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# ARF7 and ARF19 Regulate Lateral Root Formation via Direct Activation of *LBD/ASL* Genes in *Arabidopsis* [W]

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**Lateral root formation in *Arabidopsis thaliana* is regulated by two related AUXIN RESPONSE FACTORs, ARF7 and ARF19, which are transcriptional activators of early auxin response genes. The *arf7 arf19* double knockout mutant is severely impaired in lateral root formation. Target-gene analysis in *arf7 arf19* transgenic plants harboring inducible forms of ARF7 and ARF19 revealed that ARF7 and ARF19 directly regulate the auxin-mediated transcription of *LATERAL ORGAN BOUNDARIES-DOMAIN16/ASYMMETRIC LEAVES2-LIKE18* (*LBD16/ASL18*) and/or *LBD29/ASL16* in roots. Overexpression of *LBD16/ASL18* and *LBD29/ASL16* induces lateral root formation in the absence of ARF7 and ARF19. These LBD/ASL proteins are localized in the nucleus, and dominant repression of LBD16/ASL18 activity inhibits lateral root formation and auxin-mediated gene expression, strongly suggesting that these LBD/ASLs function downstream of ARF7- and ARF19-dependent auxin signaling in lateral root formation. Our results reveal that ARFs regulate lateral root formation via direct activation of *LBD/ASL*s in *Arabidopsis*.**

## INTRODUCTION

Although the branched root systems of higher plants are hidden underground, they play an essential role in whole plant growth and development. The root system of higher plants consists of an embryonic primary root and postembryonic developed lateral roots and adventitious roots. In dicot plants, lateral root formation is crucial for maximizing a root system's ability to absorb water and nutrients as well as to anchor plants in the soil. The plant hormone auxin, which mediates a variety of physiological processes (Davies, 1995), has long been known to promote lateral root formation (Torrey, 1950; Blakely et al., 1988; Laskowski et al., 1995). However, the molecular details of auxin action in this process remain largely unknown.

In *Arabidopsis thaliana*, lateral roots are initiated by the auxin-dependent local activation of pericycle cells at the xylem poles (Casimiro et al., 2001, 2003). Delineating the molecular events that lead to lateral root initiation is a prerequisite for a thorough understanding of how auxin modulates developmental functions. Control of these processes by auxin involves the complex regulation of auxin biosynthesis and transport and the ability of cells to respond to auxin in an appropriate manner. Particularly little is known about the auxin signaling cascade during the onset of lateral root initiation.

At the molecular level, auxin rapidly alters transcript levels of numerous genes (Abel and Theologis, 1996). Thus, the effects of auxin, including the promotion of lateral root formation, may be mediated directly through changes in gene expression. Transcriptional regulation of auxin-regulated genes is dependent on two related families of transcriptional regulators, AUXIN RESPONSE FACTORs (ARFs) and AUXIN/INDOLE-3-ACETIC ACIDs (Aux/IAAs). ARFs bind to the auxin response elements (AuxREs) in the promoter region of early auxin response genes and activate or repress their transcription (reviewed in Guilfoyle and Hagen, 2001). Of the 23 ARFs in the *Arabidopsis* genome (Liscum and Reed, 2002), five members with a Gln-rich sequence in the middle region (ARF5/MONOPTEROS [MP], ARF6, ARF7/NONPHOTOTROPIC HYPOCOTYL4 [NPH4], ARF8, and ARF19) function as transcriptional activators of auxin-responsive genes (Ulmasov et al., 1999; Tiwari et al., 2003; Wang et al., 2005). By contrast, Aux/IAA proteins, which are encoded by 29 genes in *Arabidopsis*, negatively modulate auxin-regulated gene expression as transcriptional repressors through heterodimerization with the ARF transcription activators (Tiwari et al., 2001, 2003, 2004).

Aux/IAA proteins are labile in planta; they are subject to proteolysis through the ubiquitin-mediated pathway (Gray et al., 2001). Importantly, auxin promotes this degradation pathway. Recent studies have revealed that binding of auxin to the F-box protein TRANSPORT INHIBITOR RESPONSE1 (TIR1) or the related AUXIN RECEPTOR F-BOX PROTEINS (AFBs) accelerates the degradation of Aux/IAAs by promoting their interaction with the $SCF^{TIR1/AFBs}$ ubiquitin-ligase complex (Dharmasiri et al., 2005a, 2005b; Kepinski and Leyser, 2005), thereby derepressing the activity of ARF transcriptional activators. Thus, auxin signaling is largely dependent on the proteolytic regulation of Aux/

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IAAs. Degradation of Aux/IAAs is dependent on the presence of a conserved domain II, which mediates interaction with the $SCF^{TIR1/AFBs}$ complex. Gain-of-function mutations in domain II of several different Aux/IAAs stabilize the corresponding proteins and cause pleiotropic defects in auxin-regulated growth and development (reviewed in Reed, 2001; Yang et al., 2004).

Given the no (or severely reduced) lateral root phenotype of several Aux/IAA gain-of-function mutants, such as *iaa14/solitary root* (*slr*) (Fukaki et al., 2002), *iaa3/short hypocotyl or suppressor of hy2* (*shy2*) (Tian and Reed, 1999), *iaa19/massugu2* (*msg2*) (Tatematsu et al., 2004), and *iaa28* (Rogg et al., 2001), it is evident that Aux/IAAs inhibit the activity of specific ARFs required for lateral root formation. Forward and reverse genetic approaches have identified ARF7 and ARF19 as key components in a developmental pathway regulating lateral root formation. *arf7 arf19* double mutants exhibit a severely reduced lateral root formation phenotype not observed in *arf7* and *arf19* single mutants, indicating that lateral root formation is redundantly regulated by these two ARF transcription activators (Okushima et al., 2005; Weijers et al., 2005; Wilmoth et al., 2005; Li et al., 2006). Furthermore, both ARF7 and ARF19 interact with IAA14/SLR, IAA3/SHY2, IAA19/MSG2, and IAA28 in yeast (Tatematsu et al., 2004; Fukaki et al., 2005; Weijers et al., 2005; our unpublished data), and their expression patterns are highly overlapped (Fukaki et al., 2002; Okushima et al., 2005). Thus, the auxin-signaling pathway originating from ARF7 and ARF19 controls lateral root formation together with several Aux/IAA transcriptional repressors, such as IAA14/SLR, IAA3/SHY2, IAA19/MSG2, and IAA28.

Identification of the genes regulated by ARF7 and ARF19 during lateral root formation is essential to reveal the extent and details of the transcriptional network. Because of their well-characterized properties as transcriptional activators of auxin-regulated genes, we were able to take advantage of genome-wide expression analysis to examine alterations of auxin-mediated gene expression of the mutant compared with the wild type. As expected, auxin-induced gene expression is globally and severely impaired in the *arf7 arf19* double mutant (Okushima et al., 2005). Based on this microarray data, we have identified >100 ARF7 and ARF19 target gene candidates. However, only a subset of these genes is expected to be direct targets, and it is not certain that all direct target genes are biologically important for lateral root formation.

Here, we describe the identification of *LATERAL ORGAN BOUNDARIES-DOMAIN/ASYMMETRIC LEAVES2-LIKE* (*LBD/ASL*) genes as direct regulatory targets of ARF7 and ARF19 in lateral root formation. Our results provide a better framework for understanding how the auxin signal is transmitted by ARF transcription factors during lateral root formation in higher plants.

## RESULTS

### ARF7 and ARF19 Are Required for the Initiation of Lateral Root Formation

Previous studies have shown that ARF7 and ARF19 play important roles in lateral root formation (Okushima et al., 2005; Wilmoth et al., 2005). However, it is not clear which stage is mediated by ARF7 and ARF19 during lateral root formation. Therefore, we examined the expression of lateral root initiation markers in primary roots of *arf7 arf19*. Expression of End199, the marker line for stage II and subsequent stages of lateral root primordium development (Malamy and Benfey, 1997), was hardly detected in 4- to 7-d-old *arf7 arf19* seedlings, whereas the number of primordia with End199 activity increased in wild-type seedlings as the primary roots lengthened (Figure 1A). These results indicate that lateral root formation is strongly inhibited at the very early stage of initiation in *arf7 arf19*. Because auxin sensitivity is severely impaired in the roots of the *arf7 arf19* double mutant (Okushima et al., 2005; Wilmoth et al., 2005), we next examined the auxin-induced pericycle division. To monitor auxin-induced pericycle cell division during synchronized lateral root initiation, we used the lateral root–inducible system (Himanen et al., 2002) and the G2/M transition–associated $Pro_{CycB1;1}$*:CycB1;1(NT)-GUS* reporter (Colón-Carmona et al., 1999), which enables the detection of cells undergoing mitosis. No *CycB1;1(NT)-GUS* expression was observed in the pericycles of either wild-type or *arf7 arf19* 3-d-old seedlings grown on medium containing 10 μM naphthylphthalamic acid (Figures 1B and 1D). However, strong β-glucuronidase (GUS) activity was detected in the pericycle cells of wild-type seedlings after they were transferred to 10 μM 1-naphthylacetic acid (NAA) for 12 h (Figure 1C), as reported previously (Himanen et al., 2002; Vanneste et al., 2005). By contrast, no GUS activity was induced in pericycle cells of *arf7 arf19* seedlings treated with NAA for 12 h (Figure 1E), indicating that 12 h of auxin treatment cannot activate pericycle cell division in *arf7 arf19*. These observations indicate that auxin-induced pericycle cell division for lateral root initiation is strongly inhibited in the *arf7 arf19* double mutant. Thus, ARF7 and ARF19 are required early in lateral root formation, probably at the initiation stage.

