Different *Polycomb* group complexes regulate common target genes in *Arabidopsis*

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***Polycomb* group (PcG) proteins convey epigenetic inheritance of repressed transcriptional states. Although the mechanism of the action of PcG is not completely understood, methylation of histone H3 lysine 27 (H3K27) is important in establishing PcG-mediated transcriptional repression. We show that the plant PcG target gene *PHERES1* is regulated by histone trimethylation on H3K27 residues mediated by at least two different PcG complexes in plants, containing the SET domain proteins MEDEA or CURLY LEAF/SWINGER. Furthermore, we identify *FUSCA3* as a potential PcG target gene and show that *FUSCA3* is regulated by MEDEA and CURLY LEAF/SWINGER. We propose that different PcG complexes regulate a common set of target genes during the different stages of plant development.**

Keywords: Polycomb group proteins; histone methylation; *PHERES1*; MEDEA; CURLY LEAF

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INTRODUCTION

The regular development of multicellular organisms depends on the stable propagation of established gene expression states during mitotic cell divisions, and in both animals and plants *Trithorax* (Trx) and *Polycomb* group (PcG) proteins carry out this task. Some Trx and PcG proteins have intrinsic histone methyltransferase activity, which is mediated by the evolutionarily conserved 130-residue SET domain (for SU[VAR]3–9, Enhancer of Zeste (E(Z)), TRX), suggesting that the maintenance of cellular memory

involves methylation of histones (Cao & Zhang, 2004). However, the enzymatic activity of the PcG SET domain protein E(Z) was detectable only if the protein was assembled with ESC, SUZ12 and p55 in the *Polycomb* repressive complex 2 (PRC2; Cao & Zhang, 2004). The PRC2 complex suppresses *Homeobox* (*Hox*) genes and other target genes by setting trimethylation marks on histone H3 lysine 27 (H3K27me3). The histone methyltransferase activity of E(Z) is essential for target gene silencing, as an E(Z)^{H703K} mutant protein, which has reduced enzymatic activity, fails to mediate *Hox* gene silencing (Müller *et al.*, 2002).

In contrast to animals, plant PcG proteins are often encoded by small gene families. In *Arabidopsis*, there are three E(Z) homologues (MEDEA (MEA), CURLY LEAF (CLF) and SWINGER (SWN); Guitton & Berger, 2005), three SUZ12 homologues (FERTILIZATION INDEPENDENT SEED2 (FIS2), VERNALIZATION2 and EMBRYONIC FLOWER2; Guitton & Berger, 2005) and five p55 homologues (MULTICOPY SUPPRESSOR OF IRA1 (MSI1–5); Hennig *et al.*, 2005). Genetic evidence suggests that there are different PcG complexes in plants, regulating different developmental pathways. One PRC2-like complex, which contains MEA, MSI1 and the ESC homologue FERTILIZATION INDEPENDENT ENDOSPERM (FIE), has been detected in flowers and seeds (Köhler *et al.*, 2003a). Interaction studies in yeast suggest that FIS2 is also part of this complex (Chanvivattana *et al.*, 2004). Mutations in the corresponding genes result in the development of seed-like structures without fertilization. If fertilization occurs, seeds that inherit a mutated maternal allele have proliferation defects in the embryo and endosperm and abort (Guitton & Berger, 2005). The only known direct target gene of MEA is the type I MADS-box gene *PHERES1* (*PHE1*); MEA regulates allele-specific expression of *PHE1* by repressing the maternal *PHE1* allele (Köhler *et al.*, 2003b, 2005). By contrast, CLF regulates leaf and floral organ development, and *AGAMOUS* (AG) and *APETALA3* (AP3) are probably direct target genes (Goodrich *et al.*, 1997; D.S. and J.G., unpublished data).

