Development 136, 2423-2432 (2009) doi:10.1242/dev.031971

LATERAL ORGAN FUSION1 and *LATERAL ORGAN FUSION2* function in lateral organ separation and axillary meristem formation in *Arabidopsis*

Dong-Keun Lee*, Matt Geisler† and Patricia S. Springer‡

Plant organs are generated from meristems throughout development. Patterning and elaboration of organ primordia occur as a result of organized cell division and expansion, processes that are likely to be controlled, in part, by meristem-derived signals. Communication between the meristem and lateral organs is crucial for meristem maintenance and organ patterning, and organ boundaries are thought to be important for mediating this communication. *Arabidopsis thaliana LATERAL ORGAN FUSION1* (*LOF1*) encodes a MYB-domain transcription factor that is expressed in organ boundaries. *lof1* mutants display defects in organ separation as a result of abnormal cell division and expansion during early boundary formation. *lof1* mutants also fail to form accessory shoot meristems. Mutations in the closely related *LATERAL ORGAN FUSION2* (*LOF2*) gene enhance the *lof1* phenotype, such that *lof1 lof2* double mutants display additional fusion defects. Genetic interactions with the *CUP-SHAPED COTYLEDON* genes *CUC2* and *CUC3* revealed a role for *LOF1* in both organ separation and axillary meristem formation. Expression of the meristem determinant *STM* was reduced in *lof1* mutant paraclade junctions and *lof1* enhanced the weak *stm-10* mutant, such that double mutants had severe defects in meristem maintenance and organ separation. Our data implicate *LOF1* and *LOF2* in boundary specification, meristem initiation and maintenance, and organ patterning.

KEY WORDS: SAM, Boundary, Organ fusion, Transcription factor, MYB, Axillary meristem, *Arabidopsis*

INTRODUCTION

The shoot apical meristem (SAM) plays a crucial role in plant development as a continuous source of new cells for organogenesis. In contrast to animals, which form organs primarily during embryogenesis, plants produce organs throughout their life cycle. The SAM contains a population of self-renewing stem cells at its center, whereas cells at the periphery are recruited into organ primordia or into stem tissue during stem elongation (Bowman and Eshed, 2000; Clark, 1997; Fletcher, 2002; Gallois et al., 2002; Long et al., 1996). A balance between maintenance of the SAM and recruitment of cells into organ primordia is crucial for precise patterning. In *Arabidopsis thaliana*, class I KNOX family homeodomain transcription factors together with *ASYMMETRIC LEAVES 1* (*AS1*) and *ASYMMETRIC LEAVES 2* (*AS2*) have key roles in regulating the balance between SAM maintenance and organogenesis (Byrne et al., 2000; Byrne et al., 2002; Lin et al., 2003; Long and Barton, 1998; Long et al., 1996; Ori et al., 2000; Semiarti et al., 2001). In the meristem, the *KNOX* gene *SHOOT MERISTEMLESS* (*STM*) represses *AS1* and *AS2* to prevent differentiation and maintain meristem fate, whereas *KNOX* genes are repressed by *AS1* and *AS2* in lateral organs, allowing for their elaboration. Genes expressed at the boundary between the SAM and lateral organs may also play a role in regulating the balance between these two reciprocally repressing domains and might function in boundary differentiation.

Department of Botany and Plant Sciences and Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA.

*Present address: Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
†Present address: Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901, USA
‡Author for correspondence (e-mail: patricia.springer@ucr.edu)

Accepted 11 May 2009

The boundary region between organ primordia and the SAM is established early during organ initiation (Aida and Tasaka, 2006; Breuil-Broyer et al., 2004; Hussey, 1971). Cells in the boundary have lower rates of cell division and are smaller than cells in either of the adjacent domains, morphological differences that are apparent soon after primordium initiation (Gaudin et al., 2000; Hussey, 1971). Boundary cells are thought to serve as a barrier to isolate populations of cells with distinct identities (Aida and Tasaka, 2006). In the shoot, boundaries located in leaf axils have the potential to form axillary meristems, making them important regulators of plant architecture. Despite the importance of boundaries, a clear understanding of the molecular mechanisms that control their identity and function is lacking.

A number of genes that are expressed specifically in organ boundaries have been described. Members of the *BLADE ON PETIOLE* (*BOP*), *CUP-SHAPED COTYLEDON* (*CUC*), *LATERAL ORGAN BOUNDARIES* (*LOB*), *LATERAL SUPPRESSOR* (*LAS*) and *REGULATORS OF AXILLARY MERISTEMS* (*RAX*) families show boundary-specific expression patterns in *Arabidopsis* and other plant species (Aida et al., 1997; Aida and Tasaka, 2006; Borghi et al., 2007; Greb et al., 2003; Ha et al., 2003; Keller et al., 2006; Müller et al., 2006; Schmitz and Theres, 2005; Shuai et al., 2002). These genes, which encode several different classes of transcription factors, act largely redundantly to control boundary formation during embryogenesis and post-embryonic development. A number of boundary genes have also been implicated in the control of axillary meristem formation.

The combined activities of the *CUC* genes, which encode NAC-domain transcription factors, function to control embryonic meristem formation and cotyledon separation (Aida et al., 1997; Hibara et al., 2006; Souer et al., 1996; Vroemen et al., 2003; Weir et al., 2004). *CUC* genes have been proposed to function as inhibitors of cell growth, an activity required for organ separation at boundaries. *CUC* genes also control meristem initiation via regulation of *STM* (Aida et al., 1997; Aida et al., 1999; Takada et al.,

2001). *LAS* and the *RAX* genes are required for axillary meristem formation, in part via regulation of *STM* expression (Greb et al., 2003; Keller et al., 2006). Double mutant analyses uncovered synergistic interactions demonstrating that *CUC* genes are also required during post-embryonic organ separation and axillary meristem formation, whereas *LAS* contributes to both organ separation and axillary meristem formation (Hibara et al., 2006). Mutations in STM cause defects in meristem maintenance and organ separation, providing further evidence of a connection between organ separation and SAM formation (Kanrar et al., 2006; Long et al., 1996). In addition, mutants that are compromised for the functions of *PENNYWISE* (*PNY*) and *POUND-FOOLISH* (*PNF*), which encode KNOX-interacting BELL homeodomain proteins, exhibit pedicel-stem fusions (Kanrar et al., 2006; Smith et al., 2004). Thus, organ separation may be closely connected to meristem identity and maintenance.

We report here the identification of two new components of organ boundary formation. *LATERAL ORGAN FUSION 1* (*LOF1*) and *LATERAL ORGAN FUSION 2* (*LOF2*) encode MYB transcription factors that function in both lateral organ separation and axillary meristem formation, in part through interaction with *CUC2, CUC3* and the *KNOX* gene *STM*.

MATERIALS AND METHODS

Plant materials

Enhancer- and gene-trap lines in the Landsberg *erecta* (L*er*) ecotype are from the Cold Spring Harbor collection (Sundaresan et al., 1995) and were identified based either on their *β-glucuronidase* (*GUS*) expression pattern (P.S.S. and R. Martienssen, unpublished results), or on the reported insertion site near *LOF1* and *LOF2*. To determine the site of *Ds* element integration, TAIL-PCR was performed as described (Rojas-Pierce and Springer, 2003). The *DsE* element in ET4016 was integrated at position –5308 and the *DsG* element in GT12154 was integrated at position –1363 relative to the start codon of *LOF1*. The *DsE* elements in ET101 and ET5681 were integrated at positions –3377 and –413, respectively, relative to the start codon of *LOF2*.

T-DNA insertion lines were isolated from the Salk Institute Genomic Analysis Laboratory collection (Alonso et al., 2003) and are in the Columbia (Col) ecotype. Homozygous mutants of *lof1-1* (Salk_025235) and *lof2-1* (Salk_064076) were selected by genomic DNA gel blot analysis and PCR-based genotyping; primers sequences used are available upon request.

For plant transformation, binary plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 (Nagel et al., 1990) and plants were transformed using the *Agrobacterium*-mediated floral dip method (Clough and Bent, 1998). *pLOB:GUS* (Shuai et al., 2002), *pSTM:GUS* (Kirch et al., 2003), *stm-10* (Kanrar et al., 2006), *cuc1-13*, *cuc2-3* and *cuc3-105* (Hibara et al., 2006) were in the Col background.