Consistent with the functions of ARF7 and ARF19 as transcriptional activators (Wang et al., 2005; Wilmoth et al., 2005), ARF7- and ARF19-GFP (for Green Fluorescent Protein) fusion proteins were localized in nuclei (Figures 1F and 1G). ARF7- and ARF19-GFP rescued the phenotype of the *arf7 arf19* double mutant when driven by their own promoters (data not shown), strongly suggesting that ARF7 and ARF19 proteins function in the nucleus.

### *LBD16*, *LBD29*, and *LBD33* Genes Are Candidate Targets for ARF7 and ARF19 in Lateral Root Formation

If ARF7 and ARF19 are transcriptional activators, as currently thought (Wang et al., 2005; Wilmoth et al., 2005), they should directly regulate auxin-induced genes rather than auxin-repressed genes. We previously identified ARF7 and ARF19 target gene candidates among the genes that are induced by auxin in wild-type seedlings but not in *arf7 arf19* (Okushima et al., 2005). Among these candidates, we focused on a set of closely related genes that encode LBD/ASL protein family members. *LBD16/ASL18*, *LBD17/ASL15*, *LBD18/ASL20*, *LBD29/ASL16*, and *LBD33/ASL24* (hereafter referred to as *LBD16*, *LBD17*, *LBD18*, *LBD29*, and *LBD33*, respectively) are induced by auxin in wild-type seedlings but not in *arf7 arf19* (Figure 2K) (Okushima et al., 2005). *LBD33* was not identified using the original criteria for auxin-induced genes. However, a more lenient significance

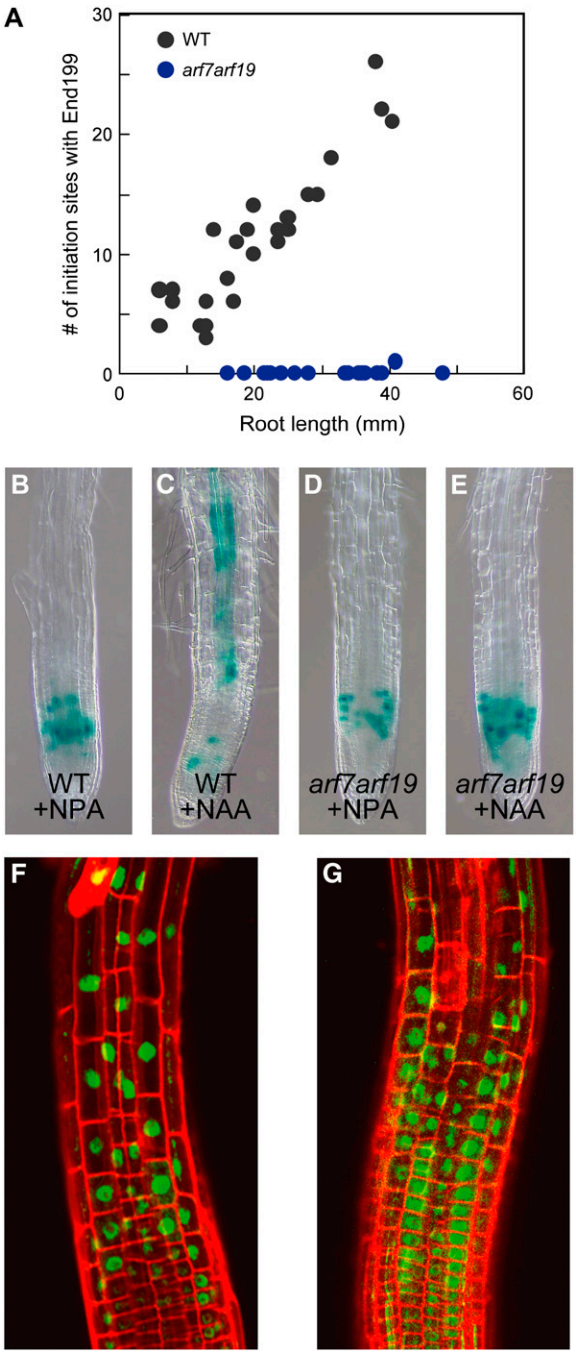


**Figure 1.** Auxin-Mediated Lateral Root Initiation Is Severely Impaired in the *arf7 arf19* Double Mutant.

**(A)** Numbers of lateral root initiation sites in wild-type and *arf7 arf19* roots. Lateral root primordia with End199 activity were counted. More than 19 samples from 4- to 7-d-old seedlings grown on agar plates were examined for each genotype. Each dot indicates the length of the primary root versus the number of initiation sites with End199 activity.

**(B)** to **(E)** Expression of the $Pro_{CycB1;1}$*:CycB1;1(NT)-GUS* reporter in roots of wild-type (**[B]** and **[C]**) and *arf7 arf19* (**[D]** and **[E]**) seedlings germinated on 10 μM naphthylphthalamic acid (NPA) and transferred 72 h after germination to 10 μM NAA for 12 h. GUS activity in the root tip was detected in all samples.

**(F)** and **(G)** Nuclear localization of ARF7-GFP and ARF19-GFP fusion proteins. Cells were counterstained with propidium iodide.

**(F)** Fluorescence in the root epidermal cells of transgenic *arf7 arf19* plants expressing ARF7-GFP under the control of the cauliflower mosaic virus (CaMV) 35S promoter.

**(G)** Fluorescence in the root epidermal cells of transgenic *arf7 arf19* plants expressing ARF19-GFP under the control of the *ARF19* promoter.

cutoff ($P < 0.01$; originally we used $P < 0.001$) placed it in the gene set of statistically valid auxin-induced genes (data not shown). Interestingly, these five *LBD* members cluster together within the same clade of the phylogenetic tree (Iwakawa et al., 2002) (Figure 2A), suggesting that they are closely related at the sequence level as well as in terms of auxin-mediated expression properties. In addition, ~2-kb promoter regions of the five candidate *LBD* genes all have TGTCTC or GAGACA sequences that may function as AuxREs, suggesting that they could be regulated directly by ARF7 and/or ARF19.

Among the five *LBD* genes, *LBD16*, *LBD29*, and *LBD33* are the most likely targets of ARF7 and ARF19 during lateral root formation, because they are expressed primarily in roots (Shuai et al., 2002) (see Supplemental Figure 1 online). In 6-d-old $Pro_{LBD16}$*:GUS* transformant seedlings, strong GUS activity was detected in root stele and lateral root primordia (Figure 2B). A similar but weaker GUS expression pattern in root stele and lateral root primordia was detected in $Pro_{LBD29}$*:GUS* seedlings (Figure 2C). In $Pro_{LBD33}$*:GUS* seedlings, GUS activity was localized in lateral root primordia (Figure 2D) and was hardly detected in root vasculature. Expression of all three reporter lines was induced in response to exogenous auxin (Figures 2E to 2J), but their induction profiles were partially distinct. For example, $Pro_{LBD16}$*:GUS* was strongly expressed in vascular tissues of the mature root region, but auxin-mediated induction was observed in the elongation zone only (Figures 2E and 2F). By contrast, auxin treatment led to increased expression of $Pro_{LBD29}$*:GUS* and $Pro_{LBD33}$*:GUS* in the steles of mature regions in primary and lateral roots (Figures 2G to 2J). The previous observation that both *ARF7* and *ARF19* are expressed in the root stele (Okushima et al., 2005; Wilmoth et al., 2005) supports the observation that auxin-mediated induction of these *LBD* genes is dependent on ARF7 and ARF19 function.