So far, it is unclear how plant PcG complexes regulate their target genes and whether different PcG complexes act on distinct targets. Our results show that PcG-mediated gene repression is

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Fig 1 | Dynamics of chromatin modifications at the *PHE1* locus during development are correlated with the expression profile of *MEA* as well as with the binding of *MEA* to promoter regions of *PHE1*. (A) Schematic diagram of the *PHE1* locus indicating the regions analysed by quantitative PCR after chromatin immunoprecipitation (ChIP). The numbers indicate the position relative to the translational start site of *PHE1*. (B) ChIP analysis of *MEA* binding to the *PHE1* locus during reproductive development. Material used: closed flowers for 0 days after pollination (DAP), open flowers for 1 DAP and siliques for 2–6 DAP. For relative enrichment values, the ratios between *PHE1* and *UBQ11* enrichments were calculated. (C) Relative *MEA* messenger RNA levels in wild-type flowers before fertilization (0 DAP) and siliques harvested at different DAP. (D) ChIP analysis of different histone modification marks at region 2 of *PHE1* using closed flowers. (E) ChIP analysis of H3K27me3 marks at the *PHE1* locus during reproductive development. Material used: closed flowers for 0 DAP, open flowers for 1 DAP and siliques for 2–6 DAP. (F) ChIP analysis of H3K27me3 at region 2 of *PHE1* in wild type (WT) and *mea/mea* mutants using closed flowers. *MEA*, *MEDEA*; *PHE1*, *PHERES1*.

established by an evolutionarily conserved mechanism and that different PcG complexes share a common subset of target genes during the different stages of plant development.

RESULTS

The *PHERES1* locus contains H3K27 methylation marks

To test whether *MEA* binding at the *PHE1* locus causes histone modifications, we analysed the *MEA*-binding and histone methylation profiles at the *PHE1* locus by performing chromatin immunoprecipitation (ChIP) experiments, using flowers harvested before pollination, open pollinated flowers, siliques at 2–3 days after pollination (DAP) and siliques at 4–6 DAP. We found preferential binding of *MEA* to *PHE1* regions 1 and 2, with a peak of *MEA* binding detected in region 2 before fertilization and a second peak in regions 1 and 2 in siliques at 2–3 DAP (Fig 1A,B). We detected two peaks of *MEA* expression: one before fertilization and the other 2 days after fertilization (Fig 1C), which shows that *MEA* messenger RNA levels correlate with *MEA* binding

to the *PHE1* locus. As *MEA* binding occurred preferentially to region 2 of *PHE1*, we investigated the presence of different histone modifications in this region using closed flowers as the input material (peak 1 in the time course). We found strong enrichment of H3K27me3 as well as weak enrichment of H3K9me2. No enrichment was detected for H3K27me2 or the euchromatic mark H3K4me2 (Fig 1D).

Despite anti-H3K27me3 antibodies also recognizing H3K27me2, and anti-H3K27me2 antibodies recognizing H3K27me3, both have a clear preference for their main substrates (Peters et al, 2002). Thus, the main methylation mark on the *PHE1* locus is H3K27me3. We analysed whether *MEA* binding correlates with the presence of H3K27me3 at the *PHE1* locus and found the strongest enrichment of H3K27me3 in closed flowers at region 2, overlapping with the peak of *MEA* binding. However, we also detected H3K27me3 at fragments 1, 3 and 4, which indicated that *MEA* also binds to these regions, but at a lower frequency. After fertilization, lower levels of H3K27me3

Fig 2 | The SET domain of MEA is necessary for *PHE1* repression. (A) Alignment of conserved parts of the SET domains of MEA, CLF, SWN, E(Z) and KYP. Black boxes indicate identical amino acids and grey boxes indicate conservative changes. The conserved histidine residues are indicated by a star. Numbers indicate the positions of amino acids. (B) Open siliques of wild-type (WT), *mea*/MEA and *mea*/MEA plants containing WT MEA (*MEA*^{wt}) or the *MEA*^{H621K} construct. (C) Western blot analysis of MEA expression in WT, *mea*/*mea* and *mea*/*mea* plants containing the *MEA*^{H621K} construct. The Coomassie stain of a replicate gel is shown as a loading control. (D) *PHE1*::*GUS* expression in *mea* seeds containing the *MEA*^{wt} or the *MEA*^{H621K} construct. Scale bars, 100 μ m. CLF, CURLY LEAF; E(Z), ENHANCER OF ZESTE; GUS, β -GLUCURONIDASE; KYP, KRYPTONITE; MEA, MEDEA; *PHE1*, *PHERES1*; SWN, SWINGER.

were detected, followed by a second peak at 2–3 DAP, also correlating with stronger MEA binding at this stage (Fig 1E). MEA expression, MEA binding to *PHE1* and enrichment of H3K27me3 at *PHE1* all had two peaks during reproductive development, prompting us to speculate that this reflects separate functions of the MEA PRC2 complex before and after fertilization.

