MGOUN3: evidence for chromatin-mediated regulation of FLC expression

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Abstract

The MGOUN3 (MGO3)/BRUSHY1 (BRU1)/TONSOKU (TSK) gene of Arabidopsis thaliana encodes a nuclear leucine–glycine–asparagine (LGN) domain protein that may be implicated in chromatin dynamics and genome maintenance. Mutants with defects in MGO3 display a fasciated stem and disorganized meristem structures. The transition to flowering was examined in mgo3 mutants and it was found that, under short days, the mutants flowered significantly earlier than the wild-type plants. Study of flowering-time associated gene expression showed that the floral transition inhibitor gene FLC was under-expressed in the mutant background. Ectopic expression of the flower-specific genes AGAMOUS (AG), PISTILLATA (PI), and SEPALLATA3 (SEP3) in mgo3 vegetative organs was also detected. Western blot and chromatin immunoprecipitation experiments suggested that histone H3 acetylation may be altered in the mgo3 background. Together, these data suggest that MGO3 is required for the correct transition to flowering and that this may be mediated by histone acetylation and associated changes in FLC expression.

Key words: Arabidopsis thaliana, BRU1, chromatin dynamics, FLC, histone modifications, MGO3, TSK.

Introduction

The continuous development of plants relies on the activity of shoot and root apical meristems (SAM and RAM). These are organized pools of dividing and progressively differentiating cells located at the stem and at the root tips, generating shoot and root tissues continuously, while maintaining their integrity. A complex network of transcription factors and signal proteins ensures the co-ordination of cell division and cell differentiation in these structures (for a review see Veit, 2004). In addition, numerous genes involved in chromatin dynamics have been shown to participate in SAM and RAM maintenance and activity (for a review see Guyomarc’h et al., 2005). Chromatin modifiers are required to generate the chromatin structure in which DNA is wrapped or to modulate its conformation during development, favouring or inhibiting the expression of specific loci in different developmental phases (for review see Hsieh and Fischer, 2005).

In Arabidopsis thaliana, the transition to flowering is controlled by four major pathways, namely the autonomous, vernalization, gibberellic-acid-dependent and long-day pathways (for a review see Boss et al., 2004). These pathways converge to induce the expression of two main targets: FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) (for reviews see Bolt et al., 2004; Parcy, 2005). Among multiple regulators involved in this control, the MADS-box floral repressor gene FLOWERING LOCUS C (FLC) emerges as a convergent point for the control of flowering time by the vernalization and autonomous pathways. Regulation of FLC expression involves putative transcription factors, RNA-processing factors, and chromatin modifiers (He and Amasino, 2005). In the autonomous pathway, homologues of histone deacetylase complex subunits FLOWERING LOCUS D (FLD) and FVE have been shown to repress FLC expression by means of histone H3 and H4.
deacetylation around the translation start site and in the first intron of the gene (He et al., 2003; Ausın et al., 2004; Kim et al., 2004). Conversely, histone H3 acetylation in FLC is associated with high expression levels of this gene (Bastow et al., 2004; Sung and Amasino, 2004). In addition, Arabidopsis relatives of subunits of the yeast RNA polymerase II (Pol II) Associated Factor 1 (PAF1) complex were recently shown to participate in FLC activation via histone H3 Lysine 4 trimethylation at the translation start site of this locus (He et al., 2004; Oh et al., 2004).

A new gene, MGOUN3 (MGO3), has previously been described, mutations of which affect both SAM and RAM organization and maintenance (Guyomarc'h et al., 2004). This gene was also identified as BRUSHY1 (BRU1) and TONSOKU (TSK) by other groups (Suzuki et al., 2004; Takeda et al., 2004). The mgo3/bru1/tsk mutants display stem fascination, aberrant phyllotaxy, perturbation in leaf and flower morphogenesis, and shorter roots. These developmental defects can be linked to the loss of the cellular organization of both SAM and RAM and perturbations in the expression pattern of some key meristem regulators such as WUSCHEL (WUS) in the SAM, SCARECROW (SCR) in the RAM, and AGAMOUS (AG) in flower meristems (Guyomarc'h et al., 2004; Suzuki et al., 2004). Defects in cell cycle progression and increased levels of cyclin B1 have also been recently reported in tsk mutant seedlings (Suzuki et al., 2005).

