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Review

The ARF family of transcription factors and their role in plant hormone-responsive transcription

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Abstract. Auxin response factors or ARFs are a recently discovered family of transcription factors that bind with specificity to auxin response elements (AuxREs) in promoters of primary or early auxin-responsive genes. ARFs have an amino-terminal DNA-binding domain related to the carboxyl-terminal DNA-binding domain in the maize transactivator VIVIPAROUS1. All but one ARF identified to date contain a carboxyl-terminal protein-protein interaction domain that forms a putative amphipathic α-helix. A similar carboxyl-terminal protein-protein interaction domain is found in the Aux/IAA class of auxin-inducible proteins. Some ARFs contain transcriptional activation domains, while others contain repression domains. ARFs appear to play a pivotal role in auxin-regulated gene expression of primary response genes.

Key words. ARFs; AuxREs; transcription factor; auxin.

Introduction

Plant growth, development, and physiology are regulated by a variety of hormones. The classical plant hormones, which include auxins, cytokinins, gibberellins, abscisic acid and ethylene, were discovered several decades ago. Recently, a number of additional molecules have been identified that might also be classified as plant hormones. These include jasmonic acid, salicylic acid, the steroid brassinolide and some small peptides like systemin. While a considerable amount is known about the biosynthesis and distribution of these hormones in plants, the receptors and signal transduction pathways for plant hormones are only beginning to be unravelled.

Auxin was discovered more than a half century ago, and its biosynthesis, transport, distribution and mode of action have been intensively investigated since its discovery. Still, little is known about the molecular mechanisms of auxin action. Auxin is known to regulate a variety of cellular processes, including cell division, cell extension and cell differentiation. Auxin is generally required to propagate plant cells grown as callus or liquid suspension cultures. At the whole plant level, auxin is known to play important roles in root formation, apical dominance, gravitropism, phototropism and senescence. The most prevalent naturally occurring auxin is indole-3-acetic acid (IAA), but a number of highly active synthetic auxins have been developed in the agricultural and horticultural industries. These include 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (1-NAA). Depending on the concentration of auxin that is applied to plants, this hormone can act as a herbicide, growth retardant or growth stimulator.

While it is well documented that natural auxins are highly important for normal plant growth and development and that application of synthetic auxins can dra-

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matically alter normal growth and development, little is known about the signal transduction pathways and targets involved in auxin action. A number of auxin-binding proteins have been identified in plants [1], but it remains controversial whether these are receptors involved in auxin signal transduction pathways. One of several approaches that is currently being used to gain insight into auxin action and signal transducton is to identify and characterize primary or early auxin-responsive genes that might initiate or contribute to auxin-induced growth and developmental responses. Primary or early auxin response genes are defined as those genes that are activated or repressed within about 2 to 20 min after a plant, excised plant organ or cultured cell is exposed to exogenous auxin. In addition, primary response genes do not require ongoing protein synthesis to elicit a response to auxin (i.e. brief pretreatment with or simultaneous application of cycloheximide and auxin does not prevent activation or repression of the gene). Transcripts of several primary response genes increase in abundance when plants or excised plant organs are exposed to cycloheximide in the absence of auxin treatment, but these increases may result from either or both transcriptional and posttranscriptional processes [2, 3].

A variety of primary auxin response genes have been identified and characterized [4]. In a few cases, the promoters of these genes have been analysed for *cis* elements that confer an auxin response, and these have been referred to as auxin response elements or AuxREs. Naturally occurring AuxREs that have been defined in the greatest detail are composite elements, consisting of a constitutive element or coupling element that lies adjacent to or overlaps with a TGTCTC element which confers auxin responsiveness in the soybean *GH3* promoter [5, 6] (D1 and D4 in fig. 1). Within a composite AuxRE, the constitutive element is defined as an element that confers constitutive expression to a minimal promoter-reporter gene. In composite AuxREs, the TGTCTC element represses the constitutive element when auxin levels are low, and the composite element is derepressed and activated when auxin levels are elevated. Recent experiments suggest that the TGTCTC element can function as an AuxRE in the absence of a coupling element if the TGTCTC element is multimerized with appropriate spacing and orientation [7, 8] (ER7 and DR5 in fig. 1). Some of these synthetic AuxREs are severalfold more active than natural AuxREs and have proven advantageous for identifying transcription factors involved in auxin-responsive gene expression.

