

# Roles and activities of Aux/IAA proteins in *Arabidopsis*

Jason W. Reed

Auxin induces various distinct developmental responses, partly by regulating gene expression. The *Aux/IAA* genes are a large gene family, many of which are induced by auxin. Work on *Arabidopsis Aux/IAA* genes has begun to reveal that they can regulate development and auxin-induced gene expression. Furthermore, auxin responses require *Aux/IAA* protein turnover. Finally, recent evidence suggests that *Aux/IAA* proteins can mediate light responses. Work in the near future should test whether *Aux/IAA* proteins are antennae that connect auxin and light signals to endogenous developmental responses.

Auxins regulate diverse cellular and developmental responses in plants, including cell division, expansion and differentiation, patterning of embryos, vasculature and other tissues, and distribution of growth between primary and lateral root and shoot meristems. This multiplicity of regulatory activities has spurred considerable interest in mechanisms of auxin signaling and response. Auxin might regulate some cellular responses (such as expansion or polarization) through direct effects on membrane or cytoskeletal functions, but it also regulates the expression of many genes whose products probably carry out most developmental responses.

Proteins of two related families called *Aux/IAA* and ARF regulate auxin-induced gene expression. The *Aux/IAA* genes encoding the *Aux/IAA* proteins are themselves regulated by auxin, and were discovered and named for this property. In this article, I discuss recent results that reveal developmental functions of *Aux/IAA* proteins, discuss how auxin and/or other signals might regulate their activity, and highlight some of the questions that might be answered in the near future\*.

## What is an *Aux/IAA* gene?

*Aux/IAA* genes were first isolated as members of a family of genes that were rapidly induced in response to auxin (indole-3-acetic acid, IAA). Additional *Aux/IAA* genes were subsequently found based on sequence similarity to known *Aux/IAA* genes<sup>1</sup> or in yeast two-hybrid assays with *Aux/IAA* proteins<sup>2</sup>. *Aux/IAA* genes have been found in dicots (pea, soybean, *Medicago truncatula*, *Arabidopsis*, tomato, tobacco and cotton), grasses (maize, rice) and pine trees. They have not been found in bacterial, animal or fungal genomes and are therefore probably unique to plants.

\*Additional details about these proteins and other aspects of auxin metabolism and signaling will appear this year in special review issues of *Plant Molecular Biology* and *Journal of Plant Growth Regulation*.

Canonical *Aux/IAA* proteins share four conserved amino acid sequence motifs called domains I, II, III and IV (Fig. 1), although several predicted proteins lacking one or more of these domains are also included in the family. *Aux/IAA* proteins also have nuclear-localization sequences and representative *Aux/IAA* protein fusions localize to the nucleus<sup>3</sup>. Because domains I–IV are conserved in multiple *Aux/IAA* proteins, they presumably have important structural or regulatory functions. Domain I is the smallest and least strictly conserved of these, and it has not been ascribed a specific biochemical activity. Domain II is highly conserved and mutations in this domain increase activity of the corresponding proteins by stabilizing them. Domain II therefore destabilizes *Aux/IAA* proteins.

Yeast two-hybrid assays have shown that domains III and IV can mediate homo- and heterodimerization between *Aux/IAA* proteins and heterodimerization between *Aux/IAA* proteins and ARF proteins (which share these domains). Domain III mutant versions of AXR3/IAA17 do not dimerize, indicating that domain III is required for dimerization<sup>4</sup>. Domain III has sequence similarity to the ArcA bacteriophage repressor family, which folds into a  $\beta\alpha\alpha$  structure, and a synthetic peptide containing domain III (but not domain IV) can fold and dimerize *in vitro*<sup>4,5</sup>. Thus, domain III probably forms a true domain in the protein structural sense and might be sufficient by itself for dimerization. Domain IV might also contribute to dimerization, and it has a functional nuclear localization signal.

The divergent amino acids between these conserved domains might simply be 'filler' loops that enable the conserved domains to adopt the correct tertiary structure, or they might mediate interactions with other proteins, thereby conferring functional specificity on different members of the family. Such functions could include specific regulatory inputs from different endogenous or environmental signals, or different interactions with transcription factors. If the divergent interdomain loops indeed confer specific functional attributes on particular *Aux/IAA* proteins then *Aux/IAA* proteins that lack such loops might have more limited and general functions than those with large loops.

