Polycomb-group proteins repress the floral activator *AGL19* in the *FLC*-independent vernalization pathway

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Polycomb-group (PcG) proteins form a cellular memory by maintaining developmental regulators in a transcriptionally repressed state. We identified a novel flowering gene that is under PcG control in *Arabidopsis*—the MADS-box gene *AGL19*. *AGL19* expression is maintained at very low levels by the PcG proteins MSI1, CLF, and EMF2, and *AGL19* is partly responsible for the early flowering phenotype of *clf* mutants. *AGL19* chromatin is strongly enriched in trimethylation of Lys 27 on histone H3 (H3K27me3) but not in H3K9me2. Repressive H3K27me3 marks were reduced by decreased CLF or MSI1 levels and by prolonged cold, suggesting that the PcG proteins MSI1 and CLF repress *AGL19* in the absence of cold. Ectopic expression of *AGL19* strongly accelerates flowering, and *agl19* mutants have a decreased response to vernalization, the promotion of flowering by prolonged cold. Epistasis analyses revealed that *AGL19* works in the poorly characterized *FLC*-independent vernalization pathway and does not require *SOC1* to function. In this pathway, prolonged cold relieves *AGL19* from PcG repression by a mechanism that requires *VIN3* but not *VRN2*. Elevated *AGL19* levels activate *LFY* and *AP1* and eventually cause flowering.

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Polycomb-group (PcG) proteins are cellular memory modules that are thought to maintain genes in a transcriptionally inactive state after an initial repression has been established (for review, see Brock and Fisher 2005). PcG proteins were first identified in insects, but are essential for normal development in both animals and plants (for review, see Schubert et al. 2005). These proteins often form large multiprotein complexes, such as the Polycomb Repressive Complex 2 (PRC2). *Drosophila* PRC2 contains four core subunits: the MSI1-like protein p55, Enhancer of Zeste [E(Z)], Extra Sex Combs (ESC), and Suppressor of Zeste 12 [Su(Z)12] (Czermin et al. 2002; Müller et al. 2002). Transcriptional repression by PRC2 relies on histone methyltransferase activity of the E(Z) subunit, which preferentially catalyzes histone H3 Lys 27 (H3K27) trimethylation (for review, see Cao and Zhang 2004).

Unlike in insects, where the PRC2 members are encoded by single-copy genes, in plants homologous PRC2 subunits are often encoded by small gene families

(Schubert et al. 2005), and several possible PRC2-like complexes have been proposed to exist in *Arabidopsis* (Chanvittattana et al. 2004; Hennig et al. 2005). However, to date, only one *Arabidopsis* PRC2 complex has been well characterized—the FERTILISATION INDEPENDENT SEED (FIS) complex (Köhler et al. 2003a). The FIS complex consists of MSI1, the E(Z) homolog MEDEA (MEA), the ESC homolog FERTILISATION INDEPENDENT ENDOSPERM (FIE), and likely the Su(Z)12 homolog FIS2 (Köhler et al. 2003a; Chanvittattana et al. 2004). This complex has specific functions during gametophyte and early seed development, including suppression of seed development in the absence of fertilization and repression of the MADS-box gene *PHERES1* (*PHE1*) (Chaudhury et al. 1997; Grossniklaus et al. 1998; Ohad et al. 1999; Köhler et al. 2003b). During sporophyte development, a second PRC2 complex, the CURLY LEAF (CLF) complex, most likely represses transcription of floral homeotic genes, such as the MADS-box gene *AGAMOUS* (*AG*). The CLF complex probably consists of MSI1, the E(Z) homolog CLF, FIE, and the Su(Z)12 homolog EMBRYONIC FLOWER2 (EMF2) (Goodrich et al. 1997; Kinoshita et al. 2001; Yoshida et al. 2001; Hennig et al. 2003; Chanvittattana et al. 2004; Katz et al. 2004).

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The third potential PRC2-like complex is the VERNALIZATION (VRN) complex. The existence of the VRN complex was hypothesized because the Su(Z)12 homolog VRN2 is required for maintaining repression of the MADS-box gene *FLOWERING LOCUS C* (*FLC*) after vernalization and for vernalization-induced H3 methylation at the *FLC* locus (Chandler et al. 1996; Gendall et al. 2001; Bastow et al. 2004; Chanvivattana et al. 2004; Sung and Amasino 2004). Because *FLC* is a very potent repressor of flowering, epigenetic control of the floral transition has recently received considerable attention (for review, see He and Amasino 2005).

The transition to flowering is controlled by diverse environmental and developmental signals, and many genes that control flowering in *Arabidopsis* have been identified (for review, see Boss et al. 2004; Ausin et al. 2005). These genes have been grouped in several genetic pathways, including the photoperiod, the vernalization, and the autonomous pathways. The photoperiod pathway responds to seasonal changes in day length, while the vernalization pathway renders *Arabidopsis* competent to flower after prolonged exposure to low temperatures; for example, during winter (for review, see Henderson and Dean 2004; Searle and Coupland 2004). These two pathways contribute to initiate flowering of winter-annual plants in the favorable conditions of spring and summer. In contrast, the autonomous pathway promotes flowering independently of environmental signals. Both the autonomous and vernalization pathways function mainly by reducing expression of the floral repressor *FLC* (Michaels and Amasino 1999, 2001; Sheldon et al. 2000; Gendall et al. 2001). Repression of *FLC* by vernalization is initiated by VERNALIZATION INSENSITIVE3 (*VIN3*) and maintained by VERNALIZATION1 (*VRN1*) and VERNALIZATION2 (*VRN2*) (Gendall et al. 2001; Levy et al. 2002; Sung and Amasino 2004). When expressed, *FLC* prevents transcription of the pathway integrators *FLOWERING LOCUS T* (*FT*) and the MADS-box gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*, or *AGL20*) (Borner et al. 2000; Lee et al. 2000; Michaels and Amasino 2001), which combine signals from several pathways to induce flowering (for review, see Parcy 2005). Vernalization releases *SOC1* from *FLC* repression, thus making *SOC1* an important component of the *FLC*-dependent vernalization pathway (Lee et al. 2000; Hepworth et al. 2002; Moon et al. 2003a). Although *FLC* plays a central role in vernalization in *Arabidopsis*, *flc*-null mutants still respond to vernalization, demonstrating that *FLC*-independent mechanisms exist in the vernalization pathway (Michaels and Amasino 2001; Sung and Amasino 2004). However, the molecular nature of these *FLC*-independent mechanisms is poorly understood.