The correlation of MEA binding and H3K27me3 at the *PHE1* locus suggests that MEA is mediating *PHE1* repression by setting H3K27me3. To test this hypothesis, we analysed H3K27me3 in closed flowers of homozygous *mea* mutants. In *mea* mutant flowers, H3K27me3 was reduced to half of the wild-type levels (Fig 1F). This indicates that MEA is indeed mediating trimethylation of H3K27 at the *PHE1* locus. However, the substantial levels of H3K27me3 remaining in *mea* mutants suggest that H3K27 in *PHE1* chromatin is also methylated by different methyltransferases.

The SET domain of MEA is necessary for *PHE1* repression

To test whether MEA-dependent H3K27me3 on *PHE1* is required for MEA function, we mutated the catalytic centre of the SET domain of MEA by introducing a histidine 621 to lysine (H621K) point mutation. The equivalent mutation of the conserved histidine in the catalytic centre of the MEA homologue E(Z) markedly impairs the histone methyltransferase activity of the E(Z) protein (Müller et al, 2002; Fig 2A). The mutated complementary DNA, driven by the *MEA* promoter, was introduced into *mea* mutant plants. No transgenic line out of 27 primary transformants showed complementation of the *mea* seed abortion phenotype

(seed abortion ratios between 45% ($\chi^2 = 2.1 < \chi^2_{0.05[1]} = 3.84$) and 55% ($\chi^2 = 1.6 < \chi^2_{0.05[1]} = 3.84$). By contrast, 5 out of 18 lines containing the wild-type MEA sequence (*MEA*^{wt}) showed complementation of the seed abortion phenotype (seed abortion ratios between 31% ($\chi^2 = 2.5 < \chi^2_{0.05[1]} = 3.84$) and 29% ($\chi^2 = 2.3 < \chi^2_{0.05[1]} = 3.84$; Fig 2B). The H621K mutation does not affect protein stability, as homozygous *mea* mutant plants expressing the *MEA*^{H621K} version contain even higher MEA levels than wild-type plants, confirming that MEA expression is regulated by a negative feedback loop (Fig 2C; Gehring et al, 2006; Jullien et al, 2006). We then addressed whether the MEA H621K mutation also impairs *PHE1* repression. We crossed three transgenic lines expressing *MEA*^{H621K} and three lines expressing *MEA*^{wt} with a *PHE1*:: β -GLUCURONIDASE (*GUS*) reporter line and assayed GUS activity in seeds. In *mea* mutant seeds of all three lines expressing *MEA*^{wt}, *PHE1*::*GUS* expression was restricted to the chalazal region of the endosperm, as in the wild type. By contrast, in *mea* mutant seeds expressing *MEA*^{H621K}, *PHE1*::*GUS* expression was detectable in the embryo and endosperm at the heart stage (Fig 2D; Supplementary Table SI online). These data indicate that the histone methyltransferase activity of MEA is necessary for normal MEA function, including *PHE1* repression.

CLF and SWN redundantly regulate *PHE1* repression

Although MEA is in part responsible for the H3K27me3 at *PHE1*, the significant residual H3K27me3 enrichment in flowers of homozygous *mea* mutants shows that MEA is not exclusively

Fig 3 | Regulation of *PHE1* repression in sporophytic tissues by CLF and SWN. (A) Chromatin immunoprecipitation (ChIP) analysis of H3K27me3 at region 2 of *PHE1* in wild type (WT), *clf* and *clf swn* mutants. Light grey bars and dark grey bars indicate samples of closed flowers and leaf samples, respectively. (B) Relative messenger RNA levels of *PHE1* in sporophytic tissues of WT, *clf*, *swn* and *clf swn* mutants. *PHE1* mRNA levels in WT seeds at 3 days after pollination indicate maximum *PHE1* expression of 1.0. Light grey bars and dark grey bars indicate samples of seeds and leaf samples, respectively. CLF, CURLY LEAF; *PHE1*, *PHERES1*; SWN, SWINGER.

