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Update on Hormone Action

How Does Auxin Turn On Genes?

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The plant hormone auxin (or IAA) plays a key role in a wide variety of growth and developmental processes. At the cellular level, auxin acts as a signal for division, extension, and differentiation during the course of the plant life cycle. At the whole-plant level, auxin plays an important role in root formation, apical dominance, tropism, and senescence. The question is how does such a simple molecule regulate such a plethora of responses within an assortment of cells, tissues, and organs of plants?

The answer to this question requires an understanding of auxin perception, signal transduction, and gene regulation. At this time, little is known about how auxin is recognized as a hormone by plant cells or what receptor molecules are involved in this recognition. Although several classes of auxin-binding proteins have been identified and characterized (for review, see Napier and Venis, 1995), it is not clear which if any of these function as receptors in signal transduction pathways that target the nucleus and regulate auxin-responsive gene expression. Likewise, the auxin signal transduction pathway involved in early auxin-regulated gene expression is currently a mystery. It is possible that one or several classes of auxin receptors and auxin signal transduction pathways exist in plant cells, and that these receptors and pathways are not uniformly distributed among different cell types and tissues. Multiple types of auxin receptors and signal transduction pathways could account for some of the diversity observed in different tissues and organs that respond to auxin in a variety of ways.

Whatever the auxin receptors and signal transduction pathways, it is clear that exogenously applied auxin can rapidly and specifically alter the expression of selected genes in different tissues and organs. Responses at the gene-expression level can be detected as early as 2 to 3 min after auxin application (for review, see Guilfoyle, 1998), and genes that are activated or repressed in this brief time are referred to as primary or early auxin-responsive genes, a number of which have been identified and characterized. These genes and their expression have been discussed in recent reviews (Abel and Theologis, 1996; Guilfoyle, 1998) and will not be elaborated on here. This *Update* focuses on *cis*-acting elements (i.e. DNA sequences that confer auxin responsiveness to a promoter) and *trans*-acting factors (i.e. transcription factors that bind to the *cis*-acting elements) involved in the regulation of plant genes that respond rapidly and specifically to auxin.

SPECIFIC DNA ELEMENTS CONFER AUXIN RESPONSIVENESS TO EARLY GENE PROMOTERS

Only a few primary auxin-responsive gene promoters have been analyzed for *cis*-acting elements that confer auxin responsiveness. The most extensively studied auxin-responsive plant gene promoters are those from the pea *PS-IAA4/5* gene (Ballas et al., 1993, 1995), the soybean *GH3* gene (Liu et al., 1994, 1997; Ulmasov et al., 1995), and the soybean *SAUR15A* gene (Li et al., 1994; Xu et al., 1997). Each of these promoters is rapidly and specifically activated in response to biologically active auxins. To define auxin-responsive *cis*-acting elements or AuxREs in the pea and soybean promoters, a variety of approaches have been used, including analysis of 5′-unidirectional deletions, internal deletions, site-directed mutations or linker scans, and gain-of-function experiments with isolated promoter elements fused to minimal promoters.

These studies have led to the identification of the *cis*-acting elements (G/T)GTCCCAT within an auxin-responsive region of the pea *PS-IAA4/5* promoter (Ballas et al., 1993, 1995) and TGTCTC within three small AuxREs of the soybean *GH3* promoter (Liu et al., 1994, 1997; Ulmasov et al., 1995). An auxin-responsive region of the *SAUR15A* promoter contained both types of these *cis*-acting elements (Li et al., 1994; Xu et al., 1997). It has been noted that the (G/T)GTCCCAT element might simply be a degenerate version of the TGTCTC element when the (G/T)GTCCCAT element takes the form of TGTCCCAT (Ulmasov et al., 1995). DNA sequence comparisons with other auxin-responsive genes have revealed that these two types of elements are found in many genes that respond to auxin (Oeller et al., 1993; Ulmasov et al., 1995; Guilfoyle et al., 1998), but in most promoters the functional significance of the two types of *cis*-acting elements remains to be assessed.