Additional functional specificity could arise from differential gene expression. *Aux/IAA* genes are expressed in tissue-specific patterns, and auxin induces different *Aux/IAA* genes to varying degrees

Jason W. Reed  
Dept Biology, University  
of North Carolina at  
Chapel Hill, Chapel Hill,  
NC 27599-3280, USA.  
e-mail:  
jreed@email.unc.edu

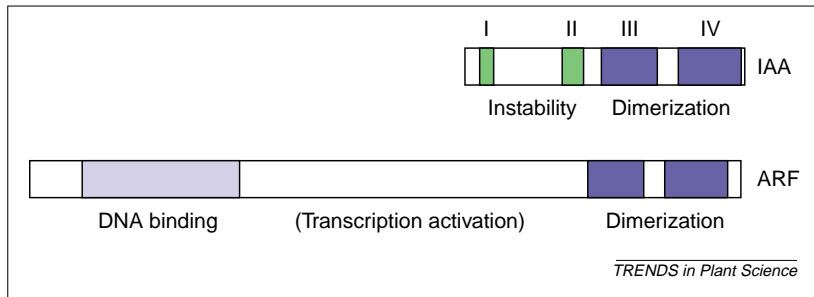


Fig. 1. Structure of Aux/IAA and ARF proteins. Conserved domains present in most Aux/IAA or ARF proteins are indicated. Domain I of Aux/IAA proteins is about nine amino acids long, domain II is ~15 amino acids long, domain III is ~40 amino acids long and domain IV is ~45 amino acids long. Sequences between the conserved domains vary in size, so that the conserved domains form between a third and two-thirds of each protein. ARF domains III and IV are similar to the corresponding domains of Aux/IAA proteins. The middle region of some, but not all, ARF proteins can activate transcription<sup>13</sup>. Some Aux/IAA and ARF proteins lack one or more of the conserved domains. For example, IAA20 lacks domain II and ARF3/ETTIN lacks domains III and IV.

and with different kinetics<sup>1</sup>. Auxin does not induce, and can even repress, other *Aux/IAA* genes<sup>6</sup>. These differences in auxin induction kinetics suggest that some *Aux/IAA* genes are part of early responses, whereas others might be secondary auxin response genes.

The completion of the *Arabidopsis* genome sequence has provided the most complete information on the *Aux/IAA* gene family for this species. *Arabidopsis* has 25 deduced genes encoding Aux/IAA proteins that have all four of the conserved domains that define the family. In addition, at least four predicted sequences bear similarity to portions of Aux/IAA proteins but lack one or two of the conserved domains. Some of the deduced sequences might be pseudogenes, because no information about expression is available for several of them. *Arabidopsis Aux/IAA* genes have been given IAA designations (such as IAA1 and IAA2) and several of them have compound names (e.g. *AXR3/IAA17*) that reflect the phenotypes of mutants in the corresponding genes. *Arabidopsis Aux/IAA* and ARF gene Accession numbers, genomic locations and protein sequence phylogenies have been compiled in two recent reviews<sup>7,8</sup>.

#### Aux/IAA proteins interact with ARF proteins

A related class of 23 *Arabidopsis* genes called ARFs encode auxin response factors<sup>9,10</sup>. Typical ARF proteins have C-terminal domains homologous to domains III and IV of Aux/IAA proteins (Fig. 1). Domains III and IV enable ARF proteins to interact with Aux/IAA proteins in yeast two-hybrid assays<sup>2,11</sup>. ARF proteins also have a highly conserved N-terminal DNA-binding domain that binds to auxin-response elements in promoters of auxin-regulated genes, and a divergent middle region that can activate transcription in some ARFs but has unknown function in others<sup>12,13</sup>. Taken together, these results suggest that Aux/IAA proteins regulate gene expression by interacting with ARF proteins to alter their activity (Fig. 2).

In light of this model, functions of ARF proteins are likely to provide insight into functions of Aux/IAA proteins that might interact with them. Mutations in genes encoding three ARF proteins indicate that they mediate auxin responses. The *ettin/arf3* mutations affect floral patterning<sup>14</sup>, *monopteros/arf5* mutations affect formation of vasculature and embryonic phyllotaxy<sup>15,16</sup>, and

*msg1/nph4/arf7* mutations decrease auxin sensitivity in the hypocotyl and leaf, and cause defective tropic responses and auxin-regulated gene expression<sup>17–20</sup>. All three of these developmental phenotypes reflect processes that depend on spatial gradients of auxin concentration<sup>21–25</sup>, suggesting that these three ARF proteins might control cell fate decisions in response to local auxin concentrations. ETTIN/ARF3 probably does not interact physically with Aux/IAA proteins because it lacks domains III and IV. However, competition among ARFs for auxin-binding promoter elements might provide an indirect means for interactions with Aux/IAA proteins.