responsible for *PHE1* methylation. Therefore, we tested whether *PHE1* is also methylated in sporophytic tissues (leaves), in which *MEA* is not expressed (Kiyosue et al, 1999). We found that H3K27me3 was also strongly enriched in *PHE1* chromatin from leaves (Fig 3A). CLF and SWN are two close homologues of *MEA*; therefore, we tested whether sporophytic H3K27me3 on *PHE1* requires one or both proteins. However, H3K27me3 at the *PHE1* locus was unchanged in *clf* leaves (Fig 3A) and in *mea/MEA;swn/swn* leaves (data not shown). By contrast, *clf swn* double mutants had much less H3K27me3 on *PHE1* than wild-type leaves (Fig 3A). Thus, CLF and SWN are redundantly needed for trimethylation of H3K27 at the *PHE1* locus. As *PHE1* is not expressed in wild-type leaves, we asked whether *PHE1* repression involves H3K27me3. We analysed *PHE1* expression in the leaves of *clf* and *swn* single mutants and in *clf swn* double mutants. *PHE1* was not expressed in the leaves of wild type, *clf* and *swn* single mutants (Fig 3B). By contrast, *PHE1* expression was upregulated in *clf swn* double mutants (Fig 3B). However, *PHE1* expression levels were much lower in *clf swn* than in wild-type siliques, which suggests that, in addition to the loss of H3K27me3, some activating signals are needed to completely activate *PHE1* expression.

MEA, CLF and SWN regulate common target genes

Our data establish that CLF and SWN repress the *MEA* target gene *PHE1* by histone methylation in sporophytic tissues. Next, we asked whether other plant PcG target genes are repressed by different PcG complexes as well. *AG* and *AP3* are probably direct target genes of CLF and SWN (Goodrich et al, 1997; D.S. and J.G., unpublished data); therefore, we analysed whether the expression of these genes is also upregulated in *mea* mutant seeds. Upregulation of *AG* and *AP3* was observed in *clf swn*, but not in the seeds of *mea/MEA* mutant plants (data not shown). However, both genes were not repressed but highly expressed in wild-type seeds at 3 DAP; thus, the *mea* mutation probably has no impact on *AG* or *AP3* expression at this developmental stage. Therefore, we tested the expression of other potential CLF and SWN targets. *clf swn* double mutants form somatic embryos, suggesting a misexpression of embryonic regulators such as *LEAFY COTYLEDON1* (*LEC1*), *LEC2* and *FUSCA3* (*FUS3*; Lotan et al, 1998; Stone et al, 2001; Chanvivattana et al, 2004). Indeed, *FUS3* was highly upregulated, and *LEC1* and *LEC2* were mildly upregulated in *clf swn* mutants (Fig 4A). Similarly, we also observed strong increased expression of a *FUS3::GUS* reporter gene in *clf swn* mutants (Fig 4B). Thus, the embryonic regulators *LEC1*, *LEC2* and *FUS3* are potential target genes of CLF and SWN. We then tested whether *LEC1*, *LEC2* and *FUS3* are also upregulated in *mea* mutant seeds. *FUS3* expression was increased in siliques of *mea/MEA* mutant plants at 2–3 DAP (Fig 4C), whereas *LEC1* and *LEC2* expression was at very low levels in both mutant and wild-type seeds (data not shown). In a parallel approach, we analysed the expression of *FUS3::GUS* and *LEC2::GUS* reporter constructs in *mea* mutant and wild-type seeds. In wild-type seeds, expression of *FUS3::GUS* was restricted to the embryo until the heart stage, whereas in *mea* mutant seeds, *GUS* expression was detectable in the endosperm as well (*mea/MEA; FUS3::GUS/FUS3::GUS*; 114:156, strongly staining seeds:weakly staining seeds; 1:1; $\chi^2 = 6.5 < \chi^2_{0.01[1]} = 6.635$, Fig 4D). By contrast, *LEC2::GUS* expression was similar in wild-type and *mea* mutant seeds and remained restricted to the embryo (data not shown). To test whether *FUS3* is a direct target gene of *MEA*, we performed ChIP experiments with anti-*MEA* and anti-H3K27me3 antibodies using flowers harvested before pollination. We detected a clear enrichment for *FUS3* with both antibodies, which demonstrates that *FUS3* is a direct target gene of *MEA* (Fig 4E,F). These data establish that a subset of plant PcG target genes is regulated by different PcG complexes during the different stages of development.

