MGOUN3, an Arabidopsis gene with TetratricoPeptide-Repeat-related motifs, regulates meristem cellular organization

Soazig Guyomarc'h1, Teva Vernoux2, Jan Traas2, Dao-Xiu Zhou1 and Marianne Delarue1,*

1 Institut de Biotechnologie des Plantes, CNRS UMR 8618, Bât. 630, Université Paris-Sud, F-91405 Orsay, France
2 Laboratoire de Biologie Cellulaire, INRA, Route de St-Cyr, F-78026 Versailles cedex, France

Received 25 June 2003; Accepted 13 November 2003

Abstract

In order to understand the functioning of apical meristems in Arabidopsis more clearly, a new mutant, mgoun3 (mgo3), affected in the structural organization and the functional regulation of both shoot and root meristems has been isolated. mgo3 plants display perturbations in leaf morphogenesis, in the spatial and the temporal formation of primordia, and frequent fasciation of the inflorescence stem. Cellular analysis showed that both cellular organization and cell identity patterning are impaired in the mutant meristems. The MGO3 gene has been isolated by positional cloning. The protein deduced from the cDNA sequence contains TetratricoPeptide Repeats (TPR) and Leucine-Rich Repeats (LRR), two motifs that are thought to act in protein–protein interactions. This gene appears to be unique in the Arabidopsis genome. Although the MGO3 protein presents TPR as in the Arabidopsis proteins HOBBIT and SPINDLY, the MGO3 motifs are more similar to those present in LGN-related proteins, which are regulators for some of the asymmetric cell divisions in animal development. These features suggest a key role for MGO3 in meristematic cell divisions and would be of interest for the comparison between plant and animal development.

Key words: Arabidopsis, fasciation, meristem, MGOUN3, TPR.

Introduction

In higher plants, organogenesis is not limited to embryonic development but continues throughout the life of the plant. This ability lies in the activity of the meristems, which are organized pools of actively dividing and undifferentiated cells. After germination, the main apical–basal axis of the plant body is built by two apical meristems: the shoot apical meristem (SAM), and the root apical meristem (RAM). These meristems fulfil two main tasks: the self-perpetuation of the meristem with the proliferation of stem cells, and the production of new organs or tissues by differentiation of some of the newly produced cells.

The highly organized activity of SAM and RAM is based on a complex structure, where expression patterns of different regulatory genes are associated (for a review see Fletcher, 2002; Traas and Vernoux, 2002). In the case of the SAM, the meristem itself can be subdivided into three different zones. The central zone (CZ) is the apical part of the meristem, and is occupied by slowly dividing cells that never differentiate. This CZ lays on the rib zone (RZ) where cells divide and eventually differentiate to participate in the elaboration of the stem. The CZ is surrounded by the peripheral zone (PZ) where cells divide actively and provide the founder cells for new primordia which emerge in a regular geometric fashion (phyllotaxy) and with a specific period between the formation of two successive organs (plastochron). Primordia are committed to develop into leaves and axillary shoots during the vegetative phase and flowers and floral organs during the reproductive phase. One striking feature of all meristematic structures is the highly conserved cell division pattern leading to a
typical organization in distinct cell layers. In the SAM, the superficial cells follow anticlinal divisions, to a depth depending on the species. In Arabidopsis, this results in two layers, L1 and L2, in which cells have the same clonal origin. These layers cover the L3, where cell division orientation is more random (Steeves and Sussex, 1989). This structural regulation has a functional importance since the expression of some regulators are based on these clonal relations (Lu et al., 1996).

The organization of the RAM involves fewer cells. Its activity and maintenance start in a quiescent centre formed by slowly dividing cells. Their daughter cells follow a specific pattern of cell divisions and progressive differentiation. Above the RAM, daughter cells undergo expansion (elongation zone) and, later on, differentiation (maturation zone) to build a regular arrangement of cell files within the root body (Benfey and Scheres, 2000). Therefore, the basic organization of the SAM and that of the RAM are similar in terms of having a central region of slowly dividing cells (Dolan et al., 1993).

Meristems are stable structures in spite of the very dynamic nature of their constituent cells, which continuously divide, grow and differentiate while they transit from one zone to another. The co-ordination of the cellular behaviour in this stable system is thought to rely on an elaborate signalling network. To understand how this is achieved, genetic analysis has been used and a number of genes regulating meristematic functions can now be exploited. In Arabidopsis, both the analysis of mutant phenotypes and the expression studies of the corresponding genes have helped to improve models of meristems functioning (Fletcher, 2002; Traas and Vernoux, 2002). In the SAM, the expression of class 1 KNOX genes such as SHOOTMERISTEM LESS (STM), KNAT1, and KNAT2, is related to the meristic identity of the cells (Dockx et al., 1995; Endrizzi et al., 1996; Lincoln et al., 1994; Long et al., 1996). At defined places in the margins of the PZ, KNOX genes expression is switched off by primordia-specific genes (Ori et al., 2000), leading to the development of new primordia. WUSCHEL (WUS) is the most precociously expressed gene during embryonic SAM development (Mayer et al., 1998). Throughout the plant’s life, WUS is expressed in a group of cells underneath the CZ, called the ‘organizing centre’. A signal is emitted from this region, which confers stem cell fate to the overlying region by a regulatory loop between the CLAVATA (CLV) genes and WUS (Brand et al., 2000; Schoof et al., 2000). The transition to flowering involves a change in the identity of the primordia arising at the flanks of the SAM. This switch is dependent on the activity of floral meristem identity genes, such as LEAFY (LFY) which is expressed at high levels in emerging flower primordia (Schultz and Haughn, 1991). In floral meristems, LFY switches on the expression of floral organ identity genes, among which AGAMOUS (AG) acts to specify stamen and carpel development (Cohen and Meyerowitz, 1991; Parcy et al., 1998). AG represses WUS expression, which results in the termination of the floral meristem, with central gynoecium primordia (Lenhard et al., 2001; Lohmann et al., 2001).