### *LBD16* and *LBD29* Are Primary Auxin Response Genes

RT-PCR analysis confirmed the auxin-mediated induction (1 μM NAA, 2.5 h) of *LBD16*, *LBD29*, and *LBD33* and its strong dependence on ARF7 and ARF19 function (Figure 2K). Considering the current model of ARF transcriptional activators as regulators of primary auxin response genes, their direct target genes would be expected to have expression properties similar to those of previously identified primary auxin response genes (e.g., several *Aux/IAA* genes). As reported previously (Abel et al., 1995), *IAA5*, one of the typical primary response *Aux/IAA* genes, was induced significantly by the protein synthesis inhibitor cycloheximide (CHX) (Figure 2K) and responded to exogenous

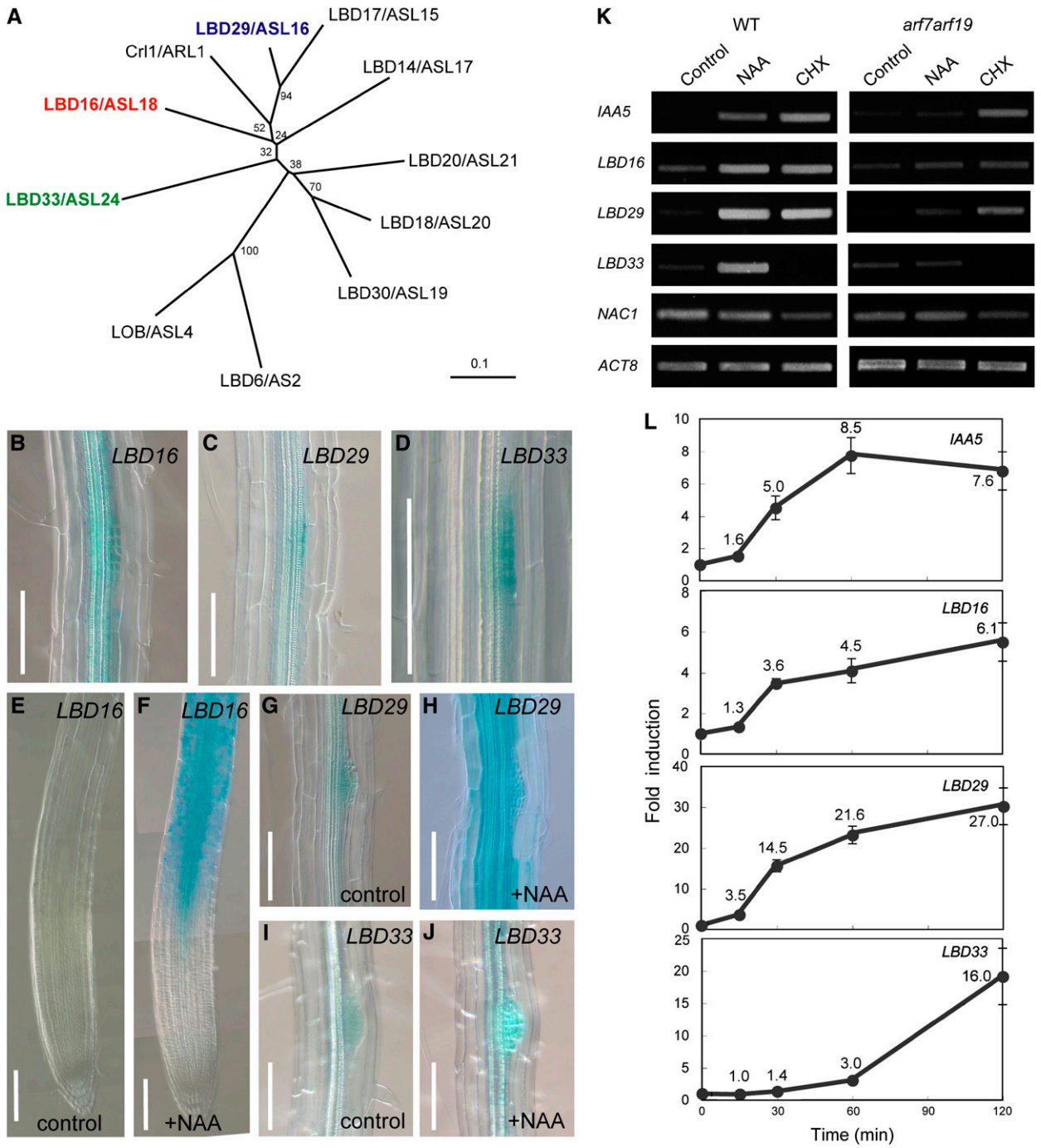


**Figure 2.** Expression Profiles of Auxin-Responsive *LBD/ASL* Genes.

**(A)** Phylogenetic analysis of 10 *Arabidopsis* LBD/ASLs and rice (*Oryza sativa*) CRL1/ARL1 based on the LOB/AS2 domain. An unrooted dendrogram was obtained using the neighbor-joining method. Bootstrap values ($n = 1000$) are indicated at the nodes of the tree.

**(B)** to **(J)** Analysis of *LBD* promoter activity in roots of 6-d-old transgenic seedlings. Seedlings were treated with (+NAA) or without (control) 1 μM NAA for 2.5 h before GUS staining (incubation time, 90 min). Bars = 100 μm.

**(B)** to **(D)** GUS expression patterns in early lateral root primordia and/or root vasculature of $Pro_{LBD16}$:*GUS* **(B)**, $Pro_{LBD29}$:*GUS* **(C)**, and $Pro_{LBD33}$:*GUS* **(D)**.

**(E)** to **(J)** Auxin-inducible expression of $Pro_{LBD16}$:*GUS* **[E]** and **[F]**), $Pro_{LBD29}$:*GUS* (**[G]** and **[H]**), and $Pro_{LBD33}$:*GUS* (**[I]** and **[J]**).

**(K)** Semiquantitative RT-PCR analysis of *IAA5*, *LBD16*, *LBD29*, *LBD33*, and *NAC1*. Total RNA was extracted from 7-d-old wild-type and *arf7 arf19* seedlings treated with or without 1 μM NAA or 10 μM CHX for 2.5 h. Transcripts were amplified by 28 cycles of PCR with gene-specific primers. The expression of *ACT8* was used as a control.

**(L)** Kinetics of mRNA accumulation in response to exogenous auxin. Wild-type *Arabidopsis* seedlings (6-d-old) were treated with 1 μM NAA for the indicated durations. Total RNA was assayed by real-time RT-PCR for the accumulation of *IAA5*, *LBD16*, *LBD29*, and *LBD33* relative to an internal *ACT8* control. Data are presented as means ± SD from three independent amplification reactions. Note that different scales are used in the graphs.

auxin within the first 30 min of treatment (Figure 2L). Interestingly, expression of both *LBD16* and *LBD29* was strongly induced by CHX in the wild type (Figure 2K) and was induced within 30 min of auxin treatment (Figure 2L). These results qualify *LBD16* and *LBD29* as primary response genes, which could be regulated directly by ARF7 and ARF19. In addition, mild but significant CHX-mediated induction of *LBD16* and *LBD29* was still observed in the *arf7 arf19* background (Figures 2K and 3B), suggesting the existence of an ARF7- and ARF19-independent pathway probably controlled by an unknown transcription regulator. By contrast, treating wild-type seedlings with CHX did not result in the induction of *LBD33* (Figure 2K), and the induction of *LBD33* was detectable only after 1 h of auxin treatment (Figure 2L), suggesting that *LBD33* belongs to a set of secondary response genes. Although not identified as a candidate target of ARF7 and ARF19 (Okushima et al., 2005), expression of *NAC1*, a positive regulator of lateral root formation downstream of auxin signaling (Xie et al., 2000, 2002; Guo et al., 2005), was examined. Under our conditions, at least 2.5 h after treatment, neither NAA nor CHX dramatically affected *NAC1* expression in either wild-type and *arf7 arf19* seedlings (Figure 2K). Thus, *NAC1* expression does not appear to be regulated by ARF7 and ARF19.

### Direct Activation of *LBD16* and *LBD29* by ARFs

To examine whether *LBD16*, *LBD29*, and *LBD33* are regulated directly by ARF7 and ARF19, we produced transgenic plant lines with inducible ARF7 and ARF19 activity. These lines were generated by introducing native promoter-driven ARF7 or ARF19 fused to the steroid binding domain of rat glucocorticoid receptor (GR) (*Pro$_{ARF7}$:ARF7-GR* or *Pro$_{ARF19}$:ARF19-GR*) into the *arf7 arf19* double mutant. Both *Pro$_{ARF7}$:ARF7-GR/arf7 arf19* and *Pro$_{ARF19}$:ARF19-GR/arf7 arf19* plants showed the typical *arf7 arf19*–like impaired lateral root formation phenotype unless treated with the steroid hormone dexamethasone (DEX). Continuous DEX treatment resulted in lateral root formation similar to that observed in *arf19* and *arf7* single mutants (Figure 3A; see Supplemental Figure 2 online), indicating that ARF-GR fusion proteins were biologically functional in a DEX-dependent manner.