Among the *Arabidopsis* homologs of PRC2 subunits, the floral transition is not only regulated by the Su(Z)12 homolog VRN2, but also by the p55 homolog MSI4 (also called FVE) (Koornneef et al. 1991; Ausin et al. 2004; Kim et al. 2004). *FVE* is part of the autonomous pathway, needed for the epigenetic repression of *FLC* via histone deacetylation. In addition to *FVE/MSI4*, *Arabidopsis*

contains four other MSI1-like proteins (MSI1–3, MSI5) (Ach et al. 1997; Hennig et al. 2003). Although MSI1-like proteins have no catalytic activity, they are often essential subunits of protein complexes controlling chromatin dynamics (for review, see Hennig et al. 2005). *Arabidopsis* MSI1, for instance, is vital for plant development, as *msi1*-null mutants are embryo lethal (Köhler et al. 2003a; Guitton et al. 2004). Strong reduction of MSI1 protein levels by cosuppression (*msi1-cs* plants) is not lethal but has severe impacts on development (Hennig et al. 2003). In this study, we performed transcriptional profiling of *msi1-cs* plants and found that the MADS-box gene *AGL19* is under control of the MSI1, CLF, and EMF2 *Arabidopsis* PcG proteins. *AGL19* is a potent floral activator, which is repressed via H3K27 trimethylation in the absence of vernalization. This work also establishes a novel role for PcG proteins as repressors of the *FLC*-independent vernalization pathway of *Arabidopsis*.

Results

AGL19 is up-regulated in *msi1-cs* plants

In order to identify genes affected by the severe reduction in MSI1 levels in *msi1-cs* plants, we performed microarray RNA profiling experiments on rosette leaves of 23-d-old wild-type and *msi1-cs* plants grown in long days (LD). This developmental stage was chosen because phenotypic alterations of *msi1-cs* rosette leaves can then first be observed (Hennig et al. 2003). Statistical analysis of the microarray data identified eight robustly down-regulated genes and 122 robustly up-regulated genes. These deregulated genes fall into diverse categories, with a large proportion being involved in histone metabolism, cell cycle, and DNA repair. The MADS-box gene *AGL19* was among the strongest up-regulated genes, with a 21-fold increase in *msi1-cs* leaves, and was the only MADS-box gene with a significantly altered expression pattern (Fig. 1A). RT-PCR confirmed that *AGL19* is usually expressed at very low levels in wild-type rosette leaves but was strongly up-regulated in *msi1-cs* leaves (Fig. 1B). *AGL19* belongs to the type II class of MADS-box genes and is phylogenetically most closely related to *AGL14* and to the floral pathway integrator *SOC1/AGL20* (Becker and Theissen 2003). So far, *AGL19* has not been extensively characterized, and its biological function is unknown. Because MADS-box transcription factors are often developmental key regulators, *AGL19* was selected for a detailed study.

AGL19 is a weakly expressed transcription factor localized in the nucleus

AGL19 was originally considered as a root-specific gene (Alvarez-Buylla et al. 2000). However, as we detected *AGL19* in wild-type rosette leaves using both microarrays and RT-PCR, we re-examined the expression pattern of *AGL19* in different organs and compared it with

Figure 1. Molecular characterization of *AGL19*. (A) Signal values from microarrays testing RNA from rosette leaves of 23-d-old *ms1-cs* and wild-type (WT) plants grown in LD. Shown are means $\pm$ ranges of two replicates. (B) Confirmation of *AGL19* up-regulation in *ms1-cs* using rosette leaves from 21-d-old plants grown in LD. Here and in all subsequent experiments, *GAPDH* was used as a control. (C) Protein blots of wild-type, *35S::MSI1* overexpressing (*ms1-oe*), and *MSI1* cosuppression plants (*ms1-cs*). Protein was extracted from leaves of 20-d-old plants. (Top panel) Ten micrograms of protein per sample was subjected to immunoblotting with affinity-purified, anti-*MSI1*-specific antiserum (Hennig et al. 2003). (Bottom panel) Ponceau red staining of the blot is shown as a loading control. (D) Expression analysis of *AGL19*, *SOC1*, and *AGL14* in LD. (S) Nine-day-old seedlings; (RL) rosette leaves from 35-d-old plants; (CL) cauline leaves from 35-d-old plants; (INF) inflorescence meristem and closed flower buds; (F) open flowers; (GS) 2-d-old germinating seeds; (R) roots. Note that PCR was done with 33 cycles for *AGL19* and *AGL14*, but with only 30 cycles for *SOC1*. (E) *AGL19* expression increases with plant age. RNA was extracted from seedlings grown in SD. Age was measured in days after germination (DAG). Note that PCR was done with 38 cycles for *AGL19*. (F) *AGL19* is strongly expressed in apices. RNA was extracted from dissected 18-d-old seedlings grown in SD. (S) Total seedlings; (A) apices only; (RL) rosette leaves only. (G) Nuclear localization of *AGL19*-GFP. (Left) Differential interference contrast image. (Right) GFP fluorescence. Bar, 50 μ m.

that of its closest phylogenetic relatives *SOC1* and *AGL14* (Fig. 1D). Although the strongest expression of *AGL19* was detected in roots, it was also detected in rosette leaves and seedlings and to a lesser extent in cauline leaves and flowers. This expression pattern overlaps widely with the expression of *SOC1*. In contrast, *AGL14* transcripts were confined to roots as previously described (Alvarez-Buylla et al. 2000). In general, *AGL19* transcripts increase with the age of the plant (Fig. 1E). *AGL19* is enriched in apices including the shoot apical meristem and developing leaf primordia in dissected 18-d-old seedlings grown in short days (SD), while it is very weakly expressed in leaves at this developmental stage (Fig. 1F). Low gene expression levels have precluded a

more detailed analysis of the *AGL19* expression pattern in the apex by in situ hybridization. Localization studies using GFP fusion proteins showed that *AGL19* is targeted to the nucleus (Fig. 1G). Together, *AGL19*, but not its close homolog *AGL14*, is expressed with a similar organ-specificity as the well-known floral integrator *SOC1*.