DISCUSSION

PHE1 chromatin is strongly enriched in H3K27me3, and also contains some H3K9me2. Both marks are involved in transcriptional repression in many eukaryotes (Martin & Zhang, 2005), and gene silencing by PRC2 in animals involves H3K27me3 (Cao & Zhang, 2004). In *Arabidopsis*, H3K27me3 localizes primarily to euchromatin, and H3K27me2 localizes to euchromatin and heterochromatin, whereas H3K9me2 localizes almost exclusively to heterochromatin (Lindroth et al, 2004). The strong enrichment of the euchromatic mark H3K27me3 on the *PHE1* locus suggests that silencing of *PHE1* occurs in euchromatin and differs from the heterochromatic silencing of transposons. In plants, methylation of H3K27 has been also reported for *FLOWERING LOCUS C* (*FLC*; Bastow et al, 2004; Sung & Amasino, 2004), which is a possible

Fig 4 | *FUS3* is regulated by CLF, SWN and MEA. (A) Relative messenger RNA levels of *FUS3*, *LEC1* and *LEC2* in *clf swn* double mutants in comparison with wild-type (WT) leaves. (B) *FUS3::GUS* expression in WT and *clf swn* mutant seedlings. Scale bars, 1 mm. (C) Relative mRNA levels of *FUS3* in silicles of WT and *mea*/MEA mutants at 3 days after pollination (light grey bars). Expression of *FUS3* in *clf swn* and WT leaves is shown as a comparison (dark grey bars). (D) *FUS3::GUS* expression in WT and *mea* mutant seeds. Scale bars, 100 μ m. (E) Chromatin immunoprecipitation (ChIP) analysis of MEA binding to the *FUS3* locus in closed flowers. (F) ChIP analysis of H3K27me3 marks at the *FUS3* locus in closed flowers. CLF, CURLY LEAF; *FUS3*, *FUSCA3*; GUS, β -GLUCURONIDASE; MEA, MEDEA; SWN, SWINGER.

target of the VRN2 PcG protein, as well as of MEA itself (Gehring et al, 2006; Jullien et al, 2006); however, it is not known whether H3K27me2 or H3K27me3 is the dominating repressive mark. Our results establish that, in the case of *PHE1*, PRC2 repression involves mainly H3K27me3 and not H3K27me2. In addition to H3K27me3, the *PHE1* locus contains H3K9me2 in closed flowers but not at later stages (data not shown). Similarly, the *FLC* locus contains H3K9me2 (Bastow et al, 2004), and it has been proposed that methylation of both H3K9 and H3K27 is required for recruiting the CMT3 methyltransferase to heterochromatic loci (Lindroth et al, 2004).

PHE1 is a target of the plant PcG protein MEA, and H3K27me3 on *PHE1* depends in part on MEA. H3K27me3 seems to be necessary for *PHE1* repression, as an H621K mutation in the catalytic centre of the SET domain of MEA causes *PHE1* de-repression. The equivalent mutation in E(Z) impairs histone methyltransferase activity and *Hox* gene silencing (Müller et al, 2002), suggesting that MEA and E(Z) operate through similar mechanisms. MEA binding was not uniform along *PHE1* chromatin but was strongest around the translation start. This is consistent with the idea that PcG silencing blocks transcription initiation in *Drosophila* (Dellino et al, 2004). Thus, repression of PcG target genes by H3K27 trimethylation seems to be conserved in animals and plants.

We show that *PHE1* is redundantly methylated by CLF and SWN in sporophytic tissues, and low levels of H3K27me3 methylation in *clf swn* double mutants correlate with upregulated *PHE1* expression. Thus, *PHE1* repression depends on MEA-containing, as well as CLF- or SWN-containing, PRC2 complexes. In addition, we identified *FUS3* as a common target gene of MEA as well as CLF and SWN, indicating that at least a subset of plant PcG target genes are under repressive control of MEA-containing, as well as CLF- and SWN-containing, PRC2 complexes. *FUS3* controls the transition from embryonic to vegetative development through terpenoid hormone signalling (Gazzarrini et al, 2004), and the functional connection between PRC2-like complexes and *FUS3* will be the subject of future investigations.

We conclude that the PcG genes MEA, CLF and SWN share at least a subset of common target genes during the different stages of plant development. MEA is required for PcG target repression during gametophyte and early seed development, whereas CLF and SWN take over this function during later sporophytic development.

METHODS

Plant material and growth conditions. The *mea* mutant used in this study was the *mea-1* allele described by Grossniklaus et al (1998). The *clf* and *swn* mutants are T-DNA insertion mutants

SALK_021003 and SALK_050195, respectively. The *PHE1::GUS* line has been described previously by Köhler et al (2003b). The *LEC2::GUS* and *FUS3::GUS* lines were kindly provided by François Parcy. Plants were grown in a greenhouse at 70% humidity and daily cycles of 16 h light at 21°C and 8 h darkness at 18°C.