Although the MGO3/BRU1/TSK protein structure does not allow any speculation about its function, the increased intrachromosomal homologous recombination, the hypersensitivity to DNA-damaging agents, and the release of transcriptional gene silencing observed in mgo3 mutants, suggested that the MGO3/BRU1/TSK protein is involved in chromatin organization and dynamics and in genome maintenance (Takeda et al., 2004). Moreover, the mutant phenotype is very close to those of fasciata1 (fasi) and fasciata2 (fas2) mutants that also show enlarged and disorganized SAM and RAM, resulting in stem fascination, irregular phyllotaxy, and a shorter root (Kaya et al., 2001; Leyser and Furner, 1992). The cellular arrangement and the expression pattern of cell-identity regulators such as WUS and SCR are misregulated in fas1 and fas2 apical meristems. The FAS1 and FAS2 genes encode subunits of the Arabidopsis thaliana counterpart of the Chromatin Assembly Factor-1 (CAF-1) complex (Kaya et al., 2001). In yeast, CAF-1 acts as a histone-chaperone complex and participates in the reconstitution of chromatin after DNA replication and repair (Polo et al., 2004).

In order to understand the function of MGO3 in Arabidopsis development better, analysis of the mgo3 genotype was continued by focusing on particular developmental pathways. In this work, it is reported that mgo3 mutants showed an early-flowering phenotype under short days as well as under-expression of FLC, associated with histone H3 hypoacetylation at this locus. These data suggest that MGO3 is required for the chromatin modifications involved in the regulation of FLC expression.

**Materials and methods**

**Plant material and growth conditions**

All three mgo3 lines used in this study were described in Guyomarc'h et al. (2004). The mgo3-1 allele is in the Landsberg erecta (Ler) background, mgo3-2 is in the Wassilewskija (WS) background, and the mgo3-4 allele is in the Columbia (Col-0) background. All mgo3 lines were back-crossed at least twice with the corresponding wild-type parent.

Plants were grown in chambers at 20 °C on soil or on sterile half-strength MS medium supplemented with 10 g l⁻¹ sucrose and 1.5% agar in short-days (8 h of light; SD) or long-days (16 h of light; LD) conditions. To assess the vernalization response, plants were first grown at 4 °C under SD for 6 weeks before being placed into SD at 20 °C. In all cases, bolting time is given as the number of days in normal growth (20 °C) SD or LD conditions.

For double mutant analysis, the flc-3 mutant (Col-0 background) was kindly provided by Richard M Amasino (Michaels and Amasino, 1999).

**Genetic characterization**

Double mutants (flc-3 mgo3-4) were selected from an F₂ population using molecular markers on individual F₂ plants. The MGO3-4 genotype was determined with primers 5'-GCTGACATTTGGAGC-CACC-3' and 5'-ACGGAATATGGATAGACACC-3', which give a PCR product for the wild-type allele but not for the mutant allele. The FLC-3 genotype was determined with primers 5'-TATC GGCG-GAGGAGAAC-3' and 5'-TAGAAAGAAATAAAGCGGAAAG-AGGA-3', which amplify a 300 bp fragment for the wild-type allele and a 196 bp fragment for the flc-3 allele because flc-3 has a 104 bp deletion (Michaels and Amasino, 1999).

**Expression analysis by RT-PCR and microarrays**

Total RNA was extracted from rosette leaves, flower buds or whole seedlings following the TRIzol® protocol (Invitrogen/Life Technologies). After quantification by spectrophotometry, RNA integrity was checked by electrophoresis.

To study gene expression in leaves, 1 μg of total RNA was reverse transcribed using the RNase H- Superscript® II DNA polymerase (Invitrogen/Life Technologies). The CDNA solution was diluted four times and 1 μl was used to test candidate gene amplification by PCR. The primers used were WUS: 5'-GTAGCCATATGCTATGGTCA-TGG-3' and 5'-ACCGGCGTAGCTGTCACGAC-3', AG: 5'-GTTGATTTGGCTACAAAGCTAACCAGA-3' and 5'-TTCACTGATACCAACATCATCGGGA-3', PI: 5'-GAGGAGGAATGGATTGGT-GA-3' and 5'-CGGCAATCATTCTCCTATT-3', Amplification with the ACTIN primers (5'-GTTAACCATTGTCGATCGTGGT-GG-3' and 5'-AAGGACCTTTAATCCTCATGTCGC-3', Charrier et al., 2002) was used as a positive control.

