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RESEARCH PAPER

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

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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

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Abbreviations: FST, flanking sequence tag; LGN, leucine, glycine, asparagine-rich protein; RAM, root apical meristem; SAM, shoot apical meristem; TPR, TetratricoPeptide Repeats.

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 meristematic 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

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 *mgoun3* (*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.

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* [L*er*], Columbia 0 [Col-0], and Wassilewskija [WS]) were obtained from the Nottingham Arabidopsis Stock Centre (NASC: http://nasc.nott.ac.uk). The *mgoun3-1* allele was obtained by activation tagging mutagenesis in the L*er* background, and it was kindly provided by John L Bowman (University of California Davis, Davis, USA). The *mgoun3-2* 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 Tasaka (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 F_1 progeny.

For complementation tests and double mutants, *fas2-1* (L*er* background; obtained from the NASC) and *mgo1-1* and *mgo2* mutants (WS background) were used. The p*AG*-I*::GUS*, *WUS::GUS* and *LFY::GUS* lines were kindly provided, respectively, by Leslie Sieburth (Deyholos and Sieburth, 2000), Thomas Laux (Groß-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 F_2 progeny.

Mapping of the mgo3 *mutation*

The F_2 progeny from the cross between Col-0 (*MGO3*/*MGO3*) and L*er* (*mgo3-1*/*mgo3-1*), and the cross between L*er* (*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×L*er* F_2 population (Jander *et al*., 2002). Annotations of genes on chromosome 3 presented by the TIGR, the MAtDB, 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.

MGO3 *sequence and expression analysis*

Total RNA from mixed leaves and inflorescences from the WS ecotype were extracted with the Plant RNeasy extraction kit (Qiagen USA, Valencia, CA), following Qiagen's instructions. To eliminate the residual genomic DNA, the RNA was treated by an RNAse-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/embl/) under the accession number AJ579629. It differs from the predicted cDNA presented by the TIGR, the MAtDB, and the Genbank databases (http://www.tigr.org, http://mips.gsf.de/proj/thal/db/, 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.cbs.dtu.dk/services/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.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 Technologies's instructions. After quantification by spectrophotometry, 15 µg of total RNA were used for northern blotting. The blot was hybridized with radiolabelled ^{32}P 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

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 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:propionic 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 *mgoun*-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 *mgoun3-1* to *mgoun3-3* (*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 lanceolated 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).

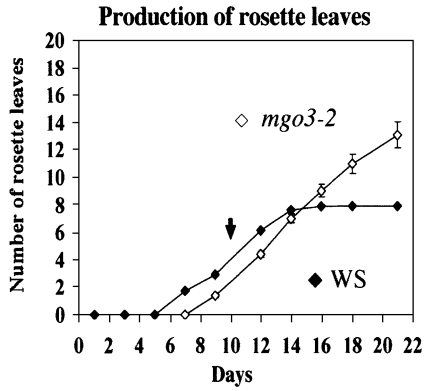

Fig. 1. Production of rosette leaves in the wild-type (WS) and *mgo3* mutant (*mgo3-2*) plants. For each genotype, 53 plants were analysed and the standard error is indicated. Note that if no delay has been observed for the radicle protrusion out of *mgo3-2* seeds, the *mgo3* seedlings present significantly fewer leaves than the wild type 10 d after germination (arrow).

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 uncompletely 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 p*AG*-I::*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