COMPOSITE AuxREs ARE FOUND IN AUXIN-RESPONSIVE PROMOTERS

The soybean *GH3* promoter possesses three AuxREs, referred to as E1, D1, and D4, that can function indepen-

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Abbreviations: ARF1, auxin response factor 1; AuxRE, auxin response elements; GRE, glucocorticoid response element; HRE, hormone response element; VP1, VIVIPAROUS1.

dently of one another (Liu et al., 1994, 1997). Fine-structure mapping of the D1 and D4 AuxREs indicated that the TGTCTC element was required but not sufficient to confer auxin responsiveness to a mimimal promoter-GUS reporter gene (Ulmasov et al., 1995). Both D1 and D4 required a constitutive or coupling element located adjacent to or overlapping the TGTCTC element, and these AuxREs were referred to as composite AuxREs (Fig. 1). E1 may also function as a composite AuxRE with a TGTCTC element in inverse orientation (Liu et al., 1997; Guilfoyle, 1998). The constitutive or coupling element in composite AuxREs is defined as an element that in isolation confers constitutive expression to a minimal promoter-GUS reporter gene and shows no response to auxin. With composite AuxREs, the TGTCTC element acts to repress the expression of the adjacent or overlapping constitutive element when auxin levels are low. When auxin levels are high, this repression is released and the composite element is activated. Composite AuxREs may represent a common feature of primary auxin-response gene promoters. TGTCTC and TGTCCCAT elements in the soybean *SAUR15A* promoter and the pea *PS-IAA4/5* promoter may also function as composite AuxREs that contain different constitutive or coupling elements than those found in composite AuxREs of the soybean *GH3* promoter.

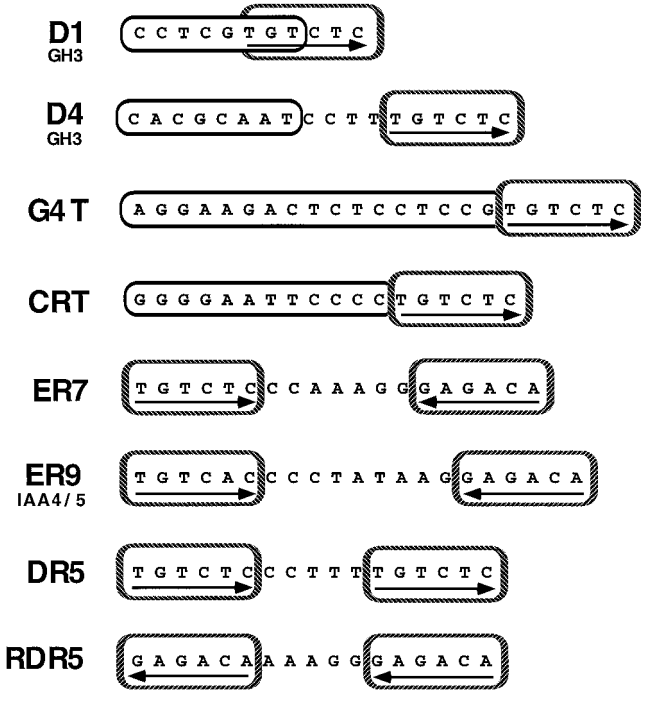

Figure 1. Composite and simple AuxREs. Each AuxRE contains a TGTCTC element (boxes with arrows) that confers auxin responsiveness. The composite D1 and D4 AuxREs are found in the soybean *GH3* promoter (Liu et al., 1994; Ulmasov et al., 1995) and consist of a constitutive element (open boxes) that lies adjacent to a TGTCTC element in D4 and overlaps with a TGTCTC element in D1. The G4T and CRT composite AuxREs consist of a heterologous constitutive element fused next to a TGTCTC element. The constitutive element in G4T is the yeast GAL4 DNA-binding site, and G4T functions as an AuxRE in the presence of a transactivator containing a GAL4 DNA-binding domain when transfected into carrot protoplasts (Ulmasov et al., 1995). The constitutive element in CRT is a chicken cRel DNA-binding domain. CRT functions as an AuxRE in carrot protoplast transient assays without an added transactivator (Ulmasov et al., 1997b). Simple AuxREs contain no apparent constitutive element and consist of palindromic or direct repeats of the TGTCTC element. ER7 is a highly active, synthetic AuxRE containing the preferred binding site for ARF1 and ARF5/IAA24 (Ulmasov et al., 1997a; Guilfoyle et al., 1998). ER9 is a palindromic AuxRE found in the pea *PS-IAA4/5* promoter (Ulmasov et al., 1997a). Synthetic direct repeats of TGTCTC function as AuxREs in either the forward (DR5) or the reverse (RDR5) orientation (Ulmasov et al., 1997b).