The TGTCTC element is found within auxin-responsive regions of a variety of auxin-responsive genes, including *GH3*, *SAUR* and *Aux/IAA* genes. Table 1 lists the positions of these elements within a few of these auxin-responsive promoters. The functional significance of the TGTCTC element has only been evaluated in the soybean *GH3* and *PS-IAA4/5* promoters (indicated by underscores in table 1). It should be noted that single copies of the TGTCTC element do not function as AuxREs unless they are within a composite element.

Some similarities between TGTCTC AuxREs and glucocorticoid or steroid hormone response elements

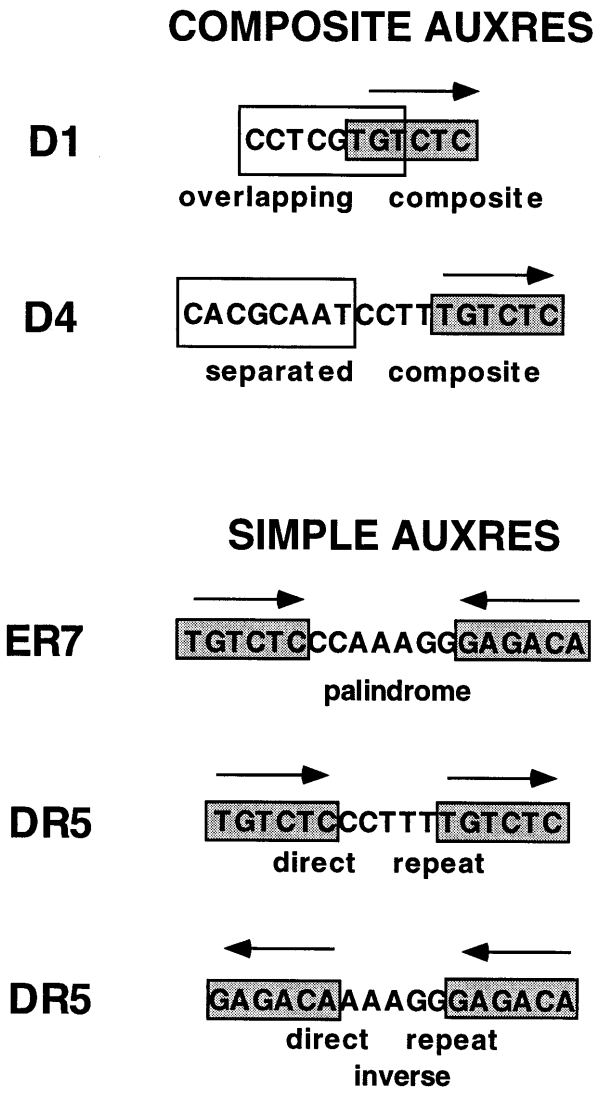

Figure 1. Composite and simple AuxREs. The composite AuxREs which are shown are found in the soybean *GH3* promoter [26, 34] and consist of a constitutive element (open box) that lies adjacent to (D4) or overlaps with (D1) a TGTCTC element (shaded box), which confers auxin responsiveness [5]. Simple AuxREs contain no apparent constitutive element and consist of palindromic (ER7) or direct repeats (DR5) of the TGTCTC element [7, 8]. Direct repeats of TGTCTC function as AuxREs in either orientation. Arrows show the orientation of the TGTCTC element in different AuxREs.

Table 1. TGTCTC-like elements in auxin-responsive regions of promoters.

Gene	Position	Sequence
GH3	(−241)	TTTTGCTGACGTG**GcGACA**
GH3	(−181)	CCTCG**TGTCTC**
GH3	(−142)	CACGCAATCCTT**TGTCTC**AATAAG
PS-IAA4/5	(−173)	**TGTCc**CAT
PS-IAA4/5	(−162)	**TGTCa**CCCCTATAAG**GAGACA**
SAUR 15A	(−142)	CATATGCCA**TGTCTC**TCAATTGG-TCCCAT
SAUR AC1	(−263)	CAACTTCA**TGTCcC**
SAUR AC1	(−218)	**TGTCTtTGAGACA**
SAUR AC1	(−161)	AACTTCT**cAGACA**CCATTTAT

TGTCTC or related elements are indicated in bold letters. Bases that differ from a perfect TGTCTC sequence within different elements are shown in lower-case letters. Some of these elements are in reverse orientation. The *GH3* and *SAUR 15* genes are from soybean [5, 7, 15, 26], the *PS-IAA4/5* gene is from pea [36], and the *SAUR AC1* gene is from *Arabidopsis* [37]. Sequences containing TGTCTC elements that have been shown to function as AuxREs in vivo as composite or simple elements are underscored [5, 7, 36].