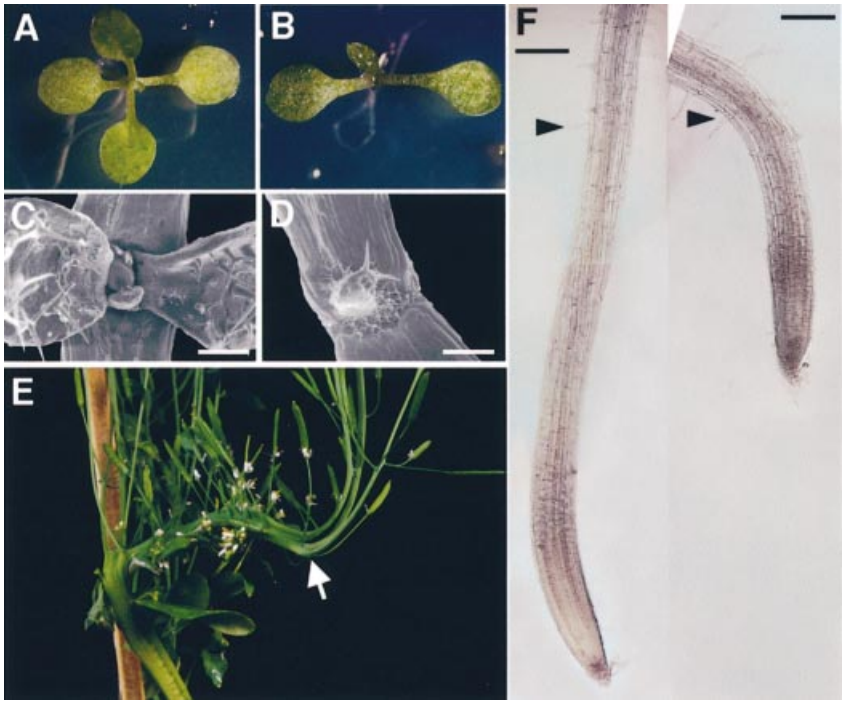

Fig. 2. Shoot organogenesis and phyllotaxy are affected in *mgo3* mutants. (A) A wild-type (WS) *Arabidopsis* seedling with two cotyledons and two opposite leaves 10 d after germination. (B) A typical *mgo3-2* seedling has just produced one short and one lanceolate leaf at the same time after germination. (C) Visualization of the shoot apex of a 6-d-old wild-type seedling by scanning electron micoscopy. 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.

Table 1. *Number of floral organs* 106 flowers were analysed for each genotype. Rare aborted flowers without carpels nor stamens have not been considered.

Genotype	Sepals		Petals		Stamens		Carpels	
	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
WS	4	4.0±0.0	4	4.0±0.0	4–6	5.1±0.1	2	2.0±0.0
mgo3-2	3–6	4.1±0.05	0–6	3.6±0.1	3–8	5.1±0.1	1–4	2.3±0.1

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

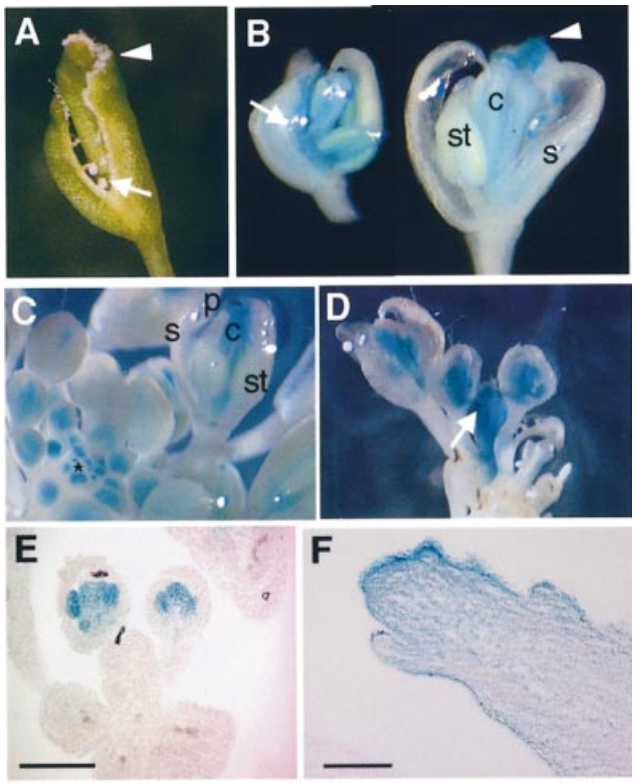

Fig. 3. The *mgo3* mutation alters the expression patterns of *AG* in the inflorescence. (A) Extremely distorted carpelloid flowers of a *mgo3-2* mutant, with twisted carpels and serrated sepals. An ectopic and serrated sepal bears ovules (arrow) and stigmatic papillae (arrowhead). (B) Carpelloid flowers of a *mgo3-2* mutant displays p*AG*-I::*GUS* expression in the ectopic ovules (arrow) and stigmatic papillae (arrowhead) on sepals. (C) A wild-type (WS) inflorescence apex expressing the p*AG*-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 p*AG*-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 p*AG*-I::*GUS* construct. Bar: 100 μm. (F) Histological section of the *mgo3* apex shown in (B). 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 p*AG*-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).

