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Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene *BELLRINGER*

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SUMMARY

Lateral organs in plants arise from the meristem in a stereotypical pattern known as phyllotaxy. Spiral patterns result from initiation of successive organs at a fixed angle of divergence but variable patterns of physical contact. Such patterns ultimately give rise to individual leaves and flowers at positions related to each other by consecutive terms in the mathematical series first described by Leonardo Fibonacci. We demonstrate that a BELL1 related homeodomain protein in *Arabidopsis*, BELLRINGER, maintains the spiral phyllotactic pattern. In the absence of *BELLRINGER*, the regular pattern of organ initiation is disturbed and lateral organs are initiated more frequently. *BELLRINGER* is also required for maintenance of stem cell fate in the absence of the regulatory genes *SHOOT MERISTEMLESS* and *ASYMMETRIC LEAVES1*. We propose a model whereby *BELLRINGER* coordinates the maintenance of stem cells with differentiation of daughter cells in stem cell lineages.

Key words: Phyllotaxy, Homeobox, Shoot apical meristem, KNOX, BELLRINGER, BREVIPEDICELLUS, SHOOT MERISTEMLESS, ASYMMETRIC LEAVES1

INTRODUCTION

The plant shoot is derived from a group of undifferentiated stem cells within the shoot apical meristem (SAM). Lateral organs such as leaves arise on the flanks of the SAM by the coordinated repression of stem cell fate, determination of founder cells, and elaboration of the incipient primordium. Early events specify the spatial and temporal positioning of leaf and flower primordia and ultimately establish shoot phyllotaxy (Greek, 'leaf order'). Later events define organ patterning along proximodistal, dorsoventral and mediolateral axes (reviewed by Kidner et al., 2002).

Initiation of successive lateral organs on the flanks of the SAM proceeds in predictable patterns generating a phyllotaxy. Spiral phyllotaxy observed in the vast majority of plants derives from the regular initiation of successive lateral organ primordia at a constant divergence angle approximating 137.5°. This pattern can be described in terms of two sets of opposing intersecting spirals or 'contact parastichies' that connect adjacent primordia (Richards, 1951; Steeves and Sussex, 1989). The number of clockwise and counterclockwise spirals in each set is characteristic of a species, and corresponds to successive numbers in the Fibonacci series (3+5, 5+8, 8+13 etc.) (reviewed by Richards, 1951). Inter-specific and developmental variation in the number of contact parastichies is thought to reflect relative rates of primordia initiation and growth (Richards, 1951).

Genetic pathways that establish and maintain phyllotaxy are yet to be identified. Potentially the site of primordium initiation is established by molecular inhibitory signals from preexisting primordia or from biophysical stresses within the shoot apex (reviewed in Green, 1999). Consistent with both of these models alterations in phyllotaxy are often associated with changes in the dimensions and organization of the SAM. For example, in the *Arabidopsis* mutants *fasciata1* (*fas1*) and *fasciata2* (*fas2*) meristem shape and patterning is irregular and phyllotaxy is disrupted (Kaya et al., 2001; Leyser and Furner, 1992). The pattern of organ initiation is also irregular in the *serrate* (*se*) mutant where the meristem is enlarged compared with wild type (Clarke et al., 1999; Ori et al., 2000; Prigge and Wagner, 2001). Reduced meristem size is associated with phyllotaxy defects in the DNA topoisomerase mutant *top1α* (Takahashi et al., 2002). Alternatively, the *abphyl1* mutant in maize has a phyllotaxy defect associated with an enlarged meristem. Additional leaves are formed in a decussate pattern rather than the normal alternate, distichous arrangement (Jackson and Hake, 1999). In contrast to these examples, *terminal ear (te1*) mutations in maize alter the rate of leaf initiation and phyllotaxy, but have relatively minor effects on the geometry of the shoot apex (Veit et al., 1998). *te1* encodes a protein related to the RNA binding protein Mei2 from *Schizosaccharomyces pombe*. *te1* is expressed in the presumptive internode in a region around the meristem excluding the site of organ initiation. This expression pattern suggests *te1* may inhibit lateral organ differentiation.

One genetic pathway required for SAM function involves

class 1 KNOX homeobox transcription factors. Recessive mutations in the *Arabidopsis* KNOX gene *SHOOT MERISTEMLESS* (*STM*) and in the closely related maize gene *knotted1* have defects in meristem function indicating a requirement for SAM initiation and/or maintenance (Barton and Poethig, 1993; Long et al., 1996; Vollbrecht et al., 2000; Vollbrecht et al., 1991). Consistent with a role in SAM function both *STM* and *kn1* are expressed in vegetative and reproductive SAMs but are down-regulated in founder cells and lateral organ primordia (Jackson et al., 1994; Long et al., 1996). *STM* maintains stem cell fate by negative regulation of the myb domain transcription factor *ASYMMETRIC LEAVES1* (*AS1*) and a member of the LOB-like transcription factor family *ASYMMETRIC LEAVES2* (*AS2*) (Byrne et al., 2000; Byrne et al., 2002; Iwakawa et al., 2002; Shuai et al., 2002). *AS1* is related to *rough sheath2* in maize and *PHANTASTICA* in *Antirrhinum*. All three genes are expressed in lateral organ primordia where they function as negative regulators of KNOX genes (Byrne et al., 2000; Ori et al., 2000; Semiarti et al., 2001; Timmermans et al., 1999; Tsiantis et al., 1999).

Arabidopsis has three additional class I KNOX genes, *BREVIPEDICELLUS* (*BP*, formerly *KNAT1*), *KNAT2* and *KNAT6*. Like *STM*, these genes are expressed in SAMs and downregulated in lateral organs, although the pattern and timing of expression differs from that of *STM* (Byrne et al., 2002; Dockx et al., 1995; Lincoln et al., 1994; Pautot et al., 2001; Semiarti et al., 2001). Mutations in *BP* alone do not cause meristem defects (Byrne et al., 2002; Douglas et al., 2002; Venglat et al., 2002). *BP* is, however, redundant with *STM* and has a role in SAM function in *as1 stm* and *as2 stm* mutant backgrounds (Byrne et al., 2002). Disruption of *KNAT2* gene expression has no phenotypic effect, probably because of redundancy with the duplicate gene *KNAT6* (Byrne et al., 2002).

We have isolated several insertion alleles of *BELLRINGER (BLR)*, a *BELL1*-like homeobox gene. Prominent defects in *blr* mutants include an increase in the number of leaves and disruption to the normal spiral pattern of primordia initiation. Genetic interactions also demonstrate that *BLR* is required for stem cell maintenance. Previously we reported that *BP* plays a role in meristem function in the absence of *AS1* and *STM* (Byrne et al., 2002). *BLR* is also necessary for meristem function in the absence of *AS1* and *STM*. BLR probably interacts directly with STM and BP in meristem function.

MATERIALS AND METHODS

Plant material and growth conditions

Mutant alleles, *blr-1* (GT7797) and *blr-2* (ET6411), were obtained from gene trap and enhancer trap lines generated as previously described (Springer et al., 1995; Sundaresan et al., 1995). The *blr-3* allele was obtained from the The Salk Institute Genomic Analysis Laboratory (SIGnAL) collection. *bel1-1*, *wus-1*, *clv1-1* and *clv3-2* were obtained from the *Arabidopsis* Biological Resource Center (ABRC). Other mutants (*as1-1*, *stm-1*, *stm-11*, *stm-2*, and *bp-2*) were obtained as described previously (Byrne et al., 2002). All mutants other than *blr-3* were in a Landsberg *erecta* background. Plants were grown either on soil or on MS medium supplemented with sucrose, with a minimum day length of 16 hours.

Plant genetics

Tests for allelism were carried out by crossing plants homozygous for *blr-2* and *blr-3* to plants homozygous for *blr-1*. F_1 plants from both crosses displayed the *blr* mutant phenotype. All other genetic interactions and phenotypic studies were carried out with the *blr-1* allele in a Landsberg *erecta* background. To generate double mutants, plants homozygous for *bp*, *as1*, *bel1*, *clv1* or *clv3* were crossed to plants homozygous for *blr*. All double mutants segregated in the F_2 progeny in a 1:15 ratio. Double *blr wus*, *blr stm-11* and *blr stm-2* mutants were generated by crossing plants homozygous for *blr* to plants heterozygous for *wus*, *stm-11* or *stm-2*. Double *blr wus* mutants segregated 1:3 in F_3 seed from *blr* mutant plants. In lines carrying either *stm-11* or *stm-2* F_3 seed from homozygous *blr* plants segregated 1:3 *stm* mutants.