Constructs

The *LOF1* coding sequence was amplified from a Col inflorescence cDNA sample. Primers contained introduced restriction sites for cloning and were designed to amplify the full coding sequence, predicted based on comparisons with related *MYB*-gene sequences. The resulting PCR product was sequenced to confirm the 1077 nt open reading frame, and subsequently subcloned into the binary vector pCL0011, containing the Cauliflower mosaic virus *35S* promoter (Lin et al., 2003) to generate *p35S:LOF1*.

The *LOF2* promoter region (–3795 from ATG to +53) was amplified from Col genomic DNA, sequenced to confirm its integrity, and cloned into the binary vector pCB308 (Xiang et al., 1999) using introduced *Sma*I sites to create an in-frame fusion to the *GUS* gene. Primer sequences are available upon request.

Growth conditions and morphological analyses

Plants were grown on soil at 23°C under either long-day (16 hours light/8 hours dark) or short-day (8 hours light/16 hours dark) conditions. To examine early paraclade development, plants were grown on soil in short days for 30 days, then transferred to long days to induce flowering. After 2, 4, 6 and 8 long days, tissue was harvested and embedded for sectioning. Examination of the number and length of accessory shoots and anatomical analyses were carried out on 30- or 40-day-old plants grown in soil under long-day conditions. All single- and double-mutant combinations containing *lof1* and *stm-10* or *cuc* mutations were grown under long-day conditions. To

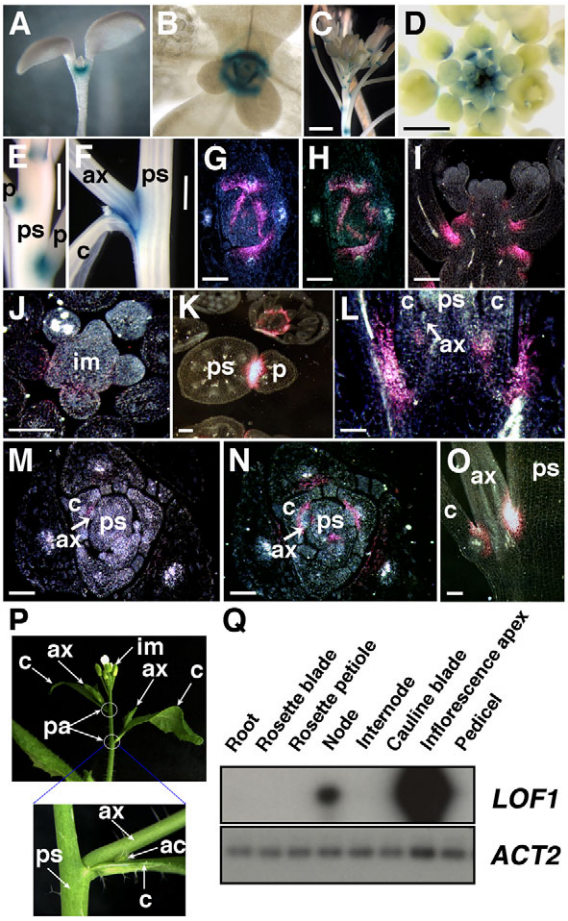

Fig. 1. Expression of *LOF1*. GUS activity in ET4016 enhancer trap line. (**A**) Side and (**B**) top view of seedling showing GUS activity in boundaries between the SAM and rosette leaves. (**C**) Side and (**D**) top view of inflorescence apex showing GUS activity in boundaries between the primary stem and pedicels and at the base of floral organs. (**E**) Pedicel-stem and (**F**) paraclade junction showing GUS activity specifically in boundaries between the primary and axillary stem and between the axillary stem and cauline leaf. (**G**,**H**) Serial cross sections of seedling apex showing GUS activity in two to three cell layers at primordia boundaries. (**I**) Longitudinal and (**J**) cross section of inflorescence apex. (**K**) Cross section of pedicel junction. Longitudinal section (**L**) and serial cross sections (**M**,**N**) showing early axillary meristem. GUS activity was detected in the junctions between the primary and axillary stems and between the axillary stem and cauline leaf. (**O**) Longitudinal section of mature paraclade junction. (**P**) Structure of paraclade junction. A paraclade junction consists of a cauline leaf, an axillary branch in the axil and an accessory bud, which forms in the junction between the axillary branch and the cauline leaf but does not typically elongate. (**Q**) RT-PCR analysis of *LOF1* expression in indicated tissues (node sample included pedicel-stem and paraclade junctions). PCR products were detected by blotting and probing with a *LOF1*-specific probe. *ACT2* was used as a loading control. Scale bars: 2 mm in C,F; 1 mm in D,E; 0.1 mm in G-O. ac, accessory branch; ax, axillary branch; c, cauline leaf; im, inflorescence meristem; p, pedicel; pa, paraclade junction; ps, primary stem.

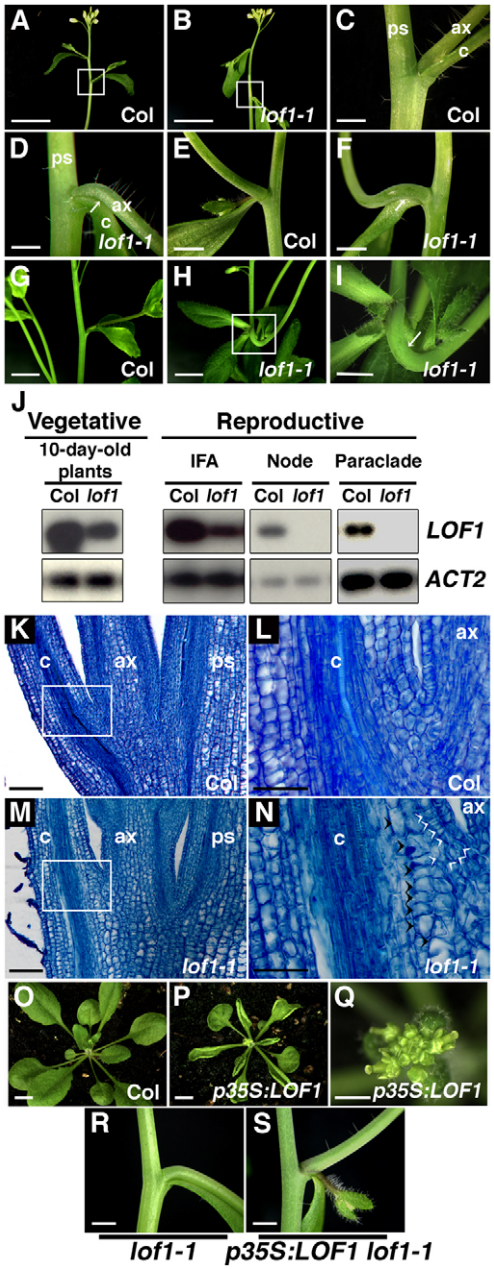

Fig. 2. Fusion phenotypes in the *lof1* mutant. (**A-I**) Morphology of paraclade junctions in Col wild-type (A,C,E,G) and *lof1-1* (B,D,F,H,I) plants at early (A-D) or mature (E-I) stages. Plants were grown in long days (A-F) or short days (G-I). Panels C, D and I represent higher-magnification views of boxed regions in A, B and H, respectively. White arrows indicate fused junctions. (**J**) RT-PCR analyses of *LOF1* transcript levels in Col wild type and *lof1-1* mutants. Total RNAs were isolated from 10-day-old vegetative shoots, inflorescence apices (IFA), pedicel nodes or paraclade junctions. RT-PCR products were detected by blotting and probing with a *LOF1*-specific probe. *ACT2* was used as a control. (**K-N**) Longitudinal sections of young paraclade junctions in Col wild type (K,L) and *lof1-1* (M,N). Plants were grown under short days for 30 days, followed by 6 days under long-day conditions. Panels L and N represent magnified views of boxed regions in K and M, respectively. Black arrowheads indicate enlarged cells, white arrowheads indicate dividing cells in *lof1*. (**O-S**) Rescue of *lof1* phenotype by *p35S:LOF1* expression. Col (O) and *p35S:LOF1* (P) 17-day-old plants. (Q) Inflorescence apex of 25-day-old *p35S:LOF1* plant. (R) Paraclade junction of 32-day-old *lof1-1* plant containing *p35S:LOF1* T-DNA, showing accessory bud formation and no fusion. Scale bars: 1 cm in A,B; 1 mm in C,D,Q,R,S; 2 mm in E,F,I; 5 mm in G,H; 0.1 mm in K,M; 0.05 mm in L,N; 5 mm in O,P. ax, axillary stem; c, cauline leaf; ps, primary stem.

assess the potential for accessory shoot formation, primary or axillary inflorescence stems of 40-day-old plants were removed and elongated accessory shoots were measured 2 weeks later.