The structures of naturally occurring composite AuxREs suggest that they might function with a variety of different constitutive or coupling elements. Composite AuxREs could potentially confer a wide range of tissue-specific and developmentally regulated expression patterns, depending on the nature of the constitutive or coupling element that functions with the TGTCTC element. In fact, novel composite AuxREs have been created by fusing foreign or heterologous constitutive elements (i.e. yeast GAL4 and chicken cRel DNA-binding sites) adjacent to the TGTCTC element (G4T and CRT in Fig. 1) (Ulmasov et al., 1995, 1997b).

SIMPLE ELEMENTS ALSO FUNCTION AS AuxREs

Results with the natural AuxREs in the soybean *GH3* promoter indicated that the TGTCTC element required a closely associated constitutive or coupling element to function as an AuxRE. The question remained, however, whether the TGTCTC element had intrinsic AuxRE activity if it was multimerized with appropriate spacing between TGTCTC repeats. Recent experiments by Ulmasov et al. (1997a, 1997b) suggest that the TGTCTC element can function as an AuxRE in the absence of a coupling element when the TGTCTC element is multimerized with appropriate spacing and orientation (ER7, DR5, and RDR5 in Fig. 1). A multimerized TGTCCCAT element has also been shown to have AuxRE activity when fused to a minimal promoter-reporter gene (Ballas et al., 1995).

When properly spaced and oriented, TGTCTC AuxREs have been shown to be severalfold more active than natural AuxREs (Ulmasov et al., 1997a, 1997b). Two copies of the TGTCTC element oriented as a palindrome or as a direct repeat are sufficient to confer auxin responsiveness to a minimal promoter-GUS-reporter gene (Ulmasov et al., 1997a, 1997b). Simple AuxREs were being created before the discovery of natural simple AuxREs; however, a TGTCTC palindrome was subsequently identified in an auxin-responsive region (domain A) of the pea *PS-IAA4/5* promoter (ER9 IAA4/5 in Fig. 1) and was shown to function as an AuxRE when fused to a minimal promoter-GUS-reporter gene (Ulmasov et al., 1997a).

TGTCTC AuxREs HAVE SIMILARITIES TO ANIMAL HREs

Some similarities between plant TGTCTC AuxREs and animal GREs or steroid HREs have been described previously (Ulmasov et al., 1997a; Guilfoyle et al., 1998). First, the TGTCTC AuxRE is similar in size and sequence to the

GRE half-site TGTTCT. Second, both AuxREs and GREs or HREs may take the form of composite elements. For example, a composite GRE may contain a TGTTCT half-site that overlaps with an AP-1 (Activator Protein-1) binding site or some other DNA-binding site, and a composite AuxRE may contain a TGTCTC element that overlaps with a G-box or some other coupling element. Third, GREs and HREs may be simple elements consisting of direct repeats or palindromes with a specific number of nucleotides separating the half-sites. Likewise, AuxREs may be simple elements consisting of direct repeats or palindromes with TGTCTC half-sites. In fact, simple palindromic AuxREs were created based upon analogy to GREs (Ulmasov et al., 1997a).

ARFs ARE TRANSCRIPTION FACTORS THAT TARGET TGTCTC AuxREs

Because simple TGTCTC AuxREs that display greater AuxRE activity than natural AuxREs can be designed, they have proven advantageous for identifying and cloning transcription factors involved in auxin-responsive gene expression. A highly active palindromic repeat of the TGTCTC element was used as bait in a yeast one-hybrid system along with an Arabidopsis cDNA expression library to clone a transcription factor referred to as ARF1 that binds with specificity to the TGTCTC element (Ulmasov et al., 1997a).