Triple *blr as1 stm-11* mutants were generated by crossing double homozygous *blr as1* plants to *as1/as1 stm-11*/+ plants. F_2 seed from *blr*/*blr as1*/*as1 stm11*/+ plants segregated a meristemless phenotype in a 1:3 ratio. Triple *bel1 as1 stm-11* mutants were generated by crossing *as1*/*as1 stm-11*/+ plants with *bel1*/*bel1*. F_3 seed from plants homozygous for *as1* and heterozygous for *stm11* and *bel1* segregated only wild type, *as1 stm11* and *as1 bel1* phenotypes in a 9:4:3 ratio.

Molecular biology

DNA extraction and manipulation were carried out using standard protocols (Sambrook et al., 1989). The Ds element copy number in lines carrying *blr-1* and *blr-2* was determined using Southern gel blot analysis as described previously (Vongs et al., 1993). The Ds-specific hybridization probe was obtained by PCR amplification of the Ds element using the primers agcccatgtaagaaatacctagcg and tgctgtactgctaagtgctgtgag. Identification of the tagged gene in *blr-1* and *blr-2* was carried out by thermal asymmetric interlaced-PCR (Liu et al., 1995). To confirm the Ds element insertion sites in *blr-1* and *blr-2*, PCR products were generated using Ds and gene-specific primers. Ds primers were acccgaccggatcgtatcggt and acggtcgggaaactagctctac. *blr-1* primers were ctgctggtcaaagacatggat and tgcatgcttaattagcaagaaat. *blr-2* primers were atcgtgcttcaaaaagacacc and gcagagaagaatcatcgtcgt. PCR products were sequenced using dye terminator cycle sequencing (Applied Biosystems).

Total RNA for northern and RT-PCR analysis was purified using Trizol reagent (Life Technologies). For northern hybridization, 20 μg of RNA was separated on a 1.4% gyloxal/DMSO denaturing gel. RNA was transferred to a membrane and hybridized using Ultrahyb buffer (Ambion). The *BLR* probe for northern analysis was a PCR product synthesized with the primers taatgtgggtcgtgggattta and aggagcatgatgatcaggaaa. RT-PCR was carried out as previously described (Byrne et al., 2002). Following DNase treatment and synthesis of complementary DNA with M-MuLV reverse transcriptase (New England Biolab) PCR reactions were performed with gene-specific primers. *BLR* primers were as above. *RBC* primers were gaacaatggcttcctctatgc and cacaaggaatccacttgttgc. PCR products were subject to Southern hybridization using gene-specific probes.

The *BLR::GUS* construct was derived as follows. A 3.9 kb genomic fragment containing the *BLR* promoter was amplified from Landsberg *erecta* using the primers ttggcacgattctgaaacacg and ctcgccggctttgttgaaga. The product was cloned into pRITA, which contains the GUS reporter gene and NOS terminator sequences (a gift from John Bowman). The resulting plasmid, pRIP3, contains an in frame fusion of the start codon of *BLR* with GUS. This gene fusion fragment was cloned into a binary vector and introduced into plants using *Agrobacterium* transformation.

Histology and microscopy

Inflorescences were prepared for sectioning by fixation in glutaraldehyde (2.5% in 0.1 M sodium phosphate buffer pH 7.0), dehydration through an ethanol series and infiltration with Histoclear prior to embedding in paraffin wax. All sections were 8 μm thick. Sections were cleared of paraffin wax using Histoclear, rehydrated to 50% ethanol, stained for 20 minutes in 0.1% safranin in 50% ethanol, rinsed in 70% ethanol, then stained for 3 minutes in 0.1% Fast Green

in 95% ethanol. The sections were then dehydrated in 100% ethanol, and moved to Histoclear. For meristem size comparisons measurement were taken from longitudinal sections of 8 wild-type and 9 *blr* plants that were 23 days old.

GUS staining was carried out as previously described (Gu et al., 1998) using a substrate solution containing 100 mM sodium phosphate pH 7, 10 mM EDTA, 0.1% Triton X-100, 0.5 mg/ml 5-bromo-4-chloro-3-indolyl β-D glucuronic acid (X-Gluc), 100 μg/ml chloramphenicol, and either 2 mM or 5 mM each of potassium ferricyanide and potassium ferrocyanide. After 24-48 hours at 37°C samples were cleared in 70% ethanol at room temperature. Samples were viewed with a Leica MZ8 microscope and images captured with a Spot RT digital camera (Diagnostic instruments).

For scanning electron microscopy (SEM) inflorescences from wild-type and *blr* plants were first fixed overnight in 2.5% glutaraldehyde, then rinsed in 0.1 M sodium phosphate buffer and dehydrated through an ethanol series (30%, 50%, 70%, 80%, 95%, 100%) prior to critical point drying using a Tousimis Auotsamdri-815. Samples were subsequently mounted on silver tape and sputter coated with gold (Emitech K550) before viewing with an Hitachi S-3500N SEM under high vacuum and with a beam accelerating voltage of 3-5 kV. Measurements of meristem radius and organ divergence angle were derived from SEM images of 16 wild-type and 16 *blr* mutant that were 18 days old. Plastochron ratios were measured from 7 wild-type and 7 *blr* plants.

In situ hybridization

In situ hybridizations were performed using digoxigenin-labeled probes (Long and Barton, 1998; Long et al., 1996). Antisense and control sense *BLR* transcripts were synthesized from the plasmid pBlue028, which carries a 531 bp fragment, encompassing the 3′ end of *BLR*, in pBluescript. Antisense *STM* transcripts were synthesized from the plasmid Meri HB1 (a gift from Kathy Barton). All sections were 8 μm thick.

Yeast 2-hybrid assay

Full-length *BLR* cDNA was amplified with the primers ggtcgacgggctgatgcatacgagcct and agcggccgcatttcaattcccccatatc. The PCR product was digested with *Sal*I and *Not*I cloned into the GAL4 transcriptional activation domain (TA) vector pBI-771 (Kohalmi et al., 1997) forming the plasmid TA-BLR. *STM* cDNA was amplified with the primers acgcgtcgacgtatggagagtggttccaac and ataagaatgcggccgcccaagtataccgagaacc. The PCR product was digested with *Sal*I and *Not*I and cloned into the GAL4 DNA-binding domain (DB) vector pBI-770. Other constructs, DB-BP, DB-KNAT4 and TA-BEL1 were kindly provided by George Haughn and are as previously described (Bellaoui et al., 2001). All plasmids were transformed into the yeast strain pJ69A using a lithium acetate/polyethylene glycol protocol (Schiestl et al., 1993).