Plasmid constructs. The MEA complementation construct was generated in pCAMBIA 3300 by fusing 3.8 kb upstream sequence of the MEA translational start to the MEA cDNA. The MEA^{H621K} mutation was created using the QuikChange Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions.

Quantitative reverse transcriptase-PCR, GUS expression and protein analysis. See the supplementary information online for details.

Chromatin immunoprecipitation. We followed the ChIP protocol of Johnson et al (2002); the details are given in the supplementary information online.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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REFERENCES

- Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* **427**: 164–167
- Cao R, Zhang Y (2004) The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr Opin Genet Dev* **14**: 155–164
- Chanvittana Y, Bishopp A, Schubert D, Stock C, Moon YH, Sung ZR, Goodrich J (2004) Interaction of Polycomb-group proteins controlling flowering in *Arabidopsis*. *Development* **131**: 5263–5276
- Dellino GI, Schwartz YB, Farkas G, McCabe D, Elgin SC, Pirrotta V (2004) Polycomb silencing blocks transcription initiation. *Mol Cell* **13**: 887–893
- Gazzarrini S, Tsuchiya Y, Lumba S, Okamoto M, McCourt P (2004) The transcription factor FUSCA3 controls developmental timing in *Arabidopsis* through the hormones gibberellin and abscisic acid. *Dev Cell* **7**: 373–385
- Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, Fischer RL (2006) DEMETER DNA glycosylase establishes *MEDEA* Polycomb gene self-imprinting by allele-specific demethylation. *Cell* **124**: 495–506
- Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G (1997) A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature* **386**: 44–51
- Guitton AE, Berger F (2005) Control of reproduction by Polycomb group complexes in animals and plants. *Int J Dev Biol* **49**: 707–716
- Grossniklaus U, Vielle-Calzada JP, Hoepfner MA, Gagliano WB (1998) Maternal control of embryogenesis by *MEDEA*, a Polycomb group gene in *Arabidopsis*. *Science* **280**: 446–450
- Hennig L, Bouveret R, Grissem W (2005) MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. *Trends Cell Biol* **15**: 295–302
- Johnson L, Cao X, Jacobsen S (2002) Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. *Curr Biol* **12**: 1360–1367
- Jullien PE, Katz A, Oliva M, Ohad N, Berger F (2006) Polycomb group complexes self-regulate imprinting of the Polycomb group gene *MEDEA* in *Arabidopsis*. *Curr Biol* **16**: 486–492
- Kiyosue T et al (1999) Control of fertilization-independent endosperm development by the *MEDEA* Polycomb gene in *Arabidopsis*. *Proc Natl Acad Sci USA* **96**: 4186–4191
- Köhler C, Hennig L, Bouveret R, Gheysels J, Grossniklaus U, Grissem W (2003a) *Arabidopsis* MSI1 is a component of the MEA/FIE Polycomb group complex and required for seed development. *EMBO J* **22**: 4804–4814
- Köhler C, Hennig L, Spillane C, Pien S, Grissem W, Grossniklaus U (2003b) The Polycomb-group protein *MEDEA* regulates seed development by controlling expression of the MADS-box gene *PHERES1*. *Genes Dev* **17**: 1540–1553
- Köhler C, Page DR, Gagliardini V, Grossniklaus U (2005) The *Arabidopsis thaliana* *MEDEA* Polycomb group protein controls expression of *PHERES1* by parental imprinting. *Nat Genet* **37**: 28–30
- Lindroth AM et al (2004) Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with CHROMOMETHYLASE3. *EMBO J* **23**: 4286–4296
- Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) *Arabidopsis* *LEAFY* *COTYLEDON1* is sufficient to induce embryo development in vegetative cells. *Cell* **93**: 1195–1205
- Martin C, Zhang Y (2005) The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol* **6**: 838–849
- Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, Miller EL, O'Connor MB, Kingston RE, Simon JA (2002) Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* **111**: 197–208
- Peters AH, Mermoud JE, O'Carroll D, Pagani M, Schweizer D, Brockdorff N, Jenuwein T (2002) Histone H3 lysine 9 methylation is an epigenetic imprint of facultative heterochromatin. *Nat Genet* **30**: 77–80
- Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ (2001) *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. *Proc Natl Acad Sci USA* **98**: 11806–11811
- Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the Phd finger protein VIN3. *Nature* **427**: 159–164