To monitor the expression of flowering-time genes, total RNA was extracted from aerial parts of seedlings and reverse transcription was performed as described above. Semi-quantitative RT-PCR was then carried out; in each case the semi-quantitative property of the amplification was checked by comparing the results obtained with one volume and twice this volume of template. The primers used were FLC: 5'-ATGTTGAGTATCGTGTGATCG-3' and 5'-AGTATA-CAAAAGCTGGCC-3', CO: 5'-CTCCTTGCCATTTTTC-3' and 5'-CTTAAACATAACGCAATCATTTC-3', FT: 5'-TACGAAAATCCGAAGTCCCA-3' and 5'-AAACTCGGGAGTGTGTA-GTTC-3', SOC1: 5'-AAATATGCAAGGATACCATGAGC-3'.
and 5'-TCTTGAAGACAAAGGTAAACCAT-3'. Amplification of the ACTIN2 cDNA (5'-CTAACGTCCTCAAGTCAAGGGCTT-3' and 5'-TTAACATGCAGAGTCTTCAAGGT-3', Bertrand et al., 2004) was used as a semi-quantitative control. The bands were scanned with Molecular Image FX Pro (Bio-Rad), and normalized to actin mRNA signals by using Bio-Rad Quantity One 1-D Analysis software.

To compare precisely gene expression between mgo3 and wild-type leaves, a transcriptome analysis was performed using CATMA arrays containing 24 576 gene-specific tags from Arabidopsis thaliana (http://www.catma.org). Total RNA was extracted from the three first rosette leaves of at least 25 14-d-old seedlings using the TRIzol® extraction kit (Invitrogen/Life technologies) followed by two ethanol precipitations, and then checked for integrity with the Bioanalyser from Agilent (Waldbronn, Germany). cRNA were produced from 2 μg of total RNA from each sample using the Message Amp aRNA® kit (Ambion). Then 5 μg of cRNA were reverse transcribed in the presence of 300 units of Superscript™ III DNA polymerase (Invitrogen/Life technologies), cy3-dUTP and cy5-dUTP (PerkinElmer Life sciences) for each slide. Three plant replicates were analysed, allowing the statistical analysis of hybridization results with respect to experimental and biological variabilities as described by Lurin et al. (2004). Functional data or predictions given by the Gene Ontology database (http://www.genontology.org) were used to classify in a functional way genes expressed differentially between mgo3 and wild-type leaves.

**Histone acetylation analysis by western blot and chromatin immunoprecipitation**

Nuclear proteins were extracted from aerial parts of 12-d-old seedlings grown under SD as described by Gendrel et al. (2002). After quantification with the Bradford method, the same amounts of protein were resolved by SDS-PAGE, and then transferred to a PVDF membrane (Bio-Rad) using a Mini-Protean® 3 Cell (Bio-Rad). Western analysis was performed using primary polyclonal antibodies raised against histones H3 acetylated on Lysine 18 (H3acK18; Cell Signaling) or on any residue between 4 and 18 (H3acK4-18; Upstate), or raised against all forms of histones H3 (Upstate), and then secondary antibodies conjugated to alkaline phosphatase. Antibody complexes were detected by chemiluminescence using the Immun-Star™ AP Substrate kit (Bio-Rad).

Chromatin immunoprecipitation experiments were performed as described by Gendrel et al. (2002), starting from 2 g of entire 12-d-old seedlings grown in vitro in SD. Antibodies raised against acetylated histone H3 (Upstate) were used for immunoprecipitation. DNA fragments associated with the immune complexes were dissolved in water and detection of target sequences was carried out by PCR using primers kindly provided by Ausin et al. (2004). Three independent experiments were performed, and PCR tests were repeated at least twice. The bands were scanned with Molecular Image FX Pro (Bio-Rad), and normalized to actin signals using Bio-Rad Quantity One 1-D Analysis software.

**Results**

**Mutations in MGO3 result in early flowering in short days**

In studies on Arabidopsis, the transition to flowering is usually dated at bolting or measured with the number of rosette and cauline leaves produced by the SAM before flowering (Pouteau et al., 2004). The strong developmental defects of the mgo3 mutants prevented us from using leaf numbers as a criterion for floral transition, and in most experiments, only the bolting day was used. In addition, these mgo3 developmental defects sometimes led to heavily fasciated plants which did not bolt or flower. This feature led us to state previously that the transition to flowering was often delayed in the mgo3 mutant (Guyomarc'h et al., 2004). However, a precise study of the mgo3 plants revealed that, in the absence of very strong developmental defects in the vegetative phase (heavy fascination, possible meristem abortion), the mgo3 mutants flowered significantly earlier than the wild-type plants. Thus, a minority of mgo3 individuals showing heavy fascination in the vegetative phase were excluded in the studies presented here.

