Auxin enters the matrix — assembly of response machineries for specific outputs
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The basic mechanism of auxin as a modulator of gene expression is now well understood. Interactions among three components are required for this process. Auxin is first perceived by its receptor, which then promotes degradation of inhibitors of auxin response transcription factors. These in turn are released from inhibition and modify expression of target genes. How this simple signaling pathway is able to regulate a diverse range of auxin responses is not as well understood, however a clue lies in the existence of large gene families for all components. Recent data indicates that diversification of gene expression patterns, protein activity, and protein–protein interactions among components establishes a matrix of response machineries that generates specific outputs from the generic auxin signal.

Introduction
The plant hormone auxin plays an important regulatory role in virtually every aspect of plant growth and development. At the core of the auxin response is the auxin receptor that interacts with and promotes the degradation of one of the two antagonistically acting transcription factors, Global analyses of gene expression following auxin action (Figure 1a). Auxin is now commonly referred to as ‘molecular glue’ as it enhances interactions between an auxin receptor and a family of transcriptional inhibitors known as Auxin/Indole-3-Acetic Acids (Aux/IAAs) [4**]. The TRANSPORT INHIBITOR RESISTANT1/AUXIN F-BOX (TIR1/AFB) auxin receptor is a subunit of the SKP1–CULLIN1–F-BOX (SCF) TIR1/AFB ubiquitin ligase complex and binding of Aux/IAAs triggers their ubiquitin-mediated degradation [5,6]. Aux/IAAs themselves bind and inhibit AUXIN RESPONSE FACTORS (ARFs) that are the DNA-binding transcription factors capable of directing the expression levels of auxin-responsive genes. As the auxin concentration in a cell directly determines the Aux/IAA protein abundance and resulting ARF activity, the pathway from auxin to changes in transcription is quite short.

Auxin controls inherently different cellular responses, including cell expansion and division, as well as changes in the developmental fate of the cell. Particularly in the latter case, auxin can promote different cell-fate specification events, depending on where the cell happens to be. For example, lateral root initiation is activated in peripheral cells [7], while root formation is promoted in basal embryo cells [8], and cotyledon initiation is triggered in apical embryo cells [9]. Yet, all these responses are disrupted in tir1/afb auxin receptor mutants [10]. Hence a major outstanding question is how specificity is generated in such a brief, simple pathway. Here we review recent literature that sheds light on how specific gene expression responses are elicited by auxin. Even though the pathway is brief, each of the three major components (TIR/AFB receptor, Aux/IAA, and ARF) is represented by a sizable gene family (Figure 1b,c). Specificity can therefore be generated by the regulation of gene expression patterns, variations in component activity, and in interactions between components. We will consider each of these in the following sections.

Auxin prepatterns — regulation of component gene expression
The TIR1/AFB auxin receptors form a small subclade of six genes (TIR1 and AFB1–5) in the Arabidopsis thaliana (Arabidopsis) F-box gene family. Of these, four (TIR1 and AFB1–3) have been shown to act as auxin receptors [5,6,10], and do so redundantly in planta [10]. There is limited diversification of the gene expression patterns and it is therefore likely that the receptor component is rather generic [10]. Despite this, the level of receptors still offers a node for globally modulating auxin sensitivity. For example, microRNA393 is induced by bacterial infection, including cell expansion and division, as well as changes in the developmental fate of the cell. Particularly in the latter case, auxin can promote different cell-fate specification events, depending on where the cell happens to be. For example, lateral root initiation is activated in peripheral cells [7], while root formation is promoted in basal embryo cells [8], and cotyledon initiation is triggered in apical embryo cells [9]. Yet, all these responses are disrupted in tir1/afb auxin receptor mutants [10]. Hence a major outstanding question is how specificity is generated in such a brief, simple pathway. Here we review recent literature that sheds light on how specific gene expression responses are elicited by auxin. Even though the pathway is brief, each of the three major components (TIR/AFB receptor, Aux/IAA, and ARF) is represented by a sizable gene family (Figure 1b,c). Specificity can therefore be generated by the regulation of gene expression patterns, variations in component activity, and in interactions between components. We will consider each of these in the following sections.