#### Developmental functions of Aux/IAA genes

Mutations in *Aux/IAA* genes have been detected in several distinct screens for plants with altered auxin response or morphology (Table 1), and characterizations of these mutants have begun to reveal developmental roles of the corresponding genes. Each of these mutations changes an amino acid in domain II (Table 1), and genetic and molecular studies indicate that they cause gain of function. For each mutation, some or all of the mutant phenotypes are dominant or semidominant, and the domain II mutant alleles *axr2-1*, *axr3-1*, *shy2-2*, *iaa28-1*, *slr-1* and *bdl-1* can each recapitulate mutant phenotypes when introduced into wild-type plants<sup>6,26,27</sup> (P. Nagpal and J.W. Reed, unpublished; H. Fukaki and M. Tasaka, pers. commun.; T. Hamann and G. Jürgens, pers. commun.). The wild-type *AXR3/IAA17* gene also confers *axr3-1*-like phenotypes when overexpressed<sup>27</sup>, although this is not true for wild-type *IAA28* or *SHY2/IAA3* genes<sup>6</sup> (Q. Tian and J.W. Reed, unpublished). Moreover, intragenic mutations with molecular characteristics of loss-of-function mutations suppress the phenotypes of the gain-of-function *axr2-1*, *axr3-1* and *shy2-2* mutations<sup>26,28–30</sup>. Several of these intragenic suppressors affect single amino acids in domains I, III or IV, confirming the importance of these domains for activity of the gain-of-function proteins.

These domain II mutations in different *Aux/IAA* genes affect various tissues and developmental responses, including root or shoot gravitropism, lateral root formation, shoot apical dominance, stem elongation, leaf expansion and leaf formation in the dark (Table 1). In general, the phenotypes can be explained in terms of reduced auxin response, and some auxin-induced genes are expressed at lower levels in *axr2-1* and *shy2-2* mutants<sup>1</sup> (Q. Tian and J.W. Reed, unpublished). However, *axr3-1* plants have some phenotypes consistent with hypersensitivity to auxin, such as ectopic expression of an auxin-regulated gene and formation of adventitious roots<sup>31</sup>. Therefore, Aux/IAA proteins might both activate and inhibit auxin responses, depending on the particular Aux/IAA protein and/or the tissue.

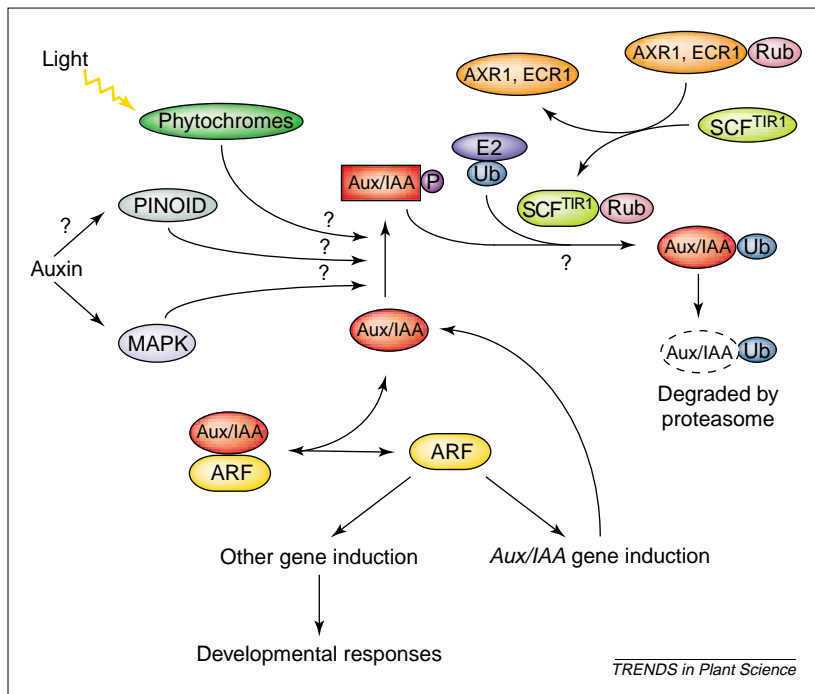


Fig. 2. Working model indicating possible input and output regulatory interactions of Aux/IAA proteins. The model is based on correlative results and is therefore probably incorrect in some particulars. Question marks indicate putative regulatory connections that would be worth testing biochemically. Putative auxin- or light-regulated phosphorylation events, ubiquitin-mediated turnover of Aux/IAA proteins and regulatory outputs acting through ARF proteins are indicated. Rub- and ubiquitin-modification pathways are indicated in abbreviated form<sup>33</sup>. A putative Aux/IAA negative feedback loop is indicated as acting through Aux/IAA inhibition of an activating ARF. Abbreviations: E2, ubiquitin-conjugating enzyme; IAA, indole-3-acetic acid (auxin); MAPK, MAP kinase cascade; P, phosphate group; Ub, ubiquitin.

