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# Auxin Response Factors

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## ABSTRACT

Auxin response factors (ARFs) are a family of transcription factors that are unique to plants and bind with specificity to auxin response elements (AuxREs) in promoters of primary or early auxin response genes. ARFs have a modular structure with an amino-terminal DNA binding domain, a centralized activation or repression domain, and, in most cases, a carboxy-terminal dimerization domain. The dimerization domain in ARF proteins is related in amino sequence to the dimerization domain in Aux/IAA proteins. Homotypic and heterotypic interactions can occur between ARF and Aux/IAA protein family members. Recent biochemical and genetic studies suggest that the ARF and Aux/IAA proteins play pivotal and concerted roles in regulating the expression of primary/early auxin-responsive genes.

**Key words:** Auxin-responsive gene expression; ARF genes; ARF proteins; Aux/IAA proteins

## INTRODUCTION

The identification of *Aux*in *R*esponse *E*lements (AuxREs) consisting of the six-bp sequence TGTCTC made it possible to design highly active synthetic AuxREs in the form of TGTCTC direct or palindromic repeats (reviewed by Guilfoyle and others 1998a; Guilfoyle 1999). A synthetic palindromic AuxRE, referred to as P3(4X), was used as bait in a yeast one-hybrid system with an *Arabidopsis* cDNA expression library to screen for transcription factors that recognize the TGTCTC element (Ulmasov and others 1997a). This screen resulted in the cloning of a novel protein that was given the name *A*uxin *R*esponse *F*actor 1 or ARF1. A second ARF, initially referred to as ARF1-binding protein (ARF1-BP), was cloned using the carboxy-terminus of ARF1 as bait in a yeast two-hybrid system (Ulmasov and others 1997a). ARF1-BP contained a carboxy-terminus that was similar in amino acid sequence to ARF1, suggesting that ARF1 and ARF1-BP might form heterodimers through carboxy-terminal domain interactions (Ulmasov and others 1997a). ARF1-BP represented only a partial sequence, and subsequent identification of a full-length cDNA clone resulted in its being renamed ARF2. At the same time, two additional ARFs, ARF3 and IAA24 or ARF5, were identified as expressed sequence tags (ESTs) or partial cDNA clones, and the sequences of full-length cDNA clones were determined (Ulmasov and others 1997a).

## ARF GENES

With the completion of the *Arabidopsis* Genome Sequencing Project, a total number of 23 *ARF* genes have been identified in this plant (Figure 1 and Table 1). One of these, *ARF23,* is likely to be a psuedogene. Based on our annotation of the GenBank sequence (GenBank accession number AC022314; BAC F9C16.11), *ARF23* contains a stop codon within a conserved region of the DNA-binding domain (DBD; see below) and lacks an ARF-related sequence carboxy-terminal to the DBD. The *ARF23* gene is located near a cluster of seven *ARF* genes on chromosome I (Figure 1). All of the genes in this cluster,

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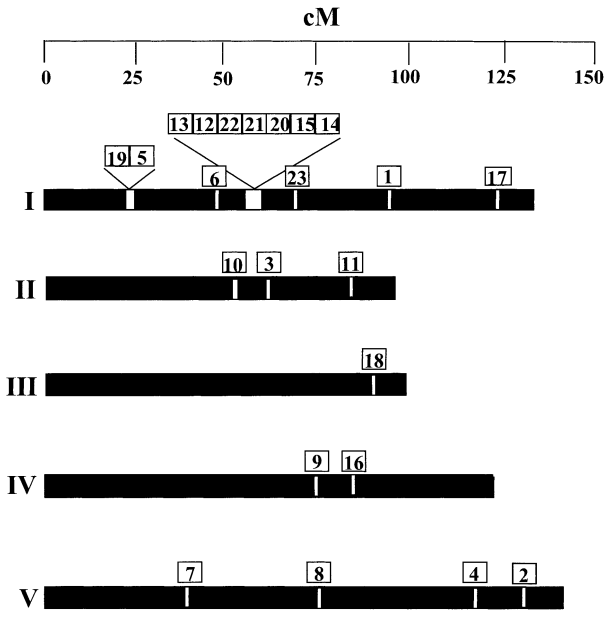



**Figure 1.** Location of ARF genes on the five *Arabidopsis* chromosomes. Approximate positions of ARFs 1 to 23 are indicated. cM is centiMorgans.

along with *ARF23,* are more closely related to one another than to other *ARFs.* It has not been shown that any of the *ARF* genes in this cluster are expressed (that is, no ESTs or mRNAs have been reported). In this regard, we have been unsuccessful in attempts to clone a cDNA for *ARF12* (unpublished results). A portion of an *ARF* gene, corresponding to the carboxy-terminal half of the DBD, is found in the mitochondrial genome of *Arabidopsis,* and this partial gene is also found in a large chunk of mitochondrial DNA that resides on chromsome II. The mitochondrial and chromosome II sequences are most closely related to the *ARF17* gene and contain a 32 bp ARF17-like insert in the DBD (see ARF DBDs below), suggesting that the *ARF*-related sequences in mitochondrial DNA may be derived from an *ARF17* gene. *ARF* genes are found in other dicots, monocots, gymnosperms, and a fern (GenBank EST databases), but are not found outside of the plant kingdom.

## ARF Proteins

ARF proteins have predicted molecular masses that range from 67 to 129 kDa (Table 1). The amino-terminal portion of each ARF protein contains a conserved DBD (Figure 2), followed by a region that is not highly conserved in amino acid sequence among the ARF proteins. This nonconserved region is referred to as the middle region of ARFs. With the exception of ARF3 and ARF17, ARF proteins contain a carboxy-terminal domain that is related in amino acid sequence to domains III and IV of Aux/IAA proteins (Ulmasov and others 1997a).