AGL19 is sufficient to promote flowering

To study the effects of ectopic *AGL19* expression, we constructed transgenic plants containing the *AGL19* cDNA under the control of the strong cauliflower mosaic virus 35S promoter (*35S::AGL19*) (Fig. 2A). Five independent transgenic lines were analyzed, all of which were extremely early flowering in LD conditions and produced two to four rosette leaves at the time of bolting (Fig. 2B; Table 1). In noninductive SD conditions, *35S::AGL19* plants were also early flowering but produced more leaves than in LD, and therefore, they remained responsive to photoperiod (Fig. 2C). In the late flowering *fve* background, the overexpression of *AGL19* had the strongest effect on flowering time as *35S::AGL19* *fve* plants flowered similarly to *35S::AGL19* plants (Table 1). Thus, overexpression of *AGL19* is sufficient to promote flowering.

agl19 mutants have a decreased vernalization response

To determine whether *AGL19* normally controls flowering time in wild-type plants, we used two *agl19* insertion alleles (*agl19-1* and *agl19-2*), in which the *AGL19* transcript was undetectable (Fig. 3A,B). The *agl19* mutants flowered normally in LD conditions, but flowered slightly later than wild type in SD (Fig. 3C). Because overexpression of *AGL19* caused phenotypes similar to overexpression of *SOC1* (Borner et al. 2000), which is regulated by the vernalization pathway, we tested whether *AGL19* was also involved in the response to vernalization. Indeed, *agl19* mutants flowered consider-

Figure 2. *AGL19* promotes the transition to flowering. (A) Expression of *AGL19* in *35S::AGL19* plants. RNA was extracted from 10-d-old seedlings grown in LD. (B) Appearance of wild-type (WT) and *35S::AGL19* plants grown for 20 d in LD. (C) Flowering time of *35S::AGL19* plants grown in LD or SD. Note that for flowering time experiments in all figures, means $\pm$ S.D. are shown for at least 14 plants.

Table 1. Effect of 35S::AGL19 on flowering time in LD

Genotype	Leaf number at bolting ^a
Wild type	11.6 ± 0.5
35S::AGL19a	3.9 ± 0.4
35S::AGL19b	3.0 ± 0.6
35S::AGL19c	2.1 ± 0.4
35S::AGL19d	2.5 ± 0.5
35S::AGL19e	2.0 ± 0
Wild type	8.2 ± 0.7
<i>fve</i>	26.6 ± 1.9
35S::AGL19a	3.0 ± 0.4
<i>fve</i> 35S::AGL19a	3.8 ± 0.4

Note that two independent flowering time experiments are separated by an empty line.

^aMean ± S.D.; *n* ≥ 14.

ably later than wild type after a 6-wk cold treatment (Fig. 3C,D). Thus, *AGL19* is not only sufficient to promote flowering when ectopically expressed, but it is also required for the promotion of flowering in response to vernalization.

Vernalization induces *AGL19* expression

Because the *agl19* mutants had a reduced vernalization response, we tested whether a prolonged cold treatment affected *AGL19* transcript levels. Indeed, *AGL19* mRNA amounts increased considerably in response to an extended cold exposure (Fig. 4A). The same increase could be seen for *SOC1* after vernalization but not for *AGL19*'s closest homolog, *AGL14*. *VRN2* and *VIN3* are two crucial mediators of vernalization in *Arabidopsis* (Chandler et al. 1996; Sung and Amasino 2004). When testing induction of *AGL19* by vernalization in *vrn2* and *vin3* mutant plants, we found that *VIN3* but not *VRN2* is required for increased *AGL19* expression after vernalization (Fig. 4B,C). However, *VRN2* is needed to attain maximum *AGL19* expression. In the absence of vernalization, *FLC* represses *SOC1* (Lee et al. 2000). To test whether *FLC* represses *AGL19* as well, we measured *AGL19* expression in *flc* seedlings. Because *AGL19* levels were not increased in *flc* but even slightly reduced, *FLC* is not required to maintain *AGL19* in a repressed state before vernalization (Fig. 4D). Next, we tested whether *AGL19* affected *FLC* expression. Neither loss nor strong ectopic overexpression of *AGL19* influenced *FLC* levels considerably (Fig. 4E). After the initial repression of *FLC* by a vernalization cold treatment, flowering will only be promoted if *FLC* repression is maintained (Gendall et al. 2001; Levy et al. 2002). Mutants such as *vrn1* have a reduced vernalization response, similar to *agl19*, due to the inability to maintain stable repression of *FLC* after a vernalization treatment (Levy et al. 2002). Therefore, we tested whether the decreased vernalization response observed in *agl19* was also caused by a derepression of *FLC*. In contrast to *vrn1*, *FLC* levels remained low after vernalization in *agl19*, indicating that

AGL19 is dispensable for the establishment and maintenance of *FLC* repression (Fig. 4G). Taken together, these results show that *AGL19* is not repressed by *FLC* in wild-type plants and that the reduced vernalization response in *agl19* mutants is not caused by elevated *FLC* levels. Thus, it is possible that *AGL19* functions in an *FLC*-independent branch of the vernalization pathway.

In order to genetically test whether *AGL19* functions in the *FLC*-independent vernalization pathway, the vernalization response of the *agl19 flc* double mutant was tested. This double mutant responded less to vernalization than either single mutant (Table 2). Thus, the *agl19* and *flc* mutants exhibit an additive rather than an epistatic relationship, confirming that *AGL19* functions in an *FLC*-independent vernalization pathway. Similar to some previous reports (e.g., Zhang and van Nocker 2002), the *flc* mutant flowered later than wild type in SD after vernalization in several independent experiments. The reasons for this effect are not known, but indirect effects mediated by poorly understood *FLC* targets and functions (McKay et al. 2003; Edwards et al. 2006) might be involved.

AGL19 function does not require *SOC1*

The *FLC*-dependent vernalization pathway functions mainly to control expression of the floral integrator *SOC1* (Lee et al. 2000; Gendall et al. 2001; Hepworth et al. 2002; Levy et al. 2002; Moon et al. 2003a, 2005). Therefore, we next investigated whether the *AGL19*-

Figure 3. *agl19* mutants have a decreased response to vernalization. (A) Position of T-DNA insertions for *agl19-1* and *agl19-2* alleles. The arrow indicates the transcription start site. The gene structure was drawn according to TIGRv5 annotation. (B) *AGL19* transcripts in wild type (WT), *agl19-1*, and *agl19-2* plants. RNA was extracted from rosette leaves of 40-d-old plants grown in LD. RT-PCR was performed with 33 cycles. (C) Effect of vernalization (Vern) on the flowering time of *agl19*. Wild-type (WT) and *agl19-1* plants were exposed to either 2-d stratification (empty bars) or to 6-wk vernalization (hatched bars) at 4°C before being transferred to either LD or SD growing conditions. (D) Comparison of decreased vernalization response in *agl19-1* and *agl19-2*. Flowering time in SD without vernalization (empty bars) or after a 6-wk vernalization treatment (hatched bars).