With the use of these inducible lines, we examined the expression profiles of *LBD16*, *LBD29*, and *LBD33*. Treatment with DEX plus NAA of *Pro$_{ARF7}$:ARF7-GR/arf7 arf19* seedlings for 4 h resulted in strong induction of *LBD16*, *LBD29*, and *LBD33* relative to mock-treated or NAA-treated controls (Figure 3B). A combined treatment with DEX, NAA, and CHX resulted in a further induction of *LBD16* and *LBD29* (Figure 3B), indicating that *LBD16* and *LBD29* may be the direct targets of transcriptional activation by ARF7. Induction of *LBD16* and *LBD29* by DEX plus NAA was detectable within 1 h of treatment, suggesting that induction occurred rapidly after ARF7 activation (data not shown). By contrast, DEX-dependent auxin-mediated induction of *LBD33* was completely inhibited by CHX treatment. These results strongly suggest that *LBD16* and *LBD29* are direct targets of ARF7, whereas *LBD33* is not induced directly by ARF7. Similar results were obtained using a *Pro$_{ARF19}$:ARF19-GR/arf7 arf19* line in the same experimental setup. However, data shown in Supplemental Figure 2B online imply only a minor contribution of ARF19 in the direct induction of *LBD16* and *LBD29* yet a significant role in the activation of *LBD29* transcription. Together, these results strongly suggest that ARF7 directly regulates both *LBD16* and *LBD29*, whereas ARF19 directly regulates both genes, but *LBD16* less so. Neither ARF7 nor ARF19 directly regulates *LBD33*.

As mentioned previously, *LBD16* contains one AuxRE in its promoter region, and *LBD29* contains two (Figure 3C). To examine whether ARF7 binds to these sequences in vitro, we performed a mobility shift assay using *LBD16* and *LBD29* promoter fragments containing AuxREs as probes. The N-terminal half of ARF7 that contains a putative DNA binding domain was expressed as a glutathione *S*-transferase (GST) fusion protein in *Escherichia coli*. Recombinant GST-ARF7 protein, but not GST alone, bound to the single *LBD16* and to both *LBD29* promoter fragments (Figure 3C; see Supplemental Figure 2C online). Similar results were obtained with crude extracts of yeast expressing full-length ARF7 rather than recombinant GST-ARF7 (data not shown). These results suggest that ARF7 binds directly to promoter regions of *LBD16* and *LBD29* to activate their expression. Similarly, recombinant ARF19 also bound the *LBD29* promoter fragments (see Supplemental Figure 2C online). Together, these results indicate that ARF7 and ARF19 regulate the auxin-mediated induction of *LBD16* and/or *LBD29* through direct binding to the promoter regions of target genes.

### Overexpression of *LBD16* and *LBD29* Rescues Lateral Root Formation of the *arf7 arf19* Mutant

To examine the biological function of LBD16 and LBD29 in lateral root formation, *LBD16* and *LBD29* were overexpressed under the control of the CaMV 35S promoter in the *arf7 arf19* mutant background (*Pro$_{35S}$:LBD16/arf7 arf19* and *Pro$_{35S}$:LBD29/arf7 arf19*). Multiple independent *Pro$_{35S}$:LBD16* lines displayed increased lateral root formation. The differences in the production of the lateral root phenotype correlate with ectopic *LBD16* gene expression (data not shown). A representative line with almost 100 times wild-type expression of *LBD16* (97.2- $\pm$ 20.2-fold) was selected for detailed studies. Although the number of lateral roots was less than in the wild type, the 12-d-old *Pro$_{35S}$:LBD16/arf7 arf19* seedlings developed lateral roots (Figure 4A), indicating that overexpression of *LBD16* partially rescues lateral root formation in the *arf7 arf19* line. We also observed that overexpression of *LBD16* in a wild-type background causes severely impaired primary root and shoot growth, suggesting the possibility that overexpression of LBD16 could modify ARF7- and ARF19-dependent pathways (our unpublished data).

To address the possibility that *LBD16* overexpression affects auxin signaling in the *arf7 arf19* mutant, we examined the root lengths and lateral root formation of *Pro$_{35S}$:LBD16/arf7 arf19* transgenic lines grown in the presence or absence of auxin. Although exogenous auxin (1 $\mu$M NAA) treatment, which strongly induces lateral root formation in wild-type seedlings, induced very few lateral roots in *arf7 arf19*, *Pro$_{35S}$:LBD16/arf7 arf19* seedlings produced many lateral roots in response to exogenous auxin (Figure 4B). In addition, primary roots of *Pro$_{35S}$:LBD16/arf7 arf19* were more sensitive to auxin than were those of the auxin-resistant *arf7 arf19* mutant (Figure 4C). These data indicate that

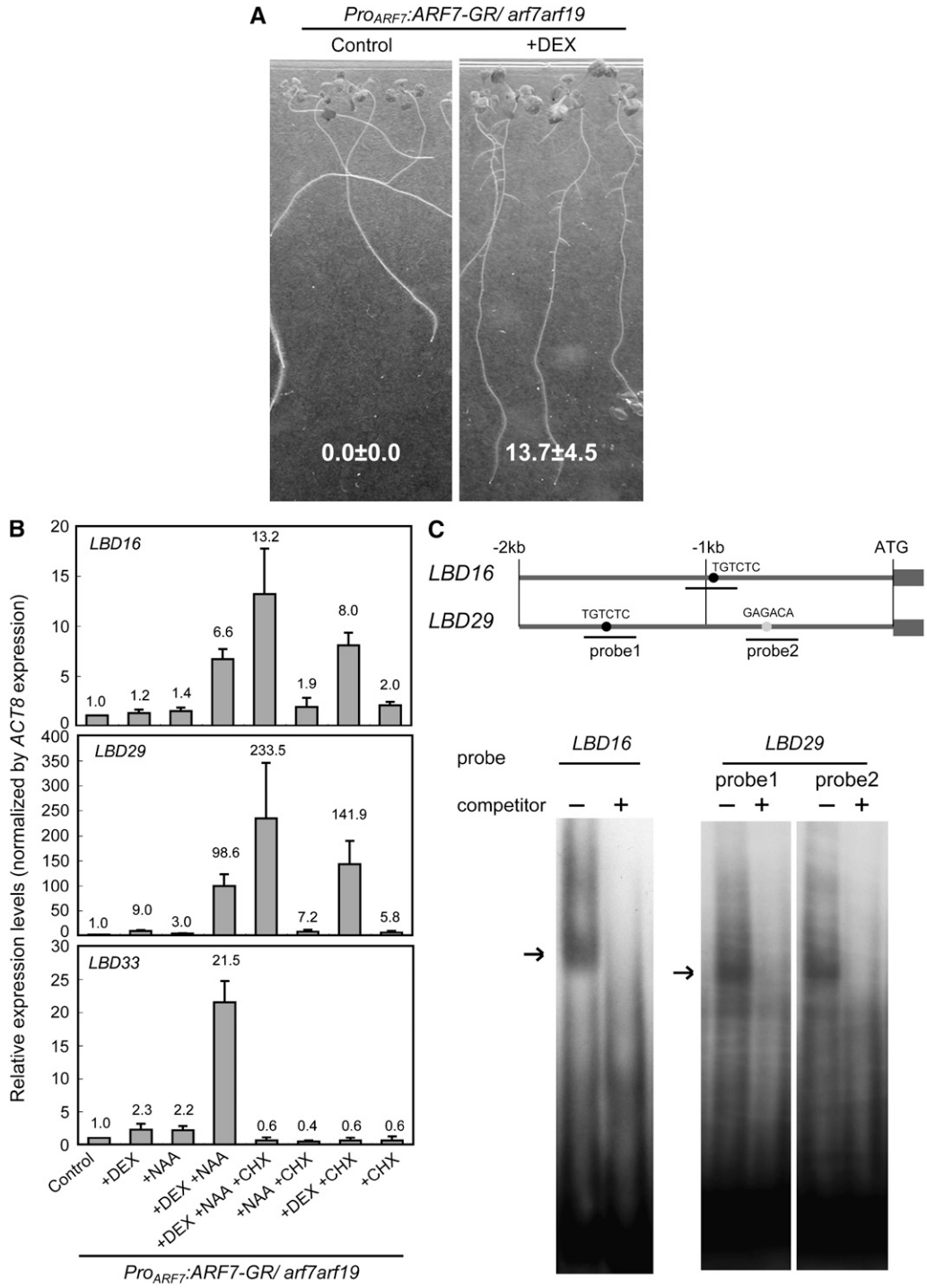


**Figure 3.** *LBD16* and *LBD29* Are Potential Direct Targets of ARF7.

**(A)** Phenotype of *Pro$_{ARF7}$:ARF7-GR/arf7 arf19* plants. DEX treatment rescues the phenotype of the *arf7 arf19* double mutant in lateral root formation.
**(B)** Effects of chemicals on the expression of *LBD* genes in the roots of *Pro$_{ARF7}$:ARF7-GR/arf7 arf19* plants. The 7-d-old seedlings were transferred to the medium with or without the indicated chemicals (1 μM NAA, 2 μM DEX, and 10 μM CHX), and roots were harvested for expression analysis after 4 h of treatment. The transcripts were analyzed by real-time PCR. The levels of *LBD16*, *LBD29*, and *LBD33* expression were normalized to *ACT8* and compared with the control condition. Data shown are averages of three biological replicates, with error bars representing SD. Note that different scales are used in the graphs.
**(C)** Electrophoretic mobility shift assays with recombinant ARF7 proteins. $^{32}$P-labeled *LBD16* and *LBD29* promoter fragments containing AuxRE sequence were used as probes. Arrows indicate the ARF7/DNA complex.