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and negatively regulates receptor abundance and hence auxin signaling, contributing to antibacterial resistance [11]. Conversely, limiting concentrations of inorganic phosphate induce TIR1 gene expression and enhance Aux/IAA degradation, releasing ARF19 and ultimately modifying the expression of genes involved in lateral root initiation [12].

With 29 and 23 respective members in Arabidopsis, the Aux/IAA and ARF components offer by far the most opportunities to generate diversity, provided that the different proteins encoded by the gene families have unique or at least quantitatively distinct functions (discussed below). No systematic analysis of gene expression patterns has been reported for either of these families in Arabidopsis, but a relatively comprehensive survey of rice Aux/IAA and ARF expression at tissue resolution suggests fairly diverse patterns of expression for both families [13]. This in turn means that different sets of Aux/IAAs and ARFs are to be expected in different tissues. Such tissue-specificity is supported by the analysis of aux/iaa gain-of-function, and arf knockout mutants in Arabidopsis. Mutations in Aux/IAA genes stabilize the protein by preventing interactions with the receptor [5,6]. Several such mutants were recovered in genetic screens and show very diverse phenotypes [14]. For example, the bodenlos (bdl)/iaa12 mutation interferes with primary root formation [15], while the solitary root (slr)/iaa14 mutation disrupts lateral root formation [16], and the short hypocotyl2 (shy2)/iaa3 mutation interferes

Auxin-dependent gene regulation and annotation of transcription components involved. (a) Auxin enhances the interaction between the SCF(TIR1/AFB) E3 ubiquitin ligase and domain II of Aux/IAA proteins, making Aux/IAA levels responsive to auxin concentration inside the cell. Aux/IAAs function to inhibit ARFs, DNA-binding transcription factors that directly modify the transcription of auxin-responsive genes. Hence, an increase in auxin concentration will release ARFs from Aux/IAA inhibition. (b) Phylogenetic relationship and annotation of the 29 Arabidopsis Aux/IAA proteins. The first two columns indicate amino acids found at positions 2 and 4 of the LxLxL motif in domain I (x1 and x2 respectively). R = arginine, C = cysteine, T = threonine, E = glutamic acid, D = aspartic acid, G = glycine, S = serine, K = lysine, A = alanine, empty boxes = no motif present. Basic amino acids are highlighted blue, cysteine yellow, acidic amino acids red, neutral amino acids gray, and polar amino acids green. A presence/absence score (green/white) for the domain II degron is shown in the third column (II). (c) Phylogenetic relationship and annotation of the 23 Arabidopsis ARF proteins. The percentage of glutamine (%Q) in the middle region (defined as the region between the last conserved amino acid in the DNA-binding domain and the first conserved position of domains III/IV) is depicted in the first column (see color legend) followed by a presence/absence score (green/white) for domains III/IV, an LxLxL motif, and the R/K-LFG-V/I/F motif. *No middle region. Note that we scored for R/K-LFG-V/I/F, where the last amino acid is hydrophobic, rather than the more stringent R/K-LFG-V [39].
with hypocotyl elongation [17]. In these cases, the differences between mutant alleles correspond to different expression patterns of the Aux/IAA genes.

Likewise, even though most arf single mutants do not have a phenotype [2], those that do are quite distinct (e.g. monopteros (mp)/arf5 [18]; arf8 [19]; arf2 [20]). In each case, this is also accompanied by differing expression patterns. Patterns of ARF accumulation are further elaborated through post-transcriptional mechanisms. It was recently shown that a trans-acting small interfering RNA (siRNA) that specifically directs ARF2–4 mRNAs acts to limit ARF3 activity to the abaxial side of the leaf [21].

Several other ARF transcripts (ARF6, 8, 10, 16, and 17) are targets of microRNA regulation [22,23].