Taken together, the gain-of-function mutant phenotypes suggest that Aux/IAA proteins can regulate many of the developmental processes governed by auxin. However, as guides to the normal functions of these genes, the phenotypes should be regarded with some caution because they might arise from levels of Aux/IAA protein activity that are higher than any reached in the normal course of development. Ideally, one should compare these results with phenotypes of loss-of-function mutations in the same genes. Unfortunately, the putative *Aux/IAA* gene null mutants that have been characterized have subtle phenotypes<sup>26,28,29</sup>, probably because of redundant activities or feedback regulatory loops that enable the mutant plants to compensate for absence of a particular Aux/IAA protein. Given the number of Aux/IAA proteins, the possible promiscuity of their dimerization interactions and the likelihood of feedback regulation, it will take considerable effort to sort out the precise functions of each one.

#### Does auxin regulate Aux/IAA protein activity?

Notwithstanding these interpretational uncertainties, the genetic results do suggest that Aux/IAA proteins regulate gene expression responses to auxin, and studies of Aux/IAA protein biochemistry have begun to suggest that they might be targets of

auxin and possibly other signals. Aux/IAA proteins have half-lives as short as 6–8 min, and the domain II mutations probably increase protein function by stabilizing the corresponding protein<sup>3,4,27</sup>. The *axr3-1* and *shy2-2* mutant plants have higher steady-state levels of the corresponding proteins than do wild-type plants, and the *axr3-1* mutation increased the half-life of AXR3/IAA17 (Refs 4,32). Similarly, fusion proteins with Ps-IAA6 or AXR3/IAA17 are degraded rapidly in plant cells, and mutations in domain II decrease this degradation<sup>27</sup> (S. Kepinski and O. Leyser, pers. commun.).

These findings might explain why several mutations that affect protein turnover reduce auxin responses<sup>33</sup>. For example, mutations in components of the SCF<sup>TIR1</sup> ubiquitin ligase complex cause auxin-resistant root growth<sup>34</sup>. An attractive model to explain these phenotypes is that SCF<sup>TIR1</sup> ubiquitinates Aux/IAA proteins and thereby targets them to the proteasome (Fig. 2). SCF<sup>TIR1</sup> might normally ubiquitinate Aux/IAA proteins that have an intact domain II. Either inactivation of the complex or mutation of domain II would then lead to higher steady-state levels of one or more Aux/IAA proteins, in turn decreasing auxin response. The cullin component of the SCF<sup>TIR1</sup> ubiquitin ligase is itself regulated by AXR1- and ECR1-dependent modification with the ubiquitin-like molecule Rub, and mutations in *axr1* also confer auxin-resistant root growth and gene induction, and have reduced apical dominance<sup>1,35,36</sup>.

The phenotypes of *axr1* and *tir1* mutants are less severe than those of gain-of-function *Aux/IAA* gene mutants, which raises the issue of whether the hypothesized stabilization of Aux/IAA proteins can fully account for the *axr1* and *tir1* phenotypes. Perhaps SCF<sup>TIR1</sup> targets only a subset of Aux/IAA proteins and other ubiquitin ligases (possibly homologous to SCF<sup>TIR1</sup>) ubiquitinate other Aux/IAA proteins. Both *AXR1* and *TIR1* are members of multigene families, suggesting that the single mutants might retain an ability to ubiquitinate Aux/IAA proteins. Another potential complication is that Aux/IAA proteins might antagonize each other, such that stabilizing multiple Aux/IAA proteins might cause more subtle phenotypes than stabilizing just one. For example, Aux/IAA proteins might inhibit each other by dimerizing, and they can also repress expression of each other's genes. Finally, in addition to stabilizing the proteins, domain II mutations might confer an added biochemical activity that is not mimicked by mutating the ubiquitination apparatus.

In light of this working model, a question of immediate interest is whether auxin regulates Aux/IAA protein turnover. Such regulation could explain how auxin activates gene expression. There is no evidence that auxin regulates Rub-conjugating or SCF<sup>TIR1</sup>-ubiquitin-ligase activities. Perhaps the most likely alternative is that auxin causes modification of Aux/IAA proteins and that such modification enables