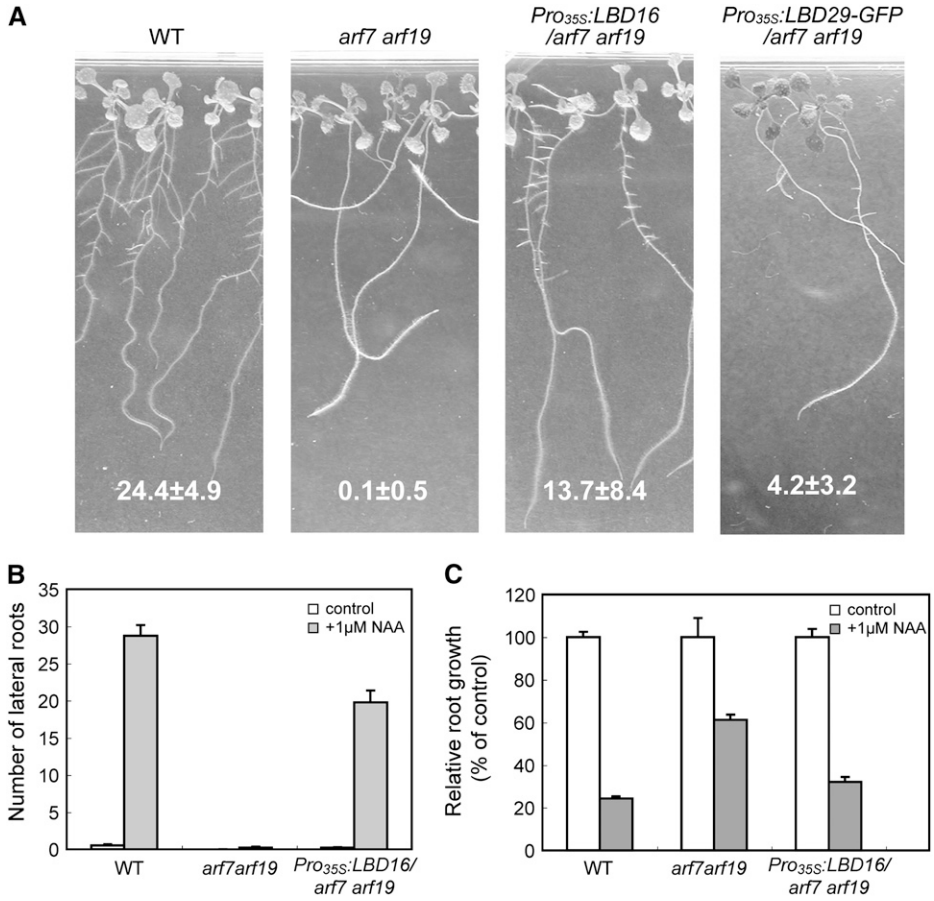


**Figure 4.** Overexpression of *LBD16* and *LBD29* Partially Suppresses the Lateral Root Phenotype of *arf7 arf19*.

**(A)** Twelve-day-old wild-type, *arf7 arf19*, $Pro_{35S}$*:LBD16/arf7 arf19*, and $Pro_{35S}$*:LBD29-GFP/arf7 arf19* seedlings. Numbers of lateral roots (means ± SD) for each genotype are shown ($n > 14$).

**(B)** and **(C)** Auxin sensitivity of wild-type, *arf7 arf19*, and $Pro_{35S}$*:LBD16/arf7 arf19* roots. Four-day-old seedlings were transferred onto plates with (gray bars) or without (white bars) 1 μM NAA, and numbers of lateral roots **(B)** and root length **(C)** were determined after 3 d of vertical growth ($n > 20$). Bars and error bars represent means + SD.

LBD16 overexpression partially restores the ability of the *arf7 arf19* mutant line to respond to exogenous auxin.

Overexpression of *LBD29* also restored lateral root formation in *arf7 arf19* but was much less effective than *LBD16*. Only a few of 40 $Pro_{35S}$*:LBD29/arf7 arf19* independent lines showed slightly increased lateral root formation (data not shown), and all of these lines exhibited only ~10-fold wild-type levels of *LBD29* expression. We also generated $Pro_{35S}$*:LBD29-GFP/arf7 arf19* lines. Among 24 independent $Pro_{35S}$*:LBD29-GFP/arf7 arf19* lines, the line with the highest transgene expression (58.5- ± 18.7-fold) was selected for detailed studies. At 12 d after germination, the *arf7 arf19* mutant did not generate lateral roots, whereas $Pro_{35S}$*:LBD29-GFP/arf7 arf19* seedlings developed a small number of lateral roots (Figure 4A). This observation indicates that overexpression of *LBD29* also has the ability to promote lateral root formation in an *arf7 arf19* mutant background, but it is much less effective than *LBD16*. Together, these results indicate that LBD16 and LBD29 induce lateral root formation in the absence of ARF7 and ARF19. We also examined the effects of *LBD33* overexpression in *arf7 arf19*, but none of 20 available transgenic lines had increased lateral root formation (data not shown).

In an attempt to identify the role of *LBD16* in wild-type plants, we obtained a T-DNA insertion mutant from the Salk collection (SALK_095791; *lbd16-1*); there are no *LBD29* knockout lines in the public collections. RT-PCR analysis confirmed that *lbd16-1* is a knockout allele of *LBD16* (data not shown). *lbd16-1* plants appeared very similar to wild-type plants at both seedling and adult stages. Root growth was inhibited to almost the same extent in both wild-type and *lbd16-1* seedlings in response to exogenous auxin (data not shown). In addition, the *lbd16-1* mutation did not affect auxin-regulated gene expression, because auxin induced *IAA5*, *IAA19*, *LBD29*, and *LBD33* to a similar degree in *lbd16-1* and wild-type seedlings (data not shown). However, the number of lateral roots was reduced slightly in 7-d-old *lbd16-1*seedlings (8.1 ± 2.8 [mean ± SD] in the wild type [$n = 54$] and 4.7 ± 2.4 in *lbd16-1* [$n = 48$]; $P < 0.001$). Although exogenous auxin induced lateral root formation in *lbd16-1* as in wild-type seedlings, there were fewer lateral roots in auxin-treated

*lbd16-1* than in the wild type (63.2 ± 15.8 [mean ± SD] in the wild type [$n = 52$] and 34.7 ± 8.8 in *lbd16-1* [$n = 31$]; $P < 0.001$). These observations imply that *LBD16* contributes to auxin-mediated lateral root formation in wild-type seedlings. The mild lateral root formation phenotype observed in *lbd16-1* may be attributable to the functional redundancy of *LBD/ASL* gene family members.

### Overexpression of LBD16 Fused to a Transcriptional Repressor Domain Inhibits Lateral Root Formation and Auxin-Induced Gene Expression

To examine the subcellular localization of LBD16 and LBD29, we generated transgenic plants that express LBD16-GFP and LBD29-GFP translational fusions under the control of the CaMV 35S promoter in the *arf7 arf19* mutant background. Among ~20 independent transgenic lines each for both constructs, three (LBD16-GFP) and one (LBD29-GFP) lines had increased lateral root formation and showed strong nuclear GFP signals in the root epidermal cells (Figures 4A, 5A, and 5B; data not shown). These data strongly suggest that LBD16 and LBD29 act in the nucleus to control lateral root formation.