Expression analysis

To examine the transcript levels of boundary genes in paraclade junctions, total RNA was isolated from a 0.5 cm region spanning the paraclade junction. cDNAs were synthesized as previously described (Lin et al., 2003) and PCR reactions were done using gene-specific primers (sequences are available upon request). Two biological replicates were conducted. RT-PCR products were detected by blotting and probing with gene-specific probes after 20 cycles of amplification. *ACT2* was used as a control with 15 cycles of amplification.

Histological analysis

For detection of GUS activity, samples were incubated in staining solution overnight (except for ET4016, which was stained for 6 hours), then transferred to 70% (v/v) followed by 100% (v/v) ethanol to clear chlorophyll (Geisler et al., 2002). Tissue was embedded using a modified in situ hybridization protocol (Lee et al., 2003; Long and Barton, 1998). Stained tissue was transferred to FAA (50% [v/v] ethanol, 5% [v/v] acetic acid, and 3.7% [v/v] formaldehyde) for 10 hours, then dehydrated and embedded. Images were captured under dark field or DIC optics using a Leica DMR microscope. For anatomical analyses, plants were fixed in FAA fixation solution for 10 hours and embedded for sectioning.

RESULTS

LOF1 is expressed in organ boundaries

Enhancer-trap line ET4016 exhibited GUS reporter activity in the boundary regions between the SAM and lateral organs during vegetative development (Fig. 1A,B). GUS activity was first detected in 4-day-old ET4016 seedlings and persisted throughout leaf development in a band of cells on the adaxial side of rosette leaf bases (Fig. 1B,G,H). No *GUS* expression was detected in roots. After flowering, GUS activity was detected at the base of floral organs, in the adaxial junction between the primary stem and pedicels, in the adaxial junction between the primary and axillary stems, in the junction between the axillary stems and cauline leaves (Fig. 1C-F) and in the junctions between the inflorescence meristem and flower primordia (Fig. 1I,J). In cauline leaf axils, GUS activity was detected both before and after axillary meristem initiation (Fig. 1L-O). In all cases, GUS activity was restricted to adaxial boundary regions and was detected in both epidermal and underlying cells (Fig. 1G-O).

The ET4016 transposant contained a single *DsE* element (data not shown) inserted 5308 bp upstream of the translation start codon of *AtMYB117* (*At1g26780*). Based on the loss-of-function phenotype (see below) we named this gene *LATERAL ORGAN FUSION 1* (*LOF1*). Attempts to detect *LOF1* transcripts using in situ hybridization were unsuccessful, perhaps due to low transcript abundance. A second transposant line, GT12154, containing a *DsG* element 1363 bp upstream of the translation start codon of *LOF1* showed a *GUS* expression pattern virtually identical to that of ET4016 (data not shown), suggesting that the enhancer trap line faithfully reports the expression of *LOF1*. In a survey of dissected

tissues, *LOF1* transcripts were only detected in RNAs isolated from nodes (pedicel and paraclade junctions; see Fig. 1P) and inflorescence apices (Fig. 1Q), consistent with the pattern of *GUS* expression in ET4016 and GT12154. Because both *Ds* insertions were between *LOF1* and *At1g26770*, we also examined the developmental expression profile of *At1g26770* reported in AtGenExpress (Schmid et al., 2005). *At1g26770* transcripts were broadly detected and present in hypocotyls, cotyledons and leaves, a pattern inconsistent with the *GUS* expression patterns in ET4016 and GT12154, suggesting that these lines accurately represent the expression of *At1g26780*/*LOF1*.

cDNA sequencing confirmed that *LOF1* produces a transcript encoding two predicted MYB DNA-binding domains and an FxDFL motif of unknown function that is common to seven *Arabidopsis* R2R3 MYB transcription factors in subgroup 21 (Stracke et al., 2001) (see Fig. S1 in the supplementary material).

LOF1 is required for organ separation

We identified a *LOF1* T-DNA insertion mutant, *lof1-1* (see Fig. S2 in the supplementary material), that showed a significant reduction in transcript accumulation (Fig. 2J; and data not shown). When grown under our standard long-day growth conditions, *lof1* plants were morphologically normal during vegetative development, but after flowering, the axillary stems produced in cauline leaf axils of the primary inflorescence (the paraclade junction; see Fig. 1P) were deflected downward compared with the wild type and the axillary stem and subtending cauline leaf were fused at their bases (Fig. 2A-D). *lof1* mutants also lacked accessory shoots, which wild-type plants produced in the junctions between the axillary stems and cauline leaves (Fig. 2E,F). *lof1* axillary branches eventually grew upward, perhaps in response to phototropism or gravitropism (Fig. 2F). When grown in short-day conditions, the *lof1* fusion was more severe; cauline leaves were fused to stems along a greater extent and mutants produced clusters of leaves resembling aerial rosettes that were not present in wild-type plants (Fig. 2G-I).

LOF1 transcript levels were slightly reduced in *lof1* vegetative shoots and the inflorescence apex compared with wild type, but undetectable in both pedicel nodes and paraclade junctions. The dramatic reduction in transcript abundance in *lof1* paraclade junctions correlates with the location of visible defects in *lof1* mutants. The lack of apparent phenotypes in other organ junctions where *LOF1* is expressed may be due to the presence of *LOF1* transcripts in those locations, or alternatively, could be due to overlapping functions of *LOF1* with redundant genes.

lof1 boundary defects result from alterations in cell division and expansion

Following synchronous induction of flowering (see Materials and methods), we examined early axillary branch development by histological analyses. No significant differences between wild-type and *lof1* plants were observed during the early stages of axillary meristem initiation, but soon after axillary stem elongation was observed, fusion between the axillary branch and cauline leaf was visible in *lof1*. At this stage, cells in the paraclade junction of wild-type plants were small, arranged in well-organized cell layers, and dividing cells were rarely observed (Fig. 2K,L). By contrast, *lof1* cell files were disrupted and enlarged cells were observed, especially near the vascular bundles (Fig. 2M,N). No evidence of epidermal cell identity was apparent in the internal fused region. Thus, *lof1* mutants exhibit defects in cell division and expansion at the boundary between cauline leaf and axillary branch, which result in failure of the two structures to properly separate.

To confirm that the lesion in *LOF1* was responsible for the observed phenotypes in *lof1*, we introduced *p35S:LOF1* into Col and *lof1* backgrounds. In Col, 32% (44/139) of *p35S:LOF1* plants exhibited a range of abnormal phenotypes, including leaves that were asymmetric, serrated or curled upward at the margin (compare