RESULTS

bellringer affects shoot architecture

In a screen for mutants affecting shoot architecture, we recovered gene and enhancer trap insertions in a gene we named *BELLRINGER*. A third allele carrying a T-DNA insertion was also identified. All three alleles produced comparable phenotypes. Compared to wild type, *blr* mutants are reduced in stature and have a bushy appearance due to precocious growth of axillary branch meristems (Fig. 1A,B). There is an increase in the number of leaves and flowers (Table 1) although time to flowering is not significantly delayed (average of 16 days in wild type compared with 18 days in *blr-1*). In addition phyllotaxy is disrupted in these mutants such

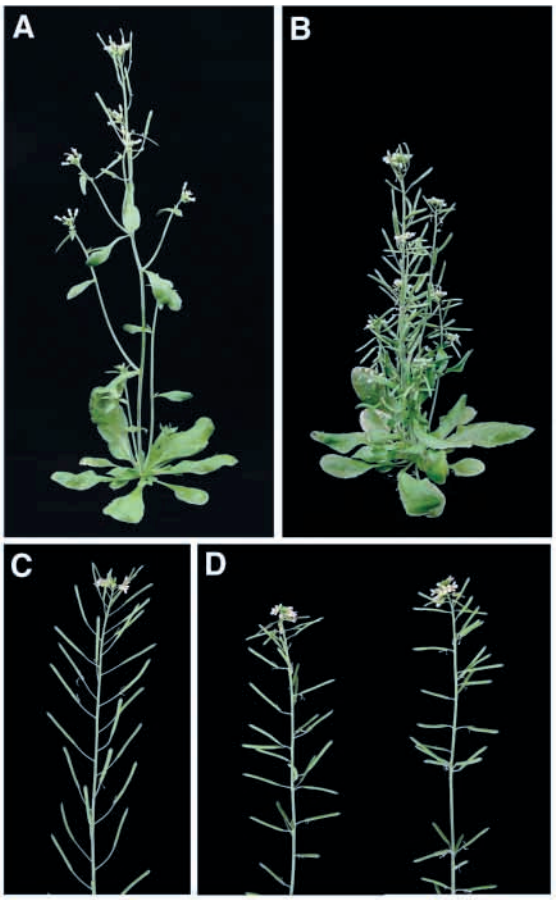

Fig. 1. *blr* mutant phenotype. (A) Wild-type Landsberg *erecta* and (B) *blr-1* mutant plants. *blr* has additional leaves and flowers, is reduced in stature and has precocious outgrowth of axillary meristems. Phyllotaxy is also disturbed. (C) In the wild-type inflorescence siliques are arranged in a regular spiral phyllotactic pattern. (D) In *blr* mutant inflorescences siliques occur in aberrant positions and internodes are irregular.

that the regular spiral arrangement of flowers on the inflorescence shoot is not strictly maintained (Fig. 1C,D). This is reflected in the relative displacement of flowers along both the radial and longitudinal axis of the stem. In the radial dimension, flowers can occur both closer together and further apart than in wild type. Inflorescence internodes are variable so that flowers occur at irregular intervals along the stem. *blr* mutants also have shorter siliques and ovary septum fusion defects (data not shown). The severity of these phenotypes is dependent on the genetic background and growth conditions. Principally the reduction in stature and loss of apical dominance are less severe under low light conditions and in a Columbia background.

Since phyllotaxy is often associated with changes in meristem size or shape, we compared meristems in wild type and *blr* mutants. In longitudinal section the size and shape of *blr* inflorescence meristems is comparable to that of wild type (Fig. 2A,B). Furthermore, the radius of *blr* mutant inflorescence meristems, estimated from SEM images of 18-day-old plants, was not significantly different from that of wild type. The average radius of wild-type meristems was 24.8 μm

Table 1. Average number of leaves and flowers in wild type and *blr* mutants

	Rosette leaves	Cauline leaves	Total leaves	Flowers
Wild type L. *erecta* (*n*=20)	6.3 (6-7)	2.5 (2-3)	8.8	29 (26-32)
blr-1 (*n*=20)	9.3 (8-11)	2.9 (2-3)	12.2	41.8 (39-47)
blr-2 (*n*=20)	8.5 (7-11)	3.0 (2-4)	11.4	39.7 (35-44)
Wild type Columbia (*n*=15)	6.9 (6-8)	2.7 (3-5)	9.4	29.3 (25-36)
blr-3 (*n*=15)	9.9 (9-12)	2.9 (2-4)	12.8	36.2 (34-40)

n, number of plants used for determining leaf and flower number.
Numbers in brackets are range of organs.
Organ numbers were determined from wild-type and mutant plants grown for the same length of time under identical growth conditions.
blr-1 and *blr-2* are in L. *erecta* background. *blr-3* is in a Columbia background.

(range 22.6-28.1 μm) while that of *blr* mutants was 23.6 μm (range 20.5-27.0 μm).

Spiral phyllotaxy can be described by two parameters, the plastochron ratio and the divergence angle (Richards, 1951). The plastochron ratio can be used to predict the number of contact parastiches and reflects alterations in the packing of lateral organs. This parameter is derived by calculating the ratio of the distance of two successive primordia from the meristem, where distances are measured from the center of the meristem to the center of each primordium. To compare initiation patterns in wild type and mutant we measured the plastochron ratio for the youngest 4-6 primordia on inflorescence apices from 18-day-old plants. Average plastochron ratios between initiating floral primordia on *blr* mutant inflorescence apices were not significantly different from wild type (1.1481 versus 1.1465). In plants with spiral phyllotaxy the divergence angle between successive primordia, measured from the center of the meristem to the middle of two successive organs, is approximately 137.5°. The average divergence angle between successive primordia in wild-type inflorescence apices was 136.72° (range 121.49°-152.31°), which was comparable with most *blr* mutant inflorescence apices at 137.52° (range 127.54°-154.91°). However, 2 out of 16 *blr* mutants had significantly reduced divergence angles between successive primordia (range 79.35°-112.13°) indicating abnormal sites of floral meristem initiation (Fig. 2C,D). This was consistent with the irregular phyllotaxy observed in mature plants (Fig. 2E-H). In agreement with these quantitative parameters, the number of clockwise and counter-clockwise contact parastichies was unchanged. However, the angle between spiral parastichies was increased, accommodating the extra organs in a tighter spiral than normal (Fig. 2G,H).

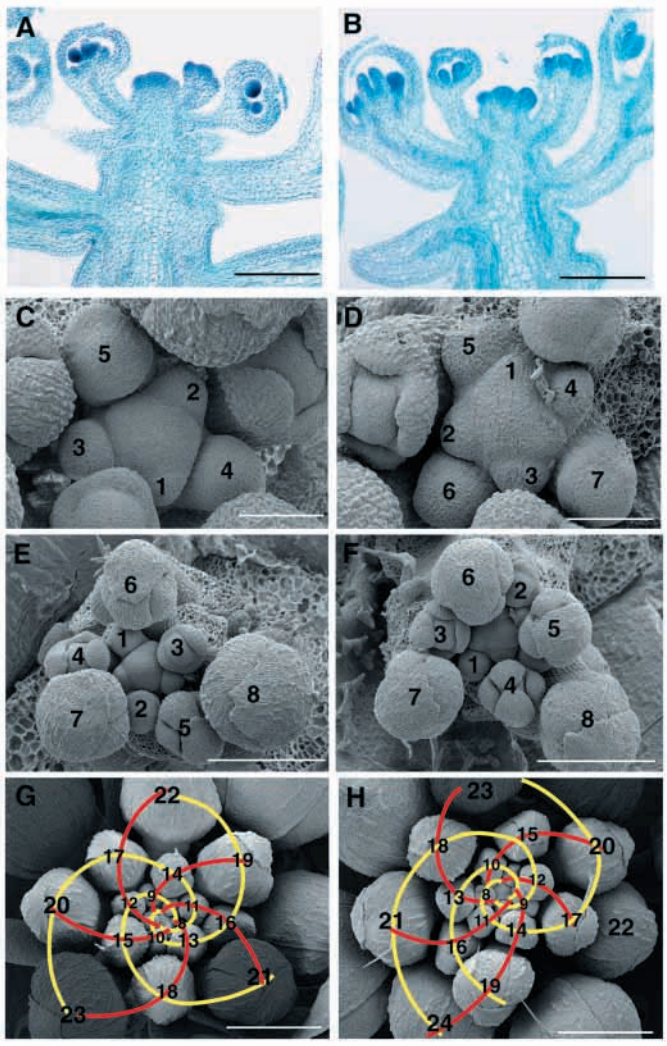

Fig. 2. Phyllotaxy defect in *blr* mutants. (A,C,E,G) Wild-type inflorescence apex; (B,D,F,H) *blr* mutant inflorescence apex. (A,B) Longitudinal sections of 23-day-old plants showing comparable size and shape of wild-type (A) and *blr* (B) inflorescence meristems. The width of the stem is also similar in wild type and *blr*. (C-F) 18-day-old plants. In wild type (C,E) organs are initiated on average 137.5° apart forming a continuous spiral. In wild type (C) the divergence angle between primordia 2 and 3 is 130.6°. In *blr* mutants (D,F) flowers can initiate in aberrant positions as in primordia 3 in D and 5 in F. In D the divergence angle between primordia 2 and 3 is 79.3°. (G,H) Inflorescence apex from 23-day-old plants. Clockwise (red) and counterclockwise (yellow) contact parastichies connecting floral primordia in a 3+5 phyllotactic pattern. Additional organs in *blr* mutants result in contact parastichies forming a tighter curve than in wild type. Numbering of each inflorescence starts at the youngest visible primordium. Scale bars: 0.5 mm (A,B), 50 μm (C,D), 200 μm (E,F) and 500 μm (G,H).