The expression domain of regulatory genes is also responsible for cell fate patterning in the RAM. For example, the SHORT-ROOT and SCARECROW genes’ expression patterns determine the endodermis and cortical identities of particular cells among the youngest daughter cells in the RAM (Helariutta et al., 2000).

Mutations affecting both SAM and RAM are of a particular interest since they may represent lesions in genes required for the fundamental organization and/or functioning of both meristems. Among them, the Arabidopsis fasciata (fas) (Leyser and Furner, 1992), hobbit (hbt) (Blilou et al., 2002), and mgoun (mgo) (Laufs et al., 1998) mutants are characterized by alterations of root and shoot organogenesis, and correspond to dramatic modifications of meristem organization or functioning. The fas mutants display pleiotropic alterations in organogenesis and morphogenesis, including misregulated phyllotaxy, fasciation of the inflorescence stem, and slow root growth compared to the wild type (Leyser and Furner, 1992). The FAS1 and FAS2 genes encode subunits of the Chromatin Assembly Factor-1 (CAF-1), involved in the epigenetic control of the expression pattern of some regulatory genes in the SAM and RAM. The fas1 and fas2 mutations lead to the misregulation of WUS and SCR expression domains and the destructuration of SAM and RAM (Kaya et al., 2001). The HBT gene is required for appropriate co-ordination of embryonic and post-embryonic cell divisions in both the shoot and the root meristems, as well as for maintenance of the balance between cell division and cell differentiation in these two structures (Blilou et al., 2002). It encodes a CDC27/Nuc2 homologue, that could act as a component of the plant Anaphase Promoting Complex, controlling the cell cycle progression and possibly differentiation through targeted proteolysis.

The mgoun1 (mgo1) and mgoun2 (mgo2) mutants were isolated using the same criteria, i.e. fewer leaves than the wild type 10 d after germination (Laufs et al., 1998). Both of them show subtle alterations in leaf morphogenesis, perturbations in the spatial and the temporal regulation of aerial organogenesis, and frequent fasciation of the shoot meristem. The SAM of mgo mutants was shown to be structurally disorganized and significantly larger than in the wild type. The MGO1 and MGO2 genes were proposed to be involved in a later step in cell fate determination, which is to enrol founder cells for organs to be produced (Laufs et al., 1998).

Despite these recent advances, the maintenance of the highly conserved cellular architecture inside meristems should involve a complex regulatory network and this is not well understood, as yet. Additional mutants need to be isolated in order to investigate the existence of
Regulation of meristem organization by MGO3

MGO3 sequence and expression analysis
Total RNA from mixed leaves and inflorescences from the WS ecotype were extracted with the Plant RNAeasy extraction kit (Qiagen USA, Valencia, CA), following Qiagen’s instructions. To eliminate the residual genomic DNA, the RNA was treated by an RNAase-free DNase I (Qiagen USA). Total RNA were quantified with a spectrophotometer, and their integrity was checked on an electrophoresis gel. Reverse-transcription was performed on 1 μg of total RNA, with M-MLV reverse-transcriptase (Promega corporation, Madison, Wisconsin, USA). The sequence of a partial MGO3 cDNA of 3.91 kbp was obtained by specific amplification of overlapping fragments using the Pfu DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), sequencing of this (Big Dye terminator kit (ABI-Prism), and construction of the contig. This sequence was deposited to the EMBL database (http://www.ebi.ac.uk/emb/) under the accession number AJ579629. It differs from the predicted cDNA presented by the TIGR, the MAiDB, and the Genbank databases (http://www.tigr.org, http://mips.gsf.de/genre/html, http://www.ncbi.nlm.nih.gov, respectively), but was in accordance with the WS genomic sequence that had also been obtained (see below).

The start codon was checked by the analysis of the genomic area by NetGene2 (http://www.ncbi.nlm.nih.gov/NetGene2/) and Genemark. hmm (http://opal.biology.gatech.edu/Genemark/) softwares. An Expressed Sequenced Tag (EST) from an Arabidopsis thaliana, ecotype Columbia, EST bank (Genbank accession number AV566787) overlapped the end of the sequenced cDNA, and extended to the end of the open reading frame and a 3’-untranslated region. The contig between the AJ579629 and the AV566787 sequences allowed the full length cDNA for the MGO3 gene to be built.

Genomic DNA was extracted from WS inflorescence apices using a classical CTAB protocol. The WS genomic sequence of the MGO3 gene was obtained by specific amplifications and sequencing of this material. This genomic sequence was compared with the cDNA sequence to deduce the MGO3 gene structure. The MGO3 protein was deduced from the full-length cDNA sequence. Prediction of amino acid repeats or functional protein patterns were made using the SMART (http://smart.embl-heidelberg.de), PSORT (http://psort.ibn.nibb.ac.jp), and SAPS (http://www.ch.embnet.org) softwares.

