An *AGAMOUS*-Related MADS-Box Gene, *XAL1* (*AGL12*), Regulates Root Meristem Cell Proliferation and Flowering Transition in Arabidopsis[1][W][OA]

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MADS-box genes are key components of the networks that control the transition to flowering and flower development, but their role in vegetative development is poorly understood. This article shows that the sister gene of the *AGAMOUS* (*AG*) clade, *AGL12*, has an important role in root development as well as in flowering transition. We isolated three mutant alleles for *AGL12*, which is renamed here as *XAANTAL1* (*XAL1*): Two alleles, *xal1-1* and *xal1-2*, are in Columbia ecotype and *xal1-3* is in Landsberg *erecta* ecotype. All alleles have a short-root phenotype with a smaller meristem, lower rate of cell production, and abnormal root apical meristem organization. Interestingly, we also encountered a significantly longer cell cycle in the strongest *xal1* alleles with respect to wild-type plants. Expression analyses confirmed the presence of *XAL1* transcripts in roots, particularly in the phloem. Moreover, *XAL1*::β-glucuronidase expression was specifically up-regulated by auxins in this tissue. In addition, mRNA in situ hybridization showed that *XAL1* transcripts were also found in leaves and floral meristems of wild-type plants. This expression correlates with the late-flowering phenotypes of the *xal1* mutants grown under long days. Transcript expression analysis suggests that *XAL1* is an upstream regulator of *SOC*, *FLOWERING LOCUS T*, and *LFY*. We propose that *XAL1* may have similar roles in both root and aerial meristems that could explain the *xal1* late-flowering phenotype.

Normal morphogenesis depends on the equilibrium between cell proliferation and differentiation (i.e. cellular homeostasis), whereas transcriptional regulatory networks reliably translate genetic information to yield specific and complex multicellular patterning. In both animals and plants, elegant models of pattern formation have suggested the existence of mechanisms that determine developmental identities in precise manners (Coen and Meyerowitz, 1991; Lawrence and Morata, 1994). Dynamic regulatory network models have substantiated the existence of these mechanisms (von Dassow and Odell, 2002; Espinosa-Soto et al., 2004). Only recently, molecular links between mechanisms that underlie cell-type specification and cell-cycle regulation have been demonstrated (Caro et al., 2007).

The MADS-box gene family encodes a large variety of transcriptional regulators of plant and animal development (Messenguy and Dubois, 2003). These transcription factors have been classified into two classes based on sequence relationships and structural features (type I and II lineages) that should have derived from at least one ancestral duplication before the divergence of animals and plants (Alvarez-Buylla et al., 2000b). Therefore, plant type I is closely related to the animal SRF factors, whereas plant type II is more similar to the MEF type of animals in their MADS domains than to

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plant type I. However, type II MADS-domain proteins of plants have three domains (I, K, C) in addition to the MADS DNA-binding domain: a small I domain that links the MADS with the dimerization K domain and the COOH domain (Riechmann and Meyerowitz, 1997).

Plant MIKC genes have been mostly characterized as regulators of the transition to flowering (Samach et al., 2000) and flower, fruit, and seed development (Bowman et al., 1991; Gu et al., 1998; Ferrandiz et al., 2000; Nesi et al., 2002; Pinyopich et al., 2003). They are fairly specific meristem- (Mandel et al., 1992; Bowman et al., 1993), cell- (Liljegren et al., 2000), or organ-identity (Yanofsky et al., 1990; Pelaz et al., 2000) genes. However, genome-wide studies suggest that most MADS-box genes are expressed at different stages of the plant's life cycle and in a variety of organs and cell types (Kofuji et al., 2003; for review, see Rijpkema et al., 2007), suggesting that these genes may have developmental roles that affect multiple tissues and organs.

Given the high sequence conservation of MADS domains of plant and animal proteins within each lineage (I and II), we hypothesized that some of their functions may also have been conserved. Animal MEF-related MADS proteins have been implicated in regulation of cellular homeostasis and linked to cell-cycle control (Lazaro et al., 2002). Therefore, we proposed that some plant MIKC genes might be important modulators of cell proliferation versus differentiation decisions. Moreover, quantitative cellular analyses of MADS-box mutants may help to further understand the role of these genes in various plant developmental processes.

We have focused on MADS-box genes expressed in the root because this organ is transparent and simple at the cellular level, enabling quantitative analyses of cell dynamics (Dolan et al., 1993; Malamy and Benfey, 1997). Indeed, the root has become a very useful system for unraveling general features of multicellular developmental mechanisms (Benfey and Scheres, 2000; Chapman et al., 2003; Wildwater et al., 2005), and specifically for understanding the links between cellular dynamics and cell-type specification during normal morphogenesis of a complex organ in vivo (Sabatini et al., 2003; Wildwater et al., 2005; Caro et al., 2007). Some components of the molecular mechanisms involved in stem-cell niche patterning and behavior (Sablowski, 2004a; Sarkar et al., 2007), as well as in the patterns of cell proliferation along morphogenetic gradients, which in the root are importantly determined by auxins, have been characterized (Sabatini et al., 1999; Galinha et al., 2007; Grieneisen et al., 2007).

In this study, we report the characterization of *AGL12* based on three alleles (two in the Columbia [Col] background and one in the Landsberg *erecta* [L*er*] background) that we have named *xaantal1* (*xal1*) due to its short-root and late-flowering phenotypes (*xaantal*: "to take longer" in Mayan), thus also renaming the *AGL12* gene *XAL1*. *XAL1* is the sister gene to the *AGAMOUS* (*AG*)-related genes that are specific for reproductive tissues. In contrast, *XAL1* was characterized as a root-specific gene (Rounsley et al., 1995). Here, we confirm that *XAL1* is indeed expressed in roots, but we report its expression also in aerial organs. Our data suggest that *XAL1* is an important regulator of cell proliferation in the root. *XAL1* mutant alleles have short roots with an altered cell production rate, meristem size, and cell-cycle duration, and thus *XAL1* is the first MADS-box gene that is shown to be involved in cell-cycle regulation. Auxins have been implicated in cell-cycle regulation (Himanen et al., 2002; Vanneste et al., 2005) and our data interestingly show that *XAL1* is induced by auxins. On the other hand, *xal1* alleles are also late flowering and our data suggest that *XAL1* could be an important promoter of the flowering transition through up-regulation of *SOC*, *FLOWERING LOCUS T* (*FT*), and *LFY*.

RESULTS

XAL1, a Sister Gene of the *AGAMOUS* MADS-Box Clade, Is an Important Regulator of Root Development

Sequence analysis of *XAL1* indicated that this gene is a member of the MADS-box transcription factor family (Fig. 1A) and recent phylogenetic analyses suggested that *XAL1* is sister to the rest of the *AG*-related genes (Martínez-Castilla and Alvarez-Buylla, 2003; Parenicová et al., 2003). However, contrary to the other members of the *AG* clade, the expression of *XAL1* is not restricted to reproductive organs because it is strongly expressed in roots (Rounsley et al., 1995; Burgeff et al., 2002). To further characterize this gene at the functional level, we isolated three *xal1* mutant alleles (Fig. 1B). The *xal1-1* allele has an *En-1* transposon insertion (Baumann et al., 1998) in the first exon of *XAL1* and the *xal1-2* allele is a T-DNA insertion in the second intron (see "Materials and Methods"), both in the Col-0 background. The third allele, *xal1-3*, is in the L*er* background and is a stable transposon mutant allele with the insertion at the end of the fourth exon (Fig. 1B).

In all three mutant alleles, the primary root was shorter than in wild-type plants. *xal1-1* seedlings showed a root length intermediate between wild type and *xal1-2* and *xal1-3* (Fig. 1B), probably due to somatic reversion of this unstable transposon allele that occurred after several generations. We performed northern-blot and reverse transcription (RT)-PCR to corroborate *XAL1* mRNA levels in roots of the three mutant alleles. RT-PCR detected low expression of *XAL1* in the *xal1-1* allele, which correlates with its intermediate phenotype, whereas the other two alleles had no expression of *XAL1* mRNA (Fig. 1C).