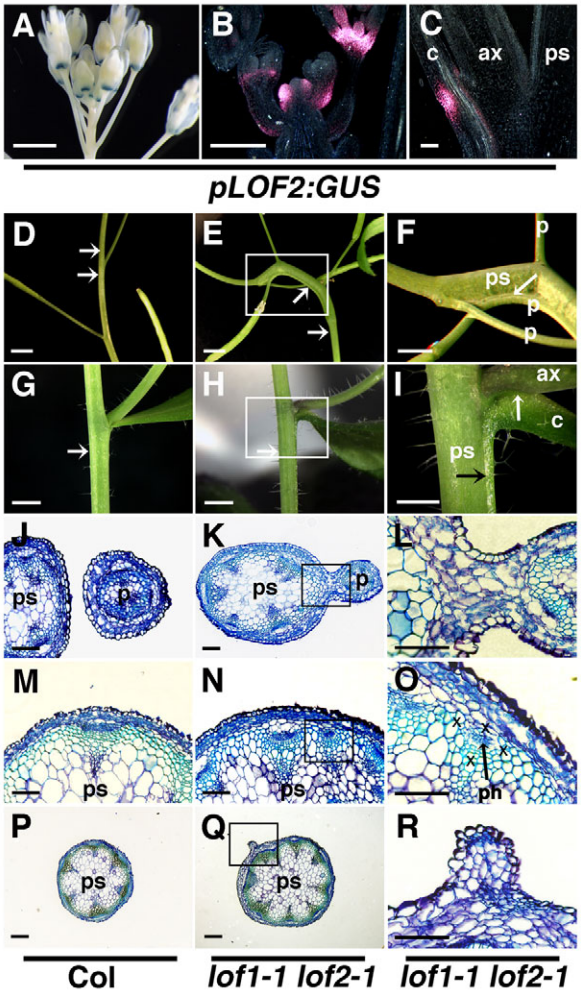

Fig. 3. *LOF2* expression pattern and enhanced phenotypes in *lof1 lof2* double mutants. (**A-C**) GUS activity in *pLOF2:GUS*. (A) Whole-mount inflorescence apex. (B) Longitudinal section of inflorescence apex. (C) Longitudinal section of paraclade junction. GUS activity was detected throughout young flower primordia and in adaxial junctions, becoming restricted to abaxial junctions later in development. (**D-I**) Inflorescence stem showing pedicel (D-F) and paraclade (G-I) junctions in wild type (D,G) and *lof1-1 lof2-1* (E,F,H,I). Panels F and I show magnified view of boxed region in E and H, respectively. White arrows in F and I indicate fused regions; black arrow in I indicates decurrent strand. (**J**,**K**) Cross sections of pedicel junction of wild type (J) and *lof1-1 lof2-1* (K) taken at the position of the upper white arrows in D and E, respectively. (**L**) Magnification of fused regions boxed in K showing enlarged cells. (**M**,**N**) Cross sections below the pedicel junction of wild type (M) and *lof1-1 lof2-1* (N) at the position of the lower white arrows in D and E, respectively. (**O**) Magnification of fused regions boxed in N showing a small vascular bundle with abnormal amphivasal pattern (xylem surrounding phloem). (**P**,**Q**) Cross sections of the primary stem below the paraclade junction in wild type (P) and *lof1-1 lof2-1* (Q) taken at the position of the white arrows in G and H, respectively. (**R**) Magnification of fused region boxed in N. Scale bars: 2 mm in A,D,E,G,H; 1 mm in F,I; 0.1 mm in B,C,J-R. ax, axillary stem; c, cauline leaf; p, pedicel; ph, phloem; ps, primary stem; x, xylem.

Fig. 2O with 2P). In general, transgenic plants had reduced organ size, which resulted in the formation of open flower buds (Fig. 2Q). In the *lof1* mutant background, *p35S:LOF1* resulted in the same range of phenotypes as in wild type and also rescued both the fusion and accessory shoot defects. Seventy-five percent (24/32) of *lof1* plants transformed with the *p35S:LOF1* construct lacked observable fusion between the axillary stem and cauline leaf and produced accessory shoots (compare Fig. 2R with 2S). The remaining 25% (8/32) of *lof1* transgenic plants failed to form accessory shoots and exhibited some degree of fusion. The observed phenotypes in *p35S:LOF1* plants suggest a role in growth limitation, consistent with the loss-of-function *lof1* phenotype. In addition, the phenotypic rescue conferred by *p35S:LOF1* indicates that the mutation in *LOF1* is responsible for the observed fusion defects.

lof1 lof2 double mutants exhibit enhanced phenotypes relative to *lof1*

Phylogenetic analyses of the MYB proteins in subgroup 21 (Stracke et al., 2001), revealed that *LOF1* is most closely related to *At1g69560*/*MYB105*, which we call *LOF2* (see Fig. S3 in the supplementary material). We therefore identified transposon and T-DNA insertions in *LOF2* to examine possible functional overlap with *LOF1*. The enhancer-trap lines ET101 and ET5681 contained a *DsE* element inserted upstream of *LOF2* (see Fig. S4 in the supplementary material). In both lines and in transgenic plants harboring a *pLOF2:GUS* construct, *GUS* expression was observed in organ boundaries (Fig. 3A-C; see Fig. S5 in the supplementary material). In early-stage flowers however, GUS activity was detected broadly throughout the primordia, including the adaxial boundaries (Fig. 3B), slightly overlapping the *LOF1* domain (see Fig. S5 in the supplementary material). Later in development, GUS activity was restricted to the abaxial pedicel junctions, the base of floral organs, the abaxial base of cauline leaves, and the junction between cauline leaves and axillary stems (Fig. 3A-C). Thus *LOF1* and *LOF2* expression had limited overlap early in development, and later expression of the two genes was distinct.

We identified a T-DNA mutant, *lof2-1*, which appears to be a null allele (data not shown). *lof2* plants were phenotypically normal, but *lof1 lof2* double mutants exhibited additional phenotypes compared with the *lof1* single mutants. Fusion between the flower pedicel and the primary inflorescence stem was observed in 11% (11/100) of *lof1 lof2* double mutants (compare Fig. 3D with 3E,F) and the cauline leaf was extended below the point of insertion and fused to the inflorescence stem forming a decurrent strand in 28% (28/100) of *lof1 lof2* plants (compare Fig. 3G with 3H,I). These additional phenotypes implicate *LOF2* in organ-boundary establishment, and suggest that *LOF1* and *LOF2* have functional overlap.

We further analyzed *lof1 lof2* double mutants by sectioning. In the wild type, sections above the pedicel-stem junction revealed two well-separated structures with intact epidermal layers (Fig. 3J), whereas *lof1 lof2* double mutants showed fusion between the pedicel and stem, with a continuous cortical cell layer (Fig. 3K,L). Cells within the fused junction were larger than cortical cells in other regions, and abnormalities in vascular pattern were observed. In wild-type plants, the vascular trace extending from the stem into the pedicel was well separated from the adjacent vascular bundle in the stem and showed clear asymmetry, with internal xylem and external phloem (Fig. 3J,M). In the *lof1 lof2* double mutants, the pedicel vascular trace formed but was not well separated from the primary vascular bundle (Fig. 3N) and displayed an abnormal amphivasal (radialized) pattern with xylem surrounding phloem (Fig. 3N,O). No vasculature was visible in the decurrent strands, which exhibited continuity between cortical layers of stem tissues and cauline leaf mesophyll (Fig. 3Q,R).

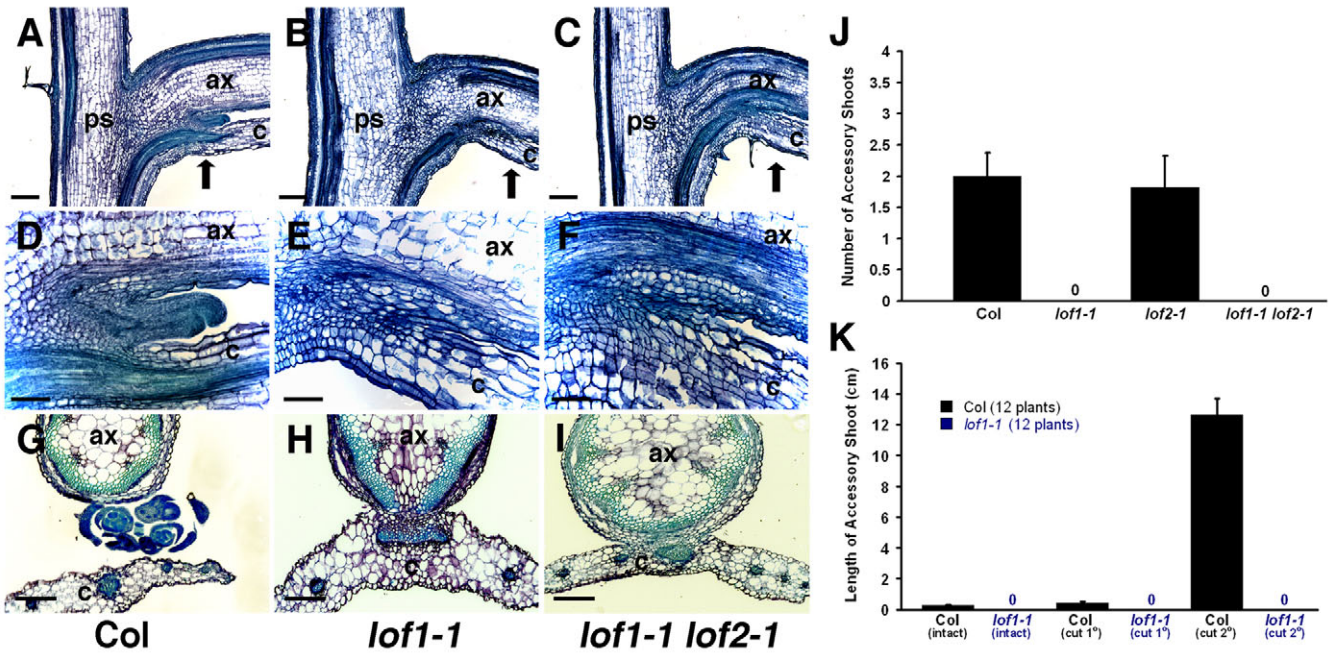

Fig. 4. Accessory shoot defects in *lof1-1* and *lof1-1 lof2-1* mutants. (**A-I**) Histological sections of paraclade junctions in 30-day-old plants. (A-F) Longitudinal and (G-I) cross sections of Col (A,D,G), *lof1-1* (B,E,H) and *lof1 lof2* double mutants (C,F,I). Arrows in A-C indicate the region magnified in panels D-F, respectively. (**J**,**K**) Accessory shoot formation in 40-day-old plants. (J) Average number of accessory shoots formed in paraclade junctions on the primary stem. (K) Accessory shoot outgrowth following removal of either primary or axillary inflorescences. Error bars indicate standard deviation. Scale bars: 0.3 mm in A-C; 0.1 mm in D-F; 0.2 mm in G-I. ax, axillary stem; c, cauline leaf; ps, primary stem.