For northern blot analysis, total RNA was extracted from different organs of wild-type plants (WS ecotype) and from mixed leaves and inflorescences from WS, mgo3-1, mgo3-2, mgo3-3, and mgo3-4 with the Trizol protocol (Life Technologies, Invitrogen Corporation, Carlsbad, CA, USA), following Life Technology’s instructions.

After quantification by spectrophotometry, 15 μg of total RNA were used for northern blotting. The blot was hybridized with radiolabelled 32P probes produced using the 2.4 kb 5’-fragment of the cDNA as a template, and the ‘Prime a gene’ labelling kit according to the manufacturer’s instructions (Promega Corporation, Madison, Wisconsin, USA). Radioactivity intensity was analysed using a Phosphoimager (STORM840, Molecular Dynamics, Amersham Biosciences Europe GmbH, Freiburg, Germany).

Histological analysis and localization of GUS activity
Inflorescences were prefixed in 90% acetone at room temperature for 20 min, rinsed in staining buffer without substrate and infiltrated with staining solution (100 mM sodium phosphate buffer, pH 7, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) under vacuum for 15 min and incubated at 37 °C for 14 h. Samples were dehydrated, fixed in formalin:propionic acid:ethanol, 10:5:70 by vol., then embedded in Paraplast Plus (Sherwood Medical Corp., St Louis, USA). Eight μm sections were performed and observed under a Zeiss light microscope.

To study embryo development in the mgo3 mutant, siliques from 6 d after fertilization (daf) to 20 daf were split longitudinally, cleared

Materials and methods
Growth conditions and genetics
Plants were grown in chambers at 21 °C under long day conditions on soil or on sterile medium according to Estelle and Somerville (1987) with 1% sucrose. Wild-type Arabidopsis ecotypes (Landsberg erecta [Ler], Columbia 0 [Col-0], and Wassilewskija [WS]) were obtained from the Nottingham Arabidopsis Stock Centre (NASC: http://nasc.nott.ac.uk). The mgo3-1 allele was obtained by activation tagging mutagenesis in the Ler background, and it was kindly provided by John L Bowman (University of California Davis, Davis, USA). The mgo3-2 allele results from T-DNA insertion mutagenesis in the WS background (INRA, Versailles, France). The mgo3-3 allele was obtained by TAG1 insertion mutagenesis in the WS background and it was kindly provided by Masao Tatuka (Nara Institute of Science and Technology, Ikoma Nara, Japan). The mgo3-4 allele corresponds to the SALK_034207 insertion line generated by the Salk Institute Genomic Laboratory (http://signal.salk.edu). It was obtained from the ABRC (http://www.arabidopsis.org/abrc) and sown first without any selection. One out of 12 plants displayed the Mgo3± phenotype. The insertion of the T-DNA in the candidate gene was checked by PCR, using primers on both the plant genomic DNA and on the T-DNA. Crosses of the mgo3-2 and mgo3-3 alleles with the SALK_034207 insertion line were performed to test the complementation of the Mgo3± phenotype in their F1 progeny.

For complementation tests and double mutants, fas2-1 (Ler background; obtained from the NASC) and mgo1-1 and mgo2 mutants (WS background) were used. The pat1-1:GUS, WUS::GUS and LFY::GUS lines were kindly provided, respectively, by Leslie Sieburth (Deyholos and Sieburth, 2000), Thomas Laux (Groû-Hardt et al., 2002), and Detlef Weigel (Blázquez et al., 1997). Analysis of the incidence of the mgo3 background on the expression pattern of these constructs was performed by crossing these GUS lines with mgo3-2 or wild-type plants, and by selecting homozygous mgo3 or wild-type individuals with the GUS transgene in the F2 progeny.

Mapping of the mgo3 mutation
The F2 progeny from the cross between Col-0 (MGO3/MGO3) and Ler (mgo3-1/mgo3-1), and the cross between Ler (MGO3/MGO3) and WS (mgo3-3/mgo3-3), were used to study genetic linkage between the mgo3 mutations and the genotype of Simple Sequence Length Polymorphism (SSLP) markers distributed on the Arabidopsis genome. New SSLP markers were defined using the CEREON database (http://www.arabidopsis.org/cereon) to narrow down the mapping in the Col-0×Ler F2 population (Jander et al., 2002). Annotations of genes on chromosome 3 presented by the TIGR, the MAiDB, and the Genbank databases (http://www.tigr.org, http://mips.gsf.de/proj/thal/db/, http://www.ncbi.nlm.nih.gov, respectively), were used to define candidate genes.

Cross-talking points among the different pathways involved. The isolation of a new mutant affected in SAM and RAM organization and activity is described here. The mgo3 (mgo3) mutant shows defects in the regulation of cell division within the SAM and the RAM. The MGO3 gene has been isolated and shown to encode a protein related to LGN-type proteins, which contain a specific Leucine, Glycine, Asparagine-rich motif, and which are involved in the regulation of some asymmetric cell divisions in animal development. Taken together, as presented in this article, these features suggest a key role for MGO3 in meristem functioning.
by an overnight incubation in chloral hydrate:glycerol:water (8:2:1, by vol.), and visualized with Normarski optics.