To test whether the shorter roots of the three alleles could be due to altered cellular organization at the root tip, we analyzed 20 roots of each mutant allele under a confocal microscope. About 30% of the plants of all three alleles showed abnormal root apical meristem (RAM) organization, with the quiescent center (QC)

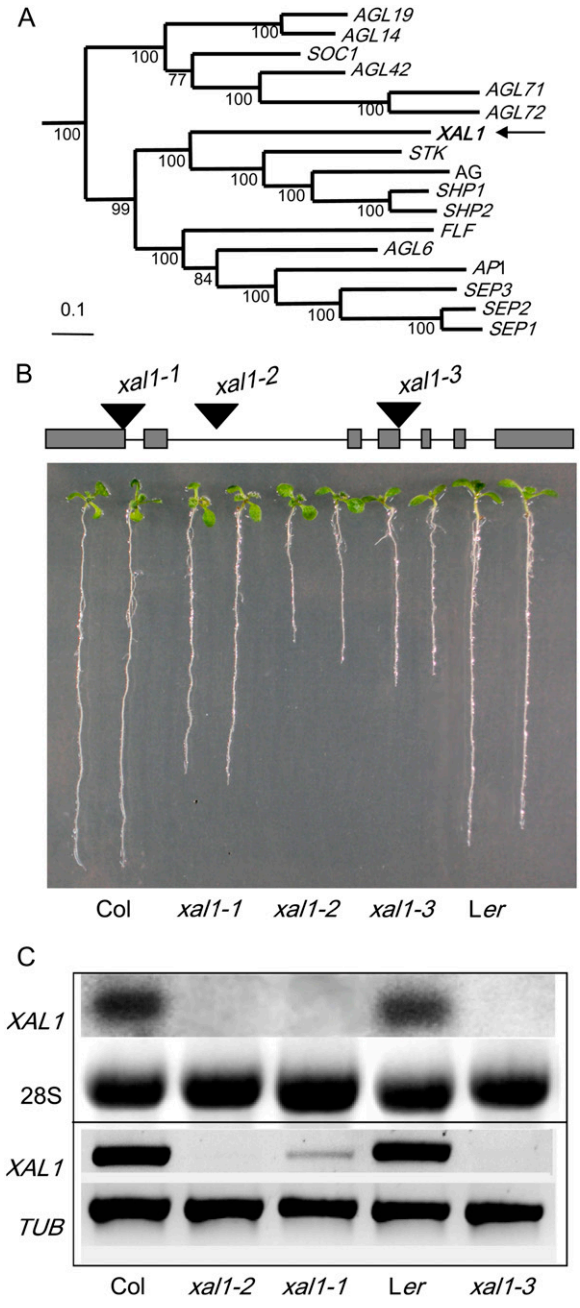

Figure 1. *XAL1* phylogeny and seedling mutant phenotypes. A, Bayesian reconstruction of the phylogenetic relationships among selected type II Arabidopsis MADS-box genes, with *XAL1* position indicated by an arrow. Numbers under the branches represent Bayesian posterior probability and can be interpreted as a measure of clade statistical support. B, Seedlings phenotype. Ten-day-old wild-type (Col-0 and L*er* ecotypes) and *xal1-1*, *xal1-2*, and *xal1-3* alleles were grown on vertical 0.2× Murashige and Skoog plates. On the top, *XAL1* gene schematic model with the sites of transposon or T-DNA insertions are shown. C, *XAL1* expression in root tissue from 14-d-old seedlings. Total RNA of both wild-type ecotypes and *xal1* alleles were subject to northern-blot hybridization (10 μg/lane; top) and semiquantitative RT-PCR. 28S and *TUBULIN* were used as internal load controls, respectively.

and columella being most affected (see examples in Fig. 2A). In a median optical section, the columella initial cells and QC cells could not be clearly recognized and the general meristem organization resembled an open-type RAM (Baum et al., 2002; Chapman et al., 2003). As a result of this disorganization, the root-cap protoderm initials giving rise to both protoderm (epidermis) and lateral root cap were abnormal in shape or could not be detected. Typical T divisions in the epidermis could be detected only in the distal portion of the RAM. This abnormal organization led to an altered columella cell differentiation. Whereas in wild-type plants these cells usually increase in length in each subsequent tier along the root axis toward the distal root end (Fig. 2A), in the affected *xal1* plants the columella cells in the root cap were of similar size along the root axis, being almost isodiametric rather than elongated as in wild-type plants (Fig. 2A; data not shown).

To further understand the observed shorter root phenotypes, we undertook quantitative cellular analyses of all *xal1* alleles. We have set up a protocol to document a series of cellular parameters geared to establish the role of root MADS-box or other types of genes in cellular homeostasis using the root as a study system (see ''Materials and Methods''; Supplemental Table S1). These analyses revealed that all three alleles have a shortened meristem with a significantly lower rate of cell production, and *xal1-2* and *xal1-3* have longer cell-cycle duration than in wild-type plants (Fig. 2B). In all cell parameters quantified, *xal1-1* showed milder phenotypes than *xal1-2* and *xal1-3* alleles (Fig. 2B; Supplemental Table S1). Therefore, *XAL1* constitutes the first MADS-box gene that affects cell-cycle duration and for which quantitative cellular data have been put forward to evaluate the role of these genes in regulating cell proliferation within the RAM.

Given that *xal1* mutants have significantly affected rates of cell production and cell-cycle duration, as well as an altered apico-basal pattern of cell behavior, *XAL1* could be regulated by auxin or *XAL1* could mediate responses to auxin in the root. Gradients and movement in the root of this plant hormone are sufficient to guide root growth by affecting cell behavior in a dose-dependent fashion (Sabatini et al., 1999; Galinha et al., 2007; Grieneisen et al., 2007). Maximal auxin levels maintain cell quiescence, intermediate levels promote cell proliferation, and lower levels induce cell elongation and differentiation (Galinha et al., 2007; Grieneisen et al., 2007).

XAL1 Is Expressed in the Phloem Tissue and *XAL1*::*GUS* Is Positively Induced by Auxins

To test whether *XAL1* responds to auxin levels, we constructed transgenic lines with a 2.8-kb *XAL1* promoter region driving the expression of GFP (*XAL1*::*GFP*; Fig. 3A) and GUS (*XAL1*::*GUS*; Fig. 3B). In the root of 8-d-old plants, GUS expression was detected in the vascular cylinder after 24-h staining, starting from

