the induction of *shy2* expression in the *axr3* mutant. This evidence suggests that a particular ratio of concentrations of two *Aux/IAA* proteins can define a distinct cellular response.

Thus, both *ARF* and *Aux/IAA* proteins seem to bear some inherent functional specificity, which might be enhanced even further by combining pairs of *ARFs* and *Aux/IAAs* that act in the same biological process. Hence, if a cell is provided with both an *Aux/IAA* that preferentially inhibits a subset of *ARFs* and an *ARF* that can activate only part of the *ARF*-dependent genes, the cell would be capable of a very precise auxin response. At least two such seemingly specialized *ARF*-*Aux/IAA* pairs exist. Embryonic root initiation requires *MP/ARF5* activity and is abrogated by a gain-of-function mutation in *BDL/IAA12*. *MP/ARF5* and *BDL/IAA12* mRNAs are co-expressed in embryos and the proteins interact in yeast [30]. Additional evidence that *MP/ARF5* is regulated by an instable protein comes from the *axr1* *rec* double mutant and from the *axr6* mutant, which both

result in a phenotype similar to that found in *mp* and *bdl* mutants [18\*,23\*]. Interestingly, the *mp bdl* double mutant fails to initiate cotyledons [34], a phenotype found in the *mp nph4* double mutant but not seen in either single mutant [26\*]. This suggests that, even though it would not normally do so, *bdl* mutant protein can repress NPH4/ARF7 in the absence of MP/ARF5, with which it can also interact in yeast two-hybrid assays [26\*]. NPH4/ARF7 also seems to be regulated by a matching Aux/IAA protein, MSG2/IAA19 [32\*]. The *nph4* phenotypes (i.e. impaired tropisms) are very similar to those caused by gain-of-function alleles of *IAA19/MSG2*. As required for a *bona fide* protein pair, NPH4/ARF7 and MSG2/IAA19 interact in yeast two-hybrid and *in vitro* pull-down assays.

### Conclusions and perspectives

The evidence discussed above shows that defined sets of ARF and Aux/IAA regulators convert the generic auxin signal into specific developmental or physiological responses. This simplified view could explain the entire complexity of auxin responses if all ARFs were to activate transcription, if all Aux/IAAs were to inhibit ARFs and if there were no feedback regulation by ARF target genes. However, this is not the case.

Upon treatment of plant cells with auxin, many genes are upregulated while others are downregulated [35]. A subset of the ARF proteins act as repressors of transcription when transiently overexpressed in carrot protoplasts [36], and these proteins may mediate auxin-dependent repression of transcription. Regulation by Aux/IAA proteins provides auxin-responsiveness to ARF-dependent expression [37,38] and possibly also to ARF repressors. It is possible that Aux/IAAs not only counteract ARF action by preventing their DNA-binding or dimerization but also by actively repressing the ARF-mediated transcription of auxin-dependent genes through a conserved motif in domain I [39\*] (Figure 1). It is difficult to explain, however, how an ARF repressor could be regulated by an Aux/IAA protein that represses transcription alone. Possibly, different Aux/IAAs act differently depending on the ARF involved.

Another complication of auxin-dependent transcriptional regulation is that Aux/IAA proteins are themselves primary auxin-response genes, enabling feedback regulation of ARF activity and making it difficult to predict the transcriptional output. To overcome this problem, it is necessary to integrate knowledge on the precise expression of all ARF and Aux/IAA proteins, their biologically relevant interactions and the dynamics of Aux/IAA degradation.

### Update

After this review had been submitted, a paper appeared that sheds light on how auxin could promote the SCF<sup>TIR1</sup>-Aux/IAA interaction. Kepinski and Leyser

[40\*\*] show that auxin treatment does not promote stable modifications to domain II of an Aux/IAA protein other than proline hydroxylation. The relevance of this modification is not obvious [6,7\*\*]. Furthermore, through a series of conditioning experiments, convincing evidence is presented showing that auxin promotes SCF<sup>TIR1</sup>-Aux/IAA interactions by modifying SCF<sup>TIR1</sup> or associated proteins, but not Aux/IAA proteins.

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