The cellular complexity of auxin response transcription factors can now only be inferred from various sources of data; appreciation of the complete picture awaits systematic gene expression analysis. Nevertheless, available evidence suggests that diversification of Aux/IAAs and ARFs gene expression patterns contributes significantly to generating specificity in auxin-dependent gene regulation.

Are all Aux/IAAs created equal?

Both Aux/IAA and ARF protein families share conserved domains. Particularly for the Aux/IAAs, these conserved domains constitute a large part of the proteins, leaving less opportunity for qualitative diversification. Three functions have been assigned to conserved Aux/IAA domains. Domain I is necessary and sufficient for transcriptional repression [24], and appears to be important in vivo since mutations in domain I of axr3/iaa17 were recovered as suppressors of the gain-of-function axr3 phenotype [25]. Recently, it was shown that domain I of a number of Aux/IAAs recruits the TOPLESS (TPL) corepressor. Closer investigation revealed that TPL binds the repression motif (LxLxL) in BDL/IAA12 and is required for BDL/IAA12 to function in hypophysis specification during embryogenesis [26].

This work suggests that the severe tpl-1 phenotype, in which the shoot pole is replaced by a second root pole, is the result of effectively removing all Aux/IAAs and that Aux/IAAs function through recruiting TPL. Conserved domain II functions as a ‘degron’ and confers auxin-dependent instability upon the protein by mediating interactions with TIR1/AFB receptors [4]. Finally, Aux/IAAs have a C-terminal region called domains III/IV that serve as an interaction domain. These domains mediate homotypic and heterotypic interactions between Aux/IAAs and ARFs.

Interestingly, there are reported cases where expression patterns alone do not account for differences in Aux/IAA function. For example when the stabilized versions of iaa7 or iaa14 are driven from the IAA19 promoter they not only confer the same phenotypes as seen in the iaa19 mutant but also result in additional, completely novel developmental phenotypes [27]. Conversely, when iaa12 is expressed from the IAA3 promoter, this protein confers only part of the phenotypes that are conferred by iaa3 [28]. This shows that there must be intrinsic differences between Aux/IAA proteins. These could cause different affinities for TPL, difference in (residual) degradation rate or different affinities for other Aux/IAAs or ARFs, but it remains to be addressed which of these factors contribute to Aux/IAA diversification. At least in principle, each could contribute: variations at the two ‘x’ positions in the TPL-interaction motif (LxLxL) [26]; Figure 1b) could certainly cause differences in affinity; the half-lives of Aux/IAAs are 8–80 min (reviewed in [29]); and there are many nonconserved positions in domains III/IV.

Strikingly, Aux/IAAs exist that lack one of the conserved domains ([30]; Figure 1b), and therefore cannot act in a canonical auxin-signaling pathway. The function of these is currently unknown, but noncanonical Aux/IAs also exist in other genomes, including rice and poplar [31,32]. The role of a subclade of three Aux/IAs , IAA20, IAA30, and IAA31, which do not contain the domain II degron has been investigated recently. As expected overexpression of these Aux/IAs leads to auxin-related phenotypes [33], which suggests that when overexpressed and/or misexpressed these aberrant Aux/IAs interfere with endogenous ARF-Aux/IAA interactions. One can as yet only speculate on the biological relevance of these permutations and what role if any they play in auxin-mediated processes. With regard to nondegradable versions of Aux/IAs this may be an additional means of tempering auxin responses by setting a threshold for the amount of auxin required for ARF activation.

