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Control of reproduction by Polycomb Group complexes in animals and plants

ANNE-ELISABETH GUITTON and FREDERIC BERGER*

Chromatin and Reproduction Group, Temasek Lifescience Laboratory, National University of Singapore, Singapore

ABSTRACT In both mammals and plants, Polycomb Repressive Complexes 2 (PRC2) are conserved and appear to be involved in the transition between vegetative or somatic and reproductive state in plants and mammals. In plants at least three different PRC2 control temporal aspects of development, and mutations in PcG cause heterochronies. Such heterochronic mutations affect the transition to flowering. During gametogenesis the Fertilization-Independent Endosperm-MEDEA-PRC2 (FIE-MEA PRC2) complex controls gametogenesis in synergy with a Retinoblastoma-dependent pathway. Several genes of the FIE-MEA pathway are imprinted as shown by their uniparental allele expression in the endosperm, the interface controlling maternal nutrition of the embryo in the seed. Imprinting is also a major feature for genes expressed in the placenta in mammals. Recent data have shown that imprinting in both placenta and endosperm likely share similar mechanisms involving cooperation between the PRC2 complexes and DNA methylation.

KEY WORDS: *polycomb, endosperm, flowering, reproduction, placenta*

Flowering plants and mammals are characterised by full maternal control of their sexual reproduction. Fertilisation occurs inside the maternal reproductive tract and the mother nurtures the embryo through a specialised interface, the placenta in mammals and the endosperm in flowering plants. The placenta develops from a specific mass of cell, the trophoblast, set apart after the first divisions of the embryo. The endosperm is the product of fertilisation of a secondary female gamete, the central cell. The central cell is genetically identical to the egg cell. In most plant species the central cell contains two copies of the maternal genome, leading to triploid endosperm. The fertilised egg cell gives rise to the embryo. As the two male gametes of a given pollen tube originate from a single mitosis, they are genetically identical and their zygotic fusion products are also genetically identical. In spite of their unique genetic identity, the embryo and the specialised maternal interface structure (endosperm or placenta) follow very divergent developmental pathways. Such divergence can only be accounted for by different controls of the genome expression potential. Epigenetic regulation involving changes in DNA methylation and in the histone code are likely mechanisms responsible for large-scale expression changes. Histone 3 modifications are mediated in part by Polycomb Group (PcG) class complexes. In plants, PcG genes have a strong impact on the control of the transition to reproductive phase and in endosperm development. In mammals there has been recent evidence that Polycomb Repressive Complexes 2 (PRC2) class of chromatin remodelling complexes control placenta development. These recent findings will be reviewed and put in perspective with recent advances obtained in plants.

PRC2 type PcG complexes control H3 methylation

DNA in eukaryotic nuclei is compacted into a structure called chromatin. The basic unit of chromatin is the nucleosome, consisting of 146 base pairs of DNA wrapped 1.8 superhelical turns around an octamer of the core histones H2A, H2B, H3 and H4 (Kornberg, 1974; Kornberg and Thomas, 1974). Chromatin is a highly dynamic polymer and its structure can be constantly modified in response to environmental or developmental signals. Basic histone tails protruding from the histone octamer have been shown to be essential regulators of such dynamics (Luger and Richmond, 1998; Wolffe and Hayes, 1999). Among proteins involved in histone code establishment and interpretation, the Polycomb (PcG) proteins have emerged as major players. Historically, both of these families were discovered in *Drosophila* (Jürgens, 1985; Lewis, 1978). PcG genes are involved in the maintenance of the segment-specific pattern of Hox genes ex-

Abbreviations used in this paper: EMF, embryonic flower; GTF, general transcription factors; HDAC, histone deactetylase; ICR, imprinting control region; PcG, Polycomb Group; PRC, polycomb repressive complex; SAM, shoot apical meristem; TBP, TATA box binding protein.