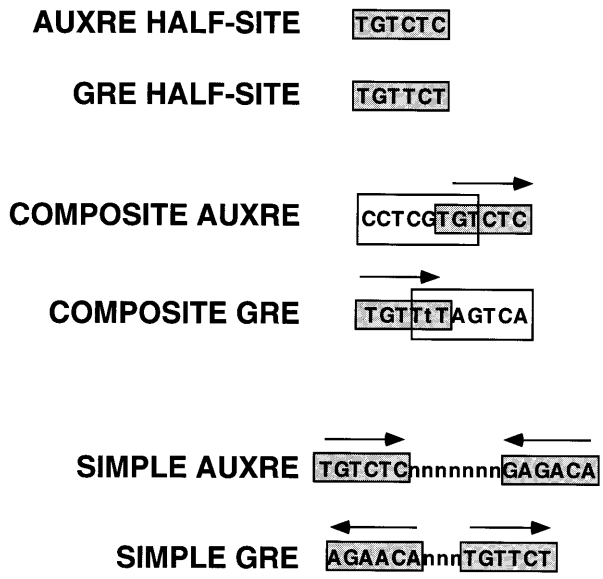

Figure 2. Comparison of AuxREs and GREs. The *cis* elements that confer auxin responsiveness and glucocorticoid responsiveness are shown as shaded boxes at the top. These are referred to as half-sites because the preferred binding sites for ARFs (i.e. transcription factors that bind to the TGTCTC element) and glucocorticoids are palindromic. Composite AuxREs and GREs contain a half-site (shaded boxes) and a coupling element (unshaded boxes). The coupling element may be a G-box in AuxREs and an AP-1 site in GREs. Palindromic repeats of TGTCTC function as AuxREs, and palindromic repeats of TGTTCT function as GREs. Spacing and orientation of the repeats are important for activity. Highly active AuxREs consist of everted repeats separated by seven or eight nucleotides, while highly active GREs consist of inverted repeats separated by three nucleotides. Arrows show the orientation of the half-sites in AuxREs and GREs.

(GREs or HREs) have been noted [7]. First, the TGTCTC AuxRE is related in sequence to the GRE half-site TGTTCT (fig. 2). Second, composite AuxREs share some similarity with composite GREs and other composite HREs found in animal genes that respond to steroid hormones [9]. GRE half-sites may overlap with other DNA-binding sites (e.g. activator protein-1 or AP-1 binding sites) in composite GREs, and TGTCTC sites may overlap with coupling elements (e.g. G-box) in composite AuxREs (fig. 2). Third, GREs and HREs may also come in the form of simple elements, consisting of direct repeats or palindromes with a specific number of nucleotides separating the half-sites [7, 9]. Likewise, synthetic AuxREs that contain direct repeats or palindromes with TGTCTC half-sites have been created [7, 8] (fig. 2), and a natural palindromic TGTCTC AuxRE has been identified in an auxin-responsive promoter [7] (see PS-IAA4/5 (−162) in table 1).

AuxREs and ARF1

ARFs or auxin response factors are transcription factors that bind to AuxREs with the consensus sequence TGTCNC [7]. ARF1 from *Arabidopsis* was the first of these transcription factors to be identified, and it was cloned using a yeast one-hybrid screen with a highly active, synthetic AuxRE containing a palindromic repeat of the TGTCTC element as bait [7].

The ARF1 protein contains 665 amino acids and has a predicted molecular mass of about 74 kDa with a pI of about 6.0. Gel mobility shift assays with truncated forms of ARF1 revealed that the DNA-binding domain was localized to the amino-terminal half of the protein (fig. 3). Within the ARF1 DNA-binding domain, a sequence stretching over about 120 amino acids [7] shows some similarity to a carboxyl-terminal sequence found in the maize transcriptional activator VIVIPAROUS1 or VP1 [10] and its relatives ABI3 in *Arabidopsis* [11], OsVP1 in rice [12] and PvAlf in bean [13]. VP1, ABI3 and OsVP1 are transcription factors that regulate abscisic acid (ABA)-responsive genes, and PvAlf is a transactivator that regulates genes that are expressed during seed formation. Although VP1 was originally thought to function as a transactivator without binding directly to DNA, recent evidence indicates that the carboxyl B3 domain, which is conserved in ABI3, OsVP1 and PvAlf and related to a portion of the ARF1 DNA-binding domain, is, in fact, a cryptic DNA-binding domain in maize VP1 [14].