Confocal microscopy and scanning electron microscopy

To analyse the cellular organization of living meristems, the fluorochrome FM1-43 (100 μg ml⁻¹ in water, directly injected in the medium) was used to stain root tip and inflorescence apices of living plantlets growing in Petri dishes with cover slips. To count cells in SAM, 6-d-old seedlings were fixed in 10:5:70 by vol. formalin:pro-pionic acid:ethanol, rehydrated, treated with RNase A and then stained with propidium iodide (5 μg ml⁻¹ in arginine 0.1 M pH 12.4, for 48 h), and washed three times with arginine 0.1 M pH 8. These stained materials were observed using a Leica TCS SP2 confocal microscope (Leica, Heidelberg, Germany). Images were analysed with Optimas 5.2 (Optimas Corporation, Bothell, WA). For scanning electron microscopy, the shoot apex of 6-d-old seedlings grown in vitro were observed with an Hitachi S-3000N scanning electron microscope (Hitachi Ltd, Tokyo, Japan).

Results

mgo3 mutation affects shoot and root development

A first mutant was isolated from a screen for morphogenesis mutants among T-DNA insertion Arabidopsis lines. It displayed a mgo3-like phenotype as described by Laufs et al. (1998). Two additional lines with a similar phenotype were kindly provided by both Masao Tasaka (Nara Institute of Science and Technology, Ikoma Nara, Japan) and John L Bowman (University of California Davis, Davis, USA). After outcrossing of the mutants with the wild type, complementation analyses showed that the three mutants represented alleles at a single locus, distinct from MGO1 and MGO2. They were named mgo3-1 to mgo3-3 (mgo3-1 to mgo3-3). All segregation analysis were consistent with a monogenic and recessive mutation conferring the Mgo3- phenotype.

In the in vitro conditions, the wild-type seeds germinated 2 d after imbibition, and the first two opposite leaves emerged 5 d later. Six to eight leaves developed before bolting. In mgo3 mutants, the first leaves emerged, on average, 2 d later than in the wild type, although no delay in germination was observed (Fig. 1). Moreover, observation of mgo3 embryos from globular to late torpedo stages did not reveal any divergence from the wild type (data not shown). The first leaves primordia, initiated by the mgo3 vegetative meristem, were usually not paired and, as development proceeded, the deregulation of the phyllotaxy and the plastochron was more and more noticeable (Fig. 2A–D). Leaf morphogenesis was also affected, since mgo3 leaves were lancedolated or misshaped. Examination by scanning electron microscopy of the leaf lower epidermis of mgo3 leaves showed that, despite occasional irregular cell arrangement, cell size and cell number were not affected in the mgo3 background, both as regards pavement cells and stomates. Counting of the cells confirmed that the cell density was similar in both genotypes: 492±24 cells mm⁻² in the WT compared with 519±29 cells mm⁻² in mgo3-2 (mean±standard error; n=20).

The transition to flowering was often delayed in the mgo3 background, and was independent of the number of rosette leaves. Eighteen days after germination (dag), this led to a large range of rosette phenotypes, from three or four small and twisted leaves to bushy and disorganized rosettes of more than 20 large leaves (Fig. 1).

The inflorescence stem built by the mgo3 plants often displayed fasciation and occasional premature termination (Fig. 2E). Fasciation could affect both primary and secondary stems. The fasciated stem extended in one plane, and the phyllotaxy of floral primordia was irregular. Multiple insertions of floral pedicels and occasional fused pedicels or flowers were noticed, revealing a misfunctioning of mgo3 floral initiation. Single flowers were also affected in their organogenesis, with variable numbers of sepals, petals, and carpels (Table 1). Moreover, the gynoecium was frequently distorted, and the septum, replum, and stigma were incompletely formed. Most affected flowers exhibited carpelloid sepals, i.e. fused sepals with aborted ovules at their margins and frequently stigmatic papillae at their top (Fig. 3A). Since the AG gene is involved in stamen and carpel identity (Bowman et al., 1989), the expression pattern of a pAG1::GUS construct was studied in these modified flowers. Detection of a GUS signal in these structures confirmed the carpel identity of the sepals in these mgo3 flowers (Fig. 3B).

Roots were also affected by the mgo3 mutation, since the primary root of the mutant grew significantly more slowly than the wild type one. Indeed, at 11 dag, the primary root measured 1.44±0.10 cm in the mgo3-2 mutant (n=24), compared with 2.43±0.11 cm in the wild type (n=31; mean±standard error). This difference was mainly due to a reduction in the size of the elongation zone as shown in Fig. 2F. Measuring of the cell length in this
zone indicated that the cell size was similar for both genotypes: 11.02±2.63 mm for WS compared with 10.80±2.40 mm for mgo3-2 (n=25). The growth of the secondary roots was affected to the same extent (data not shown).

**The organization of both the RAM and SAM is altered in mgo3 mutants**

Histological sections in the apex of mgo3-2 inflorescences revealed dramatic disorganization of their SAM (Fig. 4A, B). While the densely stained meristematic cells were clearly organized in layers in the wild-type apex, the apical cells were larger, more vacuolated, and less strictly organized in the mgo3 mutant. The different cell layers were no longer visible as in the wild-type apex. The entire meristem was significantly larger in the mutant: 105±11 µm for mgo3-2 (n=10), versus 61±5 µm for WS (n=9).

This enlargement could be observed at various stages. Using confocal microscopy, the counting of cell numbers in SAM showed that it was the same in both genotypes (59±3.5 cells in WT versus 59±6.0 cells in mgo3-2; n=20). Examination of the cell areas in the L1 of the SAM indicated that this overgrowth was mainly due to an enlargement of the meristematic cell areas in the mutant compared with the wild-type (Fig. 5). The same irregular cell shapes were observed in the RAM of all mgo3 alleles, leading to enlarged meristematic cells and a disorganization of cell files in the root tissues (Fig. 4C, D).