The 74-kD ARF1 protein contains an aminoterminal DNA-binding domain and a carboxyterminal domain related to those found in a class of proteins encoded by the auxin-responsive *Aux/IAA* genes (Fig. 2). The *Aux/IAA* gene family represents one class of early or primary auxin-responsive genes that has been identified in a variety of plants (for review, see Abel et al., 1995). A sequence stretching over about 120 amino acids within the ARF1 DNA-binding domain (Ulmasov et al., 1997a) shows some similarity to a carboxyterminal sequence in the maize transcriptional activator VP1; McCarty et al., 1991) and its Arabidopsis ABI3 relative (Giraudat et al., 1992). VP1 is a transcription factor that regulates genes expressed during seed formation and those that respond to the plant hormone ABA. The carboxyl B3 domain in VP1, which is conserved in ABI3 and related to a portion of the ARF1 DNA-binding domain, has recently been shown to function as a DNA-binding domain (Suzuki et al., 1997).

An especially intriguing feature of the ARF1 protein is the carboxyl terminus, which contains two domains (referred to as domains III and IV) that are also found in Aux/IAA proteins (Fig. 2). Many members of the Aux/IAA class of mRNAs show increases in abundance within 5 to 20 min after exogenous auxin stimulation, and this stimulation is specific for biologically active auxins (for reveiw, see Abel et al., 1995). The Aux/IAA proteins are much smaller than ARF1, generally in the range of 20 to 30 kD, and contain two additional conserved domains in their aminoterminal regions, referred to as domains I and II.

ARABIDOPSIS CONTAINS A FAMILY OF ARF TRANSCRIPTION FACTORS

ARF1 represents one member of a family of proteins containing a highly conserved aminoterminal DNA-binding domain and a carboxyterminal domain related to domains III and IV in the Aux/IAA proteins. A total of nine full-length Arabidopsis cDNA clones encoding ARF proteins have been identified to date (Guilfoyle et al., 1998). The ARF proteins range in size from 67 to 129 kD.

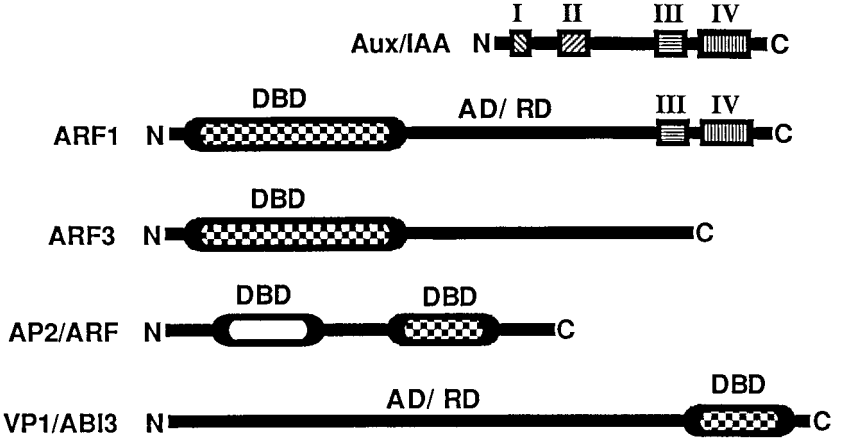

Figure 2. Proteins containing domains related to those in ARF1. Schematic diagrams are shown for Aux/IAA, ARF1, ARF3, AP2/ARF, and VP1 proteins. Aux/IAA proteins contain four conserved domains referred to as domains I, II, III, and IV (hatched boxes). ARF1 and at least seven other ARF proteins are related to Aux/IAA proteins in having similar carboxyterminal domains III and IV. ARF3 contains an aminoterminal DNA binding domain highly similar to that found in ARF1, but lacks domains III and IV in its carboxyl terminus. AP2/ARF proteins of unknown function contain both an aminoterminal AP2 or APETALA2 DNA-binding domain (DBD, open oval) (Weigel, 1995) and a carboxyterminal domain with some similarity to the DNA-binding domains in ARF1 and VP1 transcription factors. A carboxyterminal region in the maize VP1 protein functions as a DNA-binding domain (Suzuki et al., 1997) and shows some similarity to the ARF1 DNA-binding domain. DNA-binding domains in ARF proteins and related DNA-binding domains in VP1 and AP2/ARF are shown (checkered ovals). AD, Activation domains; RD, repression domains; N and C, amino and carboxyl termini, respectively.