The middle of the ARF1 protein contains a proline-rich region that is also enriched in serine and threonine residues [7]. This region represents a possible activation or repression domain because it has some sequence

similarity to other transcriptional activators or repressors that contain proline-rich motifs [15]. Within the proline-rich region of ARF1, the sequence PQ**R**N**KR**P**R** (amino acids 368–375) represents a possible SV-40 large T antigen-type nuclear localization sequence (NLS). Another possible NLS is bipartite, **RR**HLLTTG-WSVFVSS**KK**, and found in the DNA-binding domain (fig. 3).

The carboxyl-terminal domain in ARF1 is intriguing because it is related to conserved domains III and IV found in a class of proteins encoded by Aux/IAA mRNAs (fig. 3) that are rapidly elevated in abundance when seedlings or excised organs are treated with auxin [7]. Many members of the Aux/IAA class of messenger RNAs (mRNAs) show increases in abundance within 5 to 20 min after exogenous auxin stimulation, suggesting that these mRNAs are transcribed from primary or early auxin-responsive genes [16–19]. This is supported by the observation that cycloheximide treatment does not block the auxin-induced accumulation of Aux/IAA transcripts, and in fact, cycloheximide treatment itself increases the abundance of some Aux/IAA transcripts [17, 19]. The Aux/IAA proteins range in size from about 150 to 350 amino acids and contain four islands or domains (i.e. domains I, II, III and IV of about 8, 14, 17 and 40 residues in size, respectively) that are conserved in all members that make up this class of proteins [18–21]. In contrast, ARF1 contains domains III and IV, but lacks domains I and II, and ARF1 is about twice as large as the largest Aux/IAA protein (fig. 3).

Other members of the ARF family in *Arabidopsis*

A combination of experimental approaches has revealed that ARF1 is a member of a family of proteins containing a highly conserved amino-terminal DNA-binding domain that recognizes the TGTCTC element. A second member of the ARF family, ARF2, was isolated in a yeast two-hybrid screen using the carboxyl-terminal region (i.e. encompassing conserved domains III and IV) of ARF1 as bait [7]. ARF2 was originally referred to as ARF1-binding protein or ARF1-BP and represented a partial complementary DNA (cDNA) clone lacking the amino terminus. Subsequently, a full-length cDNA clone was isolated, and this encoded a protein of 859 amino acids with predicted molecular mass of about 96 kDa and a pI of 6.4 (T. Ulmasov, unpublished results). ARF2 contains an amino-terminal DNA-binding domain and a carboxyl-terminal region with domains III and IV that are highly similar to ARF1. Results with the yeast two-hybrid screen suggested that different ARFs might interact through conserved domains III and/or IV to form homodimers and heterodimers. Furthermore, the two-hybrid results suggested that ARFs might also interact with Aux/IAA proteins, because both classes of proteins contain domains III and IV within their carboxyl termini. Recent results with the yeast two-hybrid system have confirmed that ARFs and Aux/IAA proteins can interact among themselves and among one another through their conserved carboxyl-terminal domains [8, 22].

Up to now, a total of nine full-length *Arabidopsis* cDNA clones encoding ARF-like proteins have been identified by either screening cDNA libraries with amino-terminal or carboxyl-terminal cDNA probes or by using probes from database searches (T. Ulmasov, unpublished results) (fig. 3). Eight of the ARF proteins contain highly conserved amino-terminal DNA-binding domains and carboxyl-terminal domains III and IV. The remaining ARF protein, ARF3, is unique in that it contains the amino-terminal DNA-binding domain but lacks carboxyl-terminal domains III and IV. Additional ARFs are likely to be found based upon limited sequence information found in expressed sequence tag (EST) 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 a similar DNA target site.

While the ARF proteins contain highly conserved (i.e. ranging from 50 to 75% amino acid sequence identity among the ARFs that have been sequenced) amino-terminal and carboxyl-terminal regions (with the exception of ARF3, which lacks the carboxyl terminus), amino acid sequence separating these regions is poorly conserved among most ARFs. In contrast to ARF1, which contains a central region rich in proline, serine and threonine, ARF5, ARF6, ARF7 and ARF8 contain a glutamine-rich central region which is also enriched in leucine and serine residues. Some pairs of ARFs are closely related in amino acid sequence in their amino-terminal DNA-binding domain and their carboxyl-terminal domains III and IV, suggesting that different ARFs may form partners that interact more strongly with one another than with other ARFs. This may explain why only ARF2 was isolated in a yeast two-hybrid screen with the ARF1 carboxyl-terminal domains as bait, even though other ARF cDNAs existed in the cDNA library [7; T. Ulmasov, unpublished results].