Table 1. Mutations in domain II of different *Aux/IAA* genes<sup>a</sup>

<i>Aux/IAA</i> gene and mutations <sup>b</sup>	Phenotypes	Refs
<b>AXR2/IAA7</b> <i>axr2-1</i> VVGWPPVRN S	Short hypocotyl, leaves in dark, wavy leaves, no root hairs, agravitropic root and shoot	28,30,54,55
<b>AXR3/IAA17</b> <i>axr3-1</i> <i>axr3-3</i> VVGWPPVRS L G	Short hypocotyl, leaves in dark, upcurled leaves, no root hairs, agravitropic root	29,31
<b>SHY2/IAA3</b> <i>shy2-1, -2</i> <i>shy2-3</i> <i>shy2-6</i> IVGWPPVRS S E L	Short hypocotyl, leaves in dark, upcurled leaves	26,40,42,44, <sup>c</sup>
<b>SLR1/IAA14</b> <i>slr1-1</i> VVGWPPVRN S	No lateral roots, few root hairs, agravitropic root and hypocotyl	<sup>c</sup>
<b>IAA28</b> <i>iaa28-1</i> VVGWPPVRS L	Few lateral roots, decreased shoot apical dominance	6
<b>MSG2/IAA19</b> <i>msg2-1</i> <i>msg2-2</i> <i>msg2-3</i> <i>msg2-4</i> VVGWPPVCS S R L L	Agravitropic and aphototropic hypocotyl, few lateral roots	<sup>d</sup>
<b>IAA12</b> <i>bodenlos</i> VVGWPPIGL S	No embryonic root, upcurled leaves	56, <sup>e</sup>
<b>IAA18</b> <i>iaa18-1</i> VVGWPPVRS E	Long hypocotyl, fused cotyledons, short root, upcurled leaves	<sup>f</sup>
<b>SHY1/IAA6</b> <i>shy1-1</i> AVGWPPVCS R	Short hypocotyl, upcurled leaves	42, <sup>f</sup>

<sup>a</sup>Only the most dramatic phenotypes are listed. See Refs for further details.  
<sup>b</sup>Amino acid sequences of the conserved stretch of domain II, and changes caused by the listed mutations. For *shy1-1* and *iaa18-1*, the inference that the indicated mutation causes the phenotypes is based on genetic linkage only. The mutation in *shy1-1* in particular causes a conservative amino acid change, and therefore requires additional evidence.  
<sup>c</sup>H. Fukaki and M. Tasaka, pers. commun.  
<sup>d</sup>K.T. Yamamoto, pers. commun.  
<sup>e</sup>T. Hamann and G. Jürgens, pers. commun.  
<sup>f</sup>P. Nagpal and J.W. Reed, unpublished.

them to be recognized by SCF<sup>TIR1</sup>. In yeasts and animals, phosphorylation regulates ubiquitination and the subsequent turnover of many proteins<sup>33</sup>. SHY2/IAA3 can be phosphorylated *in vivo*<sup>32</sup>, although no data are available on which factor(s) regulate this phosphorylation. Both the PINOID kinase and MAP kinases have been implicated in auxin responses, and they are therefore candidates for phosphorylating and thereby possibly regulating the turnover of Aux/IAA proteins. Mutations in PINOID cause cotyledon fusions similar to those of *mp/arf5* and *iaa18-1* mutants, pin-like inflorescences resembling those of *pin1* auxin-transport-defective mutants and altered flower structure. In addition, overexpression of PINOID causes auxin insensitivity<sup>37,38</sup>. Auxin increases MAP kinase activity in *Arabidopsis* roots, and inhibitors of MAP kinase decrease auxin induction of a reporter gene construct<sup>39</sup>.

#### Do Aux/IAA proteins mediate light responses?

Several results suggest that light can also regulate Aux/IAA protein activity. For example, the red-light photoreceptor phytochrome A (phyA) can interact with two Aux/IAA proteins in the yeast two-hybrid assay<sup>40</sup> and oat phyA can phosphorylate IAA1,

SHY2/IAA3, IAA9, AXR3/IAA17 and Ps-IAA4 (from pea) *in vitro*<sup>32</sup>. PhyA also phosphorylated a fragment of SHY2/IAA3 consisting of domains I and II (Ref. 32), suggesting that phyA recognizes one of these domains. This *in vitro* activity was not light regulated. However, phytochromes move from the cytoplasm to the nucleus in response to light and phytochrome translocation could regulate phytochrome phosphorylation of (nucleus-localized) Aux/IAA proteins<sup>41</sup>.

Other data suggest that light might activate Aux/IAA proteins by stabilizing them. The domain II mutations *axr2-1*, *axr3-1*, *shy2-1* and *shy2-2* each cause seedlings to develop leaves when grown in darkness<sup>28,42-44</sup>. Also consistent with this idea, an antisense construct that decreases function of the *Arabidopsis* COP9 complex, which is required to prevent de-etiolation in darkness, decreased the turnover rate of a Ps-IAA6::luciferase protein fusion in transgenic plants<sup>45</sup>. Intriguingly, the COP9 complex removes Rub modification of cullins, the reverse enzymatic activity to that catalyzed by AXR1 and ECR1 (Refs 45,46).