The lack of an obvious loss-of-function phenotype of *LBD16* may be attributable to redundancy in gene family members. However, transgenic seedlings overexpressing LBD16 fused to the transcriptional suppression domain (SRDX) (Hiratsu et al., 2003) under the control of the CaMV 35S promoter ($Pro_{35S}$*:LBD16-SRDX*) exhibited strong morphological defects in roots and aerial organs. Interestingly, lateral root formation in $Pro_{35S}$*:LBD16-SRDX* seedlings was strongly impaired in multiple independent lines (11 of 14 independent lines in the T2 generation; Figure 5C). We did not observe any lateral root primordia before emergence in 10-d-old $Pro_{35S}$*:LBD16-SRDX* seedlings, suggesting that initiation was severely inhibited in this line (data not shown). In addition, especially in strong lines (line 6; Figure 5C), narrower and upward-curled leaves and strongly impaired root growth were observed in some seedlings (Figure 5E). Although less severe, this phenotype resembles the effects of domain II mutations in Aux/IAA proteins, such as *axr3/iaa17* (Leyser et al., 1996; Rouse et al., 1998; Reed, 2001). These observations prompted an examination of auxin-regulated gene expression in $Pro_{35S}$*:LBD16-SRDX* seedlings. We used the high *LBD16-SRDX* expression line 5 for expression analysis (63.0- ± 16.0-fold wild-type native *LBD16* level). Auxin treatment (1 μM NAA, 4 h) strongly induced the expression of *LBD29*, *LBD33*, and *IAA5* in 7-d-old wild-type seedlings but not in the *arf7 arf19* double mutant (Figure 5F). Auxin-mediated induction of *LBD29*, *LBD33*, and *IAA5* were significantly impaired in $Pro_{35S}$*:LBD16-SRDX* seedlings (Figure 5F), strongly suggesting that auxin signaling was perturbed in these plants even though they have wild-type *ARF7* and *ARF19* genes. Therefore, LBD16 may be a positive mediator of auxin signaling in *Arabidopsis* growth and development, including lateral root formation.

## DISCUSSION

Recent molecular genetic studies have identified two members of the ARF transcription activator family, ARF7 and ARF19, that redundantly regulate lateral root formation in *Arabidopsis* (Okushima et al., 2005; Weijers et al., 2005; Wilmoth et al., 2005; Li et al., 2006). Genes that trigger the lateral root initiation cascade could be regulated directly by ARF7 and ARF19, but little was known of the factors that mediate auxin-mediated lateral root initiation downstream of ARF7 and ARF19. In this study, we identified *LBD16* and *LBD29* as the direct targets of these ARFs responsible for lateral root initiation from a number of candidate genes that were screened previously by microarray-based analysis (Okushima et al., 2005). Overexpression of *LBD16* and *LBD29* partially rescues lateral root formation in *arf7 arf19*, strongly suggesting that these two related LBD/ASLs promote lateral root formation. Target gene analysis using an ARF7- and ARF19-inducible system together with electrophoresis mobility shift assay indicated that ARF7 and ARF19 are direct transcriptional regulators of *LBD16* and *LBD29*.

Thus, in our current auxin signaling cascade model for lateral root initiation (Figure 6), the auxin signal that is captured by TIR1/AFBs accelerates the degradation of Aux/IAA proteins, typified by IAA14/SLR, IAA3/SHY2, IAA19/MSG2, and IAA28, thereby derepressing the function of ARF7 and ARF19 as transcriptional activators. ARF7 and ARF19 activate the transcription of downstream target genes, including *LBD16* and *LBD29*. LBD16 also probably activates a downstream signaling network as a transcriptional regulator. In this pathway, ARF7 makes a major contribution to the activation of the downstream signaling cascade by inducing *LBD16* and *LBD29*. Activation of *LBD16* by ARF7 might be particularly important, because LBD16 is much more effective than LBD29 for generating lateral roots in the *arf7 arf19* double mutant. On the other hand, ARF19 plays an auxiliary role in the activation of *LBD*s.

Analyses using the $Pro_{CycB1;1}$*:CycB1;1(NT)-GUS* reporter construct demonstrated that auxin-induced pericycle cell division for lateral root initiation was severely impaired in *arf7 arf19* as well as in the gain-of-function mutant *iaa14/slr* (Fukaki et al., 2002; Vanneste et al., 2005). Consistently, expression of stabilized iaa14/slr mutant protein under the control of either the *ARF7* or the *ARF19* promoter also blocked lateral root formation, and IAA14/SLR interacts with ARF7 and ARF19 in yeast (Fukaki et al., 2005). These observations strongly suggest that ARF7, ARF19, and IAA14/SLR regulate lateral root initiation at the same stage. We found that dominant repression of LBD16 strongly inhibited lateral root formation, probably at the initiation stage. Induction of auxin-responsive *LBD*s, including *LBD16* and *LBD29*, is also inhibited in the *iaa14/slr* mutant that is defective in lateral root initiation (Vanneste et al., 2005). Thus, IAA14/SLR, ARF7, and ARF19 and their target genes, including *LBD16*, probably act in the initiation stage of lateral root formation. Accordingly, *LBD16* and *LBD29* are expressed mainly in the root stele and lateral root primordia in wild-type seedlings, and these expression domains overlap the region where *ARF7* and *ARF19* are expressed. Consistent with the results of microarray and RT-PCR analyses, auxin-mediated expression of $Pro_{LBD16}$*:GUS* and $Pro_{LBD29}$*:GUS* was blocked in *arf7 arf19* (data not shown), also suggesting that ARF7 and ARF19 regulate the auxin-mediated expression of these *LBD/ASL* genes. These data imply that a striking induction of *LBD16* and *LBD29* transcription by ARF7 and/or ARF19 is important to activate the downstream signal cascade for the

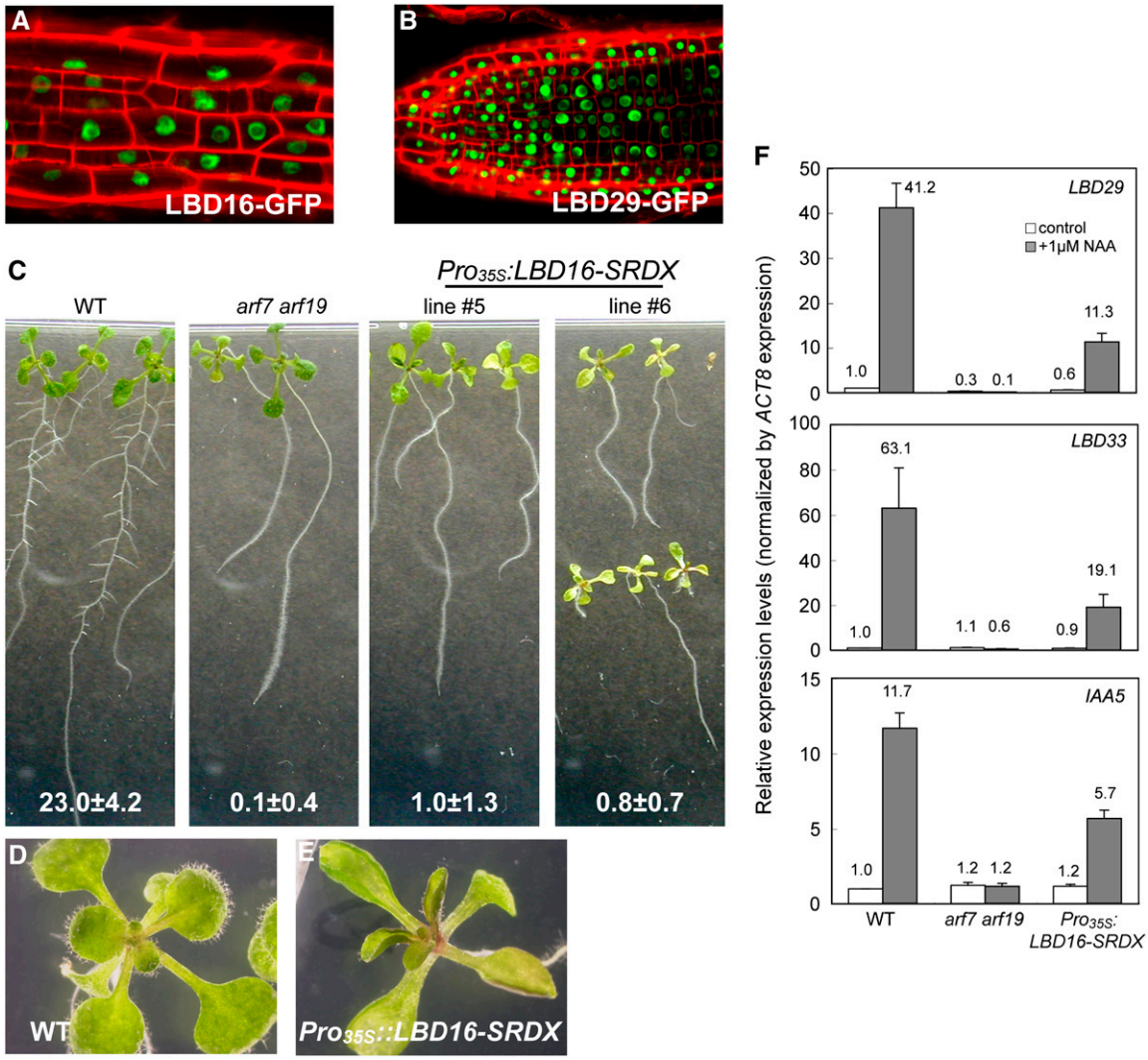


**Figure 5.** Nuclear Localization of LBD-GFP Fusion Proteins, and Dominant Repression Phenotype of LBD16.

**(A)** and **(B)** Nuclear localization of LBD16-GFP and LBD29-GFP fusion proteins. Longitudinal confocal images of *Pro$_{35S}$:LBD16-GFP/arf7 arf19* **(A)** and *Pro$_{35S}$:LBD29-GFP/arf7 arf19* **(B)** transgenic *Arabidopsis* roots are shown.