**Table 1.** *Arabidopsis thaliana* contains 23 ARF genes

| ARF | Gene ID[a] | Mol. Wt.[b] | MR compositions (%)[c] |
|---|---|---|---|
| 1 | 5080809 | 73618 | 16 S, 13 P |
| 2 | 10176918 | 95631 | 13 S |
| 3 | 3805770 | 66606 | 13 S, 12 G |
| 4 | 12744969 | 87284 | 12 S, 10 P |
| 5 | 10086486 | 99451 | 15 S, 10 Q |
| 6 | 12322119 | 103057 | 17 Q, 16 S, 10 L |
| 7 | 8071649 | 129072 | 23 Q, 12 S, 11 L |
| 8 | 9758525 | 90148 | 14 Q, 12 L, 11 S |
| 9 | 4972102 | 72278 | 18 S |
| 10 | 4432846 | 76708 | 11 S |
| 11 | 4415934 | 73809 | 20 S |
| 12 | 5091627 | 67229 | 11 S, 11 L |
| 13 | 10086460 | 70051 | 16 S |
| 14 | 8778363 | 68680 | 14 S |
| 15 | 8778352 | 67522 | 12 S, 10 L |
| 16 | 4938484 | 73979 | 14 S, 11 P, 11 L |
| 17 | 16573757 | 70937 | 15 S |
| 18 | 6850874 | 67938 | 16 S, 16 P |
| 19 | 8954059 | 120576 | 19 Q, 13 S |
| 20 | 12322942 | 68534 | 12 S, 11 L |
| 21 | 12323856 | 68765 | 12 S, 11 L |
| 22 | 12323853 | 67733 | 12 S, 11 L |
| 23 | 8778678 | 24864 | No MR (psuedogene) |

[a] *GenBank Gene identifier numbers for each ARF gene.*
[b] *The predicted molecular mass for each ARF protein.*
[c] *Any amino acid representing 10% or more of total amino acids in the middle region.*

### ARF DBDs

Gel mobility shift, DNase I footprinting, and DNA methylation interference experiments showed that full-length ARF1 protein bound with specificity to the palindromic P3(4X) AuxRE, and that the first four residues in the TGTCTC element were the most critical for ARF1 binding (Ulmasov and others 1997a). A variety of other full-length ARF proteins were also shown to bind with specificity to the P3(4X) AuxRE in gel mobility shift experiments (Ulmasov and others 1999a). On palindromic AuxREs, ARF1 bound as a dimer with each ARF protein binding to a half-site (that is, TGTCTC and GAGACA representing the two half-sites) in the palindrome (Ulmasov and others 1997a). The P3(4X) AuxRE contained four copies of the inverted repeat GAGA-