Radialized vasculature is a common hallmark of adaxial-abaxial polarity defects (Eshed et al., 2001; Kerstetter et al., 2001; McConnell and Barton, 1998); however, no other polarity defects were apparent in *lof1* or *lof1 lof2* plants.

Accessory shoot formation in *lof1*

The primary inflorescence stem of wild-type and *lof2* plants contained an average of three axillary shoots with subtending cauline leaves, and on average two of three axils contained a visible accessory shoot (Fig. 4J). Accessory shoots were never observed in *lof1* single mutants or *lof1 lof2* double mutants (Fig. 4J). In histological sections, accessory buds were clearly visible in the junctions between the axillary stem and cauline leaves of wild-type plants (Fig. 4A,D,G), whereas in *lof1* and *lof1 lof2* plants, enlarged cells were present and there was no evidence of meristem cells (Fig. 4B,C,E,F,H,I).

We also tested whether *lof1* mutants had the potential to form accessory shoots when relieved of apical dominance. Primary or axillary inflorescence apices were removed from 40-day-old plants and accessory shoot formation was evaluated 2 weeks later. In wild-type plants, removal of axillary but not primary inflorescence apices resulted in a dramatic outgrowth of accessory shoots, which do not normally elongate significantly (Fig. 4K; see Fig. S6 in the supplementary material). Thus, apical dominance originating from the axillary inflorescence normally suppresses accessory shoot outgrowth. Accessory shoots were not induced in *lof1* mutants by removal of either primary or axillary inflorescence apices, which is consistent with a complete lack of accessory shoot meristem formation in these mutants.

Expression of *STM* and boundary genes in *lof1*

A number of mutations that affect the formation of axillary meristems have been described, including those in the *LAS*, *RAX* and *CUC* families (Greb et al., 2003; Hibara et al., 2006; Keller et al., 2006; Müller et al., 2006). A common feature of these mutants is that expression of the meristem marker *STM* is reduced. To examine the relationship between *STM* and the accessory shoot defects in *lof1*, we generated *lof1* mutants expressing a *GUS* reporter driven by the *STM* promoter (Kirch et al., 2003). GUS activity was observed in paraclade junctions of *pSTM:GUS* plants before accessory shoots were visible and persisted in accessory shoot apices after their initiation (Fig. 5A,B). By contrast, no GUS activity was detected in the paraclade junctions of *lof1* mutants (Fig. 5C), although *GUS* expression was unaltered in the SAM, inflorescence meristem and floral meristem (data not shown). These data indicate that cells with meristem identity were lacking in the paraclade junctions of *lof1* mutants.

We examined the expression of boundary gene markers *LOB* and *LOF2* in *lof1* mutants using promoter:*GUS* fusion constructs. *pLOB:GUS* (Shuai et al., 2002) was expressed in wild-type organ boundaries in a pattern similar to that of *LOF1* (Fig. 5D). In the *lof1* background, GUS activity was detected in a normal pattern, although slightly expanded into the region of fusion (Fig. 5E). By contrast, *pLOF2:GUS* expression was significantly reduced in *lof1* paraclade junctions compared with wild type (Fig. 5F,G), suggesting that *LOF2* is downstream of *LOF1*, although this regulation may be indirect.

To further characterize gene expression in *lof1* mutants, transcript levels of several boundary genes were examined in dissected wild-type and *lof1* paraclade junctions by RT-PCR (see Fig. S7 in the supplementary material). Transcript levels of *LAS, RAX1, LOF2, CUC1, CUC2* and *CUC3* were reduced in *lof1* paraclade junctions, whereas *LOB* and *REV* transcript levels were unaffected and *BOP1* transcript levels appeared to be slightly elevated. These data indicate that the *lof1* mutant has defects in boundary gene expression and place *LOF1* upstream of *RAX1*, *LAS* and the *CUC* genes.

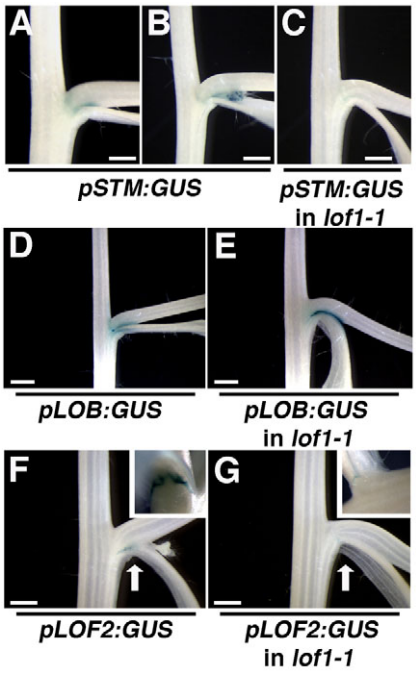

Fig. 5. Expression of meristem and boundary marker genes. (**A-C**) *pSTM:GUS* expression in paraclade junctions of wild type before (A) and after (B) accessory shoot outgrowth, and in *lof1-1* (C). (**D**,**E**) *pLOB:GUS* expression in paraclade junction of wild type (D) and *lof1-1* (E). (**F**,**G**) *pLOF2:GUS* expression in paraclade junction of wild type (F) and *lof1-1* (G). Insets in F and G are magnified views from below (arrow). Scale bars: 1 mm.

LOF1 controls boundary formation with *CUC2* and *CUC3*

Because of their demonstrated role in boundary specification, we investigated interactions between *LOF1* and the *CUC* family genes (Hibara et al., 2006). We generated double mutants between *lof1* and each of the *cuc* mutants. None of the single or double mutants had visible phenotypes during vegetative development. After flowering, the *lof1 cuc2* and *lof1 cuc3* double mutants displayed severe phenotypes. Pedicels were fused to the primary stem (Fig. 6A-F,T; see Table S1 in the supplementary material) and the plants exhibited more dramatic fusion in paraclade junctions compared with *lof1* plants (Fig. 6G-N,U; see Table S2 in the supplementary material). The double mutants exhibited additional fusion between primary and axillary stems and between cauline leaves and primary stems (decurrent strand) (Fig. 6J,K,M,N). These data indicate overlapping roles for *LOF1, CUC2* and *CUC3* to control organ separation during reproductive development.

lof1 cuc2 and *lof1 cuc3* double mutants also exhibited axillary meristem defects. In *lof1 cuc3* double mutants, 19% of axillary meristems on the primary inflorescence stem were replaced by a solitary flower (Fig. 6O,P,V; see Table S3 in the supplementary material) and 85% of cauline leaf axils on secondary/axillary branches were either barren or contained a solitary flower (Fig. 6Q-