**(C)** Twelve-day-old wild-type, *arf7 arf19*, and two independent lines of *Pro$_{35S}$:LBD16-SRDX* (lines 5 and 6) seedlings. Numbers of lateral roots (means ± SD) for each genotype are shown ($n > 16$).

**(D)** and **(E)** Closer views of aerial parts of wild-type **(D)** and *Pro$_{35S}$:LBD16-SRDX* (**[E]**; line 6) seedlings.

**(F)** Expression profiles of auxin-responsive genes in wild-type, *arf7 arf19*, and *Pro$_{35S}$:LBD16-SRDX* (line 6) seedlings. Six-day-old seedlings of the indicated genotypes were transferred to the medium with or without 1 μM NAA, and whole seedlings were harvested for real-time PCR analysis after 4 h of treatment. The levels of *LBD29*, *LBD33*, and *IAA5* expression were normalized to *ACT8* and compared with wild-type control levels. Data are presented as means ± SD from three independent amplification reactions.

induction of lateral root initiation. However, transcription of *LBD16* and *LBD29* is not only regulated by ARF7 and ARF19, because *arf7 arf19* roots have basal expression levels of these *LBD/ASL*s, just like wild-type roots. In addition, mild but significant CHX-mediated induction of *LBD16* and *LBD29* was still observed in the *arf7 arf19* background (Figures 2K and 3B), suggesting the existence of an ARF7- and ARF19-independent pathway, probably controlled by an unknown transcription regulator that is regulated by an unstable repressor. The residual ability to produce lateral roots in the older *arf7 arf19* plants implies that other ARFs also contribute to lateral root formation (Okushima et al., 2005). ARF5/MP, an essential regulator for embryonic root development, seems most likely to redundantly regulate lateral root formation via transcriptional activation of downstream target genes of ARF7 and ARF19 (Hardtke, 2006).

LBD16/ASL18 and LBD29/ASL16 are highly related members of the LBD/ASL family, which consists of 42 members in *Arabidopsis* (Iwakawa et al., 2002; Shuai et al., 2002). Several LBD/ASLs are important for lateral organ development. LBD6/AS2 and LBD36/ASL1, other related members of this family, are

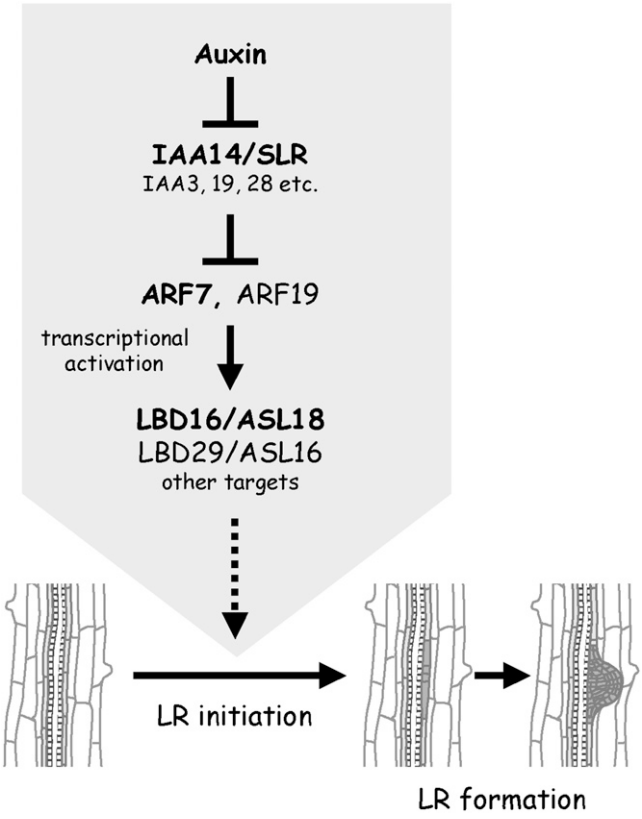


**Figure 6.** Model of the ARF7- and ARF19-Dependent Auxin Signaling Cascade for Lateral Root Formation.

Auxin accelerates the degradation of Aux/IAA proteins such as IAA14/SLR, IAA3/SHY2, IAA19/MSG2, and IAA28, thereby derepressing ARF7 and ARF19 function as transcriptional activators. ARF7 and ARF19 activate the transcription of several *LBD/ASL*s, including *LBD16/ASL18* and *LBD29/ASL16*. LBD16/ASL18 also may activate the downstream transcriptional network for lateral root (LR) initiation as a transcriptional regulator.

required for the differentiation of lateral organs in aerial plant parts, such as leaves and petals (Iwakawa et al., 2002; Chalfun-Junior et al., 2005). Maize (*Zea mays*) RAMOSA2, a potential ortholog of *Arabidopsis* LOB/ASL4, is required for the patterning of stem cells in branch meristems (Bortiri et al., 2006). Recent reports show that a rice homolog of LBD16 and LBD29, CROWNROOTLESS1/ADVENTITIOUS ROOTLESS1 (CRL1/ARL1), regulates crown root formation in rice. Mutations in *CRL1/ARL1* result in severely impaired crown root formation (Inukai et al., 2005; Liu et al., 2005). In addition, *crl1/arl1* mutants show auxin-related phenotypes in roots, including decreased lateral root number and impaired root gravitropism, and *CRL1/ARL1* gene expression is induced by auxin (Inukai et al., 2005; Liu et al., 2005). These reports indicate that the function and regulation of *LBD16*- and *LBD29*-related genes are partially conserved between monocot and dicot root development.

Several LBD/ASL proteins of *Arabidopsis* and rice are known to be localized in the nucleus and thus are thought to function in transcriptional control (Iwakawa et al., 2002; Liu et al., 2005). *LBD6/AS2* and *LBD36/ASL1* negatively regulate several *KNOTTED1-like homeobox* (*KNOX*) genes, such as *KNAT1/BP* and *KNAT2*, whose products promote stem cell proliferation in leaves (Ori et al., 2000; Semiarti et al., 2001; Lin et al., 2003; Chalfun-Junior et al., 2005). Although it is not known whether these LBD/ASLs directly control the expression of *KNOX* genes, our data using a *LBD16-SRDX* overexpressor indicates the possibility that LBD16 could function as a transcriptional regulator. We observed that LBD16-SRDX inhibits the auxin-mediated induction of not only the secondary response gene, *LBD33*, but also primary response genes, such as *LBD29* and *IAA5*. Furthermore, overexpression of *LBD16* in a wild-type background but not in an *arf7 arf19* background causes severe developmental defects in both roots and aerial parts, suggesting that LBD16 can modify ARF7- and ARF19-dependent pathways, either directly or indirectly (our unpublished data). Thus, ARF-mediated transcriptional regulation may involve a positive feedback loop through the ARF-mediated induction of *LBD16* by auxin. This is in contrast with the negative feedback loop with the ARF-mediated induction of *Aux/IAA*s. Having proposed that LBD16 has a function in auxin signaling, it would be intriguing to identify its target genes and to dissect the downstream events of LBD16 during lateral root formation. It is possible that *LBD33*, which is located downstream of ARF7- and ARF19-dependent auxin signaling but is not a direct target, could be one of the downstream genes of LBD16.