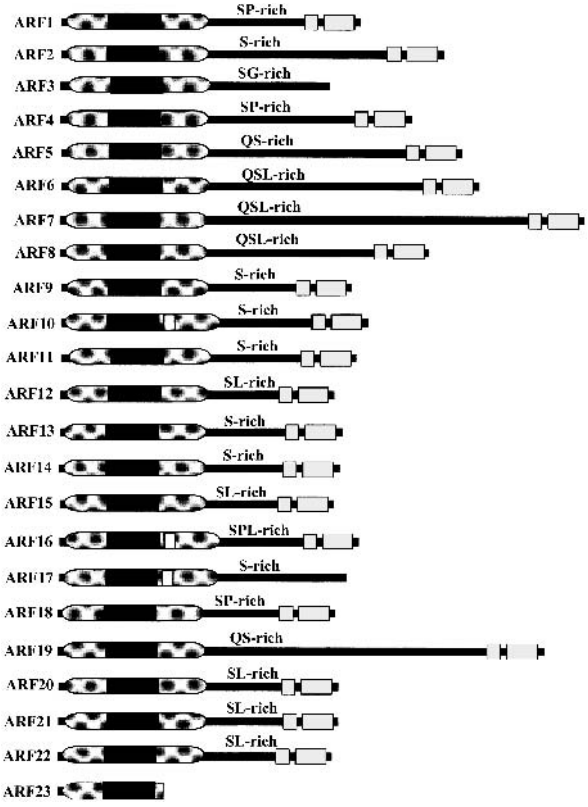


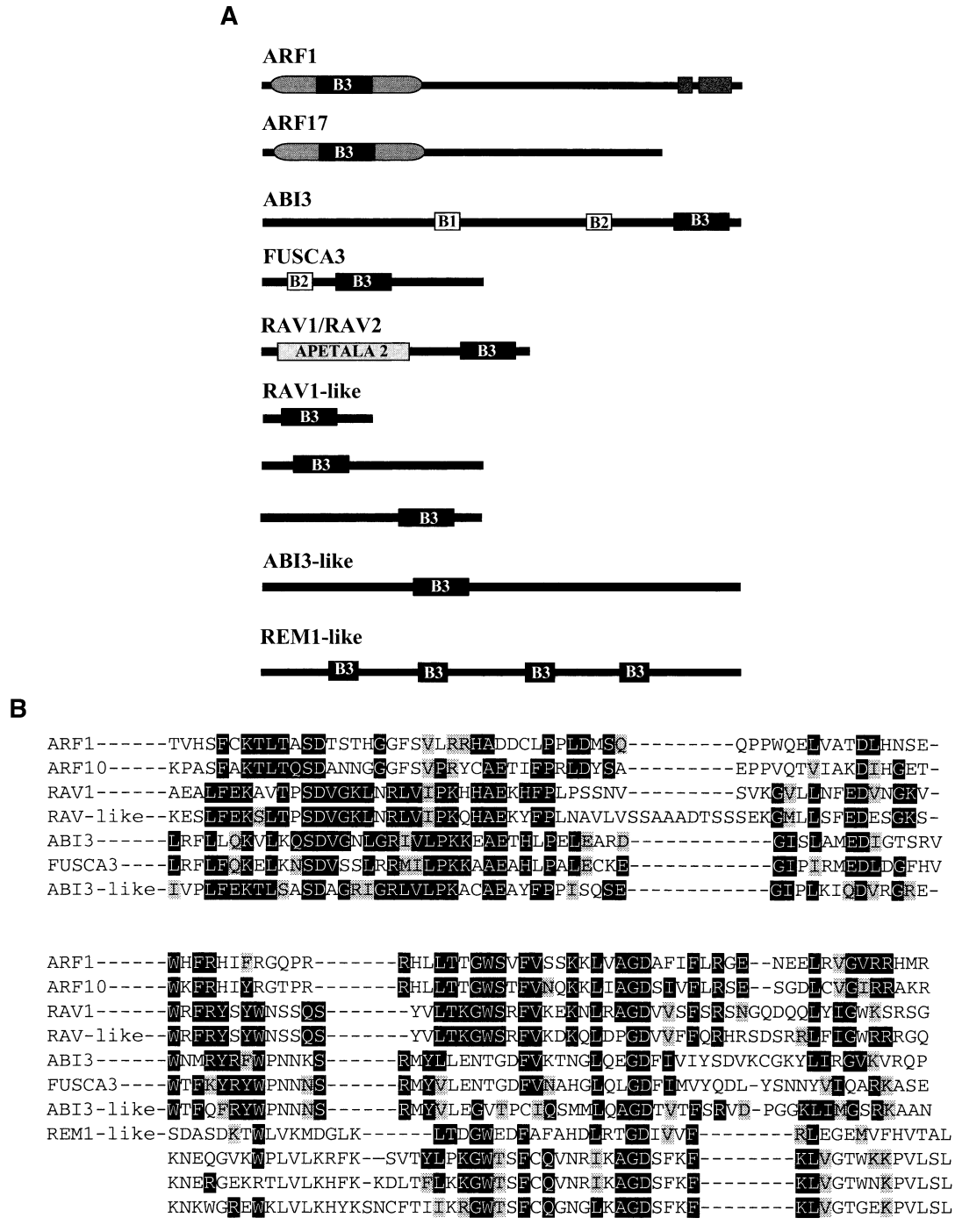

**Figure 2.** Schematic diagrams of *Arabidopsis* ARF proteins. Relative sizes of the ARFs are indicated by thick black lines. The DBDs are indicated as ovals at the amino termini, and the B3 domains are indicated as black boxes inside the ovals. The open boxes within the DBDs of ARF10, 16, and 17 represent 32 to 36 residue insertions into the DBDs of these ARFs. Middle regions are indicated with their biased amino acid sequences. The two boxes in the carboxy-termini represent domains III and IV that are also found in the Aux/IAA proteins. ARF3 and ARF17 lack domains III and IV. ARF23 is truncated and contains only a portion of the DBD with no MR or CTD.

CAactTGTCTC, with the half-sites separated by 3 bps, and spacing between the inverted repeats was 7 bps (that is, TGTCTCccaaaggGAGACA). As it turned out, ARF1 bound as a dimer only to the TGTCTCccaaaggGAGACA portion of the multimerized probe, suggesting that spacing and possibly orientation of the half-sites were important for binding. Subsequently, it was found that the spacing between the half-sites is critical for ARF1 binding, with the optimal spacing being 7 to 8 nucleotides (Ulmasov and others 1997a). For this reason, the P3(4X) probe is also referred to as the ER7(3X) probe (that is, 3 copies of the everted repeat separated by 7 bps).

Truncated versions of ARF1 were used to define the minimal DBD that was capable of binding to the P3(4X) AuxRE. The minimal DBD in ARF1 consists of about 350 amino acids, and this DBD is conserved in amino acid sequence in all of the other *Arabidopsis* ARF proteins (Ulmasov and others 1997a; Ulmasov and others 1999a). ARFs 10, 16, and 17, which are more closely related to one another than they are to other ARFs, contain an additional stretch of 32 to 36 amino acids in the latter third of their DBDs (see Figure 2). The central regions of ARF DBDs contain about 110 amino acids that are related in sequence to the B3 domain originally identified as a DBD in a truncated version of maize VIVIPAROUS1 or VP1 transcription factor (Suzuki and others 1997). Sequences related to the B3 domain are found in the *Arabidopsis* transcription factors ABI3, FUSCA3, RAV1 and RAV2 (Figure 3). ABI3 probably represents the ortholog of VP1 in *Arabidopsis* (Giraudat and others 1992). Both ABI3 and FUSCA3 also contain a conserved B2 domain (a domain originally identified in VP1) and play roles in the regulation of seed maturation (Luerssen and others 1998; Reidt and others 2000). RAV1 and RAV2 contain two unrelated DBDs, a B3-like domain and an APETELA2 domain that bind with specificity to two unrelated DNA-binding sites (Kagaya and others 1999). The types of genes regulated by RAV1 and RAV2 have yet to be identified. Curiously, a number of additional putative proteins with B3-like domains are found in the *Arabidopsis* genome databases (see Figure 3). Thus, it would appear that the B3 domain, which is unique to plants, has found its way into a number of transcription factors or putative transcription factors in plants (22 ARFs and over 50 other proteins).

## ARF Middle Regions

As noted above, the regions between the amino-terminal DBD and the carboxy-terminal domains III and IV in ARFs (or the region from the carboxy-terminal to the DBD in ARF3 and ARF17), which are referred to as the middle regions, are not highly conserved in amino acid sequences. The middle regions are, however, enriched for serine residues in all ARF proteins, and selected ARFs also contain one or two additional amino acids which are enriched in the middle regions (Figure 2 and Table 1). The most notable of these is the enrichment for glutamine residues in ARF5, −6, −7, −8, and −19. The middle regions of some ARFs are clearly more similar to one another than to other ARFs (for example, ARF7 and ARF19; ARF6 and ARF8; ARF1 and ARF2; ARF3 and ARF4), and these pairs contain short stretches of identical amino acid sequences within their middle regions. All *Arabidopsis* ARF proteins, with the exception of ARF 10, −16, −17 and those with




glutamine-rich middle regions, contain a conserved (R/K)LFGhxL (where h and x represent hydrophobic and a nonconserved amino acid, respectively) in their middle regions, but the function, if any, of this conserved motif is unknown.