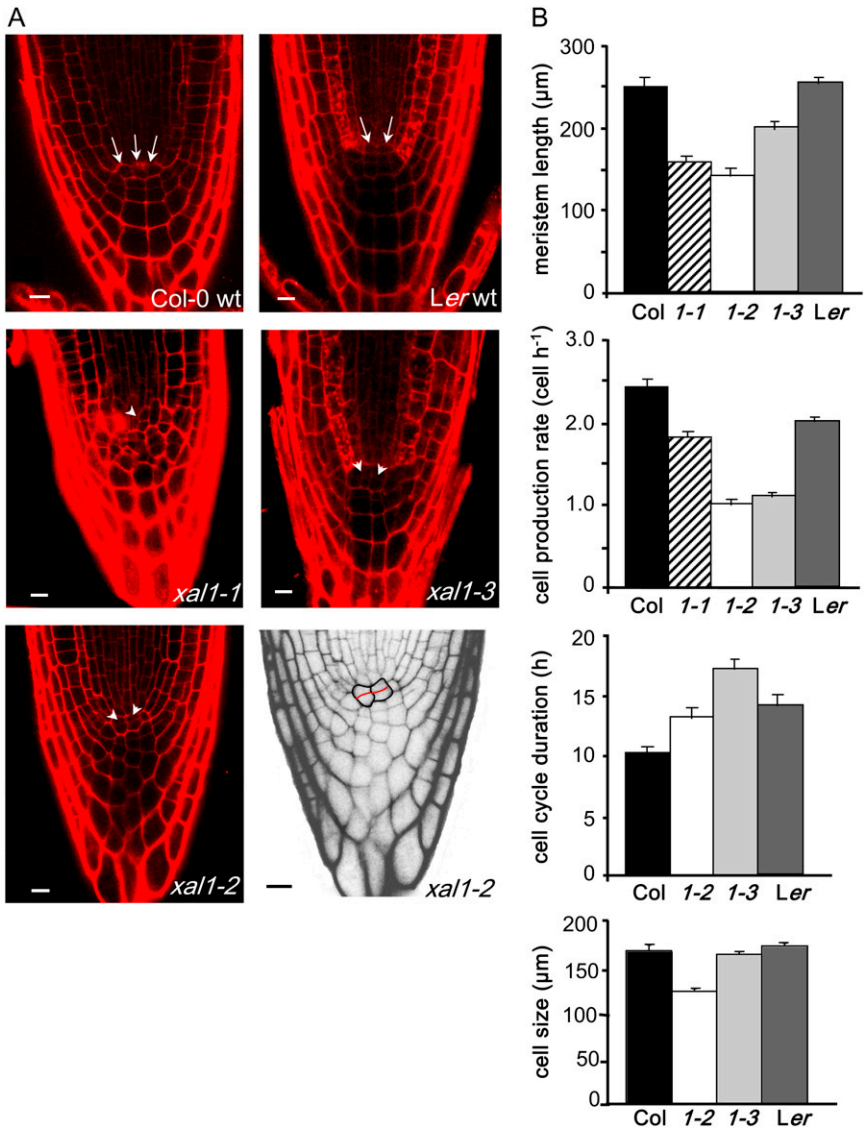

Figure 2. Root phenotype of *xal1* mutants. A, Open meristem organization in *xal1* alleles. Seven-day-old seedlings were stained with propidium iodide and analyzed by confocal microscopy. QC cells of wild type (arrows) and mutants (arrowhead) of representative phenotypes are shown (bar = 10 μm). Black-and-white zoom picture of *xal1-2* is shown to highlight abnormal periclinal divisions at the QC and deformed columella cells. B, Root cellular parameter analyses. Meristem length of 20 independent plants was measured from Col-0 and L*er* wild-type plants and *xal1-1*, *xal1-2*, and *xal1-3* alleles (*1-1*, *1-2*, *1-3*). Cell production rate, cell-cycle duration, and fully elongated cell length were obtained as described in "Materials and Methods." Bars = SES, calculated with JMP, version 5.1.1, statistical package (see data in Supplemental Table S1).

the elongation zone at the level where no signs of protoxylem differentiation were as yet detectable (Fig. 3B; data not shown). *XAL1* promoter activity in the differentiation zone was associated predominantly with protophloem cells (Fig. 3B). These results were confirmed with independent *XAL1*::*GFP* transgenic lines, which also reported the expression of the *XAL1* promoter in the root phloem in an identical pattern observed in *XAL1*::*GUS* lines (Fig. 3A). Additionally, 6.8-kb promoter constructs, as well as mRNA in situ hybridization (data not shown), revealed expression in the phloem. However, in situ data (Burgeff et al., 2002) also showed expression of *XAL1* in the root meristem that could not be recovered in the lines of these constructs, probably due to the absence of the second regulatory intron.

During lateral root formation, *XAL1*::*GUS* expression became visible only after root emergence, and the pattern was similar to that observed in the primary root (Fig. 3, C and D). This pattern of GUS activity driven by the *XAL1* promoter correlated well with a significant reduction also in lateral root length of the *xal1-1* plants compared to the wild-type plants (Fig. 3E).

Indole-3-acetic acid (IAA) treatment clearly induced GUS activity driven by the *XAL1* promoter (Fig. 3F). Interestingly, GUS expression was intensified only in the phloem tissue (Fig. 3G, left). In contrast, the *DR5*(7X)::*GUS* line in the wild-type background (Ulmasov et al., 1997) showed an expanded GUS activity domain that was found in all cell types when the roots were treated with auxins (Fig. 3G, right).

XAL1 Is a Positive Regulator of Flowering Transition That Responds to Photoperiod and Up-Regulates *SOC1*, *FT*, and *LFY*

While analyzing the *xal1* mutants, we realized that the plants were late flowering (Fig. 4A) and we de-

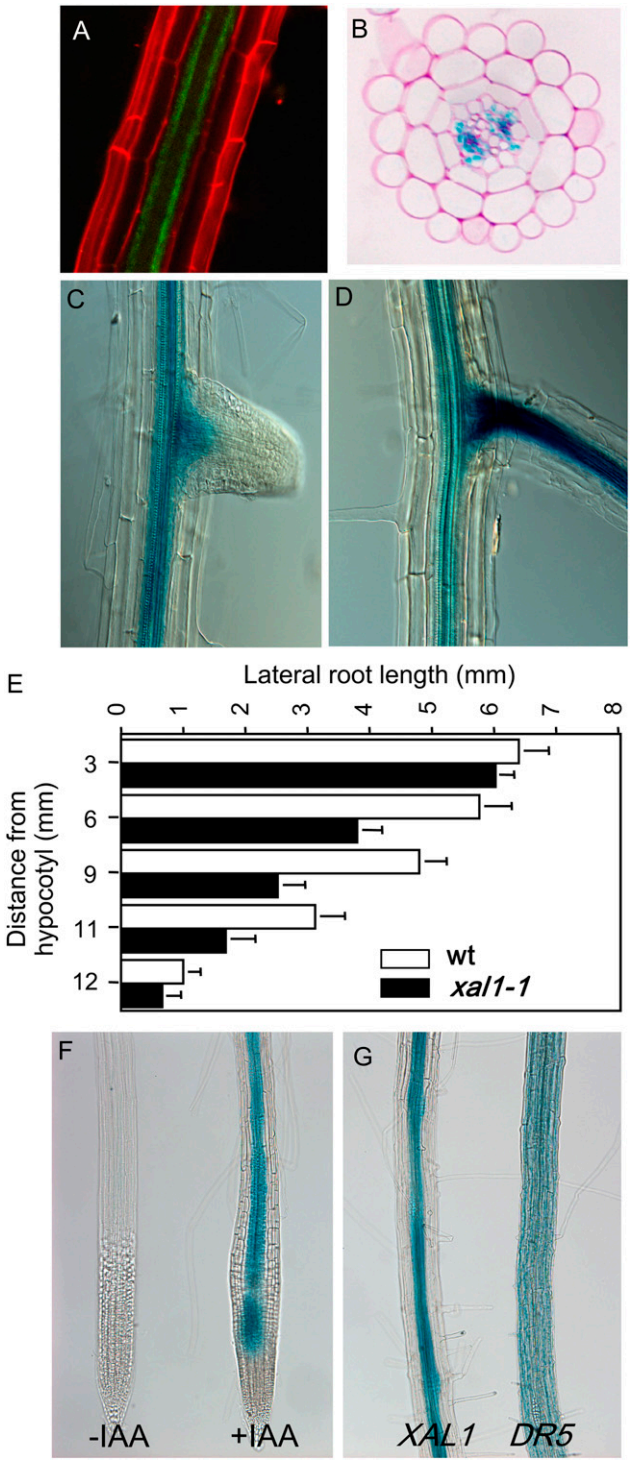

Figure 3. *XAL1* phloem expression is induced by auxins. A, Confocal image of an *XAL1*::*GFP* line taken at the protophloem plane, counterstained with propidium iodide. B, Transverse section of root *XAL1*::*GUS* line after GUS staining, counterstained with ruthenium red. C and D, *XAL1*::*GUS* expression in two different stages of lateral root development. E, Lateral root length along the primary root axis of the wild type (wt) and *xal1-1* allele ($n = 15$ plants; bars = SES). F, *XAL1*::*GUS* expression without (−IAA) and with (+IAA 2 μM). G, IAA-induced phloem GUS activity driven by the *XAL1* promoter (left) compared to the broad expression of the *DR5*::*GUS* line (right), after they were both treated with IAA (2 μM).

cided to pursue this phenotype and explore whether *XAL1* was expressed in aerial tissues. Indeed, in situ hybridization of *XAL1* mRNA revealed expression in floral meristems and also in vascular tissues in leaves (Fig. 4B). Detailed analyses of GUS activity in flower sections demonstrated that *XAL1*::*GUS* was specifically expressed in young flower meristems, subsequently becoming restricted to the nectaries (Fig. 4C), which contain phloem cells (Baum et al., 2001).

We further characterized the late-flowering phenotype of the *xal1-1* and *xal1-2* mutants both in the Col-0 background. The most striking characteristic of these mutants was the significant delay in flowering time measured by the bolting time and the total number of rosette leaves observed under long-day (LD) photoperiods (16 h/8 h) in comparison to wild-type plants (Fig. 4D).