Figure 4. *AGL19* functions in the *FLC*-independent vernalization pathway. (A) *AGL19*, *SOC1*, and *AGL14* expression in 10-d-old wild-type (WT) seedlings grown in SD with or without a 6-wk vernalization treatment (Vern). (B) *AGL19* and *SOC1* expression in 10-d-old wild-type (WT) and *vin3* seedlings grown in SD with or without a 6-wk vernalization treatment. (C) *AGL19* expression in 10-d-old wild-type (WT) (*Landsberg erecta*) and *vrn2* seedlings grown in SD with or without a 6-wk vernalization treatment. (D) Effect of *FLC* and *SOC1* on *AGL19* expression. (E) Effect of *AGL19* and *SOC1* on *FLC* expression. (F) Effect of *AGL19* on *SOC1* expression. For D–F, RNA was extracted from 10-d-old seedlings grown in LD and analyzed by Q-PCR. (G) Expression of *FLC* in wild type (WT), *agl19*, and *vrn1* after vernalization. RNA was extracted from seedlings 4, 10, and 20 d after a 2-d stratification or a 6-wk vernalization treatment. (H,I) Flowering time of wild type (WT), *agl19*, *soc1*, *35S::AGL19*, and the double mutants in LD (H) or SD (I).

containing branch of the *FLC*-independent vernalization pathway is integrated by *SOC1* as well and whether *AGL19* and *SOC1* influence each other's expression. First, we tested whether *AGL19* levels affect *SOC1* expression. However, neither loss of *AGL19* nor increased *AGL19* levels led to considerably altered *SOC1* expression in wild type (Fig. 4F). Similarly, ectopic overexpression of *AGL19* did not affect *SOC1* in *fve*, which has very low *SOC1* levels due to elevated *FLC* expression (Fig. 4F). Second, we tested whether *SOC1* levels affect *AGL19* expression. Loss of *SOC1*, which delayed flowering (Fig. 4H), reduced *AGL19* levels slightly, but ectopic overexpression of *SOC1*, which greatly accelerated flowering (Supplementary Fig. S1), strongly repressed *AGL19* (Fig. 4D). This is in contrast to *AGL24*,

which is induced by ectopic *SOC1* expression (Michaels et al. 2003). Because ectopic overexpression of *SOC1* strongly promotes flowering without increasing *AGL19* levels, *SOC1* does not function via activation of *AGL19* expression. Moreover, because ectopic overexpression of *AGL19* strongly promotes flowering without increasing *SOC1* levels, *AGL19* does not function via activation of *SOC1* expression. Rather, it is possible that *SOC1* and *AGL19* function in genetic parallel but cross-connected pathways.

To examine whether *AGL19* and *SOC1* function in the same or in parallel genetic pathways to promote flowering, the *agl19 soc1* double mutant was generated. If *AGL19* and *SOC1* act in the same pathway, then the late-flowering phenotype of the double mutant should not transgress that of the latest single mutant. However, the *agl19 soc1* double mutant flowered later than the *soc1* single mutant, and this delay was more pronounced in SD (additional 15 leaves) than in LD (additional two leaves) (Fig. 4H,I), indicating that *AGL19* and *SOC1* act in genetically different flowering pathways. Similarly, *35S::AGL19 soc1* plants were still early flowering, but showed an additive rather than an epistatic phenotype, thus confirming that *AGL19* and *SOC1* probably function in parallel pathways to promote flowering (Fig. 4H,I). Nonetheless, it is possible that *AGL19* and *SOC1* regulate common downstream targets. Indeed, expression of the floral integrator *LEAFY* (*LFY*) and of the meristem identity gene *APETALA1* (*AP1*) was increased in *35S::AGL19* and *35S::SOC1* seedlings (Supplementary Fig. S1).

Control of *AGL19* by Polycomb-group complexes

As *AGL19* expression was increased in *msi1-cs* plants, it may be repressed by a MSI1-containing *Arabidopsis* PRC2 complex, like the CLF-PRC2 complex, which functions during sporophytic development (Schubert et al. 2005). Indeed, *AGL19* was similarly up-regulated in 15-d-old *emf2* and *clf* seedlings as well as in 4-wk-old rosette leaves of *clf* and *msi1-cs* plants (Figs. 5A, 6A). Interestingly, derepression of *AGL19* in *clf* increased with plant age (Fig. 5B). These results show that MSI1, CLF, and EMF2, three likely subunits of the CLF complex, are all required to repress *AGL19* in the absence of a vernalization treatment. As *AGL19* shares similarities with *SOC1*, we tested whether CLF represses *SOC1* as well. In contrast to *AGL19*, *SOC1* expression was not

Table 2. Effect of vernalization on flowering time of *agl19* and *flc* mutants in SD

Genotype	Leaf number–vernalization ^a	Leaf number + vernalization ^a	Leaf difference ± vernalization
Wild type	58.1 ± 3.8	27.6 ± 3.2	30.5
<i>agl19</i>	59.7 ± 3.9	43.3 ± 2.4	16.4
<i>flc</i>	51.9 ± 2.3	33.1 ± 3.8	18.8
<i>agl19 flc</i>	55.2 ± 4.7	46.3 ± 1.7	8.9

^aMean ± S.D.; n ≥ 14.

Figure 5. *AGL19* is up-regulated in PcG mutants and contributes to the early flowering phenotype of *clf*. (A) Expression of *AGL19* in *clf* and *emf2*. RNA was extracted from 15-d-old seedlings grown in LD. (B) Age dependency of *AGL19* derepression in *clf*. RNA was extracted at 10, 15, and 33 d after germination. (C) Expression of *SOC1* in *clf*. RNA was extracted from 10-d-old seedlings and from rosette leaves of 21-d-old plants grown in LD. (D) *CLF*, *EMF2*, and *MSI1* expression in 10-d-old wild-type (WT) seedlings grown in SD with or without a 6-wk vernalization treatment (Vern). (E) ChIP using anti-myc antibodies and 10-d-old wild-type Landsberg *erecta* or transgenic 35S::myc-*FIE* seedlings grown in LD. Shown are PCR products of fragments II and III from the *AGL19* locus (see Fig. 6 for details) and of an unrelated *PHOSPHOFRUCTOKINASE* (*PFK*) gene. (F) Flowering time of *msi1-cs* plants in SD. (G) Flowering time of wild-type (WT), *agl19*, *clf*, and *clf agl19* plants in LD. Leaf numbers differ significantly between *clf* and *clf agl19* double mutants (t -test: $p < 0.001$). (H) Images of 22-d-old wild-type (WT), *agl19*, *clf*, and *clf agl19* plants grown in LD at 21°C. Note that all *clf* mutants are already flowering, while *agl19 clf* double mutants have only just started bolting.