At least some ARFs function as transcriptional repressors or activators when expressed from effector plasmids in protoplast transient transfection assays (Ulmasov and others 1999b). The expression of full-length ARF1 or ARF2 driven by the 35S cauliflower mosaic virus promoter results in repression of AuxRE promoter::GUS reporter genes in transfected protoplasts (Ulmasov and others 1999b; Tiwari and others, in press). On the other hand, expression of ARF5, −6, −7, or −8 results in activation of the reporter genes (see Figure 4, effector gene A). ARF19 also contains a glutamine-rich middle region and is likely to be a transcriptional activator. The activation

**Figure 3.** B3 domain proteins. **(A)** Diagrams of the B3 domains found in ARFs, other transcription factors, and putative proteins of *Arabidopsis.* The B3 domain is indicated by the black boxes. The minimal DBDs in ARF1 and ARF17 are indicated by the ovals in the amino-terminal portion of the protein. The two boxes in the carboxy-terminal portion of ARF1 represent domains related to domains III and IV in Aux/IAA proteins. ARF17 is representative of an ARF protein that lacks the carboxy-terminal domains III and IV. The transcription factors ABI3 and FUSCA3 contain two related domains, B2 and B3. The B1 domain in ABI3 is also found in maize VP1. RAV1 and RAV2 transcription factors contain an APETALA2 (AP2) DBD and a B3 DBD. RAV1-like proteins are found in a variety of forms with a B3 domain most similar to RAV1 and RAV2, but lacking an AP2 domain. ABI3-like proteins are larger than RAV-like proteins and contain a B3 domain most similar to ABI3 and FUSCA3, but lack obvious B1 or B2 domains. The REM (*Re*productive *M*eristem; Franco-Zorrilla and others 1999) and REM1-like proteins contain multiple copies of a domain related to the carboxy-terminal half of the B3 domain. **(B)** Amino acid sequence comparison of B3 domains in ARFs, RAV1, RAV-like, ABI3, FUSCA, ABI3-like, and REM1-like proteins from *Arabidopsis.* Some RAV1 and RAV2-like proteins contain an amino-terminal AP2 domain (GenBank Gen Info Identifier numbers GI: 12321505 and 7939559), while others lack the AP2 domain (sequence of the B3 domain in GI: 9758405 is shown; other RAV-like proteins are GI: 4678220, 12322912, 6899895, 3522951, 6715649, and 3695373). Some additional proteins in the size range of RAV proteins contain a much more degenerate B3 domain (GI: 10176695, 10176817, 7270187, and 8886924). ABI3-like proteins are about the same size as ABI3, but contain a centrally located B3 domain and no clear B1 or B2 domains (sequence for the B3 domain in GI: 1946371 is shown; other ABI3-like proteins are GI: 2827635 and 7268952). REM1-like proteins contain multiple copies of the carboxy-terminal half of the B3 domain (sequence for the B3 domain in GI: 9295721 is shown). There are a large number of additional *REM1*-like genes (in excess of 40) that potentially encode proteins containing a degenerate carboxy-terminal half of the B3 domain. Identical amino acids are indicated by black boxes and similar amino acids are indicated by grey boxes.

and repression is observed in both the absence and presence of auxin, but the reporter genes are more active in the presence of auxin.

The middle regions were identified as activation or repression domains by fusing them directly to the yeast GAL4 DBD and testing the chimeric proteins in protoplast transfection assays with GUS reporter genes containing GAL4 DNA-binding sites in their promoters. The middle regions of ARF5, −6, −7, and −8 function as activation domains when targeted to GAL4 DNA-binding sites by the GAL4 DBD whether or not they contain motifs III and IV (Figure 4, effectors G and I; Ulmasov and others 1999a; Tiwari and others, in press). If domains III and IV are present along with the middle region, there is still a slight auxin response with ARF5 through ARF8 (Figure 4, effector gene G; Ulmasov and others 1999b); however, the auxin response is lost if the carboxy-terminal domains are removed (Figure 4; effector gene I; Tiwari and others, in press). The carboxy-terminal domains by themselves are not activators, like the GAL4 DBD alone (Figure 4, effectors H and J; Ulmasov and others 1999b). These latter results suggest that the activation domains of ARFs are not directly responsive to auxin, as are activation domains that are not derived from ARF proteins (for example, VP16; Figure 4, effector gene K).

ARF transactivators that lack a DBD have also been shown to potentiate activation of transcription on auxin-responsive promoter genes, and this activation is dependent on both the middle region and domains III and IV (Figure 4, effectors C and D; Ulmasov and others 1999b). Furthermore, when the carboxy-terminal domain of an ARF activator is transferred to a heterologous activation domain, like VP16, activation is observed on AuxRE promoter:: GUS reporter genes (Figure 4; effector E); however, activation is not observed if the carboxy-terminal domain is derived from an ARF repressor, like ARF1 (Figure 4, effector gene F). Taken together, these results suggest that the transcriptional activation domains reside in the middle region, and that auxin-responsiveness requires the carboxy-terminal domains III and IV of ARF proteins.