Flowering time is regulated in Arabidopsis (*Arabidopsis thaliana*) by a network of signaling elements that can be assigned to at least four different pathways (Boss et al., 2004): one that promotes flowering in response to LD photoperiods, one that is essential for flowering under noninductive short-day condition (SD) and depends on the plant hormone GA, one that operates both under LD and SD conditions (also called autonomous pathway), and one that regulates flowering time in response to vernalization (Blazquez et al., 1998; Koornneef et al., 1998b; Blazquez and Weigel, 2000; Putterill, et al., 2004). In our experiments, both *xal1* mutants flowered almost concurrently as wild-type plants under SD conditions (Table I). Moreover, vernalization or GA_3 application rescued the flowering-time defects of *xal1* plants to the same extent as in wild-type plants under LD photoperiods (Table I). Thus, *XAL1* does not seem necessary for the integrity of the autonomous, GA, or vernalization pathways, but seems to be specifically necessary for the correct functioning of the photoperiod flowering pathway (Koornneef et al., 1998a; Imaizumi and Kay, 2006).

To confirm a possible genetic interaction between *XAL1* and previously characterized genetic components of the photoperiod and other integrators of flowering transition pathways (Reeves and Coupland, 2000; Moon et al., 2003), we analyzed mRNA expression of a number of genes known to be key regulators of flowering transition (Fig. 4E). First, we confirmed that *XAL1* mRNA levels were reduced in the shoot of both mutants. Indeed, *xal1-1* has drastically reduced levels of expression and in *xal1-2* we were unable to detect any mRNA expression (Fig. 4E). Interestingly, the flowering promoters *FT*, *SOC*, and *LFY* were clearly reduced at the mRNA level with respect to wild type in both *xal1-1* and *xal1-2* mutant backgrounds (Fig. 4E). In contrast, *CONSTANS* (*CO*) and *GIGANTEA* (*GI*; data not shown), which are upstream regulators in the photoperiod pathway (Mouradov et al., 2002), did not show significant alterations in mRNA expression in the *xal1* mutants in the Col-0 background. Furthermore, *XAL1* is down-regulated in the *co-1* background, although it is not totally repressed (Supplemental Fig. S1). On the

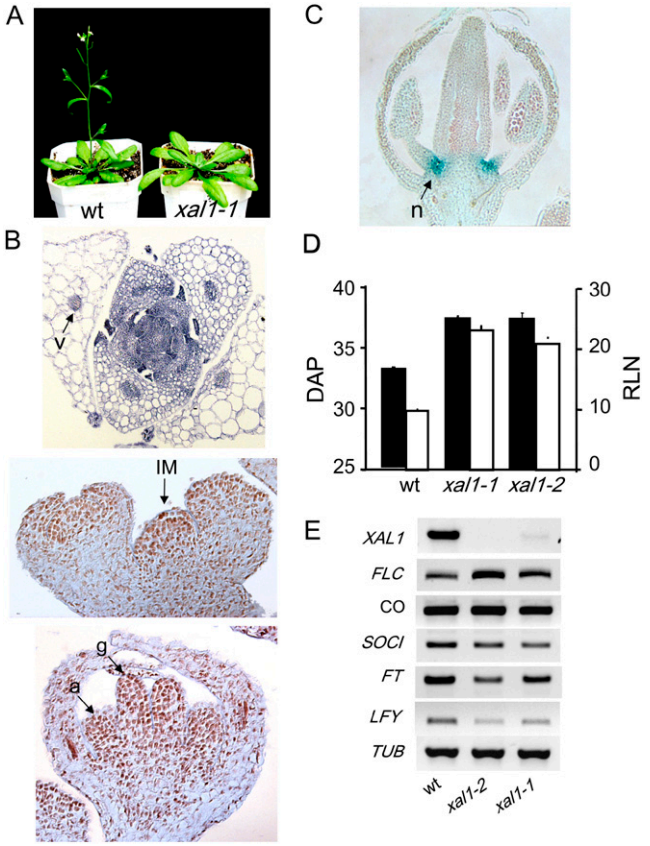

Figure 4. Flowering phenotype of *xal1-1* and *xal1-2* mutants and *XAL1* role in the photoperiod pathway. A, Late-flowering transition phenotype of the *xal1-1* mutant compared to wild-type plants. Both plants were 32 d old. B, *XAL1* mRNA in situ hybridizations. *XAL1* expression (arrows) in vascular tissue (v) of a 20 d after planting (DAP) vegetative shoot transverse section (top); in the inflorescence meristem (IM) longitudinal section (middle); and in the gynoecium (g) and anthers of a floral meristem (bottom). C, GUS expression in a floral bud longitudinal section of the *XAL1*::*GUS* line. Strong GUS staining corresponds to the nectaries (n). D, Late-flowering phenotype of *xal1* mutants. Bolting time scored by DAP at bolting (see data for LD; Table I) in black bars and total rosette leaf number (RLN) in white bars of *xal1-1* and *xal1-2* alleles compared to wild-type plants (wt). E, Comparative transcript accumulation of genes that participate in the photoperiod and integrative flowering pathways. Gene expression levels were analyzed in the shoots of 14-d-old seedlings of wild type and *xal1* mutants by RT-PCR. *TUBULIN* was included as a constitutive control. A to E, Plants were grown under LD photoperiods.

other hand, *FLC*, which is a flowering repressor and acts over *FT* and *SOC* (Michaels and Amasino, 1999; Searle et al., 2006), showed a slight up-regulation with respect to wild-type plants in both of the *xal1* mutants studied. The latter results also correlate with the late-flowering phenotypes of these *xal1* alleles.

DISCUSSION

We have shown here that the Arabidopsis MADS-box gene, *XAL1*, is required for normal root development and proper flowering transition based on mutant phenotypes of two alleles in the Col-0 background and one allele in the L*er* background. These alleles were named here *xaantal1-1*, *xaantal1-2*, and *xaantal1-3* due to their slow-growing root and late-flowering phenotypes. These results were unexpected considering that *XAL1* is a sister gene to the *AG*-related genes that are specific for reproductive tissues, and that most previously characterized MADS-box genes cluster in phylogenetic clades of genes with similar functions and expression patterns during flower, ovule, or carpel development (Rounsley et al., 1995; Alvarez-Buylla et al., 2000a). Nonetheless, previous studies for *XAL1* had already suggested that this gene could function in root development due to its high and apparently specific expression in roots (Rounsley et al., 1995; Burgeff et al., 2002). In this study, we have confirmed that *XAL1* is indeed expressed in roots, but we show that it is also expressed in aerial tissues prior to the transition to flowering and within floral meristems. In accordance with this pattern of expression, *XAL1* is also important for flowering transition.

Functional involvement in more than one tissue or developmental stage might be more common among MADS-box genes than originally believed based on the characterization of the flower-specific MADS-box genes of the A, B, and C functions (Coen and Meyerowitz, 1991). Indeed, recent studies have shown that most genes of this family are expressed in several plant tissues, organs, and developmental stages (Kofuji et al., 2003; Parenicová et al., 2003; Schmid et al., 2005). Other studies suggest that MADS-box functional specificity may depend on combinatorial protein-protein interactions (Egea-Cortines et al., 1999; Honma and Goto, 2001; de Folter et al., 2005; Kaufmann et al., 2005; Gregis et al., 2006; Sridhar et al., 2006), rather than on specific spatiotemporal expression patterns for each gene determined at the transcriptional level, as had been suggested before (Savidge et al., 1995; Alvarez-Buylla et al., 2000a).

XAL1 Is an Important Regulator of Cell Proliferation in the Root Meristem

In the root axis, three main zones with contrasting cell proliferation patterns can be distinguished: the RAM, where active cell proliferation takes place from the stem cell niche established around the QC or organizer, and two zones where cells are not proliferating, namely, the elongation and the differentiation zones (Fig. 5; Dolan et al., 1993; Ioio et al., 2007). The data summarized in this article suggest that *XAL1* is an important component of the molecular mechanisms controlling cell proliferation in the root. Consequently, the loss-of-function alleles analyzed for this gene show clear spatial alterations of cell behavior along the longitudinal axis of the Arabidopsis root with respect to wild-type plants. Our data suggest that this phenotype is indeed due to the lack of *XAL1* because we observed complementation to wild-type root phenotypes using a 35S::*XAL1* construct plasmid transformed into *xal1-1* and *xal1-2* (data not shown).