increased in *clf* mutants (Fig. 5C). Similarly, no differences were seen in *SOC1* expression in *msi1-cs* leaves compared with wild-type leaves in the microarray experiments (data not shown). Together this suggests that the CLF-PRC2 complex does not regulate *SOC1*. Because vernalization activates *AGL19*, it is possible that vernalization represses *MSI1*, *CLF*, or *EMF2*. However, we found no considerable effect of vernalization on expression of these genes (Fig. 5D). Next, we tested whether *AGL19* is a direct target of the CLF complex by chromatin immunoprecipitation (ChIP). The anti-*MSI1* antiserum is not suitable for immunoprecipitation (data not shown), and no antibodies against other PRC2 subunits are available. Therefore, we used transgenic plants that express a myc-tagged *FIE* version. Using anti-myc antibodies, *AGL19* sequences were two- to threefold enriched in precipitated chromatin from the 35S::myc-*FIE* line compared with wild type (Fig. 5E). These data suggest that the CLF-PRC2 complex directly binds to the *AGL19* locus.

Consistent with the findings that *AGL19* can promote flowering and that *AGL19* expression is increased in *msi1-cs* plants, *msi1-cs* plants flowered significantly earlier than wild type in SD (Fig. 5F). Because cosuppression of *MSI1* occurs around an age of 2–3 wk in LD, it cannot affect flowering time any more at this stage, and *msi1-cs* plants were not consistently earlier flowering than wild type in LD (data not shown). Similarly, *AGL19* expression is increased in *clf* and *emf2* mutants, and both flower earlier than wild type (Yang et al. 1995; Goodrich et al. 1997), suggesting that the overexpression of *AGL19* observed in these mutants could be partially responsible for their early flowering phenotype. In order to test this hypothesis, we constructed the *clf agl19* double mutant. Loss of *AGL19* did not abolish the *clf* leaf phenotype, but partially suppressed the early flowering phenotype (Fig. 5G,H). Thus, elevated *AGL19* levels specifically contribute to the early flowering of *clf*.

MSI1 and *CLF* epigenetically regulate the *AGL19* locus

In animals, the E(Z)-related subunit of PRC2-like complexes has H3K27 methyltransferase activity (Cao and

Figure 6. Histone methylation pattern at the *AGL19* locus. (A) Expression analysis of *AGL19* in *msi1-cs* and *clf* in rosette leaves of 4-wk-old plants grown in LD. (B) Expression analysis of *AGL19* in 10-d-old seedlings grown in SD with or without a 6-wk vernalization treatment (Vern). (C) Schematic representation of *AGL19* showing intron–exon structure (annotated according to TIGRv5) and position of the fragments used for amplification by PCR following ChIP. The arrow indicates the transcription start site. (D) Quantification of multiplex PCR products after ChIP using anti-trimethyl-histone H3K27 (H3K27me3) antiserum on 4-wk-old rosette leaves from wild-type (WT), *msi1-cs*, and *clf* plants grown in LD. Values are shown as enrichment of H3K27me3 using *AGL19* specific primers relative to *PFK* for wild-type (WT), *msi1-cs*, and *clf* plants. (E) ChIP experiment performed on 10-d-old wild-type (WT) seedlings grown in SD with or without a 6-wk vernalization treatment using antibodies against H3K27me3. Values represent mean $\pm$ range from two independent ChIP experiments.

Zhang 2004). It is likely that the *Arabidopsis* E(Z) homologs, such as CLF, function as methyltransferases as well to establish repressive H3K27 trimethylation (H3K27me₃) at target chromatin (Schubert et al. 2005). Because the PRC2 subunits CLF and MSI1 repress *AGL19*, we tested whether *AGL19* chromatin was enriched in H3K27me₃ marks. ChIP experiments using 4-wk-old rosette leaves revealed a strong enrichment of H3K27me₃ at *AGL19* (Fig. 6D). Interestingly, the H3K27me₃ mark did not evenly cover the entire locus, but peaked in the 5' region of the gene (Fig. 6C, PCR fragment II). In contrast to H3K27me₃, the heterochromatin H3K9me₂ mark, which was strongly enriched in chromatin of the *Ta2* and *Cinful-like* transposons, was not detected in *AGL19* chromatin (Supplementary Fig. S2). This indicates that in rosette leaves, the *AGL19* locus normally contains H3K27me₃ but not H3K9me₂ marks. To test whether the H3K27me₃ mark depends on MSI1 and CLF, similar experiments were performed with *msi1-cs* and *clf* plants. Expression of a *PHOSPHOFRUCTOKINASE* (*PFK*) was not changed in *msi1-cs* or *clf* plants, and *PFK* was thus suitable as an internal control in multiplex PCR (Fig. 6A). In *msi1-cs* and *clf*, H3K27me₃ at *AGL19* was greatly reduced but not completely lost (1.8-fold and threefold reduction in *msi1-cs* and in *clf*, respectively) (Fig. 6D; Supplementary Fig. S2). This decrease in H3K27me₃ observed in *msi1-cs* and *clf* was also specific for the region of *AGL19* covered by PCR fragment II. Thus, MSI1 and CLF are needed to establish repressive H3K27me₃ marks at *AGL19* chromatin in a very specific pattern, but their function is most likely partially redundant with other *Arabidopsis* proteins.

Vernalization prevents H3K27 trimethylation at the *AGL19* locus

Our experiments have established that in standard LD conditions, *AGL19* chromatin carries H3K27me₃ marks that most likely repress *AGL19* transcription. Because a prolonged cold treatment strongly activates *AGL19* expression, we tested whether such a treatment also affected the H3K27me₃ marks on *AGL19* chromatin. We performed a ChIP experiment on 10-d-old wild-type seedlings grown in SD with or without a 6-wk cold treatment. *PFK* expression was not affected by vernalization and hence was used as an internal control for multiplex PCR (Fig. 6B). In nontreated seedlings, in which no *AGL19* expression was detectable (Fig. 6B), the *AGL19* locus was highly enriched for H3K27me₃ marks specifically in the 5' region of the gene (Fig. 6E; Supplementary Fig. S2). This methylation pattern was similar to that seen in the previous ChIP experiment on wild-type rosette leaves (Fig. 6D). In contrast, when *AGL19* expression was induced after cold treatment, this H3K27me₃ mark was dramatically reduced by fivefold compared with nonvernalized seedlings (Fig. 6B,E). The overlapping pattern of H3K27me₃ observed after vernalization and in the *msi1-cs* or *clf* mutants suggests that a PRC2 complex containing MSI1 and CLF is responsible for repressing *AGL19* in the absence of vernalization.