BELLRINGER is related to the homeodomain transcription factor *BELL1*

The recessive *blr-1* mutation was found to cosegregate with a single Ds element inserted into a homeobox gene, At5g02030, located on chromosome 5 (http://cshl.genetrap.org) (The Arabidopsis Genome Initiative, 2000). This gene encodes a protein most closely related to members of the *BELL1* (*BEL1*) subclass of homeodomain transcription factors, of which there

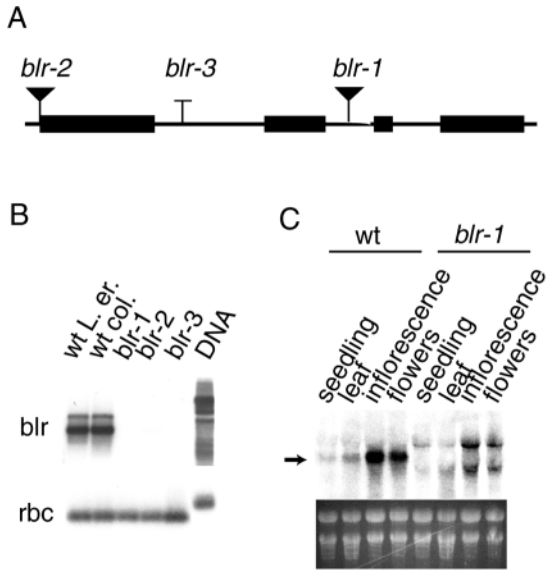

Fig. 3. *blr* mutant alleles. (A) Diagram of the *BLR* gene is with locations of Ds insertions in *blr-1* and *blr-2*, and the T-DNA insertion in *blr-3* indicated. (B) RT-PCR amplification using gene-specific primers and hybridization of products with gene-specific probes. *BLR* transcripts are detected in wild type but not in the three mutants (top panel). *RBC* transcripts were amplified as a control (bottom panel). (C) Northern hybridization detects low levels of *BLR* transcript in wild-type seedling and leaf tissue and much higher levels in the inflorescence and flowers. No wild-type transcript is detected in the *blr-1* mutant. Lower panel shows an ethidium bromide-stained gel reflecting relative amounts of RNA.

are 12 members in *Arabidopsis* (Becker et al., 2002). *BLR* comprises 4 exons and 3 introns (Fig. 3A). The Ds insertion in *blr-1* is located in the second intron whereas *blr-2* has a Ds insertion 2 bp upstream of the *BLR* ATG initiation codon. In both alleles the insertion does not activate the GUS reporter on the Ds element. The third allele, *blr-3*, carries a T-DNA insertion in the first intron of the gene. For all three alleles full-length *BLR* transcripts were not detected in homozygous mutant plants (Fig. 3B).

BELLRINGER expression pattern

The expression pattern of *BLR* in wild-type plants was examined by northern hybridization. Low levels of *BLR* transcript were detected in 8-day-old seedlings and in leaf tissue (Fig. 3C). Higher transcript levels were detected in inflorescence shoots, including the inflorescence meristem and flower buds.

BLR expression was further examined by driving GUS reporter gene expression from the *BLR* promoter. In the embryo, *BLR* expression was confined to the SAM (Fig. 4A) but weak expression could also be detected in the root tip (data not shown). In young seedlings, *BLR* was highly expressed in the SAM and could be detected in cotyledon and leaf vasculature (Fig. 4B). In the inflorescence, *BLR* was expressed in the inflorescence meristem, stem, flower pedicel and in developing flowers (data not shown).

In situ hybridization was carried out to confirm the authenticity of the *BLR::GUS* expression pattern. *BLR* expression in late heart stage embryos was confined to the SAM region between the two developing cotyledons (Fig. 4C). The expression pattern is similar to that of *STM* (Fig. 4D) (Long et al., 1996). In addition, *BLR* is expressed in the inflorescence SAM in a region similar to that of *STM* (Fig. 4E,F). *BLR* expression is initially downregulated in incipient floral primordia (Fig. 4G-I). Strong expression is subsequently

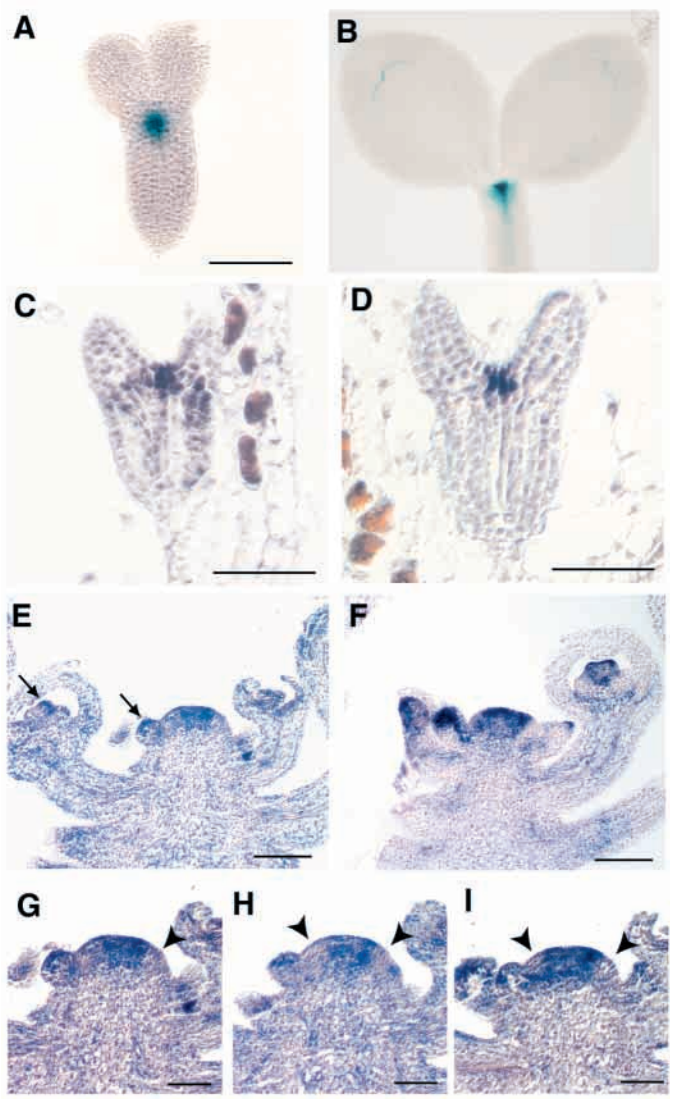

Fig. 4. *BLR* expression pattern. (A,B) Whole mounts showing GUS expression pattern from the *BLR::GUS* reporter gene. GUS activity is detected in the SAM of the embryo (A) and 8-day-old seedling (B). Weak expression is also in the vasculature of cotyledons and leaves. (C,D) In situ hybridization of wild-type embryos probed with *BLR* antisense (C), and *STM* antisense (D). *BLR* and *STM* transcripts are detected in the SAM (E,F) In situ hybridization of wild-type inflorescences probed with *BLR* antisense (E) and *STM* antisense (F). (G-I) Three consecutive serial sections of an inflorescence apex probed with *BLR* antisense. *BLR* is expressed in the SAM, in a pattern similar to that of *STM*. In initiating floral primordia *BLR* is first downregulated (arrowheads in G-I) but is subsequently detected in the central region of developing floral primordia and flowers (arrows in E). A low level of *BLR* expression is also detected at the base of developing flowers, in the pedicel and in the inflorescence stem. Scale bars: 50 μm (A,C-I).

detected in the central region of young floral primordia and the inner whorl of developing flowers (Fig. 4E,G-I). A low level of expression is also detected at the base of the developing flowers, in the pedicel and in subepidermal and core cells of the inflorescence stem. A *BLR* sense probe used as a negative control did not show a signal in embryo or inflorescence tissue (data not shown).