Arabidopsis mutants that have defects in the *ARF5*/*IAA24* gene have recently been identified. The ARF5/IAA24 protein is identical to the MONOPTEROS (MP) protein [23], which is involved in embryo axis formation and vascular development. Mutations in the *MP* gene interfere with formation of vascular strands during embryogenesis and at later times during development. Mutant *MP* plants fail to develop hypocotyls and roots. Furthermore, some of the phenotypes displayed by mutations in the *MP* gene are similar to abnormalities induced in wild-type plants in response to auxin

transport inhibitors [24]. These results are consistent with ARF5/IAA24 acting as a transcription factor in auxin responses. In another recent report, the ARF3 protein was shown to be the same as the ETT protein, which plays a role in flower development in *Arabidopsis* [25]. Mutant *ett* plants display increased numbers of sepals and petals, decreased numbers of stamens, and defects in the form of carpels and anthers. The role of auxin in these processes has not been established, but it would not be surprising that auxin might play a role in flower development and floral organ formation.

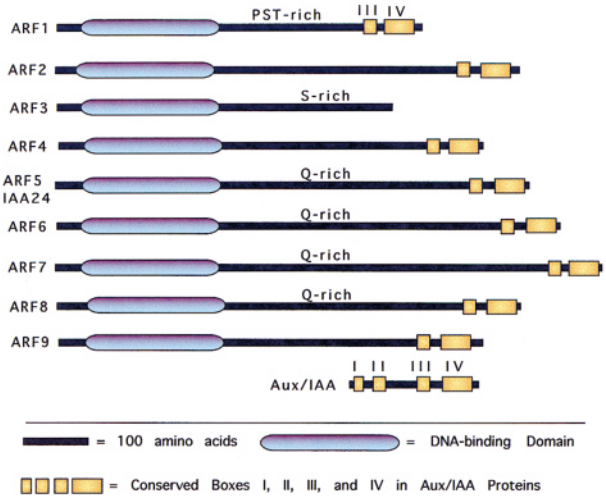

Figure 3. Diagrams of ARF protein family members in *Arabidopsis* identified to date. The amino-terminal DNA-binding domain is conserved in each ARF. All ARFs identified to date with the exception of ARF3 or ETT contain carboxyl termini with domains III and IV. Domains III and IV are also found in the carboxyl termini of Aux/IAA proteins (diagrammed below the nine ARF proteins). Aux/IAA proteins contain two additional conserved domains (I and II) which are absent in ARF proteins. The regions between the DNA-binding domains and carboxyl-terminal protein-protein interaction domains in ARFs (or the carboxyl-terminal regions in ARF3) show little sequence similarity among the ARF family members; however, these nonconserved regions are generally enriched in one or more amino acids such as proline, serine, threonine, leucine and/or glutamine. The relative sizes of the ARF and Aux/IAA protreins are indicated by the black bars.

Preferred DNA-binding sites for ARF proteins

The DNA sequence requirements for DNA binding by ARF1 and ARF5/IAA24 has been assessed by carrying out site-directed mutagenesis within TGTCTC AuxREs. Gel mobility shift assays were used to determine if mutant variants of a palindromic TGTCTC AuxRE would bind recombinant ARF proteins in vitro [7, 8]. These studies showed that the first four nucleotides, TGTC, or positions 1–4 are critically important for ARF binding to TGTCTC elements. On the other hand, some nucleotide substitutions at positions 5 and 6 are tolerated, especially at position 5. Nevertheless, positions 5 and 6 are important for ARF1 binding and AuxRE activity. The consensus sequence for a functional element is TGTCNC. Results of Ulmasov et al. [7] have shown that in vitro binding specificity of ARF1 and ARF5/ IAA24 for the TGTCTC element and variants of the TGTCTC element perfectly paralleled the in vivo auxin inducibility of the TGTCTC element and variants of the TGTCTC element in transfected carrot cells, suggesting that ARFs function as AuxRE-binding proteins in auxin-regulated gene expression.