When grown on soil in SD, plants with the mgo3-1, mgo3-2, and mgo3-4 alleles bolted significantly earlier than the wild-type (respectively 3.5, 19.7, and 6.0 d earlier than the corresponding wild-type ecotype; Table 1; Fig. 1). Vernalization still accelerated the transition to flowering in the mgo3-2 background (Table 1).

The early-flowering phenotype was also observed for these three alleles of mgo3 mutants grown under short days in vitro (Table 2). It was also apparent in LD, on soil, for the mgo3-2 allele (mgo3-2 plants bolted after 18.21±0.54 d, compared with 22.25±0.69 d for WS; n=28).

**mgo3 mutations cause under-expression of FLC and early induction of FT expression**

The early-flowering phenotype of mgo3 in SD was reminiscent of plants under-expressing FLC, a repressor of SOC1 and FT expression, or over-expressing CONSTANS (CO), a transcription factor responsible for FT and SOC1 induction in LD (Michaels and Amasino, 1999; Sheldon et al., 1999; Samach et al., 2000). RT-PCR was used to compare the expression of FLC, CO, FT, and SOC1 in mgo3-2 and wild-type seedlings grown in vitro in LD for 6, 9, or 12 d or grown in SD for 12 d or 19 d, which, in each case, roughly corresponds to the flowering inductive period for the wild-type in this study’s conditions. In both conditions, at all stages tested, levels of FLC mRNA were

**Table 1. Bolting time of wild-type and mutant plants grown under short days on soil**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Without vernalization</th>
<th>With vernalization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n days before bolting</td>
<td>n days before bolting</td>
</tr>
<tr>
<td>WS</td>
<td>21 61.87±1.33</td>
<td>21 34.52±1.12</td>
</tr>
<tr>
<td>mgo3-2</td>
<td>25 42.08±1.42</td>
<td>21 26.81±1.23</td>
</tr>
<tr>
<td>Ler</td>
<td>21 65.67±0.89</td>
<td>ND</td>
</tr>
<tr>
<td>mgo3-3</td>
<td>24 62.13±1.05</td>
<td>ND</td>
</tr>
<tr>
<td>Col-0</td>
<td>24 60.33±1.25</td>
<td>ND</td>
</tr>
<tr>
<td>mgo3-4</td>
<td>22 54.32±0.97</td>
<td>ND</td>
</tr>
<tr>
<td>ftc-3</td>
<td>24 52.42±1.02</td>
<td>ND</td>
</tr>
<tr>
<td>ftc-3 mgo3-4</td>
<td>6 55.86±1.36</td>
<td>ND</td>
</tr>
</tbody>
</table>

* a, Number of plants analysed.
  b, ND, not determined. Bolting times are shown as mean of days before bolting ± standard deviation of the mean.
dramatically reduced in mgo3-2 compared with the wild type (Fig. 2A, B). A slight up-regulation of FT expression was also detected in the mutant background both under LD (Fig. 2A) and SD (Fig. 2B). By contrast, no significant difference in CO (Fig. 2A) or SOC1 (Fig. 2A, B) expression levels was detected between the two genotypes.

A strong and slight decrease of FLC mRNA level could also be detected respectively in the mgo3-2 and in the mgo3-4 alleles compared with the wild type, when grown for 12 SD, on soil (Fig. 2C). In the Ler wild-type ecotype, the FLC gene is naturally expressed at very low levels that prevented meaningful comparison with the mgo3-1 mutant allele (Fig. 2C; see also Gazzani et al., 2003; Michaels et al., 2003).

mgo3 mutants show ectopic expression of floral meristematic genes in leaves

Defects in leaf morphogenesis in the mgo3 mutants were reminiscent of those observed when meristem-specific genes or flower-specific genes are ectopically expressed in vegetative organs (Goodrich et al., 1997; Ori et al., 2000). In addition, ectopic expression of the floral-organ identity gene AG was previously shown to cause early flowering (Mizukami and Ma, 1992; Goodrich et al., 1997). To test whether the early-flowering phenotype of mgo3 was correlated with ectopic expression of MADS-box genes in leaves, RT-PCR analysis of wild-type and mgo3 rosette leaves were performed. Transcripts of two flower-specific MADS-box genes AG and PISTILLATA (PI) were detectable in mgo3 but not in wild-type leaves (Fig. 3). No change was detected in the steady-state transcript level of a known AG regulator, WUS (Fig. 3; Lenhard et al., 2001; Lohmann et al., 2001).