Genetic interactions between *bellringer* and KNOX genes

BELL-like proteins belong to a class of homeodomain transcription factors that can interact directly with KNOX class homeodomain transcription factors (Bellaoui et al., 2001; Müller et al., 2001; Smith et al., 2002). Since protein-protein interactions between heterologous homeodomain transcription factors are required in animals (Mann and Chan, 1996) it is likely that such interactions are functionally significant in plants. We therefore investigated genetic interactions between *BLR* and the class 1 KNOX genes *STM*, *KNAT1* and *KNAT2*.

Embryos homozygous for strong *stm* alleles, including *stm-1* and *stm-11* (Fig. 5A), lack a SAM and develop cotyledons that are fused at their base (Barton and Poethig, 1993; Clark et al., 1996; Long et al., 1996). Double *blr stm-11* mutants are similar to *stm-11* mutants (Fig. 5B). However, *blr* enhances the phenotype of the weak allele *stm-2*. Single *stm-2* mutants germinate with slight fusion at the base of the cotyledons (Clark et al., 1996; Endrizzi et al., 1996). After a brief delay, vegetative shoot development is initiated (Fig. 5C). In contrast, *blr stm-2* double mutants do not form any vegetative shoot and resemble mutants of strong alleles of *stm* (Fig. 5D). This genetic interaction indicates *BLR* is required for SAM function when there is reduced *STM* activity.

The *blr* mutant phenotype shares some characteristics with *bp* mutants including reduced stature and precocious outgrowth of axillary meristems (Fig. 6A). In addition, in *bp* mutants differentiation of subepidermal chlorenchyma extending from the abaxial side of the pedicel into the adjacent stem is disrupted (Douglas et al., 2002; Venglat et al., 2002). We analyzed *blr bp* double mutants to determine if there is any interaction between these two genes. Plants homozygous for both *blr* and *bp* were much reduced in stature compared with either single mutant (Fig. 6B). In other respects the double mutant has features of both *blr* and *bp* single mutants. The number of rosette and cauline leaves in *blr bp* mutants is comparable to that of *blr* (data not shown) while flower pedicels and siliques are reduced in length and generally point downward as in *bp* (Fig. 6A,B), and subepidermal chlorenchyma below the flower is pale relative to surrounding tissue. The phenotype of the *blr bp* double mutant therefore appears additive. *blr knat2* double mutants are indistinguishable from *blr* mutants indicating that the *blr* phenotype is not influenced by loss of *KNAT2* expression (data not shown).

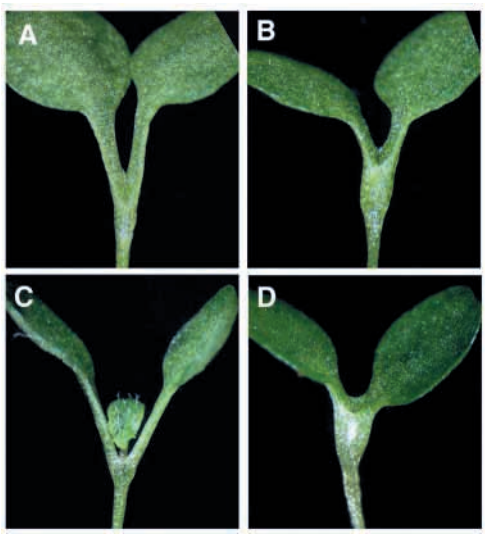

Fig. 5. *blr* enhances a weak allele of *stm*. Twelve-day-old seedlings of *stm-11* (A), *blr stm-11* (B), *stm-2* (C) and *blr stm-2* (D). *blr stm-11* double mutants are similar to *stm-11* single mutants. No vegetative shoot is produced and the base of the cotyledons are fused. Plants homozygous for the weak *stm-2* allele show limited fusion at the base of the cotyledons and initiate a vegetative shoot. The meristem defect is enhanced in *blr stm-2* double mutants which do not develop vegetative shoot structures and there is significant fusion of the cotyledons.

To further explore the possibility of genetic interactions between *BLR* and *BP* we took advantage of the conditional role of *BP* in SAM function. *BP* maintains the SAM in *as1 stm* double mutants (Byrne et al., 2002). Vegetative development in these double mutants resembles that of *as1*, whereas *bp as1 stm* mutants lack a SAM, fail to form a vegetative shoot, and are indistinguishable from *stm* single mutants (Byrne et al., 2000; Byrne et al., 2002). The phenotype of *blr as1* double mutants is additive in all respects (Fig. 6C). However, *blr as1 stm* triple mutants have severely reduced SAM function. Following germination only one or two leaves are produced (Fig. 6D). Occasionally development is resumed to form a disorganized vegetative shoot (Fig. 6E,F). *BLR* is therefore required for SAM function in the absence of *STM* and *AS1*.

In contrast to *blr* mutants where phenotypic effects are evident in the shoot, mutations in the related gene *bel1* only affect ovule development (Modrusan et al., 1994; Reiser et al., 1995; Robinson-Beers et al., 1992). *blr bel1* double mutants display a *blr* shoot phenotype and are sterile as is *bel1* (Fig. 6G,H). This demonstrates that *BEL1* is not redundant with *BLR* in shoot development. Since BEL1 protein directly interacts with BP we also investigated whether *BEL1* is required for SAM function in an *as1 stm* background. In contrast to *blr as1 stm* mutants, the triple *bel1 as1 stm* mutants form a vegetative shoot similar to *as1 stm* double mutants (data not shown). Furthermore no novel phenotype is detected in progeny of *blr as1* plants also segregating *bel1* and *stm*. Therefore *BEL1* is not required for SAM function in these contexts.

SAM function is also regulated by *WUSCHEL* (*WUS*) and the *CLAVATA* (*CLV*) genes, acting independently of KNOX genes. *WUS* is a homeodomain protein expressed in inner central zone stem cells of the SAM. Mutations in *WUS* result in loss of meristem function. *CLV1* and *CLV2* are transmembrane receptors and *CLV3* encodes a secreted peptide. Mutations in all three *CLV* genes result in a much enlarged meristem. *CLV* genes maintain meristem homeostasis by limiting *WUS* function (reviewed by Brand et al., 2001; Clark, 2001; Fletcher, 2002). We found *blr wus*, *blr clv1* and *blr clv3* double mutants are additive (not shown). Therefore, *BLR* appears to affect meristem function via a KNOX gene-specific pathway.