In addition to *Aux/IAA* gene mutations that affect de-etiolation, *msg2* mutations in *IAA19* decrease

phototropism (K.T. Yamamoto, pers. commun.). These results raise the possibility that the photoreceptor NPH1 might regulate phototropism through interactions with Aux/IAA proteins. NPH1 is a light-regulated kinase<sup>47</sup>, although there is currently no evidence that it can phosphorylate Aux/IAA proteins. Intriguingly, the kinase domain of NPH1 is related to the PINOID kinase, suggesting that this family might have a general role in auxin responses. However, NPH1 associates with the plasma membrane and therefore might not be able to interact directly with MSG2/IAA19 unless one of the proteins can shuttle between the cytoplasm and the nucleus.

If Aux/IAA proteins do mediate light responses then loss-of-function mutations in *Aux/IAA* genes should decrease light responses. Presumed null mutations in *SHY2/IAA3* and *AXR2/IAA7* cause transient increases in hypocotyl elongation rates<sup>26,28</sup>. However, these phenotypes are much subtler than those caused by mutations in photoreceptor genes. Because phyA can phosphorylate any of several different Aux/IAA proteins *in vitro*, construction and characterization of double or higher-order mutants lacking multiple potentially redundant Aux/IAA proteins might provide more convincing evidence of their importance in light responses.

#### Why does auxin regulate *Aux/IAA* gene expression?

The protein ubiquitination machinery and the multiple kinases and photoreceptors already described might regulate Aux/IAA protein stability or some other aspect of Aux/IAA protein activity. There is no evidence that Aux/IAA proteins bind specific DNA sequences and therefore the simplest model to explain how Aux/IAA proteins exert their effects is that they dimerize with ARF proteins and thereby modulate auxin-regulated gene expression. In a transient expression assay in carrot protoplasts, any of several Aux/IAA proteins could inhibit auxin-induced gene expression<sup>11</sup>, suggesting that Aux/IAA proteins can inhibit the activity of ARFs that contain an activating middle region. Also consistent with this idea, *axr2-1* and *shy2-2* mutations decrease expression of several auxin-regulated genes<sup>1</sup> (Q. Tian and J.W. Reed, unpublished). More complicated scenarios are also possible. For example, Aux/IAA proteins might dimerize with ARFs that do not contain an activation domain, and this interaction might either inhibit a repressing activity or confer some novel activity on these ARFs.

*Aux/IAA* genes were discovered based on their induction by auxin and some (such as *AXR3/IAA17*) can promote auxin responses. However, many Aux/IAA proteins actually inhibit auxin responses. These Aux/IAA-mediated negative feedbacks could have at least two purposes for plant development. They might impose a requirement for persistent (or perhaps accelerated) auxin signal input to cause a lasting developmental change. In fact, auxin induces some genes (including several *Aux/IAA* genes)

transiently<sup>1</sup>. Negative feedback loops might also enable more precise control of output level than would an undamped inductive system, which might overshoot. This would permit a close correlation between auxin level and target gene expression in different cells, and such a graded response would allow auxin to function as a morphogen in tissue patterning. Auxin gradients have indeed been implicated in patterning various tissues, including apical–basal organization in the gynoecium<sup>24</sup>, phyllotaxy in the shoot apical meristem<sup>23</sup>, cellular organization of the root meristem<sup>48</sup>, radial patterning of the vascular cambium<sup>49,50</sup> and development of leaf vasculature<sup>21,22</sup>. Such patterning might also require intercellular feedbacks so that cells in a gradient of auxin concentration could detect their relative position. Altered root meristem patterns in *axr2-1* and *axr3-1* mutants<sup>48</sup> and altered cotyledon phyllotaxy in *iaa18-1* mutant plants also suggest that Aux/IAA proteins might regulate patterning. As already discussed, several different ARFs have also been implicated in tissue patterning.

In addition to *Aux/IAA* genes themselves, the targets of Aux/IAA and ARF regulation might include genes whose products carry out developmental responses to auxin. Most of these remain to be discovered but they might include genes encoding the ethylene biosynthetic enzyme ACC synthase, transcription factors of the HD-Zip class and other early auxin response genes of the *SAUR* and *GH3* families<sup>51</sup>. We know little about the functions of most of these genes or the proteins they encode, and studies of these auxin-regulated genes should provide insight into how they might mediate developmental processes induced by auxin. Mutations in one *GH3* gene decrease responses to phytochrome A (Ref. 52) and overexpression of another *GH3* gene confers a short hypocotyl and auxin-resistant root growth<sup>53</sup>. These results suggest that GH3 proteins also mediate light responses. It is an open question whether GH3 proteins are part of the same regulatory machinery as ARF and Aux/IAA proteins or whether they act through independent pathways.