The transcriptomes of mgo3 and wild-type leaves were compared using the Complete Arabidopsis Transcriptome MicroArray (CATMA) technology (http://www.catma.org). CATMA chips containing 24,576 genes of the Arabidopsis genome were hybridized with cDNA probes synthesized from total RNA harvested from 14-d-old rosette leaves of wild-type and mgo3-2 plants in three separate experiments. Based on the statistical test (see Materials and

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**Table 2. Bolting time of wild-type and mgo3 mutant plants grown under short days in vitro**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Days before bolting</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS</td>
<td>45</td>
<td>28.33±1.03</td>
</tr>
<tr>
<td>mgo3-2</td>
<td>61</td>
<td>19.69±0.24</td>
</tr>
<tr>
<td>Ler</td>
<td>47</td>
<td>35.11±0.90</td>
</tr>
<tr>
<td>mgo3-1</td>
<td>47</td>
<td>33.98±0.60</td>
</tr>
<tr>
<td>Col</td>
<td>37</td>
<td>32.57±1.23</td>
</tr>
<tr>
<td>mgo3-4</td>
<td>40</td>
<td>26.03±0.88</td>
</tr>
</tbody>
</table>

n, Number of plants analysed. Bolting times are shown as mean of days before bolting ± standard deviation of the mean.
methods), 1.46% of the 24,576 genes were revealed as differentially expressed between the mutant and the control. The affected genes are listed under Supplementary Materials. Complete data files were deposited to ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under experiment accession number E-MEXP-196. Of the affected genes, 34% were repressed and 66% were induced. In these experiments over-expression of AG was not detected but transcript levels of two other flower-specific MADS-box genes, namely PI and SEPALLATA3 (SEP3), were significantly higher in mgo3-2 leaves compared with those of wild-type plants.

**Genetic interaction between MGO3 and FLC genes**

In order to analyse genetic interactions between MGO3 and FLC genes, a cross between mgo3-4 and null flc-3 mutants was performed. The F2 progeny of the cross, as well as the plants.

were grown under short-days on soil and the flowering time was measured. Both flc-3 and mgo3-4 single mutants flowered earlier than the wild-type Col-0 plants, and both flowered approximately at the same time compared with each other (Table 1). In the F2 progeny, flc-3 mgo3-4 double mutants were identified using molecular markers. All flc-3 mgo3-4 plants flowered earlier than the wild-type, at the same time as the mgo3-4 and flc-3 single mutants (Table 1). No new flowering phenotype was detected among the F2 progeny. Moreover, all the double mutants displayed a mgo3 phenotype with perturbations of shoot morphogenesis and organogenesis. These results are consistent with an epistasy of MGO3 over FLC with regard to the early-flowering phenotype, which suggests that FLC is one of the targets of MGO3.

**mgo3 mutations affect global histone acetylation, especially in FLC chromatin**

The recent characterization of FLC repressors and activators has shown that some of these regulatory proteins are involved in covalent modifications of FLC chromatin such as acetylation or methylation of specific histones (reviewed by He and Amasino, 2005). Previous results suggest that the MGO3 protein may be required for the stability of heterochromatin (Takeda et al., 2004). Therefore, it was asked whether the deregulation of FLC expression observed in mgo3 plants could be due to defects in epigenetic modifications of histones at the FLC locus and, particularly, loss in histone H3 acetylation.

To address this, global histone H3 acetylation levels were first compared between wild-type and mutant plants after 12 SD. Nuclear proteins were extracted and analysed by immunoblotting using antisera specific for histone H3 acetylated on lysines 4, 9, 14, or 18. For the same amount of nuclear proteins loaded, a significantly weaker signal was obtained for mgo3 compared with wild type (Fig. 4). Similar results were obtained using antibodies raised specifically against histone H3 acetylated on lysine 18 (Fig. 4). No difference in the level of histone H3 was observed between both genotypes using the antibody specific to total histone H3 (Fig. 4) suggesting that acetylation of histones H3 is reduced in the mgo3-2 plants compared with the wild type.