ARF3, the smallest member of the ARF family, is unique in that it contains the aminoterminal DNA-binding domain, but lacks carboxyterminal domains III and IV (Fig. 2). Additional ARFs are likely to be found based upon limited sequence information found in expressed sequence tag and genomic databases. Because of the high conservation in the DNA-binding domain of ARF proteins identified to date, it is likely that they all recognize the same or similar DNA target sites.

Amino acid sequences between the aminoterminal DNA-binding domain and carboxyterminal domains III and IV are poorly conserved among most ARF proteins (Guilfoyle et al., 1998). The middle of the ARF1 protein contains a Pro-rich region that is also enriched in Ser and Thr residues (Ulmasov et al., 1997a). Several other ARF proteins contain a Gln-rich central region that is also enriched in Leu and Ser residues (Guilfoyle et al., 1998). Other ARF proteins contain no particular biased amino acid sequence within their central regions.

DNA SEQUENCE REQUIREMENTS FOR ARF BINDING IN VITRO ARE IDENTICAL TO THOSE THAT CONFER AUXIN RESPONSIVENESS IN VIVO

Site-directed mutations within the TGTCTC element have revealed that positions 1 through 4 (i.e. TGTC) are critical for ARF1 and ARF5 binding in vitro (or IAA24; Guilfoyle et al., 1998) and AuxRE activity in vivo (Ulmasov et al., 1997a). On the other hand, some nucleotide substitutions at positions 5 and 6 are tolerated, especially at position 5. Positions 5 and 6 are nevertheless important for ARF1 and ARF5 binding in vitro and AuxRE activity in vivo.

Orientation and copy number of target sites also play a role in the binding of ARF to TGTCTC elements in vitro. ARF1 binds with the highest affinity to everted repeats (i.e. palindromes with an everted orientation as opposed to an inverted orientation) and with lower affinity to inverted repeats and direct repeats of the TGTCTC element (Ulmasov et al., 1997a, 1997b). Spacing between everted repeats is also important, with optimal spacing of seven or eight base pairs. The affinity of ARF1 binding to different DNA target sites in vitro is perfectly correlated with the AuxRE activity displayed by the different DNA targets in vivo. For example, composite AuxREs containing only a single copy of TGTCTC bind ARF1 with low affinity (Ulmasov et al., 1995) and are induced about 3-fold by auxin in carrot protoplast transfection assays (Liu et al., 1994; Ulmasov et al., 1995), but a single copy of the TGTCTC everted repeat binds ARF1 with greater affinity and is induced about 6-fold by auxin (Ulmasov et al., 1997a).

In natural composite AuxREs, the auxin responsiveness probably results from interactions between a factor bound to a constitutive or coupling element and ARFs. Multimerization and appropriate spacing of the TGTCTC element in simple AuxREs containing direct or palindromic repeats may allow ARFs to interact with these DNA target sites in a cooperative fashion in the absence of coupling factors (i.e. TGTCTC repeats facilitate the formation of ARF dimers, which form stable associations with the DNA target).

CONSERVED CARBOXYTERMINAL DOMAINS IN ARFs AND AUX/IAA PROTEINS FACILITATE INTERACTIONS BETWEEN THESE TWO FAMILIES OF PROTEINS

The ARF1 carboxyl terminus has been shown to interact with another ARF protein, ARF2 (originally referred to as ARF1-binding protein or ARF1-BP; Ulmasov et al., 1997a), and Aux/IAA proteins in a yeast two-hybrid system (Ulmasov et al., 1997b). This system and in vitro cross-linking studies have been used to show that Aux/IAA proteins interact with one another through their carboxyterminal domains (Kim et al., 1997). These interactions probably occur through conserved domains III and IV in the carboxyl termini of these proteins, and are likely to be dependent upon amphipathic α-helices found in and adjacent to domain III of both ARF and Aux/IAA proteins (Guilfoyle et al., 1998).