Table I. *Bolting time of xal1-1 and xal1-2 mutant plants compared to wild type (Col-0) at different flowering-transition pathways*

Days after sowing are expressed as mean ± SE and results for LD photoperiod are statistically significant. Flowering-time measurements and conditions for LD and SD photoperiods and both of them after vernalization (+VER) and gibberellin (+GA_3) treatments, respectively, are explained in "Materials and Methods."

Plant Line	Growth Conditions			
	LD	LD + VER	SD	SD + GA_3
Col-0	33.1 ± 0.4 (n = 78)	25.2 ± 0.6 (n = 37)	70.0 ± 0.7 (n = 38)	44.0 ± 0.5 (n = 21)
xal1-1	37.5 ± 0.5 (n = 48) $P < 0.0001$	28.0 ± 1.9 (n = 41)	68.1 ± 1.9 (n = 18)	–
xal1-2	37.4 ± 0.4 (n = 70) $P < 0.0001$	26.9 ± 0.5 (n = 46)	71.7 ± 0.7 (n = 27)	44.5 ± 0.5 (n = 21)

Drastically diminished levels of *XAL1* expression were correlated with altered cellular organization of the RAM, but only in one-third of the analyzed plants for the three *xal1* alleles. In these cases, we observed periclinal divisions of the QC early in root development and also lateral expansion of columella cells. However, all *xal1-2* and *xal1-3* mutant roots were shorter and had a decreased cell production rate, shorter elongated cells, and a significantly longer cell cycle that correlated with smaller meristems. Therefore, the altered cellular patterns at and around the QC in the affected plants are likely to be a consequence rather than a cause of the diminished cell production rates in the root meristem. In any case, these data suggest that type II plant MADS-box genes could be directly involved in cell-cycle regulation. The punctate pattern of mRNA in situ expression revealed for *XAL1* in the root meristematic tissues is also suggestive of a correlation of this gene expression with cell-cycle stage (Burgeff et al., 2002). In addition, *XAL1* is also involved in the regulation of cell elongation. However, this effect is apparently masked in the weaker *xal1-1* allele (data not shown; Supplemental Table S1; Figs. 2 and 5).

Future studies should further pursue the role of *XAL1* in the molecular networks controlling cell proliferation, elongation, and differentiation. Some components of such networks during root development have been characterized. *SHORT-ROOT* (*SHR*) and *SCARECROW* (*SCR*) are required for QC identity and normal root growth in addition to their role in radial patterning (Scheres et al., 1995; Di Laurenzio et al., 1996; Helariutta et al., 2000; Wysocka-Diller et al., 2000; Nakajima et al., 2001, Sabatini et al., 2003). However, because the *SHR*/*SCR* pathway specifies the entire layer surrounding provascular tissues in the root, it is necessary, but not sufficient, to define the exact position of the stem cell niche. Auxin is also an important signal of QC establishment and it regulates the *SCR* and *PLETHORA* (*PLT*) genes, which are also necessary for QC determination (Sablowski, 2004b).

WOX5 is also expressed in the QC and this gene seems to be necessary and sufficient for stem cell identity (Sarkar et al., 2007), probably with a more direct function in stem cell signaling, rather than in specifying QC identity. WOX5 protein or, most probably a downstream factor, might move to stem cells to maintain their identity (Sarkar et al., 2007). In contrast to these genes that have been shown to be important in QC specification and root growth, *XAL1* does not show a peak of expression in the QC or stem cell niche, but loss-of-function mutants in this gene also show cellular aberrations in this zone and clear alterations in cell proliferation and root growth. This suggests that this MADS-box gene could be itself a non-cell autonomous signal from more differentiated tissues (columella and vascular tissues) or control another non-cell autonomous downstream component, which could also be important for QC and stem cell behavior and thus cell production rate in the root meristem. It will be important to use genetic approaches to test whether *XAL1* functions are independent or not of *SCR*, *SHR*, and *WOX5* pathways.

Our data demonstrate that the cell production rate is lower in *xal1* mutants than in wild type, but premature cell differentiation could also contribute to the smaller meristems of *xal1* mutants. Interestingly, recent experiments have shown that cytokinins affect cell differentiation and define the root meristem by antagonizing from the transition zone a non-cell autonomous signal that could be auxin (Ioio et al., 2007). Moreover, down-regulation of cytokinins in the vascular tissue is sufficient to enlarge the root meristem by retarding the transition of cells to the elongation and differentiation zones. These results and *xal1* data presented here thus suggest that *XAL1* could be regulated and/or mediate cytokinin functions. This should be tested with genetic approaches.

Auxin Up-Regulates *XAL1* Specifically in the Root Phloem

Auxin promotes cell elongation, cell-cycle duration, and cell differentiation (Evans et al., 1994; Abel and Theologis, 1996; Himanen et al., 2002; Vanneste et al., 2005). In the root, auxin gradients and movement are sufficient to guide root growth (Sabatini et al., 1999; Galinha et al., 2007; Grieneisen et al., 2007) and affect cell behavior in a dose-dependent fashion (Galinha et al., 2007; Grieneisen et al., 2007). In concordance, auxin response or transport mutants display root-patterning defects and exogenous application of auxin induces ectopic QC and stem cells (Sabatini et al., 1999; Friml et al., 2002). Given that the *xal1* mutants analyzed here showed root phenotypes affected in these traits,

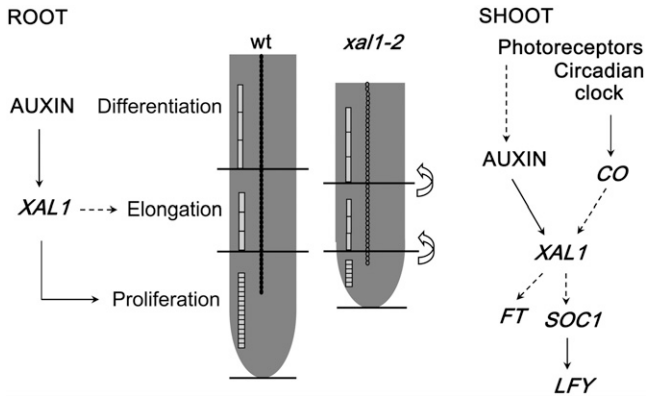

Figure 5. Model for the role of *XAL1* in root and shoot development. The MADS-box gene, *XAL1*, might mediate auxin participation in the proliferation of the root meristematic cells and the shoot meristem. In the root, *XAL1* may also be implicated in cell elongation because the *xal1-2* allele has smaller cells than wild type. Auxin may participate in the shoot meristem transition to flowering, mediating light induction of *XAL1*, which in turn may be an important promoter of downstream regulators in the photoperiod pathway. CO also induces *XAL1* expression probably by the classical photoperiod pathway. Solid arrows indicate direct proved regulation and dashed arrows suggest direct/indirect regulation.

XAL1 could mediate auxin function. Indeed, our data clearly show that *XAL1* is up-regulated after IAA treatment within the root phloem tissues, where it is normally and strongly expressed. The *XAL1* promoter also responds positively to other auxin analogs, 2,4-dichlorophenoxyacetic acid (2-4D) and naphthaleneacetic acid (NAA; data not shown). Our results thus suggest that there is a phloem-specific factor that responds to auxins and that is required for *XAL1* transcriptional up-regulation within these tissues or that *XAL1* is itself an auxin-responsive factor. The latter is supported by the presence of several auxin response elements, TGA (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) and SAUR boxes (http://www.dna.affrc.go.jp/PLACE; Higo et al., 1999) in the promoter of *XAL1*.

XAL1 could also be important for phloem cellular patterning. Careful examination of the phloem in the *xal1* mutants indicated, however, that *XAL1* does not have a key role in the morphogenesis of this tissue on its own because procambial establishment and vascular cell identity in the root are not affected in *xal1* mutants in comparison to wild-type plants.