Discussion

AGL19 is a novel floral activator

AGL19 has been identified as a novel floral activator in *Arabidopsis*, because overexpression of *AGL19* greatly accelerates the transition to flowering, while loss of *AGL19* delayed flowering after a vernalization treatment. *AGL19* belongs to the MADS-box gene family, which contains master regulatory genes essential for diverse developmental processes (Becker and Theissen 2003). Plant MADS-box genes form several phylogenetically distinct groups, and *AGL19* together with its closest homolog *AGL14* (68% amino acid identity) and the well-characterized floral activator *SOC1* (*AGL20*, 46% amino acid identity) belong to the *Tm3/SOC1* clade (Becker and Theissen 2003). Although *AGL19*, *AGL14*, and *AGL20* are closely related, their expression patterns differ. In particular, *AGL14* is exclusively expressed in roots, while *AGL19* and *SOC1* are expressed in many organs of the shoot as well. Despite the prominent expression of *AGL19* in roots, root growth and macroscopic root morphology did not differ between wild-type, *agl19*, and *35S::AGL19* plants (Supplementary Fig. S2; data not shown).

AGL19 and *SOC1* both function to promote flowering, but they have diverged significantly in terms of gene regulation and flowering pathways in which they participate. The *agl19* mutant flowered normally in LD and was only slightly late flowering in SD, indicating that under standard laboratory conditions, *AGL19* has only a minor role in the promotion of flowering. In contrast, the *soc1* mutant is significantly late flowering in both LD and SD (Borner et al. 2000; Lee et al. 2000). Because *agl19* mutants could no longer adequately accelerate flowering in response to prolonged cold treatments, *AGL19* functions in the vernalization response. While *SOC1* integrates several genetic pathways that promote flowering, including the vernalization and photoperiod pathways (Borner et al. 2000; Lee et al. 2000; Samach et al. 2000; Hepworth et al. 2002; Moon et al. 2003a), *AGL19* is mainly part of the vernalization pathway.

Similarly to ectopic expression of *SOC1*, ectopic expression of *AGL19* did not only greatly accelerate flowering but caused defects in floral development as well (Borner et al. 2000; data not shown). The fact that only *35S::AGL19* but not *agl19* plants had altered flower morphology suggests that like *SOC1*, *AGL19* is also dispensable for flower development and appears to function specifically during the floral transition after vernalization.

AGL19 regulates the floral transition in an *FLC*-independent vernalization pathway

Vernalization in *Arabidopsis* acts mainly via repression of *FLC* and subsequent up-regulation of *SOC1* (Lee et al. 2000; Gendall et al. 2001; Hepworth et al. 2002; Levy et al. 2002; Moon et al. 2003a). However, *flc*-null mutants still show a vernalization response, and thus an *FLC*-

independent vernalization pathway must exist (Michaels and Amasino 2001). Although both *AGL19* and *SOC1* are up-regulated by vernalization, only *SOC1* but not *AGL19* is repressed by *FLC*. In addition, *AGL19* levels had no effect on the amount of *FLC* transcript, and *FLC* remained repressed after vernalization in the *agl19* mutant. Thus, *AGL19* does not regulate *FLC*, and the reduced vernalization response of *agl19* mutants is not caused by deregulation of *FLC* as in the *vrn1* and *vrn2* mutants. Because *agl19 flc* double mutants had an additive effect on the vernalization response, *AGL19* and *FLC* function in genetically separate pathways. The *FLC*-containing pathway requires *VIN3* and *VRN2* (Gendall et al. 2001; Sung and Amasino 2004). The *AGL19*-containing pathway requires *VIN3* but not *VRN2*. Both elevated *AGL19* and *SOC1* levels lead to the activation of *LFY* and *AP1* to promote flowering.

Interestingly, even the *agl19 flc* double mutant showed a residual vernalization response, indicating the presence of other vernalization pathways. Such a pathway might include the MADS-box gene *AGL24*, which is known to mediate an *FLC*-independent vernalization response (Yu et al. 2002; Michaels et al. 2003). Analysis of the vernalization response of *agl19 flc agl24* triple mutants will help answer this question.

Although *AGL24* acts in an *FLC*-independent pathway, it is closely linked to *SOC1*, because these two genes positively regulate each other's expression, and overexpression of one of the two genes has little effect in the absence of the other (Michaels et al. 2003). In contrast, *AGL19* and *SOC1* act at least partially independently, because there seems to be no or even negative cross-regulation between *AGL19* and *SOC1* and overexpression of *AGL19* greatly accelerated flowering in a *soc1* background. Both *agl19 soc1* as well as *35S::AGL19 soc1* showed additive rather than epistatic interactions, indicating that *SOC1* and *AGL19* act in separate genetic pathways that converge to control the common downstream targets *AP1* and *LFY*. Thus, although *SOC1*, *AGL24*, and *AGL19* share several similarities, they clearly have different roles in the genetic network controlling flowering, and are all needed to coordinate a fully functional vernalization response.