#### What do we still need to know?

Recent progress toward discovering the functions of *Aux/IAA* genes has come from the fortuitous discovery of gain-of-function mutations in several of these genes. However, we do not know the precise developmental functions of each of these genes and analyses of loss-of-function single and multiple mutants might lead to the next advances in understanding *Aux/IAA* gene function. We would also like to know the extent to which Aux/IAA proteins function as antennae for the myriad hormonal and environmental signals that influence the basic mechanisms of cell growth, division and differentiation, and the extent to which Aux/IAA proteins can integrate multiple signals. *In vivo* biochemical studies will be essential to understand

## Acknowledgements

I thank Joe Kieber and Punita Nagpal for comments on the manuscript.

whether (and how) auxin and other signals regulate the activity of the Aux/IAA and/or ARF proteins, and might provide important insight into how auxin is perceived. Finally, global gene expression studies of

both gain- and loss-of-function mutants might reveal the regulatory targets of Aux/IAA and ARF proteins, thereby providing a clearer view of how auxin-regulated developmental processes unfold.

## References

- Abel, S. *et al.* (1995) The *PS-IAA4/5*-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *J. Mol. Biol.* 251, 533–549
- Kim, J. *et al.* (1997) Protein–protein interactions among the Aux/IAA proteins. *Proc. Natl. Acad. Sci. U. S. A.* 94, 11786–11791
- Abel, S. *et al.* (1994) Early auxin-induced genes encode short-lived nuclear proteins. *Proc. Natl. Acad. Sci. U. S. A.* 91, 326–330
- Ouellet, F. *et al.* (2001) IAA17/AXR3: biochemical insight into an auxin mutant phenotype. *Plant Cell* 13, 829–841
- Morgan, K.E. *et al.* (1999) Biochemical characterization of recombinant polypeptides corresponding to the predicted beta-alpha-alpha fold in Aux/IAA proteins. *FEBS Lett.* 454, 283–287
- Rogg, L.E. *et al.* (2001) A gain-of-function mutation in *IAA28* suppresses lateral root development. *Plant Cell* 13, 465–480
- Hagen, G. and Guilfoyle, T. Auxin-responsive gene expression: genes, promoters, and regulatory factors. *Plant Mol. Biol.* (in press)
- Liscum, E. and Reed, J.W. Genetics of Aux/IAA and ARF action in plant growth and development. *Plant Mol. Biol.* (in press)
- Guilfoyle, T. *et al.* (1998) How does auxin turn on genes? *Plant Physiol.* 118, 341–347
- Guilfoyle, T.J. *et al.* (1998) The ARF family of transcription factors and their role in plant hormone-responsive transcription. *Cell Mol. Life Sci.* 54, 619–627
- Ulmasov, T. *et al.* (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9, 1963–1971
- Ulmasov, T. *et al.* (1999) Dimerization and DNA binding of auxin response factors. *Plant J.* 19, 309–319
- Ulmasov, T. *et al.* (1999) Activation and repression of transcription by auxin-response factors. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5844–5849
- Sessions, A. *et al.* (1997) *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. *Development* 124, 4481–4491
- Przemeck, G.K.H. *et al.* (1996) Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. *Planta* 200, 229–237
- Hardtke, C.S. and Berleth, T. (1998) The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17, 1405–1411
- Watahiki, M.K. and Yamamoto, K. (1997) The *massugu1* mutation of *Arabidopsis* identified with failure of auxin-induced growth curvature of hypocotyl confers auxin insensitivity to hypocotyl and leaf. *Plant Physiol.* 115, 419–426
- Liscum, E. and Briggs, W.R. (1996) Mutations of *Arabidopsis* in potential transduction and response components of the phototropic signaling pathway. *Plant Physiol.* 112, 291–296
- Stowe-Evans, E.L. *et al.* (1998) *NPH4*, a conditional modulator of auxin-dependent differential growth responses in *Arabidopsis*. *Plant Physiol.* 118, 1265–1275
- Harper, R.M. *et al.* (2000) The *NPH4* locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial *Arabidopsis* tissue. *Plant Cell* 12, 757–770
- Sieburth, L. (1999) Auxin is required for leaf vein pattern in *Arabidopsis*. *Plant Physiol.* 121, 1179–1190
- Mattsson, J. *et al.* (1999) Responses of plant vascular systems to auxin transport inhibition. *Development* 126, 2979–2991
- Reinhardt, D. *et al.* (2000) Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12, 507–518