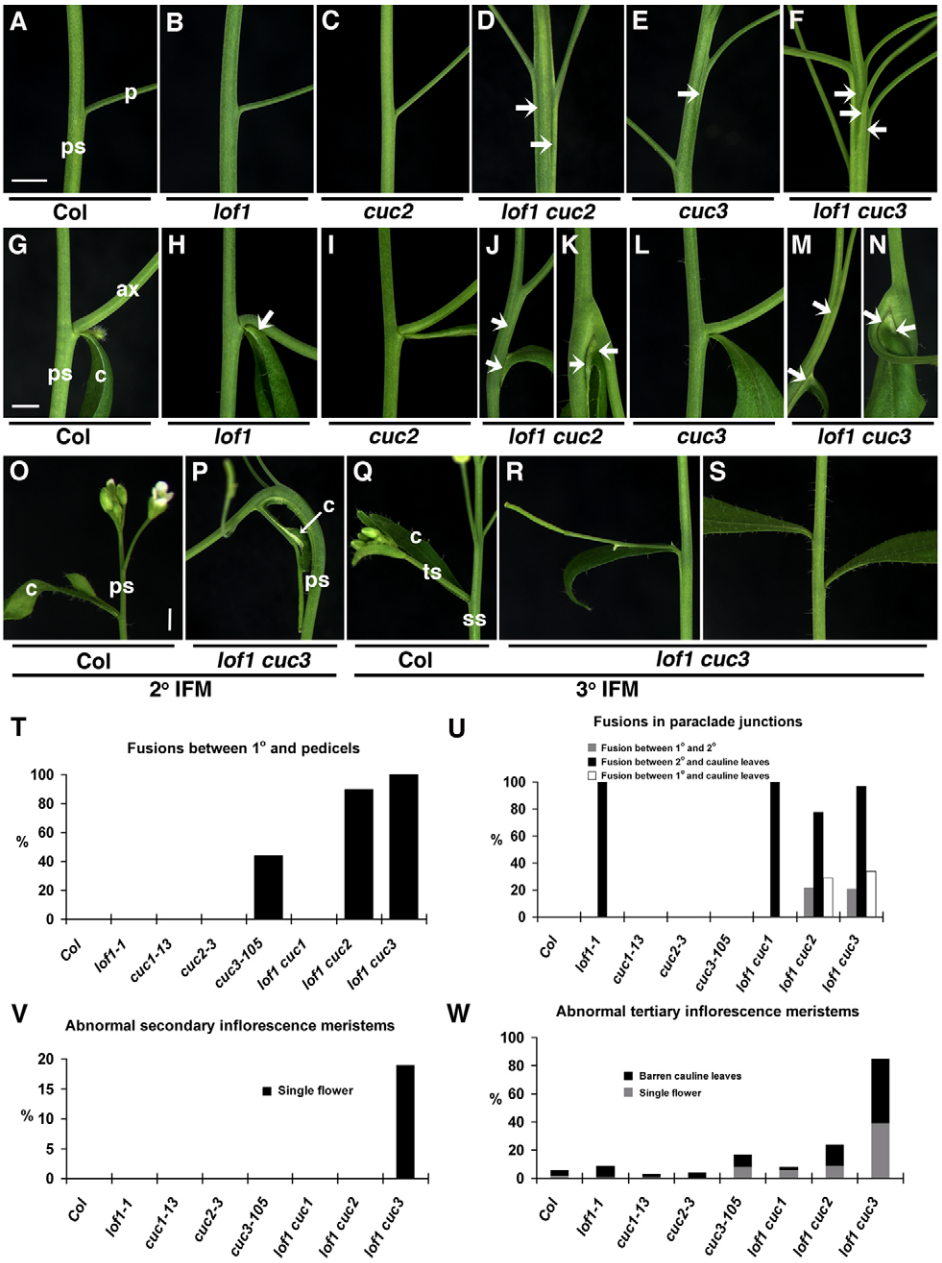

Fig. 6. Double-mutant analysis between *lof1-1* and *cuc* mutants. (**A-F**) Pedicel junctions of Col (A), *lof1-1* (B), *cuc2-3* (C), *lof1-1 cuc2-3* (D), *cuc3-105* (E) and *lof1-1 cuc3-105* (F). Arrows indicate the fused region between the primary stem and pedicels. (**G-N**) Paraclade junctions of Col (G), *lof1-1* (H), *cuc2-3* (I), *lof1-1 cuc2-3* (J,K), *cuc3-105* (L) and *lof1-1 cuc3-105* (M,N). Arrows indicate fused regions between the primary and axillary stems, between the axillary stem and cauline leaves, and between the primary stem and cauline leaves. (**O**,**P**) Cauline leaf axil on primary stem of Col (O) and *lof1-1 cuc3-105* (P). The axillary inflorescence meristem is replaced by a solitary flower in P. (**Q-S**) Cauline leaf axil on secondary/axillary stem of Col (Q) and *lof1-1 cuc3-105* (R,S) plants showing abnormal solitary flower (R) or barren axil (S). (**T**,**U**) Frequency of fusion between pedicel and stem (T) and in paraclade junctions (U). (**V**,**W**) Frequency of abnormalities in secondary/axillary inflorescence meristem (V) and the tertiary/axillary inflorescence meristem (W). Scale bars: 2 mm. ax, axillary stem; c, cauline leaf; p, pedicel; ps, primary stem; ss, secondary stem; ts, tertiary stem.

S,W; see Table S4 in the supplementary material). Similar defects were observed at lower frequencies in *cuc3* and *lof1 cuc2* plants (and to a lesser degree the other single mutants). Thus, *LOF1* and *CUC3* contribute to axillary meristem maintenance/initiation.

lof1 enhances the weak *stm-10* mutant

Having established a link between *LOF1* and meristem formation, we examined possible interactions between *LOF1* and *STM* by combining *lof1* with the weak *stm-10* allele. *stm-10* single mutants failed to maintain a SAM, stalling after formation of two or three rosette leaves (Fig. 7B; Table 1), but eventually producing additional leaves and ultimately bolting to produce abnormal flowers lacking a central carpel (Fig. 7H; and data not shown). As previously reported (Kanrar et al., 2006), some fusions were observed in mature *stm-10* plants. *lof1 stm-10* double mutants had more severe meristem defects than *stm-10* – 16% of double mutants produced no leaves and 9.8% arrested after forming a single rosette leaf (Fig. 7C; Table 1). Thus, 26% of *lof1 stm-10* double mutants exhibited dramatic defects in meristem maintenance. The remainder of double mutants terminated earlier than the *stm-10* single mutant (Fig. 7H).

lof1 stm-10 double mutants also displayed organ fusion during vegetative development – 44% of 16-day-old *lof1 stm-10* plants exhibited fusion between rosette leaf petioles (Fig. 7D; Table 1). This phenotype was never observed in *lof1* or in 16-day-old *stm-10* plants, although older (30-day-old) *stm-10* plants occasionally produced some type of rosette leaf fusion (data not shown). Analyses using cleared whole mounts or sectioning revealed the presence of two distinct vascular bundles in the fused petioles, both in the fused basal region and in the unfused apical region (Fig. 7E-G), indicating that the organ-boundary defects did not affect vascular bundle formation. Double mutant plants that did not arrest bolted after about 2 months, but terminated prematurely compared with *stm-10* single mutants (Fig. 7H). Some double

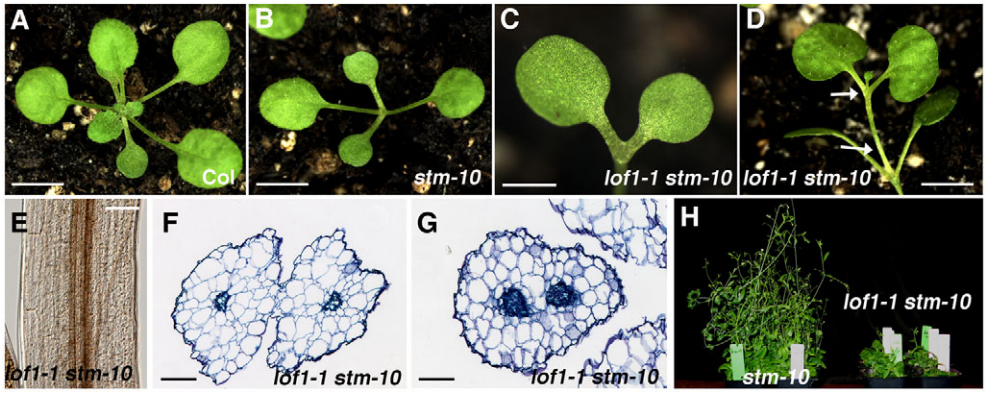

Fig. 7. Meristem and boundary defects in *lof1-1 stm-10* double mutants. (**A-D**) Fifteen-day-old plants of wild-type (A), *stm-10* (B) and *lof1-1 stm-10* (C,D) plants. (**E**) Fused petioles of *lof1-1 stm-10,* cleared with ethanol. (**F**,**G**) Cross section of fused petioles shown in D at the position of upper (F) or lower arrow (G). (**H**) Sixty-day-old *stm-10* (left) and *lof1-1 stm-10* (right) plants. Scale bars: 5 mm in A,B,D; 1 mm in C; 0.1 mm in E-G.

mutants eventually produced cauline leaves and axillary inflorescence branches that exhibited severe fusions between the primary stem and pedicels and between the axillary stems and cauline leaves compared with *stm-10* (data not shown). These data indicate that *LOF1* functions in both meristem maintenance and organ separation.