## ARF Carboxy-Terminal Domains

All ARFs but ARF3 and ARF17 contain a carboxy-terminal domain related to domains III and IV in Aux/IAA proteins (Ulmasov and others 1997a; Ulmasov and others 1999a). The carboxy-terminal domains in both ARF and Aux/IAA proteins are protein-protein interaction domains that allow the homo- and heterodimerization of ARFs and the heterodimerization among ARF and Aux/IAA proteins (Ulmasov and others 1997a b, 1999a; Kim and others 1997; Ouellet and others 2001). It has not been ruled out, however, that ARF and Aux/IAA proteins may function together as higher order multimeric complexes. In fact, there is some evidence that multimeric complexes of Aux/IAA proteins can form *in vitro* (Morgan and others 1999; Ouellet and others 2001). Domains III and IV in some ARF proteins increase the *in vitro* binding (that is, gel mobility shift assays) of these ARFs to palindromic TGTCTC AuxREs by facilitating the formation of dimers that

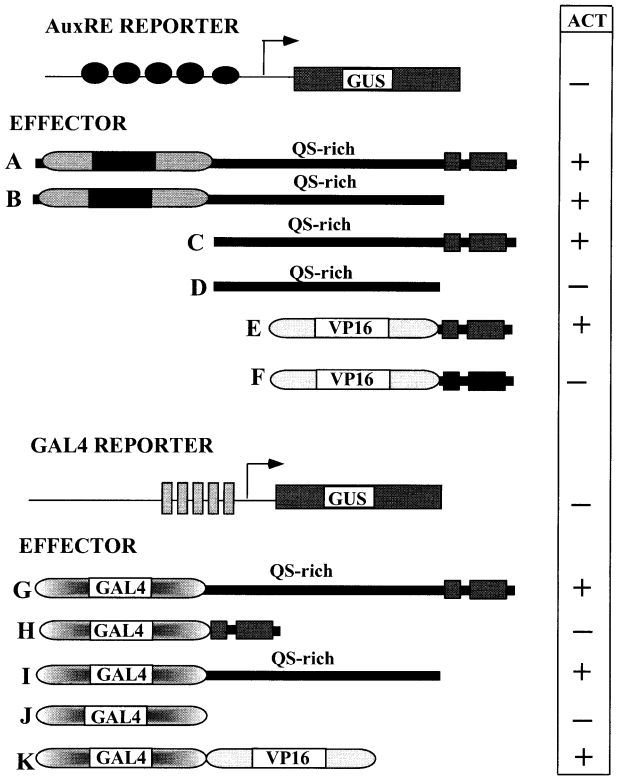



**Figure 4.** The QS-rich middle region of ARFs is an activation domain, and the carboxy-terminal domain is required for auxin-responsiveness. Generalized results from experiments with carrot protoplasts transfected with GUS reporter genes and effector genes are summarized for reporter gene activation (ACT). The two GUS reporter genes contain multiple copies of AuxREs (for example, DR5, ER7) or GAL4 binding sites. All effector plasmids contain a cauliflower mosaic virus promoter to drive their expression. Effector genes A–F were used with the AuxRE reporter gene, and effector genes G–K were used with the GAL4 reporter gene. Effector gene A is a full-length ARF activator with a middle region rich in glutamine (Q) and serine (S). Effector gene B is a carboxy-terminal truncation of effector gene A and is missing domains III and IV. Effector gene C is an amino-terminal truncation of effector gene A and is missing the DBD. Effector gene D is missing both the DBD and domains III and IV. Effector gene E contains domains III and IV of effector gene A fused to the VP16 activation domain. Effector gene F contains domains III and IV of an ARF repressor (for instance, ARF1) fused to the VP16 activation domain. Effector genes G to I have the yeast GAL4 DBD fused in frame to an ARF activator lacking its DBD (effector gene G), domains III and IV of an ARF activator (effector gene H), and the middle region of an ARF activator (effector gene I). Effector gene J is the GAL4 DBD alone. Effector gene K is the GAL4 DBD fused in frame to the VP16 activation domain. Under the ACT column, a + indicates that the effector gene functions as a activator with the reporter gene, and a – indicates that the effector gene shows no activator activity with the reporter gene.

occupy the two half sites in these AuxREs (Ulmasov and others 1999a).

## Nuclear Localization Signals

With the PSORT program for predicting subcellular localization of plant proteins, only a subset of the ARF proteins are predicted to be nuclear localized, and these include ARF 1, –2, –3, –4, –9, –10, –13, and –16. Those ARFs with Q-rich transcriptional activation domains (that is, ARF5, –6, –7, –8, and –19) are, in fact, predicted to be localized in the cytoplasm; however, the PSORT predictions may be misleading based on nuclear localization assays conducted with ARF5. Hartdke and Berleth (1998) reported that the ARF5/MP (MONOPTEROS) protein contains five putative nuclear localization signals (NLSs) and all of these reside within the DBD (which encompasses about 350 amino acids as defined for ARF1; Ulmasov and others 1997a). Two of the putative NLSs resemble the MATα-class and one resembles the bipartite class of NLSs (Raikhel 1992). Constructs containing the amino-terminal three quarters of the ARF5/MP DBD (that is, three putative NLSs) fused to the GUS reporter gene were shown to be localized to the nucleus in transient expression assays in onion epidermal cells (Hartdke and Berleth 1998). GUS fusion constructs lacking the amino-terminal three quarters of the DBD, but containing two other putative NLSs, showed less selectivity in localizing to the nucleus; nevertheless, some GUS staining was observed in nuclei as well as the cytoplasm. Nuclear localization of other ARFs has not been tested experimentally.

That ARFs may not require the DBD for nuclear localization is suggested from experiments of Ulmasov and colleagues (1999b) that showed that ARFs without DBDs can still activate or repress auxin-responsive reporter gene expression as long as they contain a functional carboxy-terminal domain. It is possible that ARFs contain NLSs outside of the DBD or lack NLSs, but can be escorted into nuclei by associating with ARFs, Aux/IAA proteins, other transcription factors, or nuclear proteins that contain functional NLSs. Another possible interpretation of the results reported by Ulmasov and coworkers (1999b) is that ARFs lacking DBDs remain in the cytoplasm and sequester Aux/IAA repressors via carboxy-terminal domain interactions, thus resulting in activation of auxin-responsive reporter genes.