AGL19 repression depends on PRC2 subunits

In the absence of vernalization, *AGL19* is lowly expressed, and *MSI1*, *CLF*, and *EMF2* are all required for repression of *AGL19*. *MSI1*, *CLF*, and *EMF2* are homologs of *Drosophila* PRC2 subunits (Ach et al. 1997; Goodrich et al. 1997; Yoshida et al. 2001), and it was suggested that these proteins together with *FIE* form an *Arabidopsis* PRC2 complex (Chanvivattana et al. 2004). Protein-protein interaction data support this model as: *MSI1* interacts with *FIE*, *FIE* interacts with *CLF*, and *CLF* interacts with *EMF2* (Köhler et al. 2003a; Chanvivattana et al. 2004; Katz et al. 2004). ChIP experiments suggested that *AGL19* is a direct target gene of the *CLF*-PRC2 complex and established that *AGL19* chromatin is enriched in H3K27me3 marks, which at least in mammals and

insects are set by PRC2 complexes (for review, see Cao and Zhang 2004). *Arabidopsis* has several homologs of PRC2 subunits and probably contains several PRC2-like complexes, which may function in different tissues or at different developmental stages (for review, see Schubert et al. 2005). PRC2-like complexes are thought to function as transcriptional repressors, and other *Arabidopsis* PcG target genes include *PHERES1* (*PHE1*), *AGAMOUS* (*AG*), and *FLC*. *MEA*, *FIE*, and *FIS2* are required to repress *PHE1* (Köhler et al. 2003b); *MSI1*, *CLF*, *FIE*, and *EMF2* are required to repress *AG* (Chen et al. 1997; Goodrich et al. 1997; Kinoshita et al. 2001; Hennig et al. 2003); and *VRN2* is required to repress *FLC* (Gendall et al. 2001). Interestingly, *PHE1*, *AG*, and *FLC* as well as *AGL19* are all MADS-box genes, supporting the notion that in contrast to metazoan PRC2 complexes, which mostly control Homeobox genes, plant PRC2 complexes often control MADS-box transcription factors. Importantly, only *AGL19*, but not *SOC1* or *AGL14*, needs *MSI1*, *CLF*, and *EMF2* to remain repressed in the absence of vernalization (this study; cf. also microarray data in Moon et al. 2003b). This suggests that either PRC2 repression was a feature of the *Tm3/SOC1* ancestor and was lost in *SOC1* and *AGL14*, or that *AGL19* acquired PRC2 repression de novo. However, plant PRC2 complexes also have non-MADS-box targets (Katz et al. 2004), and more complete lists of primary and secondary plant and metazoan PRC2 target genes are needed for more systematic comparisons.

AGL19, which is a strong activator of flowering, is up-regulated in PRC2 mutants, and *clf*, *fie*, *emf2*, and *msi1-cs* plants are all early flowering. This suggests that *CLF*, *EMF2*, *MSI1*, and *FIE* form a PRC2 complex to control flowering time. *MSI1* not only inhibits flowering by repressing *AGL19*, but also promotes flowering by contributing to normal *SOC1* expression; this function, however, is independent of *CLF* (Bouveret et al. 2006). The contribution of increased *AGL19* expression to the early flowering phenotype of *msi1-cs* plants could not be tested due to silencing of the *35S::MSI1* transgene by the *AGL19* SALK-insertion allele in the *35S::MSI1 agl19* plants (data not shown). The *35S::MSI1* transgene is required to induce *MSI1* cosuppression. In *clf* mutants, we found that *AGL19* was partially responsible for the early flowering phenotype, as the *agl19 clf* double mutant flowered later than the *clf* mutant alone. However, derepression of *AGL19* is not the major cause of the *clf* phenotype, and this is consistent with previous reports of a prominent role of ectopic *AG* expression for the *clf* phenotype (Goodrich et al. 1997). In *clf* mutants, expression of *AG* is correctly initiated in young floral meristems, but later breaks down and becomes expressed in the outer floral whorls (Goodrich et al. 1997). Similarly, *PHE1* is initially normally expressed in *fis* mutants but is subsequently ectopically expressed in the endosperm (Köhler et al. 2003b), and *FLC* is initially repressed by vernalization treatments but subsequently becomes derepressed in *vrn2* mutants (Gendall et al. 2001). Interestingly, the pattern of progressive *AGL19* derepression in *clf* mutants is strikingly similar to that of other PcG

targets in plants and *Drosophila*, where target genes are initially expressed normally but subsequently become ectopically activated (for review, see Ringrose and Paro 2004; Brock and Fisher 2005).

H3K27 trimethylation at AGL19 chromatin depends on PRC2 subunits and is regulated by vernalization

The mechanism of transcriptional repression by PRC2 complexes is not yet completely understood, but trimethylation of H3K27 appears to be of central importance in vivo (for review, see Cao and Zhang 2004). In *Arabidopsis*, H3K27me₃ marks are found mostly in euchromatin (Lindroth et al. 2004), while H3K9me₂ marks are hallmarks of silent heterochromatic genes (Soppe et al. 2002). Because *AGL19* is only enriched in H3K27me₃ but not in H3K9me₂, repression of *AGL19* is probably different from heterochromatic silencing as observed for many transposons. Instead, *AGL19* appears to be a euchromatic gene that is repressed by H3K27me₃. Interestingly, vernalization reduced H3K27me₃ marks to lower levels than either reductions of MSI1 or loss of CLF. In the case of *msi1-cs*, the ~5% residual MSI1 protein levels or one of the other four MSI1-like proteins in *Arabidopsis* could be sufficient to maintain partial H3K27me₃ at the *AGL19* locus. Similarly, partial redundancy of CLF and its homolog SWN has been observed (Chanvivattana et al. 2004). Thus, it is possible that functional redundancies attenuate the effects on *AGL19* chromatin modification in *msi1-cs* and *clf*.

H3K27me₃ was not spread uniformly across the *AGL19* locus but peaked dramatically near the transcription start site. Similarly, binding of *Drosophila* PcG proteins seems to cluster at Polycomb-responsive elements (PREs) and promoters rather than being spread uniformly over target loci (Ringrose and Paro 2004; Brock and Fisher 2005). Consistent with this local clustering, Polycomb silencing blocks transcription initiation in *Drosophila* (Dellino et al. 2004). In *Arabidopsis*, the FIS PRC2 complex was found to bind preferentially at the *PHE1* promoter (Köhler et al. 2003b), and *FLC* chromatin is enriched in H₃ methylation at the promoter and the first large intron (Bastow et al. 2004; Sung and Amasino 2004). Taken together, these results suggest that *AGL19* is repressed by a PRC2 complex containing MSI1, CLF, FIE, and EMF2 that sets H3K27me₃ marks close to the transcriptional start site, similar to the situation in other PcG target genes.

Vernalization overcomes the repressive effect of the CLF-PRC2 complex. This could be carried out by active removal of the repressive H3K27me₃ marks at *AGL19*. Because no biochemical activity that removes trimethylation is known, it is more likely that vernalization prevents the establishment and/or maintenance of H3K27me₃ by the CLF-PRC2 complex during cell proliferation. This interpretation is supported by observations that vernalization often acts on dividing cells such as those present in meristems (for review, see Ausin et al. 2005). An alternative mechanism could involve exchange of methylated histones in *AGL19* chromatin.