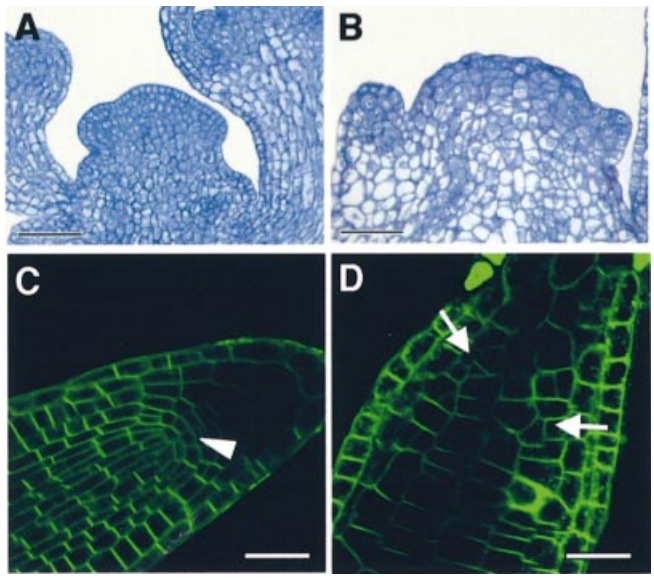

Fig. 4. The *mgo3* mutation alters the regulation of cell arrangement in both SAM and RAM. (A) Cross-sections of an inflorescence apex of a WS plant, stained with toluidine blue. The L1 and L2 layers are clearly visible. Bar: 50 μm. (B) Cross-section of a *mgo3-2* inflorescence apex, stained with toluidine 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.

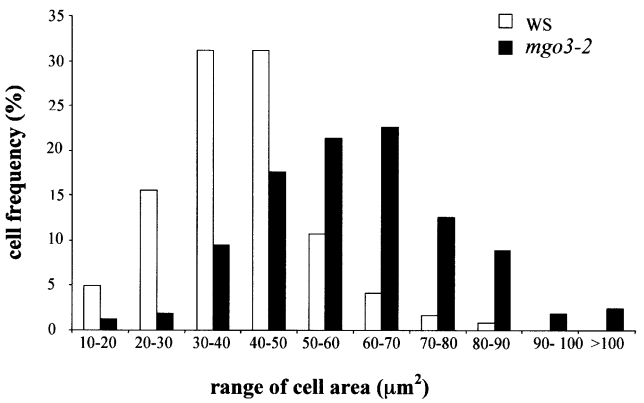

Fig. 5. Cell distribution in the L1 of SAM according to the cell area. Cell areas were determined in the L1 layer from SAM of WS and mgo3-2 plants using optical sections in confocal scanning microscopy. For each genotypes, two differents samples were analysed (see Materials and methods).

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 F_2 progeny, and their genotypes for Simple Sequence

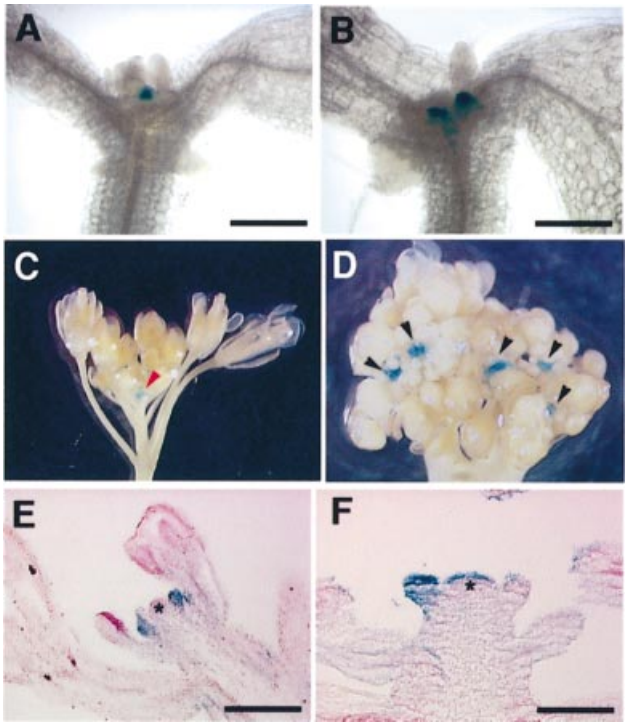

Fig. 6. The *mgo3* mutant displays fragmentation of the SAM. (A) The *WUS*::*GUS* construct is expressed in a single group of few central cells in the SAM of a 6-d-old wild-type seedling. Bar: 100 µm. (B) Two spots of *WUS*-expressing cells coexist in the SAM of an *mgo3-2* seedling at the same age. 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.