Orientation and copy number of target sites also play a role in ARFs binding to TGTCTC elements. ARF1 binds with highest affinity to everted repeats of the TGTCTC element, and binds with lower affinity to inverted repeats and direct repeats of the TGTCTC element [7, 8]. Single natural composite AuxREs containing the TGTCTC element interact only weakly with ARF1, while single copies of everted repeats (i.e. two copies of the TGTCTC element) interact strongly with ARF1. At the same time, composite elements, containing only a single copy of TGTCTC, are only weakly auxin-inducible in carrot protoplast transfection assays (i.e. two- to threefold auxin-inducible) [5, 26], while a single copy of the TGTCTC everted repeat is more highly induced by auxin in vivo (i.e. about fivefold auxin-inducible). The auxin-inducible activity of natural composite AuxREs in vivo probably results from interactions between a factor bound to a constitutive coupling element and ARFs. The multimerization of the TGTCTC element in synthetic AuxREs containing direct or palindromic repeats with appropriate spacing may allow ARFs to interact with these sites in a cooperative fashion. That ARF1 binds TGTCTC elements cooperatively is supported by the observation that single copy everted repeats appear to bind dimers of ARF1 protein (T. Ulmasov, unpublished results).

The carboxyl-terminal domain of ARFs functions in protein-protein interactions

The carboxyl-terminal region of ARF1 is intriguing because of its similarity to the conserved domains III and IV found in the carboxyl-terminal regions of the auxin-induced Aux/IAA proteins. It has been hypothesized that the Aux/IAA class of proteins are short-lived transcription factors that regulate middle or late auxin-responsive genes [27]. This hypothesis is based on the observations that the synthesis of most Aux/IAA proteins is auxin-inducible and that several members of the Aux/IAA class of proteins, when analysed as translational fusions with the GUS protein, are targeted to

