

# Auxin signalling: the beginning, the middle and the end

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The plant hormone auxin is central to the regulation of growth and development. Recent work has demonstrated that auxin signalling depends on targeted protein degradation, and in the past year this model has been strengthened. The focus is now on identifying the targets of this degradative pathway, determining how auxin influences the degradative process and linking the turnover of specific proteins to the numerous downstream responses to auxin.

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### Abbreviations

ABP1	AUXIN BINDING PROTEIN 1
ARE	auxin response element
ARF	auxin response factor
PID	PINOID
PIN	PIN-FORMED
SCF	SKP1, Cullin/CDC53, F-box protein

### Introduction

The auxin signal is perceived by plant cells and rapidly transduced into a wide variety of responses in growth and development. These include changes in the direction of growth, changes in shoot and root branching and changes in vascular differentiation. Our current understanding of the events that connect auxin to these diverse responses is very patchy. To paraphrase a famous Monty Python sketch in which a scientist is coaxed into revealing her earth-shattering theory about the brontosaurus, our understanding of auxin signalling is very very thin at one end, very very fat in the middle, and very very thin at the other end. This is, of course, rather an exaggeration but, nonetheless, we appear to know a bit more about the middle section of the auxin signalling pathway than about what goes on at either end. This review discusses recent developments in understanding this central section and its connections upstream to auxin and downstream to auxin responses.

### Auxin and protein stability

Over the past few years, the molecular analysis of auxin response mutants of *Arabidopsis thaliana* has led to a model in which auxin signalling is mediated by regulated protein degradation. This work has been recently reviewed elsewhere ([1,2]; Figure 1). In brief, wild-type auxin sensitivity depends on the function of a protein ubiquitin ligase complex of the SCF type. SCF complexes are found throughout the eukaryotes and consist of a SKP1 homologue, a Cullin/CDC53 homologue, an F-box protein and an RBX1/ROC1 homologue. Combining data from a variety of systems indicates that the RBX1/ROC1 protein, which has a

RING-H2 finger domain (common among polyubiquitin synthesising proteins), acts together with Cullin/CDC53 to catalyse the synthesis of ubiquitin polymers [3]. Cullin/CDC53 interacts with RBX1/ROC1 through its carboxyl terminus [3]. The Cullin/CDC53 carboxyl terminus is also required for nuclear localisation and can be modified by the conjugation of a ubiquitin-like protein of the NEDD8/RUB1 family [4]. NEDD8/RUB1 conjugation cannot occur without nuclear localisation and appears to increase the activity of the SCF complex [4,5]. The Cullin/CDC53 amino terminus interacts with SKP1, which acts as a scaffold to bring together the dimer formed by RBX1/ROC1 and Cullin/CDC53 with the F-box protein [3]. F-box proteins form a diverse family defined by the amino-terminal F-box, which mediates its interaction with SKP1 [6,7]. The carboxyl terminus of F-box proteins consists of one of a variety of protein–protein interaction domains, and it is this domain that is responsible for the recruitment of specific substrates to the SCF complex where they are ubiquitinated and targeted for degradation by the 26S proteasome.