Together, the vernalization pathway in *Arabidopsis* contains at least two branches—the *FLC*-dependent and the *FLC*-independent branches, both of which require the regulatory effects of PcG proteins. While VRN2 functions in repressing the floral repressor *FLC* after vernalization, the CLF complex functions in repressing the floral activator *AGL19* before vernalization. Thus, in *Arabidopsis*, different PcG proteins have been recruited to coordinate the vernalization response and to control the important developmental switch to reproductive growth.

Materials and methods

Plant material and growth conditions

Line *msi1OE2*, which ectopically expresses MSI1 and gives rise to *msi1-cs* plants, was described before (Hennig et al. 2003). To construct plants expressing an epitope-tagged version of FIE, a construct encoding an N-terminal fusion of myc to FIE was cloned under the control of the 35S promoter and transformed into wild-type (*Ler*) plants. Line 4, which expressed the myc-FIE fusion (Supplementary Fig. S1), was used for further experiments. The two null *AGL19* mutant alleles, *agl19-1* (SALK_N578786) and *agl19-2* (SALK_N516657) were obtained from the Nottingham *Arabidopsis* Seed Stock Center (NASC). The *agl19-1* allele was used for all crossings and flowering time experiments unless otherwise stated. The *fve-5*, *flc-6*, *vrn1-5*, and *clf-29* mutants are null alleles (Supplementary Fig. S1; Bouveret et al. 2006) from various collections of T-DNA insertion lines (SAIL_1167E5, SALK_41126, WiscDsLox393-396F9, and SALK_N521003, respectively) (Sessions et al. 2002; Alonso et al. 2003). All plants used in this study are in the Columbia background unless otherwise stated. Seeds of *soc1-2*, *emf2-10*, and *vrn2-1* have been described (Chandler et al. 1996; Lee et al. 2000; Chanvivattana et al. 2004) and were kindly provided by I. Lee (Seoul National University, Seoul, Korea), J. Goodrich (University of Edinburgh, Edinburgh, UK), and C. Dean (John Innes Centre, Norwich, UK), respectively.

To construct plants that ectopically overexpressed *AGL19* or *SOC1* (35S::*AGL19* and 35S::*SOC1*), the full-length coding sequences were amplified by PCR (for primers, see Supplementary Table S1). The cDNAs were inserted into binary destination vectors (*AGL19* in pH7WG2; *SOC1* in pK7WG2) (Karimi et al. 2002) downstream of the cauliflower mosaic virus (CaMV) 35S promoter. Constructs were transformed into Columbia wild-type plants, and 43 primary 35S::*AGL19* transformants were obtained, of which five (35S::*AGL19a-e*) were selected for further study. The weak line 35S::*AGL19a* was used for all crossings and flowering time experiments unless otherwise stated.

For cellular localization studies, the full-length *AGL19* cDNA was fused to an N-terminal GFP tag in vector pK7WGF2 (Karimi et al. 2002). *AGL19_pK7WGF2* was used directly for bombardment of onion cells for transient expression assays as described previously (Gendall et al. 2001).

For measuring flowering time, seeds were plated on Murashige and Skoog (MS) medium (Duchefa), stratified for 2 d at 4°C, and grown on plates for 10 d before transfer onto soil. Plants were kept in Conviron growth chambers with mixed cold fluorescent and incandescent light (110–140 μmol/m²-sec, 21°C ± 2°C) under LD (16 h light) photoperiods unless indicated otherwise. The flowering time was measured as the number of total rosette leaves longer than 0.5 cm at bolting for at least 14 plants. Graphs show means ± standard deviation. For the vernalization treatment, seeds were plated on MS medium and kept in continuous light for 1 d before being exposed to 4°C for

6 wk. After the vernalization treatment, plants were transferred to growth chambers (21°C ± 2°C) under LD or SD (8 h light) photoperiods as indicated.

RNA isolation and RT-PCR

RNA was extracted as previously described (Hennig et al. 2003). DNA-free RNA was reverse-transcribed using an oligo(dT) primer and SuperScript II reverse transcriptase (Invitrogen). Aliquots of the generated cDNA, which equaled 100 ng of total RNA, were used as the template for PCR with gene-specific primers (Supplementary Tables S2, S3). Q-PCR was performed in an ABI Prism 7700 Sequence Detection System (Applied Biosystems), using SYBR Green Master Mix reagent (Applied Biosystems) according to the manufacturer's instructions. All amplification plots were analyzed with a fluorescence signal threshold of 0.1 to obtain Ct (threshold cycle) values. Experiments were performed in duplicate with error bars representing the range. Gene expression levels were normalized to an actin (*ACT*) control gene. The average Ct value for *ACT* was 23.83 (±0.18) for all templates measured in these experiments.

Microarray hybridization and evaluation

Arabidopsis wild-type (accession Columbia) and *msi1OEc2* plants (Hennig et al. 2003) were grown for 23 d in growth chambers at 21°C under LD photoperiods. After identification of *msi1-cs* plants from the segregating *msi1OEc2* population based on phenotype, rosette leaves were harvested. Material was harvested from at least 12 plants and pooled for each sample. The experiment was repeated twice to give two independent biological replicates. Affymetrix *Arabidopsis* ATH1 GeneChips were used in the experiment (Affymetrix). Labeling of samples, hybridizations, and measurements were performed as described (Hennig et al. 2004). Signal values were derived using the GCRMA algorithm in the statistical package R (version 1.9.1). Significantly differential gene expression was detected based on the rank-product algorithm (Breitling et al. 2004). This algorithm inherently corrects for multiple testing. Genes were considered as differentially expressed if $p < 0.05$.

ChIP

ChIP was performed as previously described (Bowler et al. 2004). Anti-myc 9E11 antibodies (Eurogentec), anti-dimethyl-histone H3 Lys 9 antiserum (Upstate, catalog #07-441), and anti-trimethyl-histone H3 Lys 27 antiserum (Upstate, catalog #07-449) were used for immunoprecipitation.

Immunoprecipitated DNA was analyzed by PCR using gene specific primers for *AGL19* and primers against the control gene *PFK* (*At4g04040*) (Supplementary Table S4). Relative efficiencies of primer pairs were determined based on dilution series of input DNA (Supplementary Fig. S3B). Band intensities were quantified using Image J software. For quantitative displays of ChIP experiments, the values shown represent enrichment relative to *PFK* using the previously determined primer efficiencies. Bars represent mean and range for two independent ChIP experiments.

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Schönrock et al.

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