Next, chromatin immunoprecipitation assays were performed to detect histone H3 acetylation at the FLC locus in WS and mgo3-2 plants after 12 SD. DNA released from chromatin fragments immunoprecipitated with antibodies raised against acetylated histone H3 was analysed by PCR with primers specific to four regions of the FLC locus (regions I–IV, Fig. 5A; see also Ausín et al., 2004) covering the FLC promoter, the first exon and the first intron of the gene. No difference in immunorecovery, as judged by PCR, was detected for region IV. However, recovery and PCR using primers to the region around the FLC start codon (regions I–III) was somewhat lower in mgo3-2 than in WS. The levels of precipitation and amplification of the ACTIN2

**Fig. 3.** Expression of meristematic regulatory genes in mgo3-2 and wild-type (WS) plants. RT-PCR analysis of rosette leaves (L) or flower buds (FB) RNA with primer sets specific to AG, PI, WUS, and ACTIN genes.

**Fig. 4.** Comparison of histone H3 acetylation level in mgo3-2 and wild-type (WS) plants. Western blot analysis of nuclear proteins extracted from aerial parts of mgo3-2 or WS seedlings after 12 SD. Analysis were performed using primary polyclonal antibodies raised against histone H3 acetylated on any residue between 4 and 18 (H3 acK4-18) or on lysine 18 (H3 acK18) or against all forms of histone H3 (H3).
promoter (used as an internal control) were comparable between both genotypes (Fig. 5B, C). These results suggest that the mgo3 mutation may affect the accessibility of epitopes that reflect H3 acetylation in the proximal promoter region and in the beginning of the ORF of the FLC gene.

Discussion

MGO3 is involved in the regulation of flowering time

It has been shown that the mgo3 mutants display an early-flowering phenotype and are still responsive to day length (they flower later under SD than under LD) and to vernalization treatment. Therefore, mgo3 mutants seem to be mainly affected in the autonomous pathway of flowering-time regulation. A correlation between FLC transcript levels and the time to flowering has been established from the study of wild-type ecotypes and flowering mutants; most often the higher FLC is expressed, the later plants flower (Sheldon et al., 2000; Rouse et al., 2002; Gazzani et al., 2003; Michaels et al., 2003). Thus, the under-expression of FLC observed in the mgo3 background is likely to contribute to the early flowering of the mutant. As this is the case with the null allele flc-3, this low expression of FLC in the mgo3 background does not abrogate the vernalization response of the plants (Michaels and Amasino, 2001). The severity of the precocious-flowering phenotype is correlated with the differential of FLC expression between the wild-type and mgo3 plants: strong in mgo3-2, weak in mgo3-1, and intermediate in mgo3-4. These variations in acceleration of flowering time might be due to differences in mgo3 alleles, but also to differences in FLC alleles between the corresponding wild-type ecotypes. For instance, the relatively slight effect of mgo3 mutation on flowering time in Ler could be explained by the natural weak expression of FLC in this background, due to a transposon insertion in the first intron of the gene (Gazzani et al., 2003; Michaels et al., 2003). The fact that the mgo3 mutation accelerates the flowering transition to almost the same extent in both Ler and Col-0 backgrounds, despite the dramatic difference in strength of their respective FLC alleles, and that, conversely, the early-flowering phenotype of mgo3 mutants is much more pronounced in the WS background than in the Col-0 background, strongly suggests that MGO3 affects the activity of other flowering-time genes as well as FLC. Other members of the FLC family, such as MADS AFFECTING FLOWERING 2 (MAF2), or FLOWERING LOCUS M (FLM), or other repressors of the transition to flowering, such as SHORT VEGETATIVE PHASE (SVP) could be candidate target genes (for a review see Boss et al., 2004). In addition, the expression of downstream flower-specific genes, such as AG, PI, or SEP3, could also be up-regulated independently of FLC. However, the fact that flc mgo3-4 double mutants flower as early as flc and mgo3 single mutants does not support this hypothesis (see below).