**The expression patterns of meristematic genes are modified in mgo3 mutants**

In order to prospect functional organization of mgo3 shoot meristems, the expression pattern of meristematic genes were studied in these mutants. The strict organization of

---

**Fig. 2.** Shoot organogenesis and phyllotaxy are affected in mgo3 mutants. (A) A wild-type (WS) Arabidopsis seedling with two cotyledons and two opposite leaves 10 d after germination. (B) A typical mgo3-2 seedling has just produced one short and one lanceolate leaf at the same time after germination. (C) Visualization of the shoot apex of a 6-d-old wild-type seedling by scanning electron microscopy. The two first leaf pairs are already visible between the petioles of the cotyledons. Bar: 150 µm. (D) At the same date, an extremely short leaf has been formed by a mgo3-2 SAM, already with a differentiated trichome. Bar: 150 µm. (E) The mgo3 inflorescence stem often displays enlarging, fasciation (arrow), and dramatic perturbations of the phyllotaxy. (F) Wild-type (left) and mgo3 (right) roots (10 d). The first root hair (arrow) shows the size of the elongation zone. Bar: 100 µm.
wild-type meristems relies on a complex network of regulatory genes, that are expressed in precise areas. In correlation with the macroscopic alterations, analysis of the expression patterns of a WUS::GUS and a LFY::GUS construct in the mgo3 mutant revealed frequent fragmentation of the shoot apical meristem, leading to the multiplication of rosette centres during the vegetative phase, and the fasciation of the inflorescence stem after bolting (Fig. 6A–D). Expression of the LFY::GUS construct was occasionally observed in the inflorescence apex, revealing the transition of the inflorescence meristem itself to a floral, thus determinate, meristem (Fig. 6E, F). Misexpression of the pAG-I::GUS could also be observed in some mgo3 inflorescence apices that terminated prematurely (Fig. 3C–F), as well as in ectopic carpel features appearing in some modified flowers (see above).

**Fig. 3.** The mgo3 mutation alters the expression patterns of AG in the inflorescence. (A) Extremely distorted carpelloid flowers of a mgo3-2 mutant, with twisted carpels and serrated sepals. An ectopic and serrated sepal bears ovules (arrow) and stigmatic papillae (arrowhead). (B) Carpelloid flowers of a mgo3-2 mutant displays pAG-I::GUS expression in the ectopic ovules (arrow) and stigmatic papillae (arrowhead) on sepals. (C) A wild-type (WS) inflorescence apex expressing the pAG-I::GUS construct. The reporter gene is expressed in the central whorls of floral meristems (s: sepal, p: petal, st: stamen, c: carpel), but not in the inflorescence meristem itself (star). (D) The pAG-I::GUS construct is expressed in a flower meristem at the apex of the precociously aborted inflorescence of an mgo3-2 mutant (arrow). (E) Histological section of a WT apex expressing the pAG-I::GUS construct. Bar: 100 μm. (F) Histological section of the mgo3 apex shown in (B). Bar: 100 μm.

Fig. 4. The mgo3 mutation alters the regulation of cell arrangement in both SAM and RAM. (A) Cross-sections of an inflorescence apex of a WS plant, stained with toluidine blue. The L1 and L2 layers are clearly visible. Bar: 50 μm. (B) Cross-section of a mgo3-2 inflorescence apex, stained with toluidine blue, shows enlarged meristem and abnormal organization of cell layers. Bar: 50 μm. (C) A living wild-type RAM stained with fluorochrome FM1-43 and visualized by confocal microscopy presents a stereotyped pattern of cell divisions and differentiation around the quiescent centre (arrowhead). Bar: 25 μm. (D) A mgo3-2 RAM visualized with the same technique lacks this cell arrangement (arrows). Bar: 25 μm.

**Fig. 5.** Cell distribution in the L1 of SAM according to the cell area. Cell areas were determined in the L1 layer from SAM of WS and mgo3-2 plants using optical sections in confocal scanning microscopy. For each genotypes, two different samples were analysed (see Materials and methods).

**MGO3 positional cloning**

Cloning of the MGO3 gene was performed by a positional cloning strategy, since none of the three alleles was tagged by an insertion. To do so, a wild type Col-0 parent was crossed with a (mgo3-1/mgo3-1) Landsberg erecta plant. Homozygous mgo3 mutants were selected in the recombinant F₂ progeny, and their genotypes for Simple Sequence
Length Polymorphism (SSLP) molecular markers were studied in order to detect genetic linkages with the mgo3-1 mutation. A first genetic linkage was detected with molecular markers located at the top of chromosome 3. The mgo3-1 mutation was located at 2.5 cM from nga162 (n=120), and 11.2 cM from GAPAB (n=55). The mapping was progressively refined using SSLP markers designed for this purpose, using the genetic polymorphism between Landsberg erecta and Columbia ecotypes (CERON database, see Materials and methods). This led to the location of the mgo3-1 mutation in a region of 94 kb, containing 27 candidate genes. Several T-DNA insertion lines mutated in one of the candidate genes were available in different FST collections. One of these, from the SALK Institute Genomic Laboratory (http://signal.salk.edu/), displayed abnormalities reminiscent of the Mgo3− phenotype. This phenotype was not rescued by crossing the FST line with the mgo3-2 nor the mgo3-3 mutant, which meant that this line (SALK_534207) represented a fourth mgo3 allele named mgo3-4. In this FST line, the T-DNA is inserted in the 6th exon of the At3g18730 gene located on the MVE11 clone (AB026654) (Fig. 7A), as proven by amplification with primers located both on the gene and the T-DNA (data not shown). Sequencing of the WS gene, as well as the mgo3-2 allele, revealed that this mutant displayed a 2.6 kb deletion in the coding sequence, confirming that At3g18730 is the MGO3 gene (Fig. 7A).