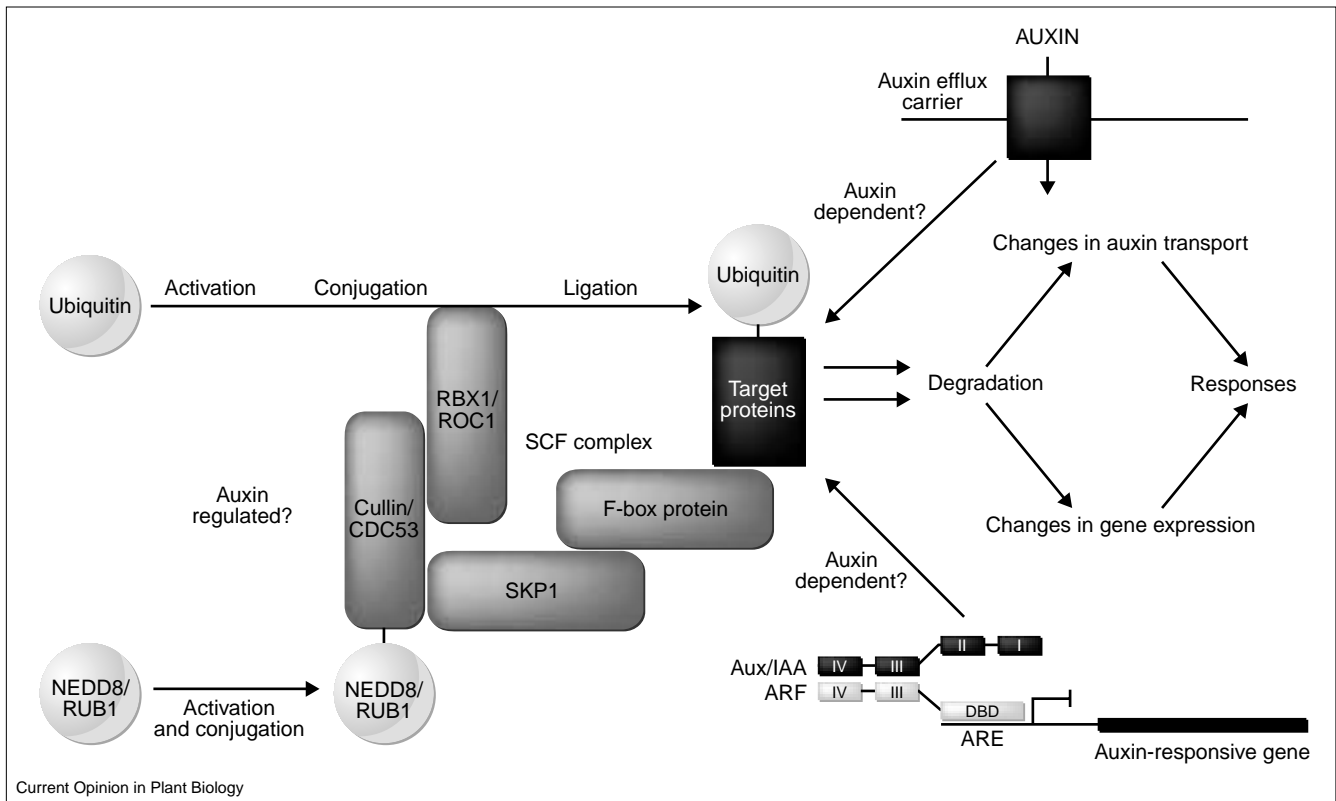
The F-box protein determines which proteins are degraded and, consequently, SCF complexes can be considered to be the hub of the degradative machinery. Three substrates feed into this complex: activated ubiquitin and activated NEDD8/RUB1 (via their respective activating enzymes and conjugating enzymes), and the target proteins destined for degradation (Figure 1).

*Arabidopsis* mutants have been recovered that are defective in several components of this system, and these share a suite of morphological phenotypes that reflect their reduced sensitivity to auxin. These mutants include *axr1*, which is defective in RUB1 activation [8,9], and *tir1*, which has a mutation in a gene encoding an F-box protein [10,11]. Neither of these mutations is likely to block the functioning of the auxin-related ubiquitination pathway completely. By analogy with other systems, blocking RUB1 conjugation is likely only to reduce the activity of SCF<sup>TIR1</sup> [12]; furthermore, there are several F-box proteins in the *Arabidopsis* genome that are similar to TIR1, and hence TIR1 could be partially functionally redundant [7]. This partial loss of SCF function in *axr1* and *tir1* mutants is supported by the observation that loss of AXR1 or TIR1 results in a similar phenotype, but the *axr1* phenotype is more severe than that conferred by *tir1*, whereas the double mutant combination has synergistic effects [10].

### Targets for the AXR1/TIR1 pathway

Mounting, although as yet unpublished, data suggest that targets for the AXR1/TIR1 degradative pathway might include the Aux/IAA proteins. Aux/IAA genes are found throughout the plant kingdom and were first identified because many of them are rapidly induced as a primary

Figure 1



SCF complex components (grey shading) and their probable involvement with potential target proteins that are conjugated to ubiquitin and degraded during auxin signalling. Degradation of auxin

efflux carriers would influence auxin distribution, and degradation of Aux/IAA proteins would influence the gene expression of auxin-responsive genes. DBD, DNA-binding domain.