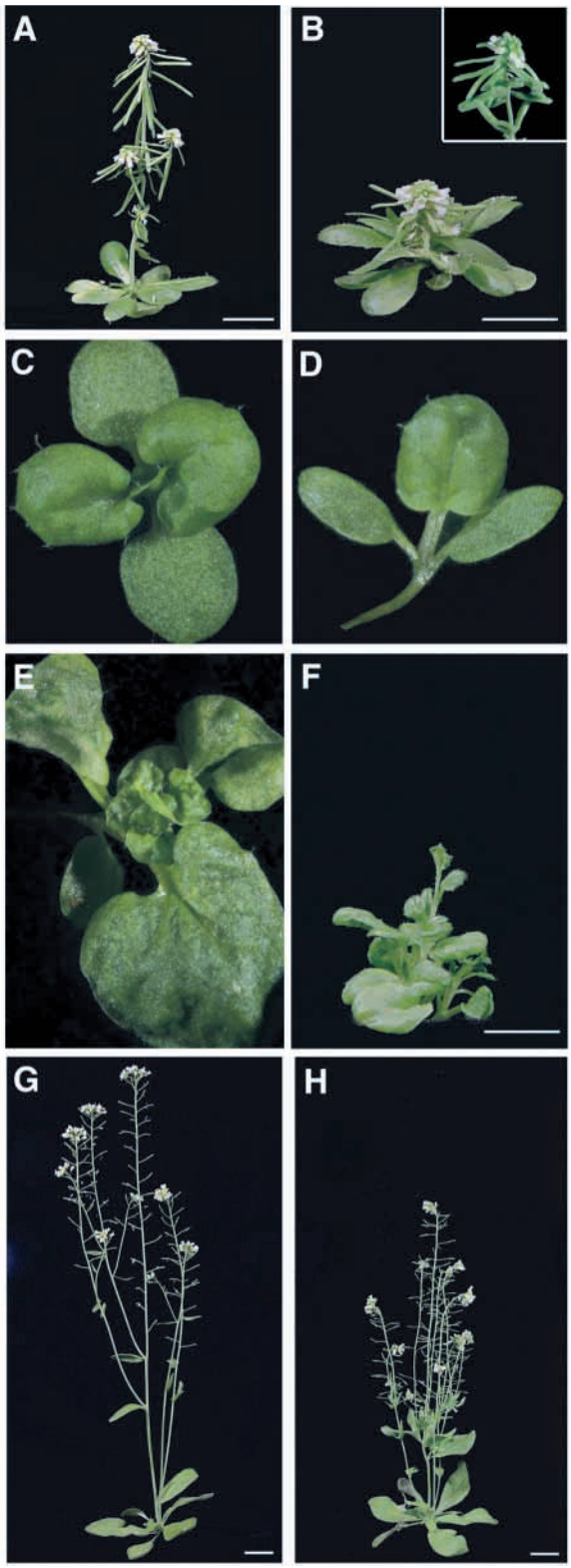

Fig. 6. *blr* interacts with *as stm1* but not with *bel1*. (A,B,F,G,H) Whole plants and (C-E) seedlings of *bp* (A), *blr bp* (B), *blr as1* (C), *blr as1 stm-11* (D-F), *bel1* (G) *blr bel1* (H). (A) The *bp* mutant is reduced in stature, pedicels are short and flowers hang down. (B) The *blr bp* double mutant is much reduced in stature compared with either single mutant. Double mutants also have additional leaves, pedicels are short and siliques tend to hang down (inset in B). (C) Twelve-day-old emerging shoot of *blr as1* seedling compared with (D) *blr as1 stm-11* sibling, with only a single vegetative leaf. (E,F) Occasionally vegetative shoot growth is resumed in *blr as1 stm-11* triple mutants. Leaves in the double and triple mutant have an *as1* phenotype. (G) The phenotype of the *bel1* mutant is confined to the ovule. Siliques do not elongate as plants fail to set seed. (H) The double *blr bel1* mutant fails to set seed as in *bel1* single mutants. The phyllotaxy is disturbed and stature is reduced as in *blr* single mutants. Scale bars: 2.5 cm (A,B,F-H).

BELLRINGER interacts directly with KNOX proteins

Genetic analysis demonstrated that *blr* enhances a weak allele of *stm* and is required for SAM function in the *as1 stm* background. Since BELL class proteins are known to interact directly with KNOX proteins (Bellaoui et al., 2001; Müller et al., 2001; Smith et al., 2002) one possibility is that BLR directly interacts with and affects the activity of STM and BP. To test this possibility a yeast two-hybrid assay was carried out (Fig. 7). Yeast strains carrying the plasmids TA-BLR and DB-BP or DB-STM were viable in the absence of histidine. In contrast, yeast carrying TA-BRL and DB-KNAT4 failed to grow in the absence of histidine. Therefore in this system BLR interacts with the class1 KNOX proteins STM and BP but not with the class 2 KNOX protein KNAT4. The negative control yeast strain carrying the plasmid TA-BLR in the presence of the DB vector showed no growth in the absence of histidine.

DISCUSSION

BLR is a BELL class gene which, like KNOX genes, are members of the TALE homeobox transcription factor family. TALE homeodomain proteins have an atypical homeodomain, characterized by three additional amino acids between the first and second helix. Several features distinguish BELL and KNOX proteins (Becker et al., 2002; Bharathan et al., 1999; Bürglin, 1997; Reiser et al., 2000). The 12 members of the BELL class share 54% amino acid identity in the homeodomain. Likewise, the 8 KNOX proteins share 54% amino acid identity in the homeodomain. However, between these two classes amino acid conservation in the homeodomain is 32%. In addition, BELL proteins have conserved SKY and BELL domains upstream of the homeodomain, whereas KNOX proteins are defined by conserved MEINOX, ELK and GSE domains upstream of the homeodomain (Bellaoui et al., 2001; Bürglin, 1997; Kerstetter et al., 1994).

BLR is strongly expressed in the embryonic SAM, and has a conditional role in SAM function as revealed by genetic interactions. Whereas *as1 stm* mutants form a vegetative shoot indistinguishable from that of *as1* single mutants, shoot development is greatly reduced in the triple *blr as1 stm* mutant. This triple mutant strongly resembles *pinhead/zwille* mutants where a solitary vegetative organ appears to consume the meristem (Lynn et al., 1999; Moussian et al., 1998). *BLR* is therefore required to maintain the SAM in the *as1 stm* background. Meristem function in *as1 stm* mutants is dependent on *BP* (Byrne et al., 2002), so that it is probable that *BP* activity is compromised in *blr*. We tested this possibility by making *bp blr* double mutants, which were additive in all respects. Thus *BP* is still functional in a *blr* mutant, meaning

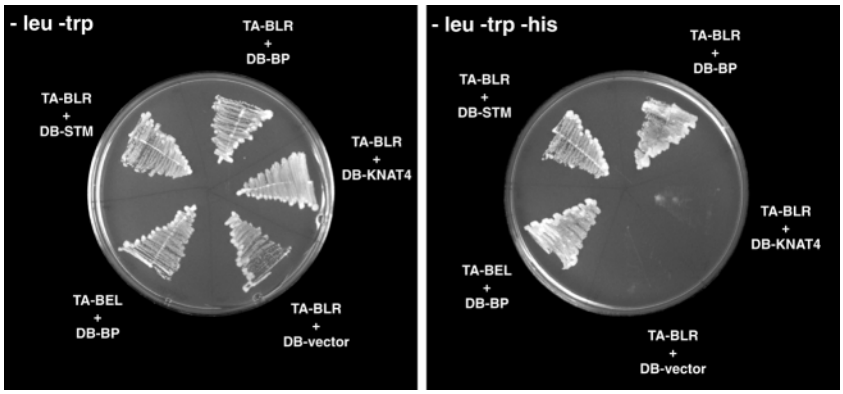

Fig. 7. BLR interacts directly with class 1 KNOX proteins. Yeast two-hybrid assay demonstrating interaction between BLR and KNOX proteins inferred through selective growth on medium lacking leucine, tryptophan and histidine (–leu –trp –his) compared with medium lacking leucine and tryptophan (–leu, –trp). All yeast strains grow on –leu –trp medium. Growth on –leu, –trp, –his medium is detected for strains carrying TA-BLR and DB-BP or DB-STM but not for strains carrying TA-BLR and DB-KNAT4 or the DB vector. Growth of a strain carrying TA-BEL1 and DB-BP is shown as a positive control.

that loss of *bp* function cannot completely explain the *blr* phenotype.

Strong alleles of *stm* are formally epistatic to *blr*, although the *blr* phenotype is difficult to observe in strong *stm*. However, a weak allele of *stm* is greatly enhanced by *blr*. This effect is far stronger than that of *bp*, which also enhances weak *stm-2* (Byrne et al., 2002). Thus it is possible that *BLR* is required for *STM* function, consistent with its expression pattern in the embryo. However, *BLR* must also have an *STM*-independent role, revealed by the strong phenotype of *blr as1 stm* triple mutants. A likely explanation is that *BLR* is required for both *BP* and *STM* function. Consistent with this possibility, yeast two-hybrid assay demonstrates the BLR protein interacts directly with both STM and BP. The requirement for *BLR* must be only partial, as *blr* has a much milder phenotype than either *bp* or *stm*, and, as noted above, *BP* is still functional in a *blr* mutant. One explanation is that *BLR* is itself partially redundant. A strong candidate would be the BELL class gene (At2g27990) most closely related to *BLR*. Conservation between these two genes is 90% within the homeodomain and 48% overall.