Unlike rice *crl1/arl1* mutants (Inukai et al., 2005; Liu et al., 2005), T-DNA insertions or RNA interference interruption of the expression of *LBD16* and *LBD29* did not show any obvious defects in plant development, including lateral root formation (this study; data not shown), whereas the dominant repression of LBD16 function strongly inhibited lateral root formation. The expression domain of *LBD16* and *LBD29* largely overlaps root stele tissues and lateral root primordia, and both are primary auxin response genes, suggesting a functional redundancy between these two related *LBD*s. Functional redundancy among LBD16, LBD29, and possibly other closely related family members may preclude genetic analysis using single mutants. *LBD/ASL*s belong to a large gene family in *Arabidopsis*, rice, and maize (Iwakawa et al., 2002; Shuai et al., 2002; Bortiri et al., 2006; Yang et al., 2006). As pointed out by Sablowski and Meyerowitz (1998) in the case of floral homeotic genes in the *MADS* family and their target *NAP* gene in the *NAC* family, it is possible that as *ARF* genes diverged during evolution their target *LBD/ASL* genes may also have duplicated and specialized.

Since the early 1990s, numerous transcription factors have been identified as master regulators of particular developmental processes in higher plants. Current efforts are now directed toward identifying the direct targets of developmentally important transcription factors to reveal whole regulatory networks, using molecular genetics, genomic, and bioinformatics approaches in both animals and plants (Samach et al., 2000; Ito et al., 2004; William et al., 2004; Levesque et al., 2006; Palaniswamy et al., 2006). Several sets of plant target genes have now been reported, like those of the MADS transcriptional factors that regulate flower development (Ito et al., 2004; de Folter and Angenent, 2006). This work represents an important step in explaining the mechanism of ARF-Aux/IAA pathway control in various auxin-regulated developmental processes of higher plants, because despite the

importance of ARFs in plant growth and development, such as ARF5/MP in early embryogenesis (Hardtke and Berleth, 1998), little is known about direct targets important for developmental pathways. Further study with the ARF7- and ARF19-inducible system will enable us to understand the signaling cascade of auxin-mediated lateral root formation and to discover how auxin controls the hidden half of higher plants.

## METHODS

### Plant Materials and Growth Conditions

*Arabidopsis thaliana* ecotype Columbia was used throughout this study. The *nph4-1 arf19-1* double mutant allele was used as the *arf7 arf19* double mutant in this study (Okushima et al., 2005). T-DNA insertion mutant *lbd16-1* (SALK_095791) was obtained from the ABRC. Seeds were germinated on Murashige and Skoog medium with 1% sucrose. Plants were grown at 23°C under continuous light conditions. For the root auxin sensitivity assay, 4-d-old seedlings were transferred to vertically oriented agar plates containing NAA. The number of lateral roots and root length were determined after an additional 3 d of growth using a dissecting microscope and ImageJ software.

### Vector Construction and Plant Transformation

$Pro_{LBD16}$*:GUS*, $Pro_{LBD29}$*:GUS*, and $Pro_{LBD33}$*:GUS* constructs were generated by fusing promoter fragments (*LBD16*, 2.5 kb; *LBD29* and *LBD33*, 2.2 kb) in front of the GUS coding sequence in the *pBI101.2* vector.

Full-length cDNAs of *ARF7*, *ARF19*, *LBD16*, *LBD29*, and *LBD33* were isolated by RT-PCR from flower or root cDNA libraries and introduced into *pDONR221* vector using Gateway BP clonase enzyme mix (Invitrogen). To construct $Pro_{35S}$*:LBD16*, $Pro_{35S}$*:LBD29*, and $Pro_{35S}$*:LBD:LBD33* plasmids, *LBD16*, *LBD29*, and *LBD33* coding regions were transferred from the *pDONR221* vectors to *pGWB2* (GWB vectors were kind gifts from Tsuyoshi Nakagawa) by Gateway LR recombination (Invitrogen). To construct $Pro_{35S}$*:ARF7-GFP*, $Pro_{35S}$*:ARF19-GFP*, $Pro_{35S}$*:LBD16-GFP*, and $Pro_{35S}$*:LBD29-GFP* plasmids, *ARF7*, *ARF19*, *LBD16*, and *LBD29* coding regions were transferred from the *pDONR221* vectors to *pGWB5* by Gateway LR recombination. To construct $Pro_{ARF7}$*:ARF7-GR* and $Pro_{ARF19}$*:ARF19-GR*, *ARF7* and *ARF19* promoter fragments (2.5 kb) were synthesized by PCR and inserted into the binary destination vector *pGWB-GR*, which contains a Gateway conversion cassette (Invitrogen) in front of the GR coding region (*pGWB-GR* was constructed by inserting the GR coding region into the *Xba*I/*Sac*I sites of *pGWB1*). ARF7 and ARF19 coding regions were subsequently transferred from the *pDONR221* vectors by Gateway LR recombination. To generate $Pro_{35S}$*:LBD16-SRDX*, the *LBD16* coding region with a transcriptional repression domain (SRDX) was generated by PCR using a primer with the SRDX (LDLDLELRLGFA) linker sequence (Hiratsu et al., 2003). This *LBD16-SRDX* cDNA fragment was introduced into *pDONR221* using Gateway BP clonase enzyme mix and was subsequently transferred to *pGWB2* by Gateway LR recombination. Details of vector construction are available upon request. The resulting vectors were introduced into *Agrobacterium tumefaciens* GV3101 (pMP90), and wild-type or *arf7 arf19* plants were transformed by floral dipping (Clough and Bent, 1998). Transformants were selected on medium containing 50 mg/L kanamycin or 20 mg/L hygromycin. Homozygous lines were identified in the T3 generation, and T3 or T4 homozygous lines were used for phenotypic and molecular analyses.

### Phylogenetic Analysis

Multiple sequence alignment based on the LOB/AS2 domain was done with ClustalW (Thompson et al., 1994) at http://www.ddbj.nig.ac.jp/search/clustalw-j.html using the neighbor-joining tree option with 1000 bootstraps. TreeView was used to generate the graphic output (Page, 1996). The actual alignment is given in Supplemental Figure 3 online.

### Histochemical GUS Assay and Microscopy

GUS staining, fixation, and whole-mount clearing preparation of roots were performed essentially as described (Malamy and Benfey, 1997), and roots were inspected with a Nikon Eclipse E800 microscope equipped with Nomarski optics. For confocal microscopy, roots were counterstained with 10 μg/mL propidium iodide and analyzed with an Olympus IX80 confocal microscope.

### RNA Extraction and RT-PCR Analysis

Total RNA was isolated from plant tissues using the RNeasy kit (Qiagen). First-strand cDNA was synthesized from 2 μg of total RNA with an oligo$(dT)_{24}$ primer and SuperScriptII reverse transcriptase (Invitrogen). Transcripts were quantified by RT-PCR or real-time RT-PCR analyses using 1/120th of the resulting cDNA as template. Real-time RT-PCR was performed with the LightCycler system (Roche) with SYBR Premix Ex Taq (TaKaRa). Primers used for analyses are listed in the Supplemental Methods online.

### Gel Mobility Shift Assay

To generate recombinant GST-ARF7 and GST-ARF19 proteins, the cDNA fragments encoding the N termini of ARF7 (amino acids 1 to 367) and ARF19 (amino acids 1 to 365) were inserted into the pGEX-2T vector and expressed in BL21 Codon Plus *Escherichia coli* cells (Stratagene). The empty pGEX-2T vector was used to generate GST alone. The production and purification of GST alone and GST fusion proteins were as described previously (Okushima et al., 2002). DNA probes were prepared by annealing complementary oligonucleotides (see Supplemental Methods online for probe sequences) and then labeled with [$^{32}$P]dCTP using the Klenow fragment of DNA polymerase I. The binding mixture contained 0.4 fmol of $^{32}$P-labeled probe, 0.5 μg of affinity-purified GST fusion protein, 1 μg of poly(dA-dT)·poly(dA-dT), 5 μg of BSA, 20 mM HEPES-KOH, pH 7.9, 50 mM KCl, 15% glycerol, and 1 mM DTT in a 20-μL reaction volume. Reactions were incubated for 30 min at room temperature and then separated on 5% native polyacrylamide gels in 0.25× Tris-borate-EDTA at 100 V for 90 min. After electrophoresis, the gels were dried and autoradiographed. For competition experiments, a 125-fold molar excess of cold double-stranded probe was added to the reaction mixture.

### Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: *ARF7* (At5g20730), *ARF19* (At1g19220), *LBD16/ASL18* (At2g42430), *LBD29/ASL16* (At3g58190), and *LBD33/ASL24* (At5g06080).

### Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure 1.** Tissue-Specific Expression of Auxin-Inducible *LBD*s.

**Supplemental Figure 2.** Regulation of *LBD*s by ARF19.

**Supplemental Figure 3.** Alignment Used for Phylogenetic Analyses.

**Supplemental Methods.**

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