response to auxin [13]. These genes can also be induced by cycloheximide, indicating that they are kept inactive by a rapidly turned-over repressor that is in some way inactivated by auxin [14]. Aux/IAA genes encode short-lived nuclear proteins that share four highly conserved domains [13,15]. Domains III and IV allow homodimerisation and heterodimerisation within the Aux/IAA family, and also between Aux/IAA proteins and members of the auxin response factor (ARF) family [16,17]. ARFs bind to auxin response elements (AREs) in the promoters of auxin-inducible genes through a carboxy-terminal DNA-binding domain, and this binding appears to be auxin-independent [17,18]. ARFs contain domains that are homologous to domains III and IV of the Aux/IAA proteins, allowing dimerisation within the ARF family as well as between ARF family and Aux/IAA family members [16]. The functional significance of ARFs in auxin signalling has been confirmed by the identification of mutations in ARF genes that confer auxin-related phenotypes such as reduced tropic growth and embryo patterning defects, as well by reduced expression of auxin-inducible genes including those encoding Aux/IAs [19,20\*].

ARFs regulate transcription as ARF-ARF dimers [17]. Overexpression of Aux/IAA proteins can block auxin-inducible transcription from an ARF-activated promoter [21].

This is most probably because dimerisation with an Aux/IAA protein prevents dimerisation with a second ARF (Figure 1). Although the transcription of *ARF* genes and the DNA binding of ARF proteins is not changed by auxin, the transcription of Aux/IAA proteins is. Therefore, Aux/IAA proteins appear to be central to the auxin response, mediating downstream auxin responses by regulating gene activity through interactions with ARFs. As the promoters of many Aux/IAA genes themselves contain ARF-binding sites, Aux/IAA proteins presumably feed back to regulate their own transcription. In this respect, it is interesting to note that a characteristic property of Aux/IAA proteins is their short half-lives, some of which are the shortest recorded for any protein being in the order of eight minutes [15,22\*\*]. This raises the possibility that Aux/IAA proteins are the short-lived repressors of their own transcription, as implicated by the cycloheximide experiments described above.

The instability of Aux/IAA proteins is required for normal auxin signalling. Semi-dominant mutations in the conserved domain II of several Aux/IAA family members have been recovered and found to increase the stability of the proteins and to confer a range of auxin-related phenotypes, including changes in the transcription of a range of

Aux/IAA genes [22••,23••,24–28]. These data support the hypothesis that Aux/IAA proteins regulate their own transcription. Dimerisation is also essential for Aux/IAA function because mutations in domain III that prevent dimerisation can suppress the effects of intragenic domain-II mutations [22••].

A second protein family that could include targets for auxin-regulated degradation is the PIN-FORMED (PIN) family of auxin efflux carriers ([29••]; Figure 1). These efflux carriers are responsible for the active and directional transport of auxin through plant tissues. The ability to pump auxin directionally in this way is required for tropic growth, regulation of root and shoot branching, and developmental patterning [30–34]. The distribution of one PIN family member, EIR1/PIN2/AGR1, has been shown to be regulated post-translationally [29••]. Accumulation of the protein is increased in the *axr1* mutant background and reduced in response to auxin addition. These data suggest a molecular mechanism for the long-recognised feedback between auxin signalling and auxin transport [35].

### The head and tail of the brontosaurus

These findings have led to a model in which auxin controls the abundance of the Aux/IAA transcriptional regulators, the auxin efflux carriers and quite possibly a range of other proteins (Figure 1). This is an attractive model because it provides an explanation as to how a single simple molecule can induce such diverse effects on different plant tissues. The specificity of auxin response is not in the auxin molecule itself but in the available targets for degradation, which are determined by cell type. The current model is, however, seriously deficient in two respects: the upstream inputs and the downstream outputs for this pathway are largely unknown.

We are at present entirely ignorant of how auxin influences the activity of the degradative machinery. There are two possibilities: either auxin could change the activity of the SCF complex or it could regulate the ability of target proteins to be recruited to it. In other SCF systems, recruitment of targets is dependent on their phosphorylation status [6]. It is, therefore, possible that auxin acts to regulate the phosphorylation of degradation targets. Certainly auxin-regulated kinase activities have been identified [36]; furthermore, genetic analysis has identified kinases and phosphatases that are required for normal auxin responses. These include the PINOID (PID) serine/threonine kinase, loss of which results in altered auxin responses [37•], and the RCN1 PP2A-type phosphatase subunit that is required for normal auxin-regulated elongation responses [38]. The phosphorylation status of SCF targets also provides a possible point for the integration of signals. Interestingly, it has recently been shown that Aux/IAA proteins can be phosphorylated by the photoreceptor phytochrome A [24]. One way in which this phosphorylation could influence Aux/IAA activity is through changes in protein stability.