Output control — ARF divergence and activity

Being larger than the Aux/IAs and having only two conserved domains that make up less than half of the protein, the ARFs are inherently more diverse. ARFs have a B3-type DNA-binding domain at their N-terminus that binds TGTCn(C) Auxin Response Elements (AuxRE) in vitro [34]. To date, only direct target genes have been identified for ARF7 and 19 [2,35]. A completely open question is whether ARFs have overlapping sets of target genes. At least to some extent this must be the case, since closely related ARFs have redundant functions that can only be uncovered in double mutants, for example [32,36]. Also, replacing ARF5/MP by the distantly related ARF16 partially complements the mp mutant defect, implying that ARF16 can bind to (some) MP targets [28]. With the identification of more ARF target genes, for example [37], we will be able to determine how different/similar ARF DNA-binding domains really are.
At their C-terminus most ARFs also have domains III/IV (Figure 1c) and these domains mediate ARF–ARF and ARF–Aux/IAA interactions. It has been proposed that ARF–ARF dimerization increases DNA-binding affinity, and as a result enhances the amplitude of auxin-dependent gene regulation [34]. This not only implicates combinatorial possibilities by interactions among coexpressed ARFs, but also suggests that Aux/IAAs might inhibit ARFs by interfering with ARF–ARF interactions (Figure 2), a model that has not received much attention so far.

The region between the conserved N-terminal and C-terminal domains has been termed ‘middle region’ and is extremely divergent. It is this region that determines the activity of the ARF. In protoplast assays, those ARFs that have a relatively glutamine-rich middle region (ARF5–8 and 19; Figure 1c) were able to activate synthetic auxin promoters resulting in their classification as activators. Correspondingly, five ARFs with less pronounced Q-enrichment in their middle regions (ARF1–4 and 9) repressed transcription [38]. On the basis of amino acid composition the remaining ARFs were somewhat arbitrarily classified as repressors despite a lack of experimental evidence. Clearly there is diversification in the middle regions of ARFs, and since these define the activity of the transcription factor, this is where the auxin-signaling output is controlled. However, there are a few difficulties with the simplified classification of ARFs as activators or repressors. Global transcriptome profiling of arf7 arf19 double mutants showed that auxin-induced changes in expression were lost for most auxin-activated (85% of 203) and auxin-repressed genes (65% of 68). This suggests that ARF7 and ARF19 have the capacity to act as both activators and repressors [2]. In fact, in addition to eight other ARFs, ARF19 contains an LxLxL motif (Figure 1c), that would potentially enable TPL recruitment. This suggests that the convenient binary

Working models for auxin-induced changes in transcription. Different models apply for auxin-activated (a,b) or auxin-repressed (c,d) transcription. (a) Under low auxin concentrations, Aux/IAAs are abundant, bind to activator ARFs (note: green middle region) through shared domains III/IV and recruit corepressor TPL through domain I, hereby repressing the transcription of a subset of auxin-responsive genes that contain AuxRE sequences in their promoters. (b) When auxin concentrations increase, Aux/IAAs are rapidly degraded and ARFs are free to activate transcription either as monomers (1) or dimers (2). Release of inhibition could also allow the formation of higher order complexes with other transcription factors including MYB77 (3). (c) Under low auxin concentrations Aux/IAAs with reduced or no affinity for TPL also inhibit ARFs with repressor activity (note: red middle region), allowing a subset of genes to be activated by other, auxin-independent transcription factors (TF). (d) Auxin-mediated degradation of Aux/IAAs enables repressor ARFs to dimerize (1) and/or complex with unknown corepressors (2; possibly TPL), ultimately repressing the transcription of these auxin-responsive genes.

Figure 2

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classification of ARFs as either activator or repressor is oversimplified. Recently another repression motif (R/K-LFGV) was identified in several transcriptional repressors [39] and also in several ARFs. Upon closer inspection of the ARF family we found that R/K-LFGV-V/I/F (where the last valine is replaced by another hydrophobic amino acid) was present in all ARFs with the exception of ARF5–8, 10, 16, 17, and 19 (Figure 1c). This motif might enable a more robust classification and might also be a recognition motif for a corepressor, potentially the SEUSS/LEUNIG complex [40]. It is notable that both repression motifs identified so far are only five amino acids long. Depending on the location in the sequence and the tertiary and quaternary structure of the ARF, one could expect that these motifs can be hidden or exposed, which could explain how ARFs could act as both activators and repressors.