***Address correspondence to:** Dr. Frederic Berger. Chromatin and Reproduction Group, Temasek Lifescience Laboratory, National University of Singapore, Singapore 117604, Singapore. Fax: +65-6872-7007. e-mail: fred@tll.org.sg

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pression initially set up by segmentation genes (Jones and Gelbart, 1990; Simon *et al.*, 1992; Struhl and Akam, 1985). PcG proteins bind hundreds of sites on polytene chromosomes. Thus it has been suggested that they have a global role in gene silencing and that they may also regulate the expression of non-homeotic genes (DeCamillis *et al.*, 1992; Rastelli *et al.*, 1993; Zink and Paro, 1989).

Two distinct Polycomb Repressive Complexes PRC1 and PRC2 have been purified and characterised in the *Drosophila* embryo. PRC1 contains the PcG proteins Polycomb (Pc), Polyhomeotic (Ph), Posterior sex combs (Psc) and Sex combs on midleg (Scm) (Shao *et al.*, 1999). The size of PRC1 complex is estimated at 2 MDa (Franke *et al.*, 1992). Two more recent studies have shown that the PRC1 complex contains a number of other factors, including dRING1, a RING finger protein (Saurin *et al.*, 2001) and general transcription factors (GTFs) (Breiling *et al.*, 2001). Furthermore, both these studies have shown that the PcG proteins Pc, Ph and Psc co-immunoprecipitate with the TATA box binding protein (TBP). The finding that general transcription factors are part of the PRC1 complex strongly suggests an interaction between PcG repression and the transcription machinery. Shao *et al.* (1999) showed that the PRC1 core complex, if preincubated with a nucleosomal array, is able to inhibit chromatin remodelling by a human SWI/SNF ATP-dependent complex. SWI/SNF complexes are able to provoke nucleosome sliding and loosen heterochromatin structure. Altogether, these data support a model in which PcG PRC1 complex protects chromatin structure against remodelling and, in parallel, directly inhibits RNA polymerase II transcription machinery by direct interaction with GTFs and TBP.

A 600kDa PRC2 complex has been purified which is biochemically distinct from the PRC1 complex (Ng *et al.*, 2000; Tie *et al.*, 2001). Two PcG proteins, Extra sex combs (ESC) and Enhancer of zeste E(Z) are part of this complex, giving it the name ESC-E(Z) (Fig. 1). Direct interaction between ESC and E(Z) has been demonstrated by independent two-hybrid and co-immunoprecipitation studies (Jones *et al.*, 1998; Tie *et al.*, 1998). Additional studies have identified new subunits of the ESC-E(Z) core complex, namely Su(z)12, a PcG zinc-finger protein (Birve *et al.*, 2001; Cao *et al.*, 2002; Muller *et al.*, 2002) and the histone binding protein p55 (Cao *et al.*, 2002; Czermin *et al.*, 2002; Muller *et al.*, 2002, Tie *et al.*, 2001) (Fig. 1). Several studies have shown that the ESC-E(Z) complex has a histone methyltransferase activity, which is necessary for the maintenance of Hox gene repression (Muller *et al.*, 2002). Histone methyltransferase activity is achieved by the E(Z) SET motif and targets H3K27 (tri-methylation) and, to a lesser extent, H3K9 (di-methylation) (Cao *et al.*, 2002; Czermin *et al.*, 2002; Muller *et al.*, 2002). The minimal protein complex required for enzymatic activity consists of E(Z), ESC and p55 (Czermin *et al.*, 2002). Association of the HDAC RPD3 to the ESC-E(Z) complex has also been described (Czermin *et al.*, 2002; Tie *et al.*, 2001) but may be weak (Muller *et al.*, 2002). However, no HDAC activity of the ESC-E(Z) complex has been reported. The identification of ESC-E(Z) complex and characterisation of its enzymatic activities highlight the intimate link between PcG proteins and establishment of the histone code.