FLC action on the transition to flowering is mainly mediated by repression of FT and SOC1 (Lee et al., 2000; Michaels and Amasino, 2001; Hepworth et al., 2002), which are positive regulators of the expression of flower-specific genes like AG and PI (for a review see Parcy, 2005). Consistent with this model, an increase in FT expression was detected in the mgo3 background, suggesting that FT may contribute to the early flowering of mgo3 mutants. However, FT expression oscillates with a circadian rhythm in wild-type plants, so time-course experiments would be necessary to gauge precisely the
effect of mgo3 mutations on FT expression. No changes were observed in SOC1 expression in the mgo3 mutants. It has been reported previously that, in addition to the down-regulation of FLC, a positive signal from the long-day pathway or from the gibberellic-acid pathway was required to stimulate SOC1 expression (Moon et al., 2003).

Ectopic expression of PI, SEP3, and AG could be due to premature FT induction but also could be linked to chromatin modifications

Over-expression of flower-specific genes during the vegetative phase is known to be associated with an early-flowering phenotype in several mutants (Goodrich et al., 1997; Kotake et al., 2003; Moon et al., 2003, see below). Therefore mRNA accumulation of AG, PI, and SEP3 genes in mgo3 leaves could be also related to the early-flowering phenotype of the mutants. Their ectopic expression in vegetative organs of the mgo3-2 mutant might be due to the premature repression of FLC and/or induction of FT expression in this mutant background. It is at present not clear why ectopic expression of AG could be detected by RT-PCR on leaves, but not by the microarray experiments on young seedlings. A variation in the severity of the phenotype between different populations or at different ages could explain this discrepancy.

Ectopic expression of AG has also been reported in leaves of curly leaf (clf), incurvata2 (icu2), embryonic flower1 and 2 (emf1 and emf2), early bolting in short days (ebs), like heterochromatin protein1/terminal flower2 (lhp1/tfl2), fertilization-independent embryo (fie), and vernalization independence4 (vip4) mutants (Chen et al., 1997; Goodrich et al., 1997; Serrano-Cartagena et al., 2000; Gomez-Mena et al., 2001; Kinoshita et al., 2001; Zhang and Van Nocker, 2002; Moon et al., 2003; Katz et al., 2004; Kotake et al., 2003). All these genes are involved in epigenetic processes regulating plant development. Most of them have been shown to influence chromatin conformation so as to repress the expression of target genes until the onset of the corresponding developmental programme, for example, flower-specific genes before the transition to flowering. Among them, emf1, emf2, lhp1/tfl2, and fie mutants also display ectopic expression of PI and emf1, emf2, lhp1/tfl2 mutants show ectopic expression of SEP3 (Kinoshita et al., 2001; Kotake et al., 2003; Moon et al., 2003). However, no repression of FLC has been reported in these mutants. These observations suggest that chromatin-mediated repression of flower-specific genes contributes to the maintenance of the vegetative phase (Reyes and Grossniklaus, 2003; Wagner, 2003). Thus, the ectopic expression of SEP3, AG, and PI in mgo3 leaves could result from FLC under-expression and/or from specific alterations of their chromatin-mediated repression, independently of FLC.

The mgo3 mutation may be responsible for the loss of targeted histone H3 acetylation at the FLC locus

The mgo3 mutation has been shown here to be associated with a reduced recovery of acetylated histone H3. In addition, preliminary evidence has been presented that loss of histone H3 acetylation may occur around the translational start of the FLC gene. This region, corresponding to the 5′-untranslated region, the first exon, and the beginning of the first intron of the gene, was previously shown to be crucial for FLC expression and regulation (Sheldon et al., 2002), and it is now known that this is mediated by histone modifications. Histone H3 lysine 4 trimethylation, histone H3 lysine 36 dimethylation, and histone H3 and H4 acetylation are associated with high FLC expression, whereas histone H3 and H4 deacetylation and H3 lysine 9 and 27 dimethylation correlate with low FLC expression (reviewed in He and Amasino, 2005; Zhao et al., 2005). These results suggest that MGO3 is required for normal histone acetylation at the FLC locus, and that MGO3 might be a chromatin-associated protein required for the regulation of FLC expression during development. The fact that the mgo3 mutation is epistatic to the fc1 mutation suggests that MGO3 function is necessary for correct functioning of the FLC gene. This is consistent with a role in FLC regulation during development via chromatin modifications. It is likely, however, that other loci might be subjected to histone H3 hypoacetylation in the mgo3-2 background, which could lead to the pleiotropic phenotype of the mutant. This is consistent with the transcriptome analysis showing that a broad range of genes is affected in mgo3-2. All the developmental alterations, including the early-flowering phenotype, could be side-effects of major perturbations in chromatin dynamics, as suggested by Komeda (2004).

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References