Although the results discussed above have shown that ARF and Aux/IAA proteins can interact with one another, the functional consequences of these interactions remain to be investigated. In general, Aux/IAA proteins are short-lived, nonabundant proteins encoded by early auxin-responsive genes (for review, see Abel and Theologis, 1996), and are hypothesized to be transcription factors that regulate middle or late auxin-responsive genes (Abel et al., 1994). This hypothesis is partially based on the observations that the synthesis of many Aux/IAA proteins is auxin inducible and that several members of the Aux/IAA class of proteins are targeted to the nucleus (Abel et al., 1994).

Based on the above information and on the predicted secondary structure of the amino acid sequence in and around domain III of Aux/IAA proteins, it has been proposed that domain III is part of a DNA-binding motif related to the amphipathic $\beta\alpha\alpha$-fold found in β-ribbon DNA-binding domains of prokaryotic Arc and MetJ repressor proteins (Abel et al., 1994). At this point, however, there are no data available supporting a role for Aux/IAA proteins as DNA-binding proteins. In this regard, it has been shown that Aux/IAA proteins fail to bind TGTCTC AuxREs (Ulmasov et al., 1997b), suggesting that if Aux/IAA proteins are DNA-binding proteins, then their DNA target site(s) must be different from the TGTCTC AuxRE. Furthermore, the carboxyterminal region of ARF1, which includes domains III and IV, plays no apparent role in ARF1 binding to TGTCTC AuxREs (Ulmasov et al., 1997a). Although it is unlikely that Aux/IAA proteins bind TGTCTC AuxREs directly, they may still function on AuxREs by binding to other DNA-binding proteins (e.g. ARFs).

ARF, AUX/IAA, AND COUPLING FACTORS INTERACT WITH ONE ANOTHER IN REGULATING AUXIN-RESPONSIVE GENE EXPRESSION

When thinking about how auxin regulates genes, it is important to consider not only ARF transcription factors that recognize and bind to AuxREs, but also Aux/IAA proteins and those proteins that bind to the constitutive or

coupling elements in AuxREs. In simple AuxREs the selection of the DNA target site and the AuxRE activity may depend upon which particular ARF and Aux/IAA proteins interact with one another. Proteins that bind to the constitutive or coupling element in composite AuxREs may also influence the auxin response and the ARF protein that targets the TGTCTC element. The various ARF-Aux/IAA combinations may be dependent upon the affinities of different ARF and Aux/IAA proteins for one another, but may also be determined by the concentration and distribution of different ARFs and Aux/IAA proteins in different tissues and cells. The same holds true for ARF interactions with proteins that bind constitutive or coupling elements in composite AuxREs.

Because there are likely to be more than 9 ARF proteins and more than 15 Aux/IAA proteins in Arabidopsis, hundreds of combinations might be possible with these 2 classes of proteins. Assuming that productive interactions can occur among the carboxyterminal domains in a variety of ARF and Aux/IAA proteins, many potential combinations of ARF-ARF homodimers and heterodimers, Aux/IAA-Aux/IAA homodimers and heterodimers, and ARF-Aux/IAA heterodimers are possible. Figure 3 shows a few examples of possible ARF and Aux/IAA protein-protein interactions that might occur when ARFs are bound to AuxREs or are not bound to their DNA target sites. Although a large number of potential combinations is possible, it is likely that tissue-specific and developmental expression patterns and different affinities between carboxyl termini among the ARFs and Aux/IAA proteins would restrict the number of homodimers or heterodimers that function in vivo. The large number of combinations that is possible with ARF and Aux/IAA proteins is reminiscent of the large number of combinations that is possible with the steroid hormone-receptor superfamily in animals (Mangelsdorf and Evans, 1995).