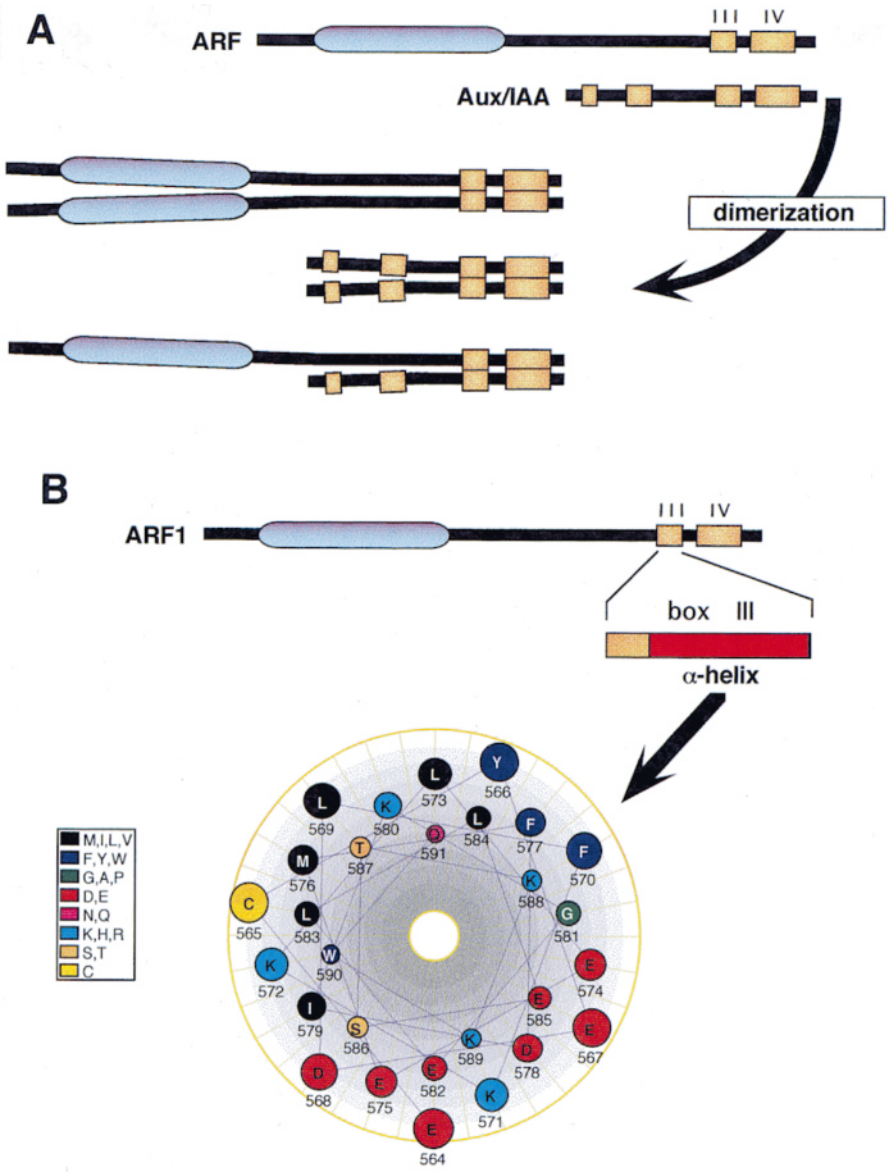

Figure 4. Protein-protein interactions between ARFs and Aux/IAA proteins. (*A*) Schematic diagram of possible interactions between an ARF and an Aux/IAA protein through their conserved carboxyl-terminal domains III and IV. Examples of an ARF homodimer an Aux/IAA homodimer and an ARF-Aux/IAA heterodimer are shown. In addition to the examples shown, different ARFs can form $ARF_{(x)}$-$ARF_{(y)}$ heterodimers and different Aux/IAA proteins can form $Aux/IAA_{(x)}$-$Aux/IAA_{(y)}$ heterodimers. (*B*) Diagram of the amphipathic α helix found in and adjacent to domain III of the ARF1 carboxyl terminus (box III, α helix). Similar amphipathic α helices are found in other ARF and Aux/IAA proteins. Related amino acids (e.g. acidic, basic etc.) are color-coded.

the nucleus [27, 28]. Nuclear localization signals have been identified by functional tests in a few of Aux/IAA proteins [28]. It has been proposed that domain III in Aux/IAA proteins is part of a motif related to the amphipathic $\beta\alpha\alpha$ fold found in β-ribbon DNA-binding domains of prokaryotic Arc and MetJ repressor proteins [27]. At this point, however, there is no direct evidence that supports a role for Aux/IAA proteins as DNA-binding proteins. In the ARF1 protein, the amino terminus contains the DNA-binding domain, and domains III and IV in the carboxyl-terminal region of ARF1 play no apparent role in ARF1 binding to TGTCTC AuxREs [7]. Furthermore, recombinant Aux/IAA proteins fail to bind TGTCTC AuxREs [8]. Thus, if amino acids in and around domain III in Aux/IAA proteins make up a DNA-binding domain with similar-

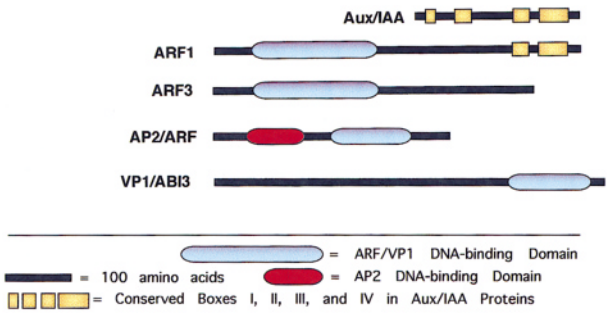

Figure 5. Proteins containing domains related to those in ARFs. Aux/IAA and ARF proteins are related to one another in having similar carboxyl-terminal domains III and IV. ARF3 lacks domains III and IV in its carboxyl terminus, but contains an amino-terminal DNA-binding domain highly similar to that found in other ARF proteins. Proteins of unknown function (labelled as AP2/ARF) contain both an amino-terminal AP2 or APETALA2 DNA-binding domain and a carboxyl-terminal domain with similarity to the DNA-binding domains in ARF and VP1 transcription factors [33]. The AP2 DNA-binding domain is also found without the ARF DNA-binding domain in a number of transcription factors that have unknown functions or play roles in floral organ specification and ethylene-responsive transcription [35]. VP1/ABI3 are transcription factors involved in embryogenesis and ABA-regulated expression, and contain a carboxyl-terminal domain related to the amino-terminal DNA-binding domain in ARF proteins. Amino (N) and carboxyl (C) ends of the proteins are indicated.

ity to β-ribbon DNA-binding domains in prokaryotic Arc and MetJ repressors, the DNA- binding site recognized by this domain must be different from the TGTCTC AuxRE.