Downstream molecular mechanisms that integrate the cellular effects of auxins and other plant hormones, such as cytokinins, in different spatiotemporal domains during root development are not fully understood. Our data suggest that *XAL1* could be one component of such mechanisms. Interestingly, it has recently been suggested that the *PLT1* and *PLT2* genes, which depend on auxin and auxin response factors for expression, could be the read-out of the root auxin gradient (Galinha et al., 2007). The *XAL1* role on cell behavior along the root axis could be related to *PLT1* and *PLT2* function or it could be part of an independent mechanism. The latter seems to be the case given that *XAL1* expression does not overlap with that of the *PLT1* and *PLT2*, which have a gradient-type expression pattern similar to that of auxins with a peak of expression at the QC (Aida et al., 2004). However, other *PLETHORA* (*PLT3* and *BBM*) genes have strong mRNA expression in the columella stem cell layer and the provascular tissues and could partially overlap with *XAL1* expression (Galinha et al., 2007).

XAL1 Is a Promoter of the Floral Transition and Participates in the Photoperiod Pathway

Interestingly, *XAL1* is not only important for root development, but is also expressed in aerial tissues and is an important component of the photoperiod pathway of flowering transition, functioning as a flowering promoter in Col-0 Arabidopsis (Reeves and Coupland, 2000; Mouradov et al., 2002). The diminished mRNA levels of the three flowering promoters, *SOC*, *FT*, and *LFY* in the *xal1-1* and *xal1-2* mutant backgrounds are consistent with this interpretation. *FT* and *SOC* act as floral integrators of several pathways, whereas *LFY* is a flower meristem identity gene that positively responds to *FT* and *SOC1* (Blazquez and Weigel, 2000; Ng and Yanofsky 2000; Moon et al., 2003; Corbesier and Coupland, 2006). None of the other three flowering-transition pathways was affected in these mutant alleles (Table I).

In contrast to several key components of the photoperiod pathway (e.g. *CO*, *GI*, *CRYPTOCHROME2* [*CRY2*], and *FT*; Koornneef et al., 1998a; Simpson and Dean, 2002; Komeda, 2004), the *xal1* late-flowering phenotype under LD photoperiods can be recovered to a wild-type phenotype following vernalization (Michaels and Amasino, 2000). In agreement with this, the MADS-box flowering repressor *FLC* (Michaels and Amasino, 1999; Rouse et al., 2002) is up-regulated in *xal1* backgrounds. Therefore, our data suggest that *XAL1* could be downstream of *CO* and *GI* and upstream of *SOC*, *FT*, and *LFY*. However, complementation of *co* and *gi* mutants with *XAL1* overexpression constructs, and conversely the overexpression of *SOC1* in the *xal1* mutant backgrounds, should be pursued in the future to confirm the proposed role of *XAL1* in the photoperiod pathway.

There are two possibilities to reconcile the root data for the *xal1* mutants with their phenotypes in flowering transition. One possibility is that, given the recently proposed role for auxin response factors in flowering (Ellis et al., 2005; Okushima et al., 2005), *XAL1* is a mediator of auxin signaling and participates in the regulation of cell behavior in root and shoot meristems, thus altering their transitions (Fig. 5). The second possibility is that *XAL1* has different roles in root and aerial meristems as part of different complexes with other MADS-box proteins, or being a downstream component of different signaling mechanisms.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) wild-type, *xal1-1*, and *xal1-2* plants, *co-1*, and the *DR5*(7X)::*GUS* auxin reporter line (Ulmasov et al., 1997) are in the Col-0 genetic background, whereas *xal1-3* is in the L*er* ecotype. Seedlings were grown on vertical plates with 0.2× Murashige and Skoog salts and 1% Suc. Plants were grown in climate chambers at 22°C. The photoperiods (110 μE m^{-2} s^{-1}) were established at 16 h of light followed by 8 h of dark for LD photoperiods and 8 h of light followed by 16 h of dark for SD photoperiods.

Identification of Mutant Alleles

The *xal1-1* allele was identified by screening for *En-1* insertions among a collection of Arabidopsis plants carrying approximately 50,000 independent insertions of the autonomous maize (*Zea mays*) transposable element (Baumann et al., 1998). The collection was screened in pools using the *En-1* transposon primer En205 (5′-AGAAGCACGACGGCTGTAGAATAGGA-3′) and the internal *XAL1* primers OEAB141 (5′-GGTCGTGGTTCTTCTTCTGCT-3′) and OEAB143 (5′-CATTTCATCTTCACACCAAC-3′). The *xal1-2* homozygous line was isolated from the Nottingham Arabidopsis Stock Centre T_2 generation stock N429367 (former GK_306H03 from the GABI-Kat collection). Plants 100% resistant to sulfadiazine were further confirmed by PCR using the following primers: GK T-DNA (5′-CCCATTTGGACGTGAATGTAGACAC-3′) and specific *XAL1* primers NASC12-LP (5′-ACCCAAACGTCAAATCATCAG-3′) and NASC12-RP (5′-CTTCATTCCGAAACACAATGC-3′). The *xal1-3* allele was identified by screening on a two-component system mutagenized collection based on the maize mobile/transposon *Spm* as described by Speulman et al. (1999).

Microscopy

Plant material for light microscopy was prepared as previously described by Malamy and Benfey (1997). Roots were visualized under an Olympus BX60 microscope. Confocal images were acquired on an inverted Zeiss LSM 510 Meta microscope with a 63× water immersion objective after root was stained with 10 μg mL^{-1} propidium iodide.

Quantitative Analysis of Cellular Parameters of Root Growth

Length of the meristem was determined for the cortex cells as the distance between the root-body/root-cap junction to the level where cells started to elongate, according to Casamitjana-Martinez et al. (2003). The length of the elongation zone was taken as the distance between the proximal meristem border and the location of the most distal root-hair bulge. The average cycle time for cortical cell production in plants growing between 7 to 8 d was done, using the rate of cell production (Ivanov and Dubrovsky, 1997). The duration of the cell cycle (T) was calculated for each individual root using the following equation: $T = (\ln 2\, N_m\, l_e)\, V^{-1}$, where N_m is the number of meristematic cells in one file of the cortex, l_e are the fully elongated cell length calculated as the average length of 10 fully elongated cortex cells in the same root, and V is the root growth rate calculated as μm h^{-1}. N_m in 7- and 8-d-old roots (a period during which the rate of the root growth was estimated) was similar in both the wild-type and mutant plants, which enabled us to consider root growth to be at steady state and apply the method described above. The rate of cell production was estimated as $V(l_e)^{-1}$ (Baskin, 2000). Statistical Student's t test or the Tukey-Kramer test (depending on the sample size) was analyzed by the JMP program, version 5.1.1.

Reporter Lines

For *XAL1*::*GUS* and *XAL1*::*GFP* constructs, a 2.8-kb or 6.8-kb promoter and the 5′ untranslated region were obtained from a Lambda genomic DNA library and cloned into pGEM-T vector (Promega) as a *Sal*I-*Xba*I fragment. This fragment was subcloned into the pBI101 binary vector and the mGFP5-ER to generate the *XAL1*::*GUS* and the *XAL1*::*GFP* lines, respectively. Arabidopsis Col-0 ecotype plants were transformed using the floral-dip method (Clough and Bent, 1998). The transgenic lines were selected based on their kanamycin resistance and the expression analysis was carried out on T_3 homozygous lines.

Hormone Treatments and GUS Reaction

XAL1::*GUS* and *DR5*(7X)::*GUS* seedlings were grown for 7 d in hormone-free medium plates and then transferred to growth medium supplemented with 2 μM of the following hormones: IAA, NAA, and 2,4-D for 24 h. After hormone treatment, *DR5*(7X)::*GUS* and *XAL1*::*GUS* seedlings were subjected to GUS staining during 40 min at room temperature and 5 h at 37°C, respectively. Stained plants were cleared and visualized under a microscope.

In Situ Hybridization and Histochemical Analysis

Inflorescence and bud flowers from wild-type and *xal1-1* were subjected to in situ hybridization (Drews et al., 1991). Digoxigenin-labeled *XAL1* probes were synthesized using a 113-bp cDNA template amplified with 5′-ATA-AAGCCTGTGGAACTTC-3′ and 5′-TAAGTACACACCACACTTG-3′ primers, cloned in pGEM-T Easy vector.