The predicted MGO3 protein contains Leucine-rich repeats and TetratricoPeptide Repeats

The ATG codon for the At3g18730 gene was predicted to be at position 36879 on the MVE11 clone (AB026654) by Netgene2 (http://www.cbs.dtu.dk/services/NetGene2/) and Genemark.hmm (http://opal.biology.gatech.edu/GeneMark/) analysis of this genomic area. A partial MGO3 cDNA of 3.91 kb starting from this ATG codon was sequenced from RT-PCR in the WS ecotype (accession number AJ579629, see Materials and methods). This sequence could be joined to an overlapping Expressed Sequence Tag (EST) from an Arabidopsis thaliana, ecotype Columbia, EST bank (Genbank accession no. AV566787) containing the stop codon of the open reading frame. Therefore, the full-length cDNA is 3933 bp long and encodes a predicted protein of 1311 residues. The gene is about 7 kb long, and contains 18 introns (Fig. 7A). This protein does not present any significant similarity to any other plant protein. However, the MGO3 protein presents two types of protein motifs, each of them sharing similarities with two distinct classes of animal proteins. Its N-terminal region contains five TetratricoPeptide Repeats (TPR) (Fig. 7B). These TPR are a degenerate 34-amino acid region that can be repeated up to 16 times and are thought to be protein–protein interaction units (Das et al., 1998; Goebl and Yanagida, 1991). They have been identified in various biological kingdoms, however, they seem to be rare in plants. A few functional interactions mediated by TPR have been characterized in plants, for example, in protein translocation to peroxisomes (Gurvitz et al., 2001), in the inhibition of gibberellin sensing by the protein SPINDLY (Tseng et al., 2001), and in the control of cell cycle progression by HOBBIT (Biliou et al., 2002). However, while TPR are present in these plant proteins, the MGO3 TPR are not sufficiently similar to them to allow their detection by a BLAST search. The features present in MGO3 are more similar to the TPR characteristic of LGN proteins in animals. These LGN proteins belong to a family identified in mammals and insects that are characterized by highly conserved TPR, in which leucine (L), glycine (G), and asparagine (N) are particularly abundant, hence their name. These proteins take part in embryonic development via the control of cell polarity establishment and asymmetric cell divisions (Du et al., 2002). The 34 amino acids of the first MGO3 TPR share 64% similarity with a TPR of

Fig. 6. The mgo3 mutant displays fragmentation of the SAM. (A) The WUS::GUS construct is expressed in a single group of few central cells in the SAM of a 6-d-old wild-type seedling. Bar: 100 μm. (B) Two spots of WUS-expressing cells coexist in the SAM of an mgo3-2 seedling at the same age. Bar: 100 μm. (C) The LFY::GUS construct is responsible for the staining of only the youngest flower buds in a wild-type inflorescence apex (arrow head), while (D), an mgo3-2 inflorescence is organized from several and distinct apices at the tip of one inflorescence stem (arrows head). Bar: 100 μm. (E) Cross-section of a wild-type inflorescence apex with LFY::GUS expression in the youngest flower meristems, but not in the inflorescence meristem itself (star). Bar: 100 μm. (F) Cross-section of an mgo3-2 inflorescence apex showing LFY::GUS expression in the apical meristem (star), suggesting the premature determination of the SAM as a flower meristem. Bar: 100 μm.
the Mus LGN-related protein (71% over the 31 central amino acids, Fig. 7C), versus 60% between the 34 amino acids of the fifth MGO3 TPR and a TPR motif of an Arabidopsis kinesin-related protein (accession number NP192822). By contrast, the best scores obtained when comparing any one of the MGO3 TPRs to some of others plant proteins are between 50% and 53% similarity with SPINDLY or HOBBIT TPR units. The MGO3 protein also displays a C-terminal domain containing probably 4 Leucine-Rich Repeats (LRR) (Fig. 7B). This motif, widespread in animal and plant proteomes, is often involved in protein–protein interactions (Buchanan and Gay, 1996). It is often present, usually in numerous copies, in proteins taking part in the reception of extracellular signals, such as CLAVATA1 (CLV1) for example (Clark et al., 1997), or in the transduction of this signal in the cell. This region of MGO3 is most similar to LRR domains in mammalian ribonuclease inhibitors or mammalian anti-bodies, but two of the LRR units identified in MGO3 are 63% and 68% similar to CLV2 LRRs.

The MGO3 cDNA has also been sequenced in the mgo3-2 background. The results confirm an important deletion in
the cDNA leading to a truncated protein containing the first 187 residues with two TPRs.

**MGO3 is expressed in most of the wild-type organs**

*MGO3* expression was analysed in different wild-type organs by northern blot hybridizations with the first 2.4 kb of the cDNA as a probe (see Materials and methods). In all organs tested (root, young and mature rosette leaves, cauline leaves, inflorescence apex and flower buds, open flowers, green siliques and stems), an accumulation of mRNA was detected without significant differences in level (data not shown).