BLR is more distantly related to the BELL class gene *BEL1*. The *blr bel1* double mutant phenotype indicates a lack of functional overlap between these two genes. The phenotype of *bel1* mutants is restricted to the ovule where the morphology of the outer integument is abnormal while the inner integument is completely absent (Modrusan et al., 1994; Robinson-Beers et al., 1992). Consistent with this phenotype *BEL1* expression in the ovule is restricted to the region where integuments initiate (Reiser et al., 1995). However, *BEL1* is also expressed in vegetative tissues and roots. The lack of other plant phenotypes suggests *BEL1* shares genetic redundancy. Similarly, BEL1 interacts physically with BP, yet the *bel1* phenotype indicates that it is not required for BP function in the inflorescence. We have demonstrated that *BEL1* is not required for SAM function in *as1 stm*, indicating it has no effect on BP activity in the embryonic or vegetative SAM. Again the apparent lack of *BEL1* genetic interactions with *BP* is possibly the result of redundancy. Candidates for such redundancy are two genes, *BLH2* and *BLH4*, most closely related to *BEL1* (Becker et al., 2002).

Mutations in *BLR* result in phyllotaxy defects including both an increase in the number of lateral organs and displacement of organs along the stem. Although mechanisms governing phyllotactic patterning are still to be elucidated, early surgical experiments have shown that leaf primordia are positioned in response to preexisting primordia (Snow and Snow, 1931). This influence may be mediated by production of a diffusible inhibitor or may be biophysical in nature. A model where biophysical forces regulate phyllotaxy is supported by studies demonstrating induction of leaf formation by local concentration of the cell wall protein expansin (Fleming et al., 1997; Pien et al., 2001; Reinhardt et al., 1998). However, organ positioning can also be influenced by auxin. Chemical inhibition of polar auxin transport and mutations affecting auxin transport, including *pinformed* and *pinoid* in *Arabidopsis*, result in failure to initiate lateral organ outgrowth (Benjamins et al., 2001; Christensen et al., 2000; Vernoux et al., 2000). Primordial outgrowth in such cases can be induced at sites of localized auxin concentration increase (Reinhardt et al., 2000; Vernoux et al., 2000). Organs can be induced at any position around the circumference of the meristem, and also within a restricted region along the apical-basal axis of the SAM.

The phenotypic defects in *blr*, including aberrant organ initiation patterns may in part due to disruption in auxin signalling. Consistent with this proposal, *blr* mutants display reduced stature and loss of apical dominance typical of reduced auxin signalling (Lincoln et al., 1990). Interestingly, phyllotaxy defects are also observed in plants expressing a constitutively active form of a RHO GTPase that may be involved in mediating plant responses to hormones such as auxin (Li et al., 2001). The inflorescence phenotype in this case resembles that of *bp* mutants.

The increase in organ number and organ displacement indicates *blr* mutants are no longer fully responsive to inhibitory signals from preexisting organs such that distances between organs are not maintained. Aberrant initiation along the apical-basal axis of the SAM potentially contributes to variable internode lengths. Alternatively, regular partitioning of cells between organs and internodes is affected. In this respect *blr* resembles *te1* in maize, which has also been interpreted as a phyllotaxy mutant (Veit et al., 1998).

Despite the phyllotactic defects in *blr* there is no appreciable difference in the size of the SAM compared with wild type. This may be coincident with more peripheral zone cells being specified as organ founder cells. Alternatively, in *blr* mutants an increase in recruitment of cells into lateral organs is offset by an increase in the number of stem cells, together maintaining SAM size. In this case *BLR* normally delays

differentiation of stem cells in the SAM and slows their propagation. In each case, the function of *BLR* in delaying specification of lateral organs is consistent with *BLR* expression in peripheral cells of the inflorescence meristem, but not in initiating primordia.

Stem cell lineages expand according to the Fibonacci series when daughter cells are delayed from acquiring stem cell fate, raising the possibility that stem cells are responsible for phyllotactic patterns (Klar, 2002). In this respect, *BLR* fulfills a postulated stem cell function required for Fibonacci progression (Klar, 2002), in that *BLR* dictates how long daughter cells require to differentiate in the stem cell lineage. However, this model remains controversial and is yet to be substantiated (Fleming, 2002). For example, stem cell lineages are multicellular in higher plants, such that extensive co-ordination in the meristem would be required for stem cell lineages to regulate organ initiation in this way. The effects of the *blr* mutation on phyllotactic pattern are intriguing in this context and will be examined further.

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REFERENCES

Barton, M. K. and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana* – an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* **119**, 823-831.

Becker, A., Bey, M., Bürglin, T. R., Saedler, H. and Theissen, G. (2002). Ancestry and diversity of *BEL1*-like homeobox genes revealed by gymnosperm (*Gnetum gnemon*) homologs. *Dev. Genet. Evol.* **212**, 452-457.

Bellaoui, M., Pidkowich, M. S., Samach, A., Kushalappa, K., Kohalmi, S. E., Modrusan, Z., Crosby, W. L. and Haughn, G. W. (2001). The Arabidopsis BELL1 and KNOX TALE homeodomain proteins interact through a domain conserved between plants and animals. *Plant Cell* **13**, 2455-2470.

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P. and Offringa, R. (2001). The PINOID protein kinase regulates organ development in *Arabidopsis* by enhancing polar auxin transport. *Development* **128**, 4057-4067.

Bharathan, G., Janssen, B. J., Kellogg, E. A. and Sinha, N. (1999). Phylogenetic relationships and evolution of the KNOTTED class of plant homeodomain proteins. *Mol. Biol. Evol.* **16**, 553-563.

Brand, U., Hobe, M. and Simon, R. (2001). Functional domains in plant shoot mersitems. *BioEssays* **23**, 134-141.

Bürglin, T. R. (1997). Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res.* **25**, 4173-4180.

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. *Development* **129**, 1957-1965.

Christensen, S. K., Dagenais, N., Chory, J. and Weigel, D. (2000). Regulation of auxin response by the protein kinase PINOID. *Cell* **100**, 469-478.

Clark, S. E. (2001). Meristems: start your signaling. *Curr. Opin. Plant Biol.* **4**, 28-32.

Clark, S. E., Jacobsen, S. E., Levin, J. Z. and Meyerowitz, E. M. (1996). The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis*. *Development* **122**, 1567-1575.

Clarke, J. H., Tack, D., Findlay, K., van Montagu, M. and van Lijsebettens, M. (1999). The *SERRATE* locus controls the formation of the early juvenile leaves and phase length in *Arabidopsis*. *Plant J.* **20**, 493-501.

Dockx, J., Quaedvlieg, N., Keultjes, G., Kock, P., Weisbeek, P. and Smeekens, S. (1995). The homeobox gene *ATK1* of *Arabidopsis thaliana* is expressed in the shoot apex of the seedling and in flowers and inflorescence stems of mature plants. *Plant Mol. Biol.* **28**, 723-737.

Douglas, S. J., Chuck, G., Dengler, R. E., Pelecanda, L. and Riggs, C. D. (2002). *KNAT1* and *ERECTA* regulate inflorescence architecture in Arabidopsis. *Plant Cell* **14**, 547-558.

Endrizzi, K., Moussian, B., Haecker, A., Levin, J. Z. and Laux, T. (1996). The *SHOOT MERISTEMLESS* gene is required for maintenance of undifferentiated cells in *Arabidopsis* shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE*. *Plant J.* **10**, 101-113.

Fleming, A. J. (2002). Plant mathematics and Fibonacci's flowers. *Nature* **418,** 723.

Fleming, A. J., McQueen-Mason, S., Mandel, T. and Kuhlemeier, C. (1997). Induction of leaf primordia by the cell wall protein expansin. *Science* **276**, 1415-1418.

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

Green, P. B. (1999). Expression of pattern in plants: combining molecular and calculus-based biophysical paradigms. *Am. J. Bot.* **86**, 1059-1076.

Gu, Q., Ferrándiz, C., Yanofsky, M. F. and Martienssen, R. (1998). The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125**, 1509-1517.