Given the size and heterogeneity of ARFs, there is ample opportunity for the diversification of ARF regulation by post-translational modification as well as by selective interactions with other proteins (other than Aux/IAAs and ARFs). Hardly anything is known about either of these phenomena. However, database searches reveal several consensus sites for kinases and predicted SMALL UBIQUITIN-RELATED MODIFIER (SUMO)-ylation motifs in ARFs and it has recently been shown that ARF2 is a substrate of the BRASSINOSTEROID INSENSITIVE2 (BIN2) kinase [41]. By phosphorylating ARF2, BIN2 inhibits both DNA-binding and repression activity. Also the transcription factor MYB77 was found to interact in vitro with a number of ARFs via their C-terminal domain and was subsequently confirmed to bind ARF7 in planta. The promoter of IAA19 whose expression is downregulated in arf7/npk4-1 and myb77-1, contains MYB factor-binding motifs and multiple AuxREs in proximity suggesting that both proteins bind DNA [42].

Synthesis — updated models of auxin-dependent transcription

In regard to the three major components in auxin-dependent transcription, most heterogeneity and hence specificity is among the Aux/IAA and ARF transcriptional regulators. Here, at the business end of auxin signaling, one could envisage a highly dynamic interplay of Aux/IAAs, the degradation machinery, TPL and other corepressors, ARFs, modifying enzymes, transcriptional cofactors, and the target sites in the DNA. It will be challenging to synthesize a model that encapsulates all these components, and mathematical modeling might be required to grasp the dynamic behavior of this system. Nonetheless, even though few core components are involved in the brief auxin-signaling pathway, sufficient divergence must exist to accommodate all different auxin responses seen throughout plant life.

Two issues emerge from this discussion. First, it appears that auxin response is conditioned by a prepattern of Aux/IAA and ARF genes, which considering the intrinsic differences between family members will lead to at least quantitatively distinct auxin responses between different cells. Second, we lack models that explain how auxin can promote repression of gene expression. The TPL-based corepression that is brought upon ARFs when in complex with an Aux/IAA predicts that genes will be actively repressed in the absence of auxin but fails to explain how an Aux/IAA–ARF based system could operate in auxin-induced gene repression. As mentioned, auxin-dependent repression of a number of genes is lost in the arf7 arf19 mutant; however, from this it cannot be deduced that auxin-dependent repression involves Aux/IAA proteins. We investigated publicly available microarray datasets [3] for example of Aux/IAA-dependent down-regulation of auxin-responsive genes and found a subset of genes whose repression is lost upon stabilization of IAA7 or IAA17. It is therefore likely that an Aux/IAA–ARF module operates in transcriptional repression. The most likely scenario to explain auxin-dependent repression through Aux/IAAs would involve Aux/IAAs interfering with repressor ARF dimerization or higher order complex formation (Figure 2). In any event in vivo studies of ARF–ARF and Aux/IAA–ARF interactions, as well as the identification of physiological targets of a number of ARFs, should help clarify this issue.

Conclusions and perspectives

Auxin is a structurally simple molecule, yet it elicits many different responses in plants. The signal transduction pathway has three major components, a ubiquitin ligase/receptor, ARF transcription factors, and their Aux/IAA inhibitors. Here we have reviewed how specificity in the output of auxin signaling can be generated by distinct regulation and the unique properties of the members of the Aux/IAA and ARF transcription factor families. Even though we are only scratching the surface of the potential complexity encoded within these families, substantial specialization is already apparent. Multiple layers of regulation include gene expression patterns, post-translational protein modifications, and protein–protein interactions, and all contribute to the generation of specificity in auxin response. It is evident that further dissection requires the isolation of physiological target genes whose function is required for the auxin-controlled processes. These, rather than synthetic promoters, should serve as biological models in which the activity and unique properties of Aux/IAAs, ARFs, and their domains can be rigorously tested.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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