DISCUSSION

LOF1 and *LOF2* function redundantly to regulate boundary formation. The domains of *LOF1* and *LOF2* expression overlap in boundaries between the inflorescence meristem and young floral primordia, between the axillary stem and cauline leaves, and at the base of floral organs, but diverge later in development. *LOF2* transcripts are reduced in *lof1* mutants, indicating that *LOF1* contributes to *LOF2* expression. The *lof1* mutant displays two phenotypes consistent with a function in organ boundaries: organ fusion and reduced axillary meristem activity. Fusion between the axillary stem and cauline leaf in *lof1* mutants is a novel phenotype that, to our knowledge, has not been reported for other boundary mutants. Additional fusions – between stem and pedicels, primary and axillary stems, primary stem and cauline leaves, and rosette leaf petioles – that were observed in *lof1* double mutant combinations indicate that *LOF1* functions broadly in organ separation. In addition, *LOF1* has a role in meristem initiation or maintenance, as *lof1* mutants failed to produce accessory meristems and enhanced the meristem defects of *cuc3* and *stm-10* mutants.

Organ separation and meristem formation during embryogenesis

Mutations in a number of boundary-specific genes result in organ fusion. During embryogenesis, the *CUC* genes function redundantly to control organ separation, such that single mutations in *CUC1*, *CUC2* or *CUC3* cause only subtle defects, whereas any combination of two *cuc* mutations results in fusion along the cotyledon margins and a dramatic cup-shaped cotyledon phenotype (Aida et al., 1997). *CUC2* and *CUC3* also function to specify boundaries during post-embryonic development and have roles in axillary meristem formation (Hibara et al., 2006; Raman et al., 2008). The fusion phenotypes in the *cuc* mutants might result from both organ-organ and organ-meristem fusions (Aida and Tasaka, 2006). Organ-organ fusions may give rise to cup-shaped cotyledons, whereas organ-meristem fusions may generate cotyledon petiole fusions and could also affect meristem maintenance or initiation by alteration of *KNOX* gene expression (Aida et al., 1999).

lof1 enhanced the weak *stm-10* mutant, a somewhat surprising finding given that *GUS* expression was not detected in embryos of either ET4016 or GT12154, which report *LOF1* expression. Although we cannot exclude a low level of *LOF1* expression in the embryo, a more likely explanation is that *LOF1* has a function in post-embryonic SAM maintenance. Consistent with this idea, meristem-maintenance defects were observed in *lof1 stm-10* plants later in development. In addition, whereas *lof1* enhanced *cuc2* and *cuc3,* the double mutants did not show cotyledon fusion or meristem arrest, which might be indicative of embryonic function. Similarly, *las cuc* double mutants did not show enhanced cotyledon fusion (Hibara et al., 2006; Raman et al., 2008). These data suggest that *LOF1* and *LAS* do not significantly contribute to cotyledon separation during embryogenesis.

LOF1 and organ separation during post-embryonic development

CUC2, *CUC3* and *LAS* function redundantly in organ separation. Although no single mutant displays organ fusions during vegetative development, *cuc2 cuc3* and *cuc3 las* double mutants produce rosette-leaf fusions (Hibara et al., 2006). By contrast, *lof1 cuc* double mutants did not show vegetative organ fusion. In *lof1 stm-10* double mutants, petioles were fused on their adaxial faces rather than laterally and the meristem was apically shifted to the site of petiole separation. These phenotypes may indicate that the *lof1 stm-10* fusions were between the meristem and forming leaves, which could result in an apical shift in meristem position. The observed meristem loss in some *lof1 stm-10* plants is also consistent with fusion events involving the meristem. Together our data suggest that *LOF1* probably does not play a major role in organ-organ separation during vegetative development, but does contribute to organ-meristem separation.

Table 1. Summary of phenotypes observed in 16-day-old *stm-10* and *lof1-1 stm-10* plants

	Number of rosette leaves								
Genotype	0	1	2	3	4	5	6	7	8
Col (*n*=100)	–	–	–	–	–	–	–	82	18
stm-10 (*n*=35)	–	–	28	7	–	–	–	–	–
lof1-1 stm-10 (*n*=81)	13	8	51*	8*	1	–	–	–	–

*28/51 plants arrested with two rosette leaves and 8/8 plants arrested with three rosette leaves in *lof1 stm-10* also exhibited severe fusion between rosette leaf petioles.

The *CUC* genes and *LAS* also function in organ separation during reproductive development. *las* single mutants occasionally exhibited fusion between primary and axillary stems under short-day growth conditions (Greb et al., 2003). Although the day-length dependence of these phenotypes is not understood, it is noteworthy that the *lof1* phenotype was also more severe under short-day conditions, suggesting a day-length-dependent aspect to boundary formation. Combining any two of the mutants *cuc2, cuc3* and *las* results in fusion between the primary and axillary stem and/or between the stem and pedicels, indicating that these three genes function redundantly to control organ separation (Hibara et al., 2006). *LOF1* and *LOF2* also function in organ separation during reproductive development – *lof1* single mutants have a novel fusion between the axillary stem and cauline leaf, and additional fusions resulted when *lof1* was combined with *lof2*, *cuc2* or *cuc3*. By contrast, *LOB* appears to function in a pathway distinct from *LOF1* and *LOF2*, despite the similar expression pattern. *LOB* expression was unaffected in the *lof1* mutant background and genetic interactions were not observed in *lof1 lob* or *lof2 lob* double mutants (data not shown).

Why do the boundary mutants have defects in organ separation? Examination of *Arabidopsis cuc1 cuc2* mutants and the petunia mutant *nam*, which both have fused cotyledon petioles, revealed that meristem cells were replaced by enlarged cells (Aida et al., 1997; Souer et al., 1996). Similar defects were observed in *lof1* – boundary cells were enlarged and exhibited abnormal cell division patterns. The enlarged cells in *lof1* boundaries may prevent or delay separation of the axillary branch and the subtending cauline leaf. Aberrant cell expansion also coincided with a downward deflection of axillary stems in *lof1*. Thus, changes in cellular organization are a common feature of mutants that exhibit fusion defects.

LOF1 and axillary meristem formation

lof1 mutants fail to form accessory meristems, have no detectable expression of the meristem marker *STM* in the paraclade junction, and reduce transcript accumulation of many, but not all, boundary markers. The relationship between defects in axillary meristem formation and organ fusion is not entirely clear, however, as the two phenotypes are not always correlated. *las* and *rax* mutants produce very few axillary meristems, but do not exhibit fusion (Greb et al., 2003; Keller et al., 2006; Müller et al., 2006), whereas loss-of-function *lob* mutants exhibit fusion between cauline leaves and axillary stems, but produce normal accessory shoots (W. C. Lin and P.S.S., unpublished data). Thus, *LOF1* function in axillary meristem initiation or maintenance may be independent of its function in organ separation. The ability of *lof1* to enhance the weak *stm-10* phenotype also supports a role for *LOF1* in meristem initiation or maintenance. It is possible that *LOF1* acts in the boundary to regulate the activity of meristem genes. Alternatively, the boundary may indirectly contribute to meristem function, perhaps by acting as a physical barrier.

Acknowledgements

We thank the *Arabidopsis* Biological Resource Center for providing the *lof1* and *lof2* alleles; Rob Martienssen (Cold Spring Harbor Laboratory, New York) for Gene Trap lines; Harley Smith (University of California, Riverside) for *stm-10* seeds; Masao Tasaka and Mitsuhiro Aida (Nara Institute of Science and Technology) for *cuc1-13*, *cuc2-3* and *cuc3-105* seeds; and Wolfgang Werr (Institute of Developmental Biology, University of Cologne) for *pSTM:GUS* seeds. We thank Jeff Long (Salk Institute, San Diego, CA) for attempts to detect *LOF1* by in situ hybridization and Panya Kim (University of California, Riverside) for cDNA samples. We thank Harley Smith and members of the Springer lab for helpful discussions. This research was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service United States Department of Agriculture, grant number 2003-35304-13369 and by the Department of Energy, grant number DE-FG02-05ER15649, to P.S.S.

Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/136/14/2423/DC1

References

Aida, M. and Tasaka, M. (2006). Morphogenesis and patterning at the organ boundaries in the higher plant shoot apex. *Plant Mol. Biol.* **60**, 915-928.

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: an analysis of the *cup-shaped cotyledon* mutant. *Plant Cell* **9**, 841-857.

Aida, M., Ishida, T. and Tasaka, M. (1999). Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* **126**, 1563-1570.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H. M., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R. et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**, 653-657.

Borghi, L., Bureau, M. and Simon, R. (2007). *Arabidopsis JAGGED LATERAL ORGANS* is expressed in boundaries and coordinates *KNOX* and *PIN* activity. *Plant Cell* **19**, 1795-1808.

Bowman, J. L. and Eshed, Y. (2000). Formation and maintenance of the shoot apical meristem. *Trends Plant Sci.* **5**, 110-115.