## ARF Gene Expression

The gene expression patterns of *ARF1* through *ARF9* have been examined by northern blotting (Hardtke

and Berleth 1998; Ulmasov and others 1999a). Based on this analysis, these *ARF* genes are ubiquitously expressed in mature organs, including roots, rosette leaves, cauline leaves, flowers, and siliques. The genes are also expressed in suspension culture cells. *ARF* transcripts do not increase in abundance in plants or seedlings treated with auxin or the protein synthesis inhibitor, cycloheximide (Ulmasov and others 1999a; Nemhauser and others 2000; G. Hagen and J.J. Guilfoyle, unpublished results). This contrasts with the auxin-responsiveness and cycloheximide-responsiveness of most *Aux/IAA* genes (Abel and others 1995), and suggests that, although ARF and Aux/IAA proteins are related to one another in their carboxy-terminal domains, regulation of *ARF* and *Aux/IAA* gene expression is fundamentally different.

Although Northern blotting experiments did not reveal distinct patterns of gene expression for the ARF proteins examined, *in situ* hybridization with *ARF3* and *ARF5* probes has demonstrated some temporal and developmental cell and tissue-specific gene expression patterns for these two *ARF* genes (Hardtke and Berleth 1998; Sessions and others 1997). With *in situ* hybridization, *ARF5/MP* transcripts were detected in all subepidermal cells of early globular embryos (Hardtke and Berleth 1998). As embryogenesis progressed, the *ARF5/MP* expression pattern became more restricted and confined to provascular tissues of differentiating vascular strands in embryos that were nearing maturity. Likewise, the pattern of *ARF5/MP* expression in developing shoots, leaves, and floral organs started out broadly, but became restricted to developing or differentiated vascular tissues as the organs matured. *ARF5/MP* transcripts were also only detected in the vasculature of mature roots. With *ARF3/ETTIN, in situ* hybridization was carried out during flower development (Sessions and others 1997). These studies showed that the *ARF3/ETTIN* gene was not uniformily expressed in floral meristems and developing floral organs, but was expressed in a complex pattern within defined regions of floral meristems and developing organs (that is, stamens, petals, and gynoecia). Like observations made with the *ARF5/MP* gene, expression of the *ARF3/ETTIN* gene became localized to procambial and vascular strands as developing floral organs matured. The *in situ* hybridization studies, while limited to two *ARF* genes, suggest that at the resolution of cells and tissues, *ARFs* may display highly specific patterns of gene expression during plant development. In the future, *in situ* hybridization with other *ARF* probes and analysis of *ARF* promoter::reporter gene constructs should reveal more about the temporal and developmental patterns for *ARF* gene expression.

## ARF Mutants

Mutant screens for defects in floral organ development, vascular tissue continuity, and photomorphogenesis and differential growth have led to the identification of mutant genes for *ARF3/ETTIN, ARF5/MP,* and *ARF7/NPH4,* respectively (Sessions and others 1997; Hardtke and Berleth 1998; Harper and others 2000). *ettin* or *ett* mutant plants display defects only in the flowers, with increased numbers of sepals and petals and decreased numbers of stamens (Sessions and others 1997). Defects are also observed in the structures of anthers and gynoecial tissues (Sessions and others 1997; Sessions and Zambryski 1995; Sessions 1997). Vascular patterning and anatomy are altered in *ettin* gynoecia, but are normal in sepals, petals, and stamens. The tissue-specific expression patterns along with the *ettin* mutant phenotypes suggest that ARF3/ETTIN plays an important role in patterning development within groups of cells found in floral meristems and floral organs (Sessions and others 1997). *ettin* mutant phenotypes associated with gynoecium patterning are mimicked by the transient application of N-1-naphthylphthalamic acid (NPA) to developing *Arabidopsis* flowers of wild-type plants (Nemhauser and others 2000). This latter study indicates that disruption of polar auxin transport leads to defects in floral organ development, especially in regard to the gynoecium, and suggests that auxin may function as a morphogen in patterning the gynoecium. Regulation of selected auxin response genes by the ARF3/ETTIN transcription factor during gynoecial development is likely to be required for normal tissue patterning in this organ.

Mutations in the *ARF5/MP* gene result in defects in the formation of vascular strands and with the initiation of the body axis in early stage embryos (Berleth and Jurgens 1993; Przemeck and others 1996; Hardtke and Berleth 1998). Severe *mp* mutants lack centrally located provascular cells within the basal domain of the embryo, and the primary root and hypocotyl are absent in embryos and seedlings. Postembryonic development can continue in some *mp* mutant plants that are able to form adventitous roots, and these plants have organs (for example, leaves) with reduced vasculature and incompletely differentiated and interconnected vascular strands. Inflorescences have spike-like structures, resembling those observed with *pin formed* mutant plants that are impaired in polar auxin transport or

with wild-type plants that have been treated with auxin transport inhibitors (for instance, NPA). Regulation of auxin response genes by the transcriptional activator ARF5/MP may involve localized gradients in auxin concentrations or an apical-basal auxin flux, and the MP transcription factor may be required for normal vascular differentiation and hypocotyl/root axis formation.

*nph4* mutant plants show defects in hypocotyl and root phototropism as well as hypocotyl gravitropism (Liscum and Briggs 1996). Defects in apical hook and phytochrome-dependent hypocotyl curvature are also observed in these mutant plants (Stowe-Evans and others 1998). Interestingly, *nph4* mutant plants are resistant to exogenously applied auxin, and a number of early auxin response genes (that is, *SAUR, GH3,* and *Aux/IAAs*) show highly reduced expression levels in both the absence and presence of exogenous auxin. Identification of *ARF7* as the mutant gene responsible for *nph4* phenotypes indicates a role for the NPH4 transcription factor in regulating auxin response genes required for differential growth (Harper and others 2000).