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 (CEREON 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 (Blilou *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

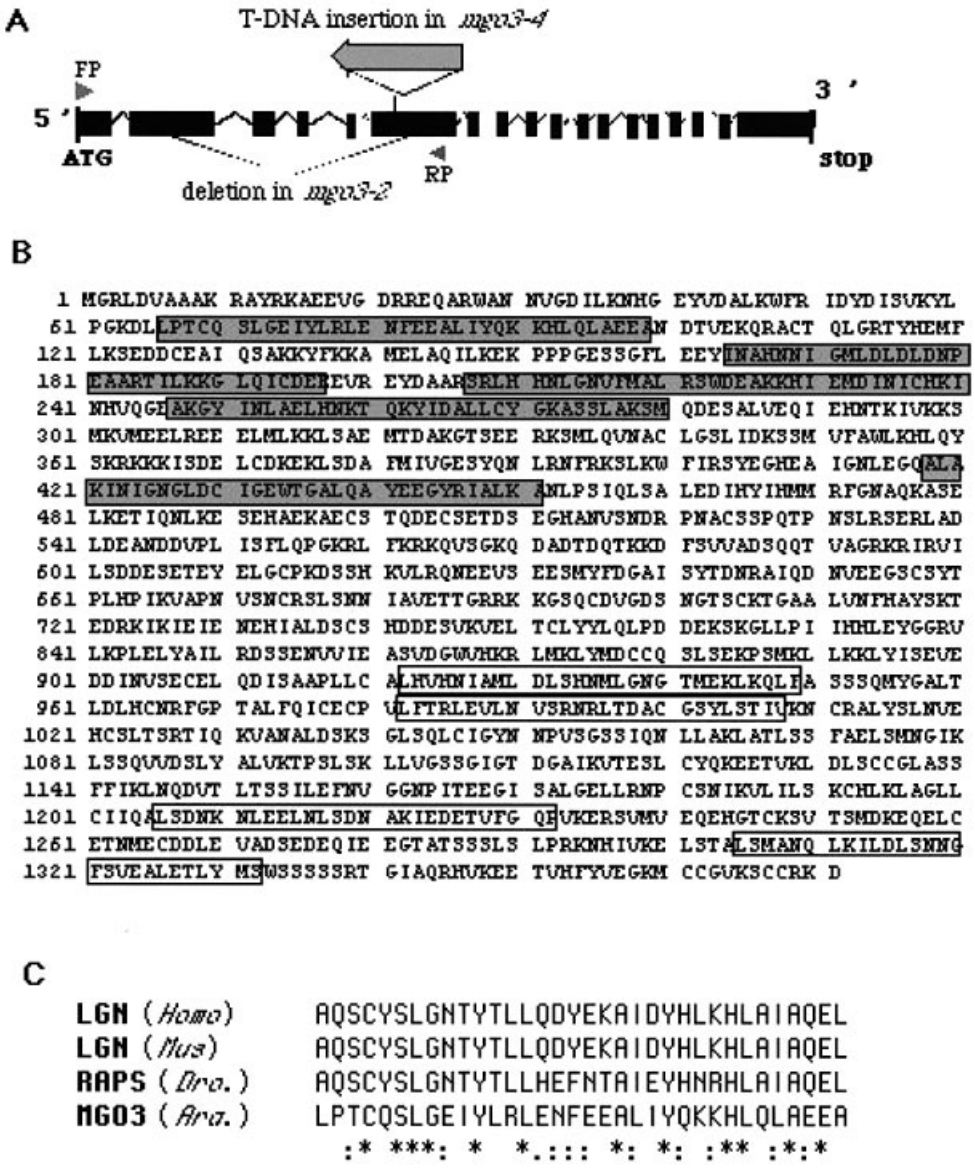

Fig. 7. The *MGO3* gene encodes a predicted protein of 1311 amino acids with similarities with TPR and LRR containing proteins in animals. (A) Predicted structure of the *MGO3* gene. FP, RP: location of the primers used to generate the probe for northern blot hybridization. (B) Predicted MGO3 protein, with five putative TetratricoPeptide Repeats (grey box) and four potential Leucine-Rich-repeats (white box). (C) Alignment of the first TPR of the predicted MGO3 protein with a characteristic LGN motif present in the LGN or LGN-related mosaic proteins from *Homo sapiens* (*Homo*), *Mus musculus* (*Mus*), and *Drosophila melanogaster* (*Dro.*).

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 antibodies, 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 F_2 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 F_2 progeny of the *mgo2 mgo3* cross displayed an enhanced *mgoun*-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 F_2 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.