Breuil-Broyer, S., Morel, P., de Almeida-Engler, J., Coustham, V., Negrutiu, I. and Trehin, C. (2004). High-resolution boundary analysis during *Arabidopsis thaliana* flower development. *Plant J.* **38**, 182-192.

Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A. and Martienssen, R. A. (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**, 967-971.

Byrne, M. E., Simorowski, J. and Martienssen, R. A. (2002). *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in *Arabidopsis*. *Development* **129**, 1957-1965.

Clark, S. E. (1997). Organ formation at the vegetative shoot meristem. *Plant Cell* **9**, 1067-1076.

Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735-743.

Eshed, Y., Baum, S. F., Perea, J. V. and Bowman, J. L. (2001). Establishment of polarity in lateral organs of plants. *Curr. Biol.* **11**, 1251-1260.

Fletcher, J. C. (2002). Shoot and floral meristem maintenance in Arabidopsis. *Annu. Rev. Plant Biol.* **53**, 45-66.

Gallois, J. L., Woodward, C., Reddy, G. V. and Sablowski, R. (2002). Combined SHOOT MERISTEMLESS and WUSCHEL trigger ectopic organogenesis in *Arabidopsis*. *Development* **129**, 3207-3217.

Gaudin, V., Lunness, P. A., Fobert, P. R., Towers, M., Riou-Khamlichi, C., Murray, J. A. H., Coen, E. and Doonan, J. H. (2000). The expression of *D-cyclin* genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the *cycloidea* gene. *Plant Physiol.* **122**, 1137-1148.

Geisler, M., Jablonska, B. and Springer, P. S. (2002). Enhancer trap expression patterns provide a novel teaching resource. *Plant Physiol.* **130**, 1747-1753.

Greb, T., Clarenz, O., Schafer, E., Müller, D., Herrero, R., Schmitz, G. and Theres, K. (2003). Molecular analysis of the *LATERAL SUPPRESSOR* gene in *Arabidopsis* reveals a conserved control mechanism for axillary meristem formation. *Genes Dev.* **17**, 1175-1187.

Ha, C. M., Kim, G. T., Kim, B. C., Jun, J. H., Soh, M. S., Ueno, Y., Machida, Y., Tsukaya, H. and Nam, H. G. (2003). The *BLADE-ON-PETIOLE 1* gene controls leaf pattern formation through the modulation of meristematic activity in *Arabidopsis*. *Development* **130**, 161-172.

Hepworth, S. R., Zhang, Y. L., McKim, S., Li, X. and Haughn, G. (2005). BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in *Arabidopsis*. *Plant Cell* **17**, 1434-1448.

Hibara, K., Karim, M. R., Takada, S., Taoka, K., Furutani, M., Aida, M. and Tasaka, M. (2006). *Arabidopsis CUP-SHAPED COTYLEDON3* regulates postembryonic shoot meristem and organ boundary formation. *Plant Cell* **18**, 2946-2957.

Hussey, G. (1971). Cell division and expansion and resultant tissue tensions in shoot apex during formation of a leaf primordium in tomato. *J. Exp. Bot.* **22**, 702-714.

Kanrar, S., Onguka, O. and Smith, H. M. S. (2006). *Arabidopsis* inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. *Planta* **224**, 1163-1173.

Keller, T., Abbott, J., Moritz, T. and Doerner, P. (2006). *Arabidopsis REGULATOR OF AXILLARY MERISTEMS1* controls a leaf axil stem cell niche and modulates vegetative development. *Plant Cell* **18**, 598-611.

Kerstetter, R. A., Bollman, K., Taylor, R. A., Bomblies, K. and Poethig, R. S. (2001). *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* **411**, 706-709.
Kirch, T., Simon, R., Grunewald, M. and Werr, W. (2003). The *DORNROSCHEN/ENHANCER OF SHOOT REGENERATION1* gene of *Arabidopsis* acts in the control of meristem cell fate and lateral organ development. *Plant Cell* **15**, 694-705.
Lee, D. K., Ahn, J. H., Song, S. K., Choi, Y. D. and Lee, J. S. (2003). Expression of an expansin gene is correlated with root elongation in soybean. *Plant Physiol.* **131**, 985-997.
Lin, W. C., Shuai, B. and Springer, P. S. (2003). The *Arabidopsis LATERAL ORGAN BOUNDARIES*-domain gene *ASYMMETRIC LEAVES2* functions in the repression of *KNOX* gene expression and in adaxial-abaxial patterning. *Plant Cell* **15**, 2241-2252.
Long, J. A. and Barton, M. K. (1998). The development of apical embryonic pattern in *Arabidopsis*. *Development* **125**, 3027-3035.
Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* **379**, 66-69.
McConnell, J. R. and Barton, M. K. (1998). Leaf polarity and meristem formation in *Arabidopsis*. *Development* **125**, 2935-2942.
Müller, D., Schmitz, G. and Theres, K. (2006). *Blind* homologous *R2R3 Myb* genes control the pattern of lateral meristem initiation in *Arabidopsis*. *Plant Cell* **18**, 586-597.
Nagel, R., Elliott, A., Masel, A., Birch, R. G. and Manners, J. M. (1990). Electroporation of binary Ti plasmid vector into *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. *FEMS Microbiol. Lett.* **67**, 325-328.
Ori, N., Eshed, Y., Chuck, G., Bowman, J. L. and Hake, S. (2000). Mechanisms that control *knox* gene expression in the *Arabidopsis* shoot. *Development* **127**, 5523-5532.
Raman, S., Greb, T., Peaucelle, A., Blein, T., Laufs, P. and Theres, K. (2008). Interplay of miR164, *CUP-SHAPED COTYLEDON* genes and *LATERAL SUPPRESSOR* controls axillary meristem formation in *Arabidopsis thaliana*. *Plant J.* **55**, 65-76.
Rojas-Pierce, M. and Springer, P. (2003). Gene and enhancer trap for gene discovery. In *Plant Functional Genomics*, vol. 236 (ed. E. Grotewold), pp. 221-240. Totowa, NJ: Humana Press.
Schmid, M., Davison, T. S., Henz, S. R., Pape, U. J., Demar, M., Vingron, M., Scholkopf, B., Weigel, D. and Lohmann, J. U. (2005). A gene expression map of *Arabidopsis thaliana* development. *Nat. Genet.* **37**, 501-506.
Schmitz, G. and Theres, K. (2005). Shoot and inflorescence branching. *Curr. Opin. Plant Biol.* **8**, 506-511.
Semiarti, E., Ueno, Y., Tsukaya, H., Iwakawa, H., Machida, C. and Machida, Y. (2001). The *ASYMMETRIC LEAVES2* gene of *Arabidopsis thaliana* regulates formation of a symmetric lamina, establishment of venation and repression of meristem-related homeobox genes in leaves. *Development* **128**, 1771-1783.
Shuai, B., Reynaga-Pena, C. G. and Springer, P. S. (2002). The *LATERAL ORGAN BOUNDARIES* gene defines a novel, plant-specific gene family. *Plant Physiol.* **129**, 747-761.
Smith, H. M. S., Campbell, B. C. and Hake, S. (2004). Competence to respond to floral inductive signals requires the homeobox genes *PENNYWISE* and *POUND-FOOLISH*. *Curr. Biol.* **14**, 812-817.
Souer, E., vanHouwelingen, A., Kloos, D., Mol, J. and Koes, R. (1996). The *No Apical Meristem* gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* **85**, 159-170.
Stracke, R., Werber, M. and Weisshaar, B. (2001). The *R2R3-MYB* gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* **4**, 447-456.
Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J. D. G., Dean, C., Ma, H. and Martienssen, R. (1995). Patterns of gene-action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Dev.* **9**, 1797-1810.
Takada, S., Hibara, K., Ishida, T. and Tasaka, M. (2001). The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development* **128**, 1127-1135.
Vroemen, C. W., Mordhorst, A. P., Albrecht, C., Kwaaitaal, M. A. and de Vries, S. C. (2003). The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in *Arabidopsis*. *Plant Cell* **15**, 1563-1577.
Weir, I., Lu, J. P., Cook, H., Causier, B., Schwarz-Sommer, Z. and Davies, B. (2004). *CUPULIFORMIS* establishes lateral organ boundaries in *Antirrhinum*. *Development* **131**, 915-922.
Xiang, C., Han, P., Lutziger, I., Wang, K. and Oliver, D. J. (1999). A mini binary vector series for plant transformation. *Plant Mol. Biol.* **40**, 711-717.