PRC2 PcG genes and complexes are conserved in mammals and in plants

Homologues of all major *Drosophila* PcG genes have been cloned in mammals. A distinct ESC-E(Z)-like complex is composed of EZH2, EED, p55 homologs RbAp46 and RbAp48 and SU(Z)12 (Cao *et al.*, 2002; Kuzmichev *et al.*, 2002). As in *Drosophila*, there is some evidence that Eed/EED can directly interact with Enx2/EZH1 and Enx1/EZH2 (Jones *et al.*, 1998; Sewalt *et al.*, 1998; van-Lohuizen *et al.*, 1998) but not with PRC1 members HPC2 or BMI-1 (Sewalt *et al.*, 1998) and that Eed and Enx proteins are found in a complex distinct from the PRC1 complex that contains Mph1 (van-Lohuizen *et al.*, 1998) (Fig. 1). Recently, interaction between SU(Z)12 and EZH2 has been shown (Yamamoto *et al.*, 2004). Whether it is the case in *Drosophila* is not known but Su(z)12 is detected in the 600 kDa ESC-E(Z) complex (Ng *et al.*, 2000, Tie *et al.*, 2001). An association of EED with HDAC1 and HDAC2 has been reported (van-der-Vlag and Otte, 1999) but, as is the case in fly, may be weak or transient (Kuzmichev *et al.*, 2002). The homolog of Pho, Yin Yang 1 (YY1) is part of EED-EZH complexes in both mouse and human (Satijn *et al.*, 2001) and directly interacts with EED. This striking conservation of protein interactions and complex formation is reinforced by the finding that EED-EZH complex has also a H3K27-specific Histone Methyltransferase activity on H3-Lys27 (Cao *et al.*, 2002; Kuzmichev *et al.*, 2002).

Thus, PcG complexes are very well conserved in terms of composition and molecular function between *Drosophila* and mammals. However, the duplication of almost all PcG genes in mammals allows variations in complex composition, depending on cell or tissue type (Lessard and Sauvageau, 2003b). For example, HPH2 is expressed in all tissues tested, whereas HPH1 is expressed only in testis, ovary and thymus (Gunster *et al.*, 1997)

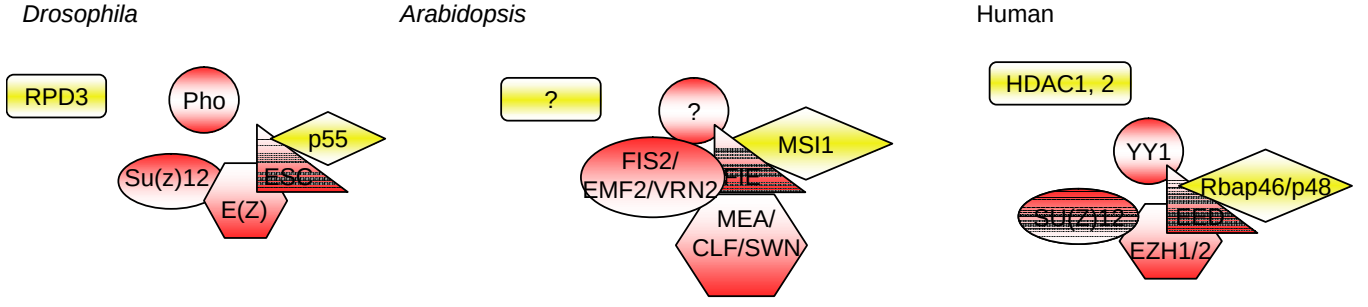

Fig. 1. Polycomb repressive complex 2 (RC2) PcG complexe homologues. *Several homologues of PcG genes exist in* Drosophila*, mammals and* Arabidopsis. *They are all associated in stereotypical complexes. The homologues are indicated in the same box. Mammalian and* Arabidopsis *complexes do not necessarily contain all the homologues that are represented in the same box.*

where it could participate to a new complex for new functions.