Some ARF proteins function as transcriptional activators in plant protoplasts transfected with ARF-effector plasmids, whereas other ARF proteins appear to function as transcriptional repressors (Guilfoyle et al., 1998). Depending on the ARF monomers, homodimers, or heterodimers that bind to AuxREs in vivo, it should be possible to achieve either activation or repression on TGTCTC AuxREs. On the other hand, ARF proteins can also interact with Aux/IAA proteins. Overexpression of Aux/IAA proteins from effector plasmids in protoplast transient assays has been shown to result in specific repression of TGTCTC AuxRE promoter-GUS reporter genes (Ulmasov et al., 1997b). Repression by Aux/IAA proteins may result by preventing ARFs from interacting with one another, with co-activators, or with DNA target sites. It is possible that Aux/IAA proteins function as general repressors of transcription from auxin-responsive promoters containing TGTCTC-type AuxREs, the binding sites for ARF proteins. Perhaps the Aux/IAA proteins that are synthesized rapidly after auxin stimulation may function by buffering or down-regulating the transcriptional activity of the ARF-transcription factors on early genes.

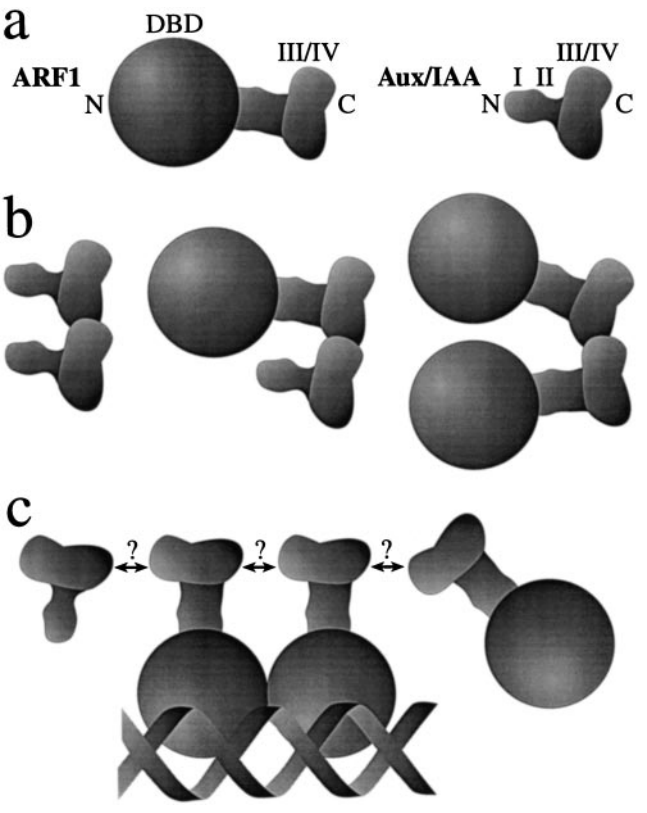

Figure 3. Protein-protein interactions between ARF1 and an Aux/IAA protein. a, Schematic diagrams for ARF1 and an Aux/IAA protein. The ARF1 DNA-binding domain (DBD) is represented by the large, circular structure. Domains III and IV are represented by the globular structure in the carboxyterminal regions of ARF1 and the Aux/IAA protein. Aminoterminal domains I and II are also indicated in the Aux/IAA protein. N and C, Amino and carboxyl termini, respectively, of ARF1 and Aux/IAA proteins. The illustrations of ARF1 and Aux/IAA are diagrammatic and are not based on structural information. b, Associations between carboxyterminal domains in ARFs and Aux/IAA proteins have been shown to occur in the absence of DNA binding (Kim et al., 1997; Ulmasov et al., 1997a, 1997b). c, Associations between ARFs and Aux/IAA proteins might also occur when ARFs are bound to AuxREs. The diagram shows possible interactions (?) between ARF and/or Aux/IAA carboxyterminal domains that might occur when an ARF1 homodimer binds to a palindromic AuxRE (e.g. ER7, indicated by the double helix). The DNA-binding domains and carboxyterminal domains of DNA-bound ARFs might both interact when ARF dimers bind palindromic AuxREs. Alternatively, carboxyterminal domains of DNA-bound ARFs might be free to bind other ARF or Aux/IAA proteins that are not bound to DNA. Depending on which interactions occur, activation or repression of an AuxRE results.