For flower histochemical analysis, samples were processed according to the protocol described in Blazquez et al. (1998). For histological root analysis, GUS-stained samples were dehydrated through ethanol/histoclear series until they were substituted with 100% histoclear (National Diagnostics). Finally, material was embedded in Paraplast+ (Oxford Labware). Transversal sections of 8-μm-thick GUS-positive root samples were counterstained with 0.1% ruthenium red (Scheres et al., 1994).

Expression Analysis by Northern Blot and RT-PCR

Wild-type and mutant seedlings were grown for 14 d on Murashige and Skoog plates under LD conditions. Total RNA was isolated from root or shoot tissue separately using TRIzol reagent (Invitrogen). Semiquantitative RT-PCR was performed from two different experiments, each time with duplicates. PCR amplification conditions and sequence primers are described in Supplemental Table S2. RNA-blot hybridization was performed with 10 μg of total RNA per lane with a gene-specific 3′ probe, amplified with the following primers: 5′-GGATGTTATGCTTCAAGAAATTC-3′ and 5′-CCAAATAATC-CATAAATTCAAAAC-3′.

Flowering-Time Measurements

The bolting time was measured as the days after seed sowing required for the stem to develop 1 cm long under either photoperiod condition. Total number of rosette leaves included fully expanded and not fully expanded leaves. For experiments involving vernalization, seeds were plated on Murashige and Skoog medium and kept under dark for 6 weeks at 4°C and then transferred to soil and grown under LD conditions until flowering. To examine GA_3 effects on flowering time, 100 μM GA_3 solution was sprayed once a week starting 30 d after sowing and continued until bolting. Data expressed as mean ± SE were analyzed by the JMP program, version 5.1.1.

Phylogenetic Analysis

We performed a Bayesian reconstruction of the phylogenetic relationships among selected type II Arabidopsis MADS-box genes using the whole cDNAs. Bayesian methods with MrBayes according to Huelsenbeck and Ronquist (2001) were used with a Markov chain Monte Carlo exploration of the tree likelihood surface. Four independent Markov chains (three heated) were used according to the Metropolis coupled scheme. The codon substitution model used was that of Goldman and Yang (1994). Four independent runs of 2,500,000 generations each were performed, and every 100th tree was saved. After checking for Markov chain convergence, we discarded the first 15,000 trees and used the remaining trees to calculate Bayesian posterior probabilities of the clades. Results from every independent run were similar.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number NC_003070.5.

Supplemental Data

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

Supplemental Figure S1. *XAL1* expression in *co-1* background.

Supplemental Table S1. Quantitative analysis of root development in *xal1-2* and *xal1-3* strong alleles and their respective control wild-type plants.

Supplemental Table S2. List of the oligonucleotides used for RT-PCR experiments.

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LITERATURE CITED

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol **111:** 9–17

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell **119:** 109–120

Alvarez-Buylla ER, Liljegren SJ, Pelaz S, Gold SE, Burgeff C, Ditta GS, Vergara-Silva F, Yanofsky MF (2000a) MADS-box gene evolution beyond flowers: expression in pollen, endosperm, guard cells, roots and trichomes. Plant J **24:** 457–466

Alvarez-Buylla ER, Pelaz S, Liljegren SJ, Gold S, Burgeff C, Ditta GS, Ribas de Pouplana L, Martinez-Castilla L, Yanofsky MF (2000b) An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. Proc Natl Acad Sci USA **10:** 5328–5333

Baskin TI (2000) On the constancy of cell division rate in the root meristem. Plant Mol Biol **43:** 545–554

Baum SF, Eshed Y, Bowman JL (2001) The *Arabidopsis* nectary is an ABC-independent floral structure. Development **126:** 4657–4667

Baum ST, Dubrovsky JG, Rost TL (2002) Apical organization and maturation of the cortex and vascular cylinder in Arabidopsis thaliana (Brassicaceae) roots. Am J Bot **89:** 908–920

Baumann E, Lewald J, Saedler H, Schultz B, Wisman E (1998) Successful PCR-based reverse genetic screen using an *En-1* mutagenised *Arabidopsis thaliana* population generated via single-seed descent. Theor Appl Genet **97:** 729–734

Benfey PN, Scheres B (2000) Root development. Curr Biol **10:** R813–R815

Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. Plant Cell **10:** 791–800

Blazquez MA, Weigel D (2000) Integration of floral inductive signals in *Arabidopsis*. Nature **404:** 889–892

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell (Suppl) **16:** S18–S31

Bowman JL, Alvarez J, Weigel D, Meyerowitz EM, Smyth DR (1993) Control of flower development of *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development **119:** 721–743

Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes of *Arabidopsis*. Development **112:** 1–20

Burgeff C, Liljegren SJ, Tapia-Lopez R, Yanofsky MF, Alvarez-Buylla ER (2002) MADS-box gene expression in lateral primordia, meristems and differentiated tissues of *Arabidopsis thaliana* roots. Planta **214:** 365–372

Caro E, Castellano MM, Gutierrez C (2007) A chromatin link that couples cell division to root epidermis patterning in Arabidopsis. Nature **447:** 213–217

Casamitjana-Martinez E, Hofhuis HF, Xu J, Liu CM, Heidstra R, Scheres B (2003) Root-specific *CLE19* over-expression and the *sol1/2* suppressors implicate a CLC-like pathway in the control of *Arabidopsis* root meristem maintenance. Curr Biol **13:** 1435–1441

Chapman K, Groot EP, Nichol SA, Rost TL (2003) Primary root growth and the pattern of root apical meristem organization are coupled. J Plant Growth Regul **21:** 287–295

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735–743

Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. Nature **353:** 31–37

Corbesier L, Coupland G (2006) The quest for florigen of recent progress. J Exp Biol **57:** 3395–3403

de Folter S, Immink RGH, Kleffer M, Parenicova L, Henz SR, Weigel D, Bussher M, Kooiker M, Colombo L, Kater MM, et al (2005) Comprehensive interaction map of the *Arabidopsis* MADS box transcription factor. Plant Cell **17:** 1424–1433

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. Cell **86:** 423–433

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organisation of the Arabidopsis thaliana root. Development **119:** 71–84

Drews GN, Bowman JL, Meyerowitz EM (1991) Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. Cell **65:** 991–1002

Egea-Cortines M, Saedler H, Sommer H (1999) Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus*. EMBO J **18:** 5370–5379

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) *AUXIN RESPONSE FACTOR1* and *AUXIN RESPONSE FACTOR2* regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development **132:** 4563–4574

Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER (2004) A regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. Plant Cell **16:** 2923–2939

Evans ML, Ishikawa H, Estelle MA (1994) Responses of Arabidopsis roots to auxin studied with high temporal resolution—comparison of wild-type and auxin-response mutants. Planta **194:** 215–222

Ferrandiz C, Liljegren SL, Yanofsky MF (2000) negative regulation of *SHATTERPROOF* genes by *FRUITFULL* during *Arabidopsis* fruit development. Science **289:** 436–438

Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, et al (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. Cell **108:** 661–673

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B (2007) PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. Nature **449:** 1053–1057

Goldman N, Yang Z (1994) A codon-based model of nucleotide substitution for protein-coding DNA sequences. Mol Biol Evol **11:** 725–736

Gregis V, Sessa A, Colombo L, Kater MM (2006) *AGL24, SHORT VEGETATIVE PHASE*, and *APETALA1* redundantly control *AGAMOUS* during early stages of flower development in *Arabidopsis*. Plant Cell **18:** 1373–1382

Grieneisen VA, Xu J, Marée AFM, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature **449:** 1008–1013

Gu Q, Ferrandiz C, Yanofsky MF, Martienssen R (1998) The *FRUITFULL* MADS-box genes mediates cell differentiation during Arabidopsis fruit development. Development **125:** 1509–1517

Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, Hauser MT, Benfey PN (2000) The SHORT-ROOT gene controls radial patterning of the Arabidopsis root through radial signaling. Cell **101:** 555–567

Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic Acids Res **27:** 297–300

Himanen K, Boucheron E, Vanneste S, de Almeida Engler J, Inzé D, Beeckman T (2002) Auxin-mediated cell cycle activation during early lateral root initiation. Plant Cell **14:** 2339–2351

Honma T, Goto K (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature **409:** 525–529