The same probe was used to detect the *MGO3* mRNA by northern blot hybridization in the four *mgo3* mutants background (Fig. 8). The result shows that the gene is transcribed in each mutant, and confirms the deletion in the *mgo3-2* mutant. The fact that the *MGO3* gene is transcribed in each mutant is not inconsistent with the hypothesis of null alleles, since the probe is large and overlaps the deletion area in the *mgo3-2* allele and the insertion area in *mgo3-4* allele. Therefore, it could hybridize with chimeric *MGO3* mRNA, encoding a non-functional protein.

**MGO3 interacts with other regulators of SAM activity**

The *mgo1 mgo3* double mutant was indistinguishable from both single mutants in the F2 progeny from the cross between two homozygous single mutants. If no embryo lethality occurs, this suggests that the two proteins MGO1 and MGO3 could be involved in the same molecular pathway. Conversely, about 1/16th of the F2 progeny of the *mgo2 mgo3* cross displayed an enhanced *mgo3*-like phenotype, with completely aborted SAM and RAM (Fig. 9). The fact that the double mutants are blocked in their early development could suggest that MGO2 and MGO3 are likely to act within two distinct pathways. In the F2 progeny from the *fas2 mgo3* cross, some individuals displayed an enhanced Mgo3-like phenotype in which the SAM is blocked after the formation of a single distorted leaf (data not shown) suggesting that FAS2 and MGO3 functions are also independent.

**Discussion**

*mgo3* mutation affects both structural and functional organization of SAM and RAM

The *mgo3* mutants were collected in the same phenotypical screen of T-DNA insertion mutants as *mgo1* and *mgo2*, following the criteria of displaying fewer leaves than the wild type 10 d after germination. Each of them presents perturbations in leaf morphogenesis, in the spatial and the temporal regulation of primordia formation, and frequent fascination of the inflorescence stem (Laufs et al., 1998). These modifications are likely to be specific to post-embryonic development, since no change in the development of *mgo3* embryos and no delay in germination have been noticed. Microscopy analysis of the mutant’s apex has revealed an abnormal enlargement of the SAM associated with a disrupted cellular and subcellular organization. These observations suggest that the *MGO3* gene is involved in the control of the pattern of cell differentiation and/or cell proliferation within different regions of the SAM. The morphological analyses on *mgo3* roots suggested that the RAM activity is affected in a similar fashion since its fails to orchestrate cell division and/or cell differentiation correctly. Although *MGO3* is expressed in all organs tested, molecular interactions with specific factors in meristematic cells could trigger this particular action of MGO3 on SAM and RAM functioning.

Modifications in the expression pattern of regulatory genes such as *WUS*, *LFY*, and *AG*, have also been observed. In particular, the occurrence of more than one spot of *WUS* expressing cells in the vegetative SAM, and of *LFY* expressing cells in the inflorescence, reveals that the enlarged SAM in *mgo3* mutants is constituted from a variable number of juxtapositioned meristems as described in *mgo1* and *mgo2* mutants (Laufs et al., 1998). Therefore, the fascination could be the result of the continuous fragmentation of the existing SAM, or the ectopic formation of new meristems, instead of the enlargement of one central meristem as observed in the *clavata* mutants (Clark et al., 1993, 1995).

In the SAM, numerous feed-back loops link one gene expression pattern to another. In particular, *WUS* and *AG* interact in the control of vegetative, inflorescence and/or floral meristem dynamics (Lenhard et al., 2001; Lohmann et al., 2001; Schoof et al., 2000). Thus, the modifications of *WUS* expressing domains can be associated with modifications in the expression patterns of other genes, such as *AG* in the *mgo3* SAM. Conversely, ectopic *AG* expression in the inflorescence apex can trigger *WUS* extinction and premature termination of the inflorescence meristem, as occasionally observed in *mgo3* apices.

As in *mgo3*, *mgo1* and *mgo2* SAM are structurally disorganized, and significantly larger than in the wild type. The enhanced phenotype displayed by the *mgo2 mgo3* double mutant suggests that both genes are involved in

[Fig. 8. Expression of *MGO3* in the *mgo3* mutants. Northern blot hybridization using the 5′ half of the *MGO3* cDNA as a probe, on transcripts from the wild-type (WS) and *mgo3* alleles. Low: ribosomal RNA visualization on the ethidium-bromide stained gel before blotting.]

Regulation of meristem organization by MGO3  681

Downloaded from https://academic.oup.com/jxb/article/55/397/673/594207 by guest on 16 February 2022
distinct regulatory processes. Conversely, the epistatic phenotype of the \textit{mgo1 mgo3} double mutant suggests that \textit{MGO1} and \textit{MGO3} act in the same genetic pathway. This model of genetic interaction is in accordance with the additive phenotype observed in the \textit{mgo1 mgo2} double mutant (Laufs \textit{et al}., 1998), involving \textit{MGO1} and \textit{MGO2} proteins in two parallel pathways controlling the same process. However, it is not known at this stage whether the different alleles are null alleles. Despite the fact that each of the four \textit{mgo3} mutations leads to the same phenotype, it cannot be ruled out that the \textit{MGO3} protein could still have partial activity, particularly in \textit{mgo3-1} background where the size and abundance of \textit{MGO3} RNA is unaffected.