In addition to fly and mammals, conservation of PcG genes and the PRC2 complex, is also obvious in plants. Several PcG genes have been cloned in *Arabidopsis* thaliana. In *Arabidopsis*, the first PcG gene identified was *CURLY LEAF* (*CLF*), homologous to E(Z) (Goodrich *et al.*, 1997). E(Z) homologues in *Arabidopsis* constitute a family of three genes, *CLF*, its closest relative *SWINGER* (*SWN*) (Chanvivattana *et al.*, 2004) and MEDEA (*MEA*) (Grossniklaus *et al.*, 1998); (Luo *et al.*, 1999). *Arabidopsis* genome contains a single homologue of ESC, *FERTILISATION INDEPENDENT ENDOSPERM* (*FIE*) (Ohad *et al.,* 1999; Luo *et al.*, 1999). As ESC and E(Z) proteins interact in *Drosophila*, direct interaction between FIE and MEA has been tested. Two-hybrid studies, confirmed by GST pull-down assays, showed that FIE and MEA are indeed able to directly bind each other *in vitro* (Luo *et al.*, 2000; Spillane *et al.*, 2000; Yadegari *et al.*, 2000). *In vivo* interaction was recently demonstrated (Bracha-Drori *et al.*, 2004). The FIE-MEA complex has been isolated and also shown to contain MSI1 (Kohler *et al.*, 2003a), homologous to the *Drosophila* histone binding protein p55 (Hennig *et al.*, 2003). Homologues of Su(z)12, the fourth conserved member of PRC2 complexes are encoded by a family of three genes, *FERTILISATION INDEPENDENT SEED 2* (*FIS2*) (Luo *et al.*, 1999), *EMBRYONIC FLOWER 2* (*EMF2*) (Yoshida *et al.*, 2001) and VERNALISATION2 (VRN2) (Gendall *et al.*, 2001). It was shown recently that each of the *Arabidopsis* Su(z)12 protein are able to interact through the conserved VEFS domain with the conserved cysteine rich C5 domain in each member of the E(Z) family (Chanvivattana *et al.*, 2004). Although only the FIE-MEA complex has been biochemically isolated and is present in the female gametes and in developing endosperm in the seed, distinct PRC2 complexes can be envisaged as some members have non-overlapping patterns of expression. Functional analysis of *CLF* and *SWN* shows a large degree of redundancy whereas distinct functions have been ascribed to *EMF2* and *VRN2* during vegetative life. Hence it can be proposed that the Su(z)12 members are essential for the specific association of PRC2 with a selected set of target genes (Chanvivattana *et al.*, 2004). It is thus possible to conclude to a strong conservation of PRC2 complexes core members ESC, E(Z), Su(z)12 and p55. In some organisms as the nematode C. elegans, Su(z)12 has been lost during evolution and strikingly the C5 domain of the E(Z) homologue MES2 has not been conserved (Holdeman *et al.*, 1998); (Chanvivattana *et al.*, 2004). The molecular mass of the FIE-MEA complex is still higher than can be accounted for by the four conserved core members of PRC2 complexes (Kohler *et al.*, 2003a). A large family of HDAC is present in *Arabidopsis*, yet no member has been identified in the FIE-MEA complex. Also, there is no obvious homologue of Pho and Yin Yang 1 (YY1).

PRC2 complexes control the transition through developmental phases in plants

Flowering plant life cycle is characterised by alternation of several phases corresponding to a series of transition towards reproduction. After seed germination, the seedling emerges and develops into a rosette. Rosette development is the result of leaf formation, without elongation of shoot internodes. The stem cells forming the shoot apical meristem (SAM) are responsible for leaf initiation, whereas the opposite root apical meristem is at the origin of root growth. Vegetative development ends as a result of integration of endogenous and environmental signals. This corresponds to floral transition of the vegetative SAM to an inflorescence SAM identity. A second major transition is the conversion of the inflorescence SAM into a specialised flower meristem. A third major transition takes place within the sexual organs of flowers, when meiosis is initiated leading to the haploid gametophytic phase. Gametes are produced by specialised haploid structures, the gametophytes. Gametophyte development is arrested at gamete maturation and the double fertilisation process initiates the subsequent sporophytic phase, namely seed development, requiring coordinated development of the embryo and the endosperm. All of these developmental phase transitions have to be tightly regulated in order to ensure the integrated development of the plant. We will present how PcG proteins are essential in such processes.

Control of flowering time by vernalisation requires a PcG protein and involves histone modifications

In winter annual plants, an essential pathway controls flowering time: vernalisation. Vernalisation is described as 'the acquisition or acceleration of the ability to flower by a chilling treatment' (Chouard, 1960). This process allows flowering only when the cold season has passed. Vernalisation requires cellular memory processes, as plants often flower weeks after they have been exposed to cold. The meristem is the centre of cold memory (Metzger, 1988; Schwabe, 1954). A central gene in the integration of vernalisation signal is the MADS box gene *FLOWERING LOCUS C (FLC)*. High