Huelsenbeck JP, Ronquist F (2001) Mr Bayes: Bayesian inference of phylogeny. Bioinformatics **17:** 754–755

Imaizumi T, Kay SA (2006) Photoperiodic control of flowering: not only by coincidence. Trends Plant Sci **11:** 550–558

Ioio RD, Linhares FS, Scacchi E, Casamitjana-Martinez E, Heidstra R, Costantino P, Sabatini S (2007) Cytokinins determine Arabidopsis root-meristem size by controlling cell differentiation. Curr Biol **17:** 678–682

Ivanov VB, Dubrovsky JG (1997) Estimation of the cell-cycle duration in the root apical meristem: a model of linkage between cell-cycle duration, rate of cell production, and rate root growth. Int J Plant Sci **158:** 757–763

Kaufmann K, Melzer R, Theißen G (2005) MICK-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene **347:** 183–198

Kofuji R, Sumikawa N, Yamasaki M, Kondo K, Ueda K, Ito M, Hasebe M (2003) Evolution and divergence of the MADS-box gene family based on genome-wide expression analyses. Mol Biol Evol **20:** 1963–1977

Komeda Y (2004) Genetic regulation of time to flower in *Arabidopsis thaliana*. Annu Rev Plant Biol **55:** 521–535

Koornneef M, Alonso-Blanco C, Blankestijn-de VH, Hanhart CJ, Peeters AJM (1998a) Genetic interactions among late flowering mutants of *Arabidopsis*. Genetics **148:** 549–563

Koornneef M, Alonso-Blanco C, Peeters AJM, Soppe W (1998b) Genetic control of flowering time in *Arabidopsis*. Annu Rev Plant Physiol Plant Mol Biol **49:** 345–370

Lawrence PA, Morata G (1994) Homeobox genes: their function in *Drosophila* segmentation and pattern formation. Cell **78:** 181–189

Lazaro JB, Bailey PJ, Lassar AB (2002) Cyclin D-cdk4 activity modulates the subnuclear localization and interaction of MEF2 with SRC-family coactivators during skeletal muscle differentiation. Genes Dev **16:** 1792–1805

Liljegren SJ, Ditta GS, Eshed Y, Savidge B, Bowman JL, Yanofsky MF (2000) *SHATERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. Nature **404:** 766–770

Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development **124:** 33–44

Mandel MA, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature **360:** 273–277

Martínez-Castilla LP, Alvarez-Buylla ER (2003) Adaptive evolution in the *Arabidopsis* MADS-box gene family inferred from its complete resolved phylogeny. Proc Natl Acad Sci USA **100:** 13407–13412

Messenguy F, Dubois E (2003) Role of MADS box proteins and their cofactors in combinatorial control of gene expression and cell development. Gene **316:** 1–21

Michaels S, Amasino R (1999) *FLOWERING LOCUS C* encodes a novel MADS-domain protein that acts as a repressor of flowering. Plant Cell **11:** 949–956

Michaels SD, Amasino RM (2000) Memories of winter: vernalization and the competence to flower. Plant Cell Environ **23:** 1145–1153

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I (2003) The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J **35:** 613–623

Mouradov A, Cremer F, Coupland G (2002) Control of flowering time: Interacting pathways as a basis for diversity. Plant Cell (Suppl) **14:** S111–S130

Nakajima K, Sena G, Nawy T, Benfey PN (2001) Intercellular movement of the putative transcription factor SHR in root patterning. Nature **413:** 307–311

Nesi N, Debeaujon I, Jond C, Stewart AJ, Jenkins GI, Caboche M, Lepiniec L (2002) *TRANSPARENT TESTA16* locus encodes the ARABIDOPSIS B-SISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. Plant Cell **14:** 2463–2479

Ng M, Yanofsky MF (2000) Three ways to learn the ABCs. Curr Opin Plant Biol **3:** 47–52

Okushima Y, Mitina I, Quach HL, Theologis A (2005) AUXIN REPONSE FACTOR 2 (ARF2): a pleiotropic developmental regulator. Plant J **43:** 29–46

Parenicová L, de Folter S, Kieffer M, Horner DS, Favalli C, Busscher J, Cook HE, Ingram RM, Kater M, Davies B, et al (2003) Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in *Arabidopsis*: new openings to the MADS world. Plant Cell **15:** 1–15

Pelaz S, Ditta G, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral identity functions require *SEPALLATA* MADS-box genes. Nature **405:** 200–203

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature **424:** 85–88

Putterill J, Laurie R, Macknight R (2004) It's time to flower: the genetic control of flowering time. Bioessays **26:** 363–373

Reeves PH, Coupland G (2000) Response of plant development to environment: control of flowering by day. Curr Opin Plant Biol **3:** 37–42

Riechmann JL, Meyerowitz EM (1997) MADS domain proteins in plant development. Biol Chem **378:** 1079–1101

Rijpkema AS, Gerats T, Vandenbussche M (2007) Evolutionary complexity of MADS complexes. Curr Opin Plant Biol **10:** 32–38

Rounsley SR, Ditta G, Yanofsky MF (1995) Diverse roles for MADS-box genes in *Arabidopsis* development. Plant Cell **7:** 1259–1269

Rouse DT, Sheldon CC, Bagnall DJ, Peacock J, Dennis ES (2002) *FLC*, a repressor of flowering, is regulated by genes in different inductive pathways. Plant J **29:** 183–191

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, et al (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell **24:** 463–472

Sabatini S, Heidstra R, Wildwater M, Scheres B (2003) SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. Genes Dev **17:** 354–358

Sablowski R (2004a) Plant and animal stem cells: conceptually similar, molecularly distinct? Trends Cell Biol **14:** 605–611

Sablowski R (2004b) Root development: the embryo within? Curr Biol **14:** R1054–1055

Samach A, Onuchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. Science **288:** 1613–1616

Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T (2007) Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature **446:** 811–814

Savidge B, Rounsley SD, Yanosfky MF (1995) Temporal relationship between the transcription of the two *Arabidopsis* MADS-box genes and the floral organ identity genes. Plant Cell **7:** 721–733

Scheres B, Di Laurenzio L, Willemsen V, Hauser MT, Janmaat K, Weisbeek P, Benfey PN (1995) Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis. Development **121:** 53–62

Scheres B, Wolkenfelt H, Willemsen V, Terlou M, Lawson E, Dean C, Weisbeek P (1994) Embryonic origin of the Arabidopsis primary root and root meristem initials. Development **120:** 2475–2487

Schmid M, Davison TS, Heinz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU (2005) A gene expression map of *Arabidopsis thaliana* development. Nat Genet **37:** 501–506

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes Dev **20:** 898–912

Simpson GG, Dean C (2002) Arabidopsis, the Rosetta stone of flowering time? Science **296:** 285–289

Speulman E, Metz PLJ, van Arkel G, Hekkert BTL, Stiekeman WJ, Pereyra A (1999) A two-component enhancer-inhibitor transposon mutagenesis system for functional analysis of the *Arabidopsis* genome. Plant Cell **11:** 1853–1866

Sridhar VV, Surendrarao A, Liu Z (2006) APETALA1 and SEPALLATA3 interact with SEUSS to mediate transcription repression during flower development. Development **133:** 3159–3166

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell **9:** 1963–1971

Vanneste S, De Rybel B, Beemster GT, Ljung K, De Smet I, Van Isterdael G, Naudts M, Iida R, Gruissem W, Tasaka M, et al (2005) Cell cycle progression in the pericycle is not sufficient for SOLITARY ROOT/IAA14-mediated lateral root initiation in *Arabidopsis thaliana*. Plant Cell **17:** 3035–3050

von Dassow G, Odell GM (2002) Design and constraints of the *Drosophila* segment polarity module: robust spatial patterning emerges from intertwined cell state switches. J Exp Zool **294:** 179–215

Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Blilou I, Korthout H, Chatterjee J, Mariconti L, Gruissem W, Scheres B (2005) The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in Arabidopsis roots. Cell **123:** 1337–1349

Wysocka-Diller JW, Helariutta Y, Fukaki H, Malamy JE, Benfey PN (2000) Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. Development **127:** 595–603

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldman KN, Meyerowitz EM (1990) The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. Nature **346:** 35–40