The ARF1 carboxyl terminus has also been shown to interact with Aux/IAA proteins in a yeast two-hybrid system [7, 8], and these interactions probably occur through conserved domains III and IV in the carboxyl termini of these proteins. These interactions may be facilitated by amphipathic α helices found in and adjacent to domain III of both ARF and Aux/IAA proteins (figs 4 A, B). The interactions between ARFs and Aux/IAA proteins in yeast two-hybrid systems suggest the possibility that such interactions may play a role in auxin-regulated gene expression. The consequence of ARF binding to TGTCTC AuxREs may depend upon a particular ARF's interactions with the same ARF, other ARFs or Aux/IAA proteins. For example, ARFs may act as repressors or activators depending on which partner an ARF binds to through its carboxyl-terminal domain (i.e. domains III and IV), and auxin may somehow influence which protein-protein interactions occur. The various ARF-Aux/IAA combinations may not only be influenced by different binding specificities, but may also be determined by the concentration and distribution of different ARFs and Aux/IAA proteins in different tissues and cells. While little is known about the expression patterns of ARFs and Aux/IAA proteins, it is likely that tissue, developmental and temporal specificity in the expression patterns for about 10 ARFs and some 20 Aux/IAA proteins may play a role in controlling the wide range of auxin-responsive gene expression. Based on the expression patterns of a few *Aux/IAA* promoter-reporter genes in transgenic plants [29, 30] and northern analysis of 14 Aux/IAA family members [18, 19], there is reason to believe that different Aux/IAA proteins are expressed in tissue- and developmental-specific programmes.

Figure 4A shows some interactions that might be possible between a single ARF and a single Aux/IAA protein. Assuming that productive interactions can occur among the carboxyl-terminal domains in a variety of ARF and Aux/IAA proteins, many potential combinations of homodimers or heterodimers are possible. Although the final number of ARFs and Aux/IAA proteins has not been determined, if there were just 10 ARFs and 20 Aux/IAA proteins, the number of possible dimer combinations would come to several hundred. If higher-order multimers can form, the possible combinations become much greater. While such a large combination of potential interactions is possible, it is likely that tissue-specific and developmental expression patterns as well as different affinities between carboxyl termini among the ARFs and Aux/IAA proteins would preclude such a wide variety of combinations. This remains to be tested, however.

The consequences of ARF and Aux/IAA interactions could vary depending upon the partners that interact. For example, some ARFs function as transcriptional activators in plant protoplasts transfected with ARF effector plasmids, while other ARFs function as transcriptional repressors (T. Ulmasov, unpublished observations). Depending on the ARF homodimers or heterodimers that form in vivo, it should be possible to achieve either activation or repression on TGTCTC AuxREs. On the other hand, Aux/IAA interactions with ARFs may result in repression on TGTCTC AuxREs by preventing ARFs from interacting with one another, with coactivators or with DNA target sites. Recently, some evidence has been obtained that supports a possible role of ARF and Aux/IAA interactions in auxin-responsive gene expression. Overexpression of Aux/IAA proteins in carrot protoplast transient assays has been shown to result in specific repression of TGTCTC AuxRE promoter-β-glucuronidase (GUS) reporter genes [8]. Thus, Aux/IAA proteins may act as general repressors of transcription from auxin-responsive promoters containing TGTCTC-type AuxREs, the binding sites for ARF proteins. The consequences of Aux/IAA interactions among themselves is less obvious, but such interactions might titrate out Aux/IAA proteins and prevent their interactions with ARF proteins. This might allow interactions between ARF proteins and result in derepression/activation of auxin-responsive genes that contain TGTCTC AuxREs.

A superfamily of plant transcription factors with ARF-like DNA-binding domains

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 DNA-binding domain in VP1 and ABI3 transcription factors and an uncharacterized family of proteins that contain both an AP-2 (APETALA2) domain [31] and a domain related to the ARF and VP1/ABI3 DNA-binding domains [32, 33; and GenBank accession numbers Z37232] (fig. 5). In addition, ARF3, lacks the conserved carboxyl-terminal Aux/IAA-like domains III and IV [7, 25]. We have identified a number of other proteins in *Arabidopsis* EST and genomic database searches that contain sequences related to the ARF1/VP1 DNA-binding domain, and these may represent other classes of transcription factors. Based on all of this information, the ARF-like 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. The family of ARF-like DNA-binding proteins in plants may be comparable to the steroid hormone receptor superfamily of transcription factors in animals. Steroid hormone receptors contain a conserved Zn-finger DNA-binding domain that can recognize subtle differences in DNA target sites when different members of this family homodimerize or heterodimerize on simple hormone response elements (HREs) or interact with coupling factors on composite elements. It is possible the ARF DNA-binding domain will recognize different types of DNA target sites when different members of the ARF family form homodimers and heterodimers on simple AuxREs or interact with coupling factors on composite AuxREs. Future studies on the expansiveness and evolution of the family of transcription factors containing an ARF-like DNA-binding domain will undoubtedly provide new insight into transcriptional regulation in plants.

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