## Model for the Roles of ARF and AUX/IAA Proteins in Regulating Auxin Responsive Gene Expression

A substantial amount of preliminary evidence implicates ARF and Aux/IAA proteins as transcription factors that regulate primary/early auxin response genes. This evidence comes from genetic, biochemical, and molecular biological experimental approaches. The precise details/mechanisms for the transcriptional regulation of auxin response genes by ARFs and Aux/IAA proteins is still a mystery because of the large number of possible interactions (that is, dimerization or multimerization) that might occur among the 22 ARF and 29 Aux/IAA proteins. Additional transcription factors that bind to composite elements along with ARF proteins must also play a role in auxin-responsive gene transcription. These transcription factors may target specific ARFs to different types of composite AuxREs (reviewed by Guilfoyle and others 1998b; Guilfoyle 1999).

Based on available evidence, we have formulated the following model (that is, extensions of previous models discussed in Guilfoyle and others 1998a, 1998b; Guilfoyle 1998) for the regulation of primary/early auxin response genes that contain TGTCTC AuxREs (see Figure 5). When auxin concentrations are low within cells or tissues, primary/early auxin response genes are repressed. Repression could result from dimerization (for simplicity, we restrict our discussion to dimers, but it is possible that higher order multimers might function in place of dimers) of Aux/IAA repressors with ARF transcriptional activators. Heterodimerization might occur between Aux/IAA proteins and ARFs that are unbound (Ulmasov and others 1997a; Kim and others 1997; Ouellet and others 2001) or bound to TGTCTC AuxRE target sites. Dimerization of an Aux/IAA protein with an ARF transactivator that is not bound to its DNA target site might prevent the ARF complex from binding to its target AuxRE, while dimerization of an Aux/IAA protein to a DNA-bound ARF transactivator might result in active repression of transcription by the Aux/IAA protein. Transfection experiments with protoplasts have shown that overexpression of some 20 different Aux/IAA proteins results in repression of auxin-responsive reporter genes (Ulmasov and others 1997b; Tiwari and others in press), but the mechanism for this repression has not been determined.

Selected ARFs could be recruited to specific composite TGTCTC AuxREs by interacting with coupling factors that bind to the specific composite AuxRE. Selective binding to TGTCTC AuxREs by ARFs could also be dictated by the cell/tissue-specific expression patterns of individual ARFs or coupling factor proteins. Selective Aux/IAA and ARF interactions might be dictated by their cell/tissue-specific expression patterns as well as their relative affinities for one another (that is, there may be selectivity in the types of ARF-Aux/IAA heterodimers/multimers that form).

When auxin concentrations become elevated, primary/early auxin response genes are rapidly derepressed/activated, as early as 2 to 5 minutes after exogenous auxin application (Abel and others 1995; Hagen and Guilfoyle 1985; McClure and others 1989). We propose that an early event in derepression might be the dissociation of Aux/IAA repressors from their ARF counterparts, concomitant with or prior to degradation of the Aux/IAA proteins by the ubiquitin/proteosome pathway (Figure 5; see Gray and Estelle 2000; Worley and others 2000). Phytochrome(s) might play a role in marking Aux/IAA proteins for degradation via the proteasome pathway by phosphorylating Aux/IAA proteins at specific sites in their amino-terminal regions (Colon-Carmona and others 2000). The phosphorylaton of Aux/IAA proteins by the kinase activity associated with phytochromes might provide a link between light signaling and auxin signaling pathways (Reed and others 1998; Tian and Reed 1999; Colon-Carmona and others 2000). Another protein kinase that could play a role in targeting Aux/IAA proteins