- Nemhauser, J.L. *et al.* (2000) Auxin and ETTIN in *Arabidopsis* gynoecium morphogenesis. *Development* 127, 3877–3888
- Kaufman, P.B. *et al.* (1995) Hormones and orientation of growth. In *Plant Hormones* (Davies, P.J., ed.), pp. 547–571. Kluwer Academic Publishers
- Tian, Q. and Reed, J.W. (1999) Control of auxin-regulated root development by the *Arabidopsis thaliana* *SHY2/IAA3* gene. *Development* 126, 711–721
- Worley, C.K. *et al.* (2000) Degradation of Aux/IAA proteins is essential for normal auxin signaling. *Plant J.* 21, 553–562
- Nagpal, P. *et al.* (2000) *AXR2* encodes a member of the Aux/IAA protein family. *Plant Physiol.* 123, 563–573
- Rouse, D. *et al.* (1998) Changes in auxin response from mutations in an *AUX/IAA* gene. *Science* 279, 1371–1373
- Timpte, C. *et al.* (1994) The *axr2-1* mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. *Genetics* 138, 1239–1249
- Leyser, H.M.O. *et al.* (1996) Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. *Plant J.* 10, 403–413
- Colón-Carmona, A. *et al.* (2000) Aux/IAA proteins are phosphorylated by phytochrome *in vitro*. *Plant Physiol.* 124, 1728–1738
- Gray, W.M. and Estelle, M. (2000) Function of the ubiquitin–proteasome pathway in auxin response. *Trends Biochem. Sci.* 25, 133–138
- Gray, W.M. *et al.* (1999) Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev.* 13, 1678–1691
- Timpte, C. *et al.* (1995) The *AXR1* and *AUX1* genes of *Arabidopsis* function in separate auxin-response pathways. *Plant J.* 8, 561–569
- del Pozo, J.C. and Estelle, M. (1999) The *Arabidopsis* cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15342–15347
- Christensen, S.K. *et al.* (2000) Regulation of auxin response by the protein kinase PINOID. *Cell* 100, 469–478
- Bennett, S.R.M. *et al.* (1995) Morphogenesis in pinoid mutants of *Arabidopsis thaliana*. *Plant J.* 8, 505–520
- Mockaitis, K. and Howell, S.H. (2000) Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of *Arabidopsis* seedlings. *Plant J.* 24, 785–796
- Soh, M.S. *et al.* (1999) Regulation of both light- and auxin-mediated development by the *Arabidopsis* *IAA3/SHY2* gene. *J. Plant Biol.* 42, 239–246
- Reed, J.W. (1999) Phytochromes are Pr-apatetic kinases. *Curr. Opin. Plant Biol.* 2, 393–397
- Kim, B.C. *et al.* (1996) Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2*. *Plant J.* 9, 441–456
- Kim, B.C. *et al.* (1998) Photomorphogenic development of the *Arabidopsis* *shy2-1D* mutation and its interaction with phytochromes in darkness. *Plant J.* 15, 61–68
- Reed, J.W. *et al.* (1998) Suppressors of an *Arabidopsis thaliana* *phyB* mutation identify genes that control light signalling and hypocotyl elongation. *Genetics* 148, 1295–1310
- Schwechheimer, C. *et al.* (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF<sup>TIR1</sup> in mediating auxin response. *Science* 292, 1379–1382
- Lyapina, S. *et al.* (2001) Promotion of NEDD8–CUL1 conjugate cleavage by COP9 signalosome. *Science* 292, 1382–1385
- Christie, J.M. *et al.* (1998) *Arabidopsis* *NPH1*: a flavoprotein with the properties of a photoreceptor for phototropism. *Science* 282, 1698–1701
- Sabatini, S. *et al.* (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* 99, 463–472
- Tuominen, H. *et al.* (1997) A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. *Plant Physiol.* 115, 577–585
- Uggla, C. *et al.* (1998) Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. *Plant Physiol.* 117, 113–121
- Guilfoyle, T.J. (1999) Auxin-regulated genes and promoters. In *Biochemistry and Molecular Biology of Plant Hormones* (Hooykaas, P.J.J. *et al.*, eds), pp. 423–459. Elsevier
- Hsieh, H.-L. *et al.* (2000) *FIN219*, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of *Arabidopsis* development. *Genes Dev.* 14, 1958–1970
- Nakazawa, M. *et al.* (2001) *DFL1*, an auxin-responsive *GH3* gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. *Plant J.* 25, 213–221
- Timpte, C.S. *et al.* (1992) Effects of the *axr2* mutation of *Arabidopsis* on cell shape in hypocotyl and inflorescence. *Planta* 188, 271–278
- Wilson, A.K. *et al.* (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Mol. Gen. Genet.* 222, 377–383
- Hamann, T. *et al.* (1999) The auxin-insensitive *bodenlos* mutation affects primary root formation and apical–basal patterning in the *Arabidopsis* embryo. *Development* 126, 1387–1395