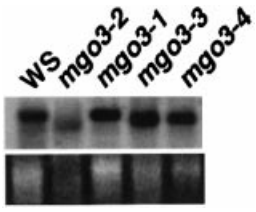

Fig. 8. Expression of *MGO3* in the *mgo3* mutants. Northern blot hybridization using the 5′ half of the *MGO3* cDNA as a probe, on transcripts from the wild-type (WS) and *mgo3* alleles. Low: ribosomal RNA vizualization on the ethidium-bromide stained gel before blotting.

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 fasciation 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. Althought *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 fasciation 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

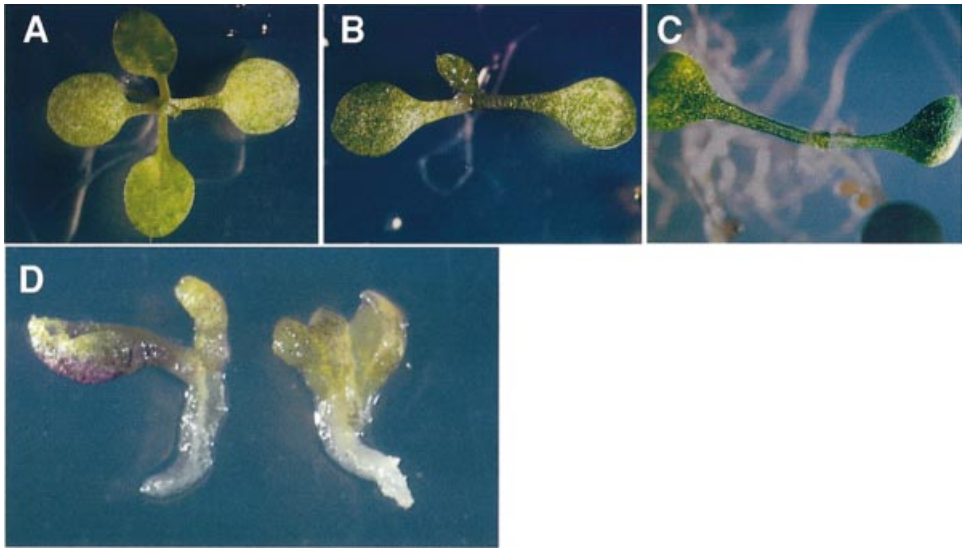

Fig. 9. Enhanced phenotypes of the *mgo2 mgo3* double mutant 10 d after germination (D) compared with wild-type (A), *mgo3* (B), and *mgo2* (C) seedlings.

distinct regulatory processes. Conversely, the epistatic phenotype of the *mgo1 mgo3* double mutant suggests that *MGO1* and *MGO3* act in the same genetic pathway. This model of genetic interaction is in accordance with the additive phenotype observed in the *mgo1 mgo2* double mutant (Laufs *et al*., 1998), involving MGO1 and 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 *mgo3* mutations leads to the same phenotype, it can not be ruled out that the MGO3 protein could still have partial activity, particularly in *mgo3-1* background where the size and abundance of MGO3 RNA is unaffected.

MGO3 represents a new type of regulatory gene of apical meristems in plants

The MGO3 protein does not share any significant similarity with other plant proteins althought 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 MGO3 TPRs are more closely related to those found in animal LGN-related proteins. These proteins have been characterized in Human and *Drosophila*, where they are key regulators of some asymmetric cell divisions (Parmentier *et al*., 2000). In the neuroblast of *Drosophila*, the LGN-related PARTNER OF INSCUTEABLE (PINS) colocalizes with INSCUTEABLE (INS), a factor asymmetrically distributed in the cell (Parmentier *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 *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 MGO3 function in the meristem? The sequence similarity between the MGO3 and the LGN proteins is restricted to the first MGO3 TPR, but is more significant than any other comparison of MGO3 TPR to those of other proteins. Taking into account the cellular disorganization of SAM and RAM in the *mgo3* mutant, it is tempting to make the hypothesis that this could be linked to a functional similarity. According to this hypothesis, 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 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 MGO3 in transmitting or transducing information in meristematic cells. In the *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

inhibitory signal for cell proliferation (Clark *et al*., 1995, 1997). The LGN-related animal proteins do not display such LRRs, and yet, the MGO3 repeats are most similar to the LRR domains of mammalian proteins. These results suggest interesting evolutionary relations between the MGO3 plant meristem protein and animal developmental regulators.

MGO3 as a regulator of meristematic 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 fulfils 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.

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