Iwakawa, H., Ueno, Y., Semiarti, E., Onouchi, H., Kojima, S., Tsukaya, H., Hasebe, M., Soma, T., Ikezaki, M., Machida, C. et al. (2002). The *ASYMMETRIC LEAVES2* gene of *Arabidopsis thaliana*, required for formation of a symmetric flat leaf lamina, encodes a member of a novel family of proteins characterized by cysteine repeats and a leucine zipper. *Plant Cell Physiol.* **43**, 467-478.

Jackson, D. and Hake, S. (1999). Control of phyllotaxy in maize by the *abphyl1* gene. *Development* **126**, 315-323.

Jackson, D., Veit, B. and Hake, S. (1994). Expression of maize *KNOTTED1* related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* **120**, 405-413.

Kaya, H., Shibahara, K., Taoka, K., Iwabuchi, M., Stillman, B. and Araki, T. (2001). *FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. *Cell* **104**, 131-142.

Kerstetter, R., Vollbrecht, E., Lowe, B., Veit, B., Yamaguchi, J. and Hake, S. (1994). Sequence analysis and expression patterns divide the maize *knotted1*-like homeobox genes into two classes. *Plant Cell* **6**, 1877-1887.

Kidner, C. A., Timmermans, M. C. P., Byrne, M. E. and Martienssen, R. A. (2002). Developmental genetics of the angiosperm leaf. In *Advances in Botanical Research: Volume 38* (ed. J. A. Callow), pp. 191-234. London: Academic Press.

Klar, A. J. S. (2002). Fibonacci's flowers. *Nature* **417**, 595.

Kohalmi, S. E., Nowak, J. and Crosby, W. L. (1997). A practical guide to using the yeast 2-hybrid system. In *Differentially Expressed Genes in Plants: A Bench Manual* (ed. E. Hansen and G. Harper), pp. 63-82. London: Taylor and Francis.

Leyser, H. M. O. and Furner, I. J. (1992). Characterisation of three shoot apical meristem mutants of *Arabidopsis thaliana*. *Development* **116**, 397-403.

Li, H., Shen, J.-J., Zheng, Z.-L., Lin, Y. and Yang, Z. (2001). The Rop GTPase switch controls multiple developmental processes in Arabidopsis. *Plant Physiol.* **126**, 670-684.

Lincoln, C., Britton, J. H. and Estelle, M. (1990). Growth and development of the *axr1* mutants of *Arabidopsis*. *Plant Cell* **2**, 1071-1080.

Lincoln, C., Long, J., Yamaguchi, J., Serikawa, K. and Hake, S. (1994). A *knotted1*-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. *Plant Cell* **6**, 1859-1876.

Liu, Y. G., Mitsukawa, N., Oosumi, T. and Whittier, R. F. (1995). Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J.* **8**, 457-463.

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.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M. K. (1999). The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. *Development* **126**, 469-481.

Mann, R. S. and Chan, S.-K. (1996). Extra specificity from *extradenticle*: the partnership between HOX and PBX/EXD homeodomain proteins. *Trends Genet.* **12**, 258-262.

Modrusan, Z., Reiser, L., Feldmann, K. A., Fischer, R. L. and Haughn, G. W. (1994). Homeotic transformation of ovules into carpel-like structures in Arabidopsis. *Plant Cell* **6**, 333-349.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G. and Laux, T. (1998). Role of the *ZWILLE* gene in the regulation of central shoot meristem cell fate during *Arabidopsis* embryogenesis. *EMBO J.* **17**, 1799-1809.

Müller, J., Wang, Y., Franzen, R., Santi, L., Salamini, F. and Rohde, W. (2001). *In vitro* interactions between barley TALE homeodomain proteins suggest a role for protein-protein associations in the regulation of *Knox* gene function. *Plant J.* **27**, 13-23.

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.

Pautot, V., Dockx, J., Hamanta, O., Kronenbergera, J., Grandjeanc, O., Jublota, D. and Traas, J. (2001). *KNAT2*: Evidence for a link between Knotted-like genes and carpel development. *Plant Cell* **13**, 1719-1734.

Pien, S., Wyrzykowska, J., McQueen-Mason, S., Smart, C. and Fleming, A. (2001). Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proc. Natl. Acad. Sci. USA* **98**, 11812-11817.

Prigge, M. J. and Wagner, D. R. (2001). The Arabidopsis *SERRATE* gene encodes a zinc-finger protein required for normal shoot development. *Plant Cell* **13**, 1263-1279.

Reinhardt, D., Mandel, T. and Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* **12**, 507-518.

Reinhardt, D., Witter, F., Mandel, T. and Kuhlemeier, C. (1998). Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. *Plant Cell* **10**, 1427-1437.

Reiser, L., Modrusan, Z., Margossian, L., Samach, A., Ohad, N., Haughn, G. W. and Fischer, R. L. (1995). The *BELL1* gene encodes a homeodomain protein involved in pattern formation in the *Arabidopsis* ovule primordium. *Cell* **83**, 735-742.

Reiser, L., Sanchez-Baracaldo, P. and Hake, S. (2000). Knots in the family tree: evolutionary relationships and functions of knox homeobox genes. *Plant Mol. Biol.* **42**, 151-166.

Richards, F. J. (1951). Phyllotaxis: its quantitative expression and relation to growth in the apex. *Philos. Trans. R. Soc. Lond.* **235**, 509-564.

Robinson-Beers, K., Pruitt, R. E. and Gasser, C. S. (1992). Ovule development in wild-type Arabidopsis and two female-sterile mutants. *Plant Cell* **4**, 1237-1249.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Press.

Schiestl, R. H., Manivasakam, P., Woods, R. A. and Gietz, R. D. (1993). Introducing DNA into yeast by transformation. *Methods: A companion to methods in enzymology* **5,** 79-85.

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., Boschke, I. and Hake, S. (2002). Selective interaction of plant homeodomain proteins mediates high DNA-binding affinity. *Proc. Natl. Acad. Sci. USA* **99**, 9579-9584.

Snow, M. and Snow, R. (1931). Experiments on phyllotaxis I. The effect of isolating a primordium. *Philos. Trans. R. Soc. Lond.* **B221**, 1-43.

Springer, P. S., McCombie, W. R., Sundaresan, V. and Martienssen, R. A. (1995). Gene trap tagging of *PROLIFERA*, an essential *MCM2-3-5*-like gene in *Arabidopsis*. *Science* **268**, 877-880.

Steeves, T. A. and Sussex, I. M. (1989). *Patterns in Plant Development.* Cambridge: Cambridge University Press.

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.

Takahashi, T., Matsuhara, S., Abe, M. and Komeda, Y. (2002). Disruption of a DNA topoisomerase I gene affects morphogenesis in Arabidopsis. *Plant Cell* **14**, 2085-2093.

The Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**, 796-815.

Timmermans, M. C., Hudson, A., Becraft, P. W. and Nelson, T. (1999). ROUGH SHEATH2: a Myb protein that represses knox homeobox genes in maize lateral organ primordia. *Science* **284**, 151-153.

Tsiantis, M., Schneeberger, R., Golz, J. F., Freeling, M. and Langdale, J. A. (1999). The maize *rough sheath2* gene and leaf development programs in monocot and dicot plants. *Science* **284**, 154-156.

Veit, B., Briggs, S. P., Schmidt, R. J., Yanofsky, M. F. and Hake, S. (1998). Regulation of leaf initiation by the *terminal ear 1* gene of maize. *Nature* **393**, 166-168.

Venglat, P., Dumonceaux, T., Parnell, L., Rozwadowski, K., Babic, V., Keller, W., Martienssen, R. A., Selvaraj, G. and Datla, R. (2002). The *Arabidopsis BREVIPEDICELLUS* gene is an important regulator of pedicel and internode development. *Proc. Natl. Acad. Sci. USA* **99**, 4730-4735.

Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P. and Traas, J. (2000). *PIN-FORMED 1* regulates cell fate at the periphery of the shoot apical meristem. *Development* **127**, 5157-5165.

Vollbrecht, E., Reiser, L. and Hake, S. (2000). Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. *Development* **127**, 3161-3172.

Vollbrecht, E., Veit, B., Sinha, N. and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* **350**, 241-243.

Vongs, A., Kakutani, T., Martienssen, R. A. and Richards, E. J. (1993). *Arabidopsis thaliana* DNA methylation mutants. *Science* **260**, 1926-1928.