Further upstream, the site of auxin perception is still a matter of considerable debate. The best candidate auxin receptor is AUXIN BINDING PROTEIN 1 (ABP1). ABP1 was first identified because of its ability to bind auxin specifically and with physiologically relevant affinity (reviewed in [39]). Most ABP1 is retained in the endoplasmic reticulum but a small amount appears to escape and act at the cell surface [40]. There is now good evidence that ABP1 mediates downstream auxin responses. Expression of *ABP1* in transgenic cell lines and plants can alter auxin sensitivity in a variety of responses, including cell expansion [41] and guard-cell potassium currents [42].

More recently, an insertion in the *Arabidopsis ABP1* gene has been identified, allowing analysis of the null mutant phenotype for the first time [43•]. Plants that are homozygous for this insertion die in the early globular phase of embryogenesis. This phenotype certainly demonstrates that *ABP1* is an essential gene but further analysis will be required to determine whether binding of auxin to ABP1 is linked to the proteolytic pathway, the Aux/IAA proteins or the auxin efflux carriers.

There is considerable evidence for multiple sites of auxin perception and there is no reason to suppose that ABP1 is the only auxin receptor. Additional possible receptors include the auxin efflux carriers themselves. In this context, it is interesting to note that glucose perception in yeast is mediated by a receptor with homology to glucose transporters via an SCF-dependent proteolytic pathway [44].

Events downstream of the potential SCF<sup>TIR1</sup> targets are also unclear. Changes in the stability of various Aux/IAA proteins result in a gene-specific pattern of morphological phenotypes, such as changes in root and shoot branching, cell elongation defects, light responses and defects in tropic growth [25–28]. How these phenotypes arise is unclear. There is good evidence that Aux/IAA proteins regulate their own transcription but additional genes in this regulatory loop are comparatively uncharacterised. This reflects a widespread problem in developmental biology in which key transcriptional regulators are shown to regulate their own transcription but further targets are harder to find. Additional auxin-responsive genes have been identified and many have AREs in their promoters, implicating ARFs and Aux/IAAs in their regulation. The functional importance of some of these genes in mediating auxin responses is now beginning to be established. For example, the *DFL1* gene of *Arabidopsis* influences auxin sensitivity and stem elongation. *DFL1* is encoded by a member of the *GH3* family of genes and, characteristically for this family, it is auxin-inducible and has AREs in its promoter [45]; however, the biochemical function of GH3s is not clear.

More direct links have been forged between elongation and the activity of the expansin family of cell wall proteins, which catalyse cell wall loosening [46]. Links between auxin and expansin expression are being uncovered in a

variety of systems. For example, addition of auxin and of expansin protein to shoot apical meristems stimulates leaf outgrowth [47,48\*]. *In situ* hybridisation shows that expansin transcripts accumulate at the presumptive sites of leaf initiation and these expansins are auxin-inducible [49,50]; however, it is likely that auxin can also regulate expansin activity at the post-transcriptional level through its effects on cell wall pH [46].

## Conclusions

Progress in understanding auxin signalling has been startling in recent years, resulting in a working model for its mode of action. Furthermore, important advances in understanding the very earliest steps in auxin perception have been made, providing tools to characterise the roles of ABP1 and PIN family members in auxin perception. Linking these pathways to the diversity of auxin responses is now a key challenge. A satisfying development here is the convergence of scientists working on auxin signalling with those working on particular aspects of plant biology in which auxin plays a role, resulting in a gradual linking of previously disparate parts of the puzzle. I hope it will not be long before our fragmented picture will be as dead as a dinosaur.

## Update

Recent work has demonstrated that the COP9 signalosome interacts with SCF<sup>TIR1</sup> and is required for efficient auxin signalling [51\*\*]. Its role appears to be in RUB1 deconjugation, suggesting that conjugation–deconjugation cycles may be important in SCF function.

## Acknowledgement

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