ARFs ARE MEMBERS OF A SUPERFAMILY OF TRANSCRIPTION FACTORS CONTAINING A NOVEL DNA-BINDING DOMAIN

Identification of the DNA-binding domain in ARF proteins has revealed a new class of transcription factors that appear to be unique to plants. The ARF DNA-binding domain shares some sequence similarity to the B3 DNA-binding domain in VP1 transcription factors and an uncharacterized family of proteins that contain both an AP-2

(APETALA2) DNA-binding domain (Weigel, 1995) and a domain related to the ARF/VP1 DNA-binding domain (Okamuro et al., 1997; and accession no. Z37232) (Fig. 2). ARF3 lacks the conserved carboxyterminal Aux/IAA-like domains III and IV (Ulmasov et al., 1997a; Sessions et al., 1997). There are a number of other proteins in the Arabidopsis expressed sequence tag and genomic databases that contain sequences related to the ARF1/VP1 DNA-binding domain, and these may represent other classes of transcription factors. The ARF1/VP1 DNA-binding domain may be present in a superfamily of transcription factors that use different variations of this domain to regulate transcription of hormone-responsive genes, developmentally regulated genes, and possibly other types of genes that are expressed in various cell types and during different stages of growth and development in plants.

GENETIC AND BIOCHEMICAL APPROACHES ARE PROVIDING NEW INSIGHTS INTO HOW AUXIN TURNS ON GENES

New insights into auxin-regulated gene expression have recently been unveiled. We are beginning to understand how auxin regulates genes through defined *cis*-acting elements and how a new class of *trans*-acting factors target these *cis*-acting elements. Other likely players involved in this regulation have been revealed by protein-protein interaction studies. The identification of AuxREs and some of the proteins that interact on AuxREs provides a starting point for identifying additional components of auxin-regulated gene expression and signal transduction pathways.

The recent identification of Arabidopsis mutants that have defects in specific ARF and Aux/IAA proteins provides strong genetic evidence that both classes of proteins play roles in auxin responses. ARF5/IAA24 has recently been shown to be identical to the MP (MONOPTEROS) protein (Hardtke and Berleth, 1998), which appears to play an important role in the formation of the embyro axis and in the development of vascular strands. Mutations in the *MP* gene interfere with the formation of vascular strands during and after embyrogenesis, and mutant plants fail to develop hypocotyls and roots. Furthermore, some of the phenotypes displayed by *MP* mutant plants are similar to abnormalities induced in wild-type plants that have been treated with auxin-transport inhibitors (Przemeck et al., 1996).

In another recent study the ARF3 protein was shown to be the same as the ETT protein in Arabidopsis (Sessions et al., 1997). The ETT protein plays a role in flower development and floral-organ patterning, and *ett* mutations result in increased numbers of sepals and petals, decreased numbers of stamens, and defects in the form of carpels and anthers. The role, if any, that auxin might play in flower development and floral organ formation is not clear. However, it is possible that ETT, like MP, is important for vascular tissue development, since *ETT* gene expression is detected during vascular tissue formation in several floral organs (Sessions et al., 1997). Auxin has long been thought to be a key player in vascular tissue formation and differentiation (Shininger, 1979), and it may be that at least some ARFs function as transcription factors that regulate early or primary auxin-responsive gene expression required for vascular patterning and development.

Genetic screens for plants that have increased resistance to exogenous auxin or ethylene have yielded a number of auxin-responsive mutants. One of these is *axr3*, which has increased apical dominance and adventitious rooting, decreased root elongation, and agravitropic roots (Leyser et al., 1996). Mutant phenotypes are partially restored to wild type by exogenous treatment with cytokinin. The protein encoded by the *AXR3* gene is identical to IAA17, an Aux/IAA protein (Rouse et al., 1998). Results with *axr3* mutant plants suggest that IAA17 plays a role in auxin signaling.

As genetic and biochemical/molecular approaches converge, we are likely to learn much more about how auxin turns on genes and regulates growth and development. At the same time, many challenges lie ahead. To sort out the specific interactions that occur in vivo between the families of ARF and Aux/IAA proteins and the consequences of these interactions in terms of different auxin responses will require considerable effort. Other players at the gene-expression level are most certainly involved, and these will need to be identified and characterized. The signal transduction pathway, starting with the auxin receptor and progressing to the activation of auxin-responsive genes, needs to be elucidated. The further identification of target genes for ARF-transcription factors should provide information on genes and gene products that play important roles in auxin action.

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