\textbf{MGO3 represents a new type of regulatory gene of apical meristems in plants}

The \textit{MGO3} protein does not share any significant similarity with other plant proteins although it contains TPR units in the N-terminal region and LRR domains in its C-terminal end. Among various TPR motifs found in different species, the \textit{MGO3} TPRs are more closely related to those found in animal LGN-related proteins. These proteins have been characterized in Human and \textit{Drosophila}, where they are key regulators of some asymmetric cell divisions (Parmentier \textit{et al}., 2000). In the neuroblast of \textit{Drosophila}, the LGN-related PARTNER OF INSCUTEABLE (PINS) colocalizes with INSCUTEABLE (INS), a factor asymmetrically distributed in the cell (Parmentier \textit{et al}., 2000). This asymmetric distribution of PINS is necessary to observe a difference in size between the two daughter cells, but not to give two distinct cell fates. That means that the distribution of PINS influences the position of the new cell membranes rather than the asymmetric distribution of determination factors. Consistent with this, a mammalian PINS protein has been shown to bind the factor NuMA, a regulator of microtubule arrangement at the spindle, and to regulate the mitotic spindle organization and orientation, in interaction with INS (Du \textit{et al}., 2001). These data suggest a key role of these LGN-related proteins in a network that links the orientation of cell division to the asymmetric distribution of determination factors in the dividing cell, at least in some specific asymmetric divisions. The TPR domains are the most conserved region of these proteins, suggesting the functional importance of these motifs.

Could this be related to \textit{MGO3} function in the meristem? The sequence similarity between the \textit{MGO3} and the LGN proteins is restricted to the first \textit{MGO3} TPR, but is more significant than any other comparison of \textit{MGO3} TPR to those of other proteins. Taking into account the cellular disorganization of SAM and RAM in the \textit{mgo3} mutant, it is tempting to make the hypothesis that this could be linked to a functional similarity. According to this hypothesis, \textit{MGO3} could directly or indirectly determine cell wall orientation in the meristematic cells, in accordance with the asymmetric distribution of determination factors, such as WUS in the SAM. Doing so \textit{MGO3} would contribute to maintain the functional zonation of the meristems throughout successive cell divisions.

The presence of LRR motifs, usually involved in protein–protein interactions (Buchanan and Gay, 1996), is consistent with a role of \textit{MGO3} in transmitting or transducing information in meristematic cells. In the \textit{Arabidopsis} SAM, the CLAVATA1 membrane receptor displays such motifs that are required for the sensing of the CLAVATA3 peptide, and thus the transduction of a
MGO3 as a regulator of meristic activity?

The structural and functional disorganization observed in mgo3 meristems suggest defects in the maintenance of the balance between cell division and cell differentiation within the apical dome. First, cells in the SAM of the mgo3 mutant are larger and more vacuolated compared with the wild type, which suggests a premature loss of meristematic identity. Second, the large range of variations observed in the areas of the meristematic cells in the mgo3 background suggest that the co-ordination of cell proliferation and cell expansion is impaired in the mutant. This could explain the overgrowth of the SAM because of cell enlargement and the fragmentation of its functional unity due to the accidental loss of meristematic identity. Moreover, the differentiation of too many cells could affect the meristem maintenance and lead to occasional abortion. It is unlikely that MGO3 participates directly in the control of cell growth since cell size in the root elongation zone and in differentiated leaves were not affected by the mgo3 mutation. The only difference in cell size was observed for the meristematic cells and is likely to be due to defects in meristematic cell identity.

The hbt and fas mutants are other interesting mutants affected in the maintenance of the meristem structural and functional organization. The HBT protein is believed to play a role in cell cycle progression and the regulation of cell structural dynamics through targeted proteolysis (Blilou et al., 2002). The HBT protein presents 10 TPR, a protein motif also identified in the MGO3 protein. Thus, one hypothesis would be an involvement of MGO3 in cell cycle progression. In this case, the variation in cell size observed in the SAM of mgo3, could be due to an alteration of cell cycle synchronization. Moreover, a modification of the number of cells available for primordium initiation could explain the defects in leaf and flower morphogenesis observed in the mutants.

At last, large-scale expression regulators, such as chromatin modifiers, can also be necessary for the maintenance of meristem organization, as shown by the fas1 and fas2 mutations (Kaya et al., 2001). By affecting these kinds of epigenetic regulation, the mgo3 mutation could trigger the misexpression of some regulatory genes of apical meristems and lead indirectly to their structural disorganization.

The localization of the MGO3 protein in dividing cells, as well as the identification of some of its functional partners, would help to determine whether MGO3 is directly involved in the control of cell division or if it fulfills an upstream function for its regulation in meristems and developing primordia. If MGO3 is revealed to act in meristematic cell divisions, like LGN-related proteins in animal embryonic growth, this would be of great interest for the comparison between plant and animal development.

Acknowledgements

We are grateful to F Parcy for fruitful discussions and his providing Leslie Sieburth’s and Detlef Weigel’s seeds under their authorization. We thank S Domenichini, O Grandjean, Spencer Brown, and Christel Talbot for their technical help on microscopy, and I Gy for the sequencing. The microscopy done in the facility of the CNRS campus in Gif-sur-Yvette (France) was supported by the Institut Fédératif de Recherche 87 and the Conseil Général de l’Essonne (programme ASTRE). We thank also B Charrier and M Hodges for critical reading of the manuscript. We thank the Salk Institute Genomic Analysis Laboratory for providing the sequence-indexed Arabidopsis T-DNA insertion mutants. Funding for the SIGnAL indexed insertion mutant collection was provided by the ‘National Science Foundation’.

References

Clark SE, Running MP, Meyerowitz EM. 1995. CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development 121, 2057–2067.