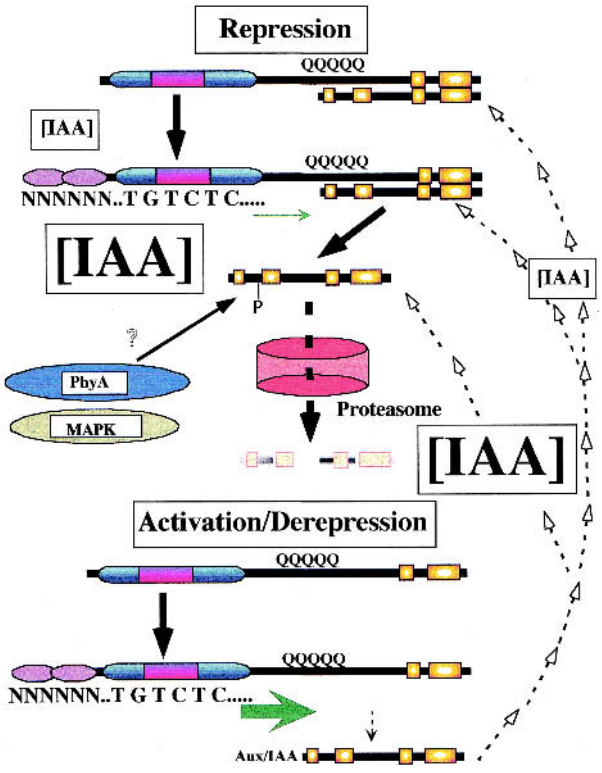



**Figure 5.** Model for the roles of ARF and Aux/IAA proteins in regulating early auxin response genes. When auxin concentrations are low, ARF activators are associated with Aux/IAA repressors through their conserved carboxy-terminal dimerization domains, and early auxin response genes are repressed. ARF and Aux/IAA dimers might form on or off AuxRE target sites. When auxin concentrations are high, Aux/IAA repressors dissociate from ARF activators, allowing the ARFs to reach their AuxRE targets or become active on their DNA targets, and early auxin response genes (for example, *Aux/IAA, GH3,* and *SAUR* genes) are derepressed/activated. Early response genes are continuously transcribed as long as auxin concentrations remain high. Concomitant with or subsequent to their dissociation from ARFs, Aux/IAA proteins are possibly phosphorylated by phytochrome (PhyA) or a MAP kinase (MAPK) and marked for degradation by the ubiquitin-proteasome pathway (? indicates that the protein kinase, if any, that marks the Aux/IAA proteins for degradation is unclear). It is also possible that some other posttranslational modification of Aux/IAA proteins besides phosphorylation is required for targeting the Aux/IAA proteins to the proteasome. For simplicity, the E3 ubiquitin ligase $SCF^{TIR1}$ (Gray and Estelle 2000) and the COP9 signalosome (Schwechheimer and others 2001), which play roles in targeting Aux/IAAs to the proteasome for degradation, are not included in the diagram. Rapid degradation of Aux/IAA proteins via the ubiquitin pathway would prevent them from reassociating with ARF activators as long as auxin concentrations remain elevated. When auxin concentrations become low, Aux/IAA proteins are degraded less rapidly and increase in abundance, eventually reaching levels sufficient to complex with ARF activators and repress transcription by a feedback mechanism. Green arrows represent relative levels of transcription. NNNNNN represents the coupling element in a composite AuxRE, and the purple ovals represent putative coupling transcription factors. High and low auxin concentrations are indicated by the large and small bracketed IAA, respectively.

to the proteasome is a MAP kinase that is rapidly induced by auxin (Mockaitis and Howell 2000).

Once the Aux/IAA proteins dissociate from the ARF transcription factor (that is, if the ARF is bound to its target site), activation might be further potentiated by the binding of additional ARF transcriptional activators (by dimerization/multimerization through domains III and IV) to the DNA-bound ARF (Ulmasov and others 1999b). Our model does not explain what role ARF transcriptional repressors such as ARF1 and ARF2 might play in regulating auxin response genes; however, the ARF repressors could repress transcription in a fashion similar to the Aux/IAA proteins (that is, by binding to ARF transcriptional activators and repressing their activator function). Alternatively, ARF repressors may function on auxin response genes that are down-regulated when auxin concentrations are high. It is also possible that some auxin response genes contain AuxREs that are occupied by ARF repressors when auxin concentrations are low, and these are displaced from their AuxRE target sites by ARF activators when auxin concentrations are high.

At least some *Aux/IAA* genes contain TGTCTC AuxREs, and these genes might be regulated by auxin as described above, resulting in increased abundance of the *Aux/IAA* mRNAs and proteins at high auxin concentrations. As the Aux/IAA proteins increase in abundance in response to auxin, they might feed back on their own genes as well as other genes containing TGTCTC AuxREs to down-regulate their expression. This would create a feedback loop, resulting in a transient derepression of these early response genes and their subsequent repression. This type of feedback repression would only occur when auxin returns to low concentrations; otherwise, the Aux/IAA proteins would be degraded as rapidly as they are produced. This latter assumption is made because in the continued presence of high exogenous auxin concentrations, early response genes remain activated for hours (Abel and others 1995; Hagen and others 1984; McClure and Guilfoyle 1989; Theologis and others 1985; Walker and Key 1982). In this model, the Aux/IAA proteins would play a pivotal role in regulating early response genes by dimerizing with ARFs which are bound or unbound to TGTCTC AuxRE target sites. Dimerization of Aux/IAA repressors with ARF acti-

vators would confer repression on early/primary auxin response genes when auxin concentrations are below a theshhold required for derepression. Derepression of early response genes would involve an auxin-dependent enhancement of Aux/IAA protein degradation through the ubiquitin-proteasome pathway.

## Future Directions

Many questions remain unanswered about the regulation of primary/early auxin response genes. How are selected ARFs, Aux/IAA proteins, and other transcription factors targeted to specific AuxREs? Do Aux/IAA proteins possess intrinsic repressor activity or do they prevent ARFs from functioning on their target sites by some other means? Is there selectivity in the types of Aux/IAA and ARF homo- and heterodimers (or multimers) that can form and does this determine the types of genes that respond to auxin? What role, if any, do Aux/IAA homodimers/multimers or Aux/IAA heterodimers/multimers play in auxin-responsive transcription? How important are the temporal and tissue-specific expression patterns of the ARF and Aux/IAA proteins in dictating the types of interactions that occur in cells and tissues? Do ARFs themselves bind auxin, or what is the receptor that binds to auxin and transduces a signal to the nucleus to activate or repress primary/early auxin response genes?

One especially important question is, What is/are the site(s) of auxin action in the transition from repression to derepression/activation of early auxin response genes? Several sites are possible targets for auxin action. Auxin could regulate the binding of ARFs to their DNA target sites or could exert an effect on the activation domain of ARF transcriptional activators by regulating interactions of these ARFs with co-activators or the general transcription machinery. Auxin might also have an effect on the ARF and Aux/IAA dimers that form, favoring the dissociation of Aux/IAAs from ARF transcriptional activators under high auxin concentrations. Lastly, auxin might regulate the degradation of Aux/IAA proteins, resulting in rapid degradation under high auxin concentrations. The latter two possibilities may be interconnected, in that depletion of Aux/IAA proteins following auxin treatment would likely upset the balance of interactions that take place among Aux/IAA and ARF proteins. While there is some evidence suggesting that auxin does not alter the life times of Aux/IAA proteins (Abel and others 1994), the connection between auxin action and the ubiquitin pathway, based on genetics (Gray and Estelle 2000) and biochemistry (Worley and others 2000), make these latter possibilities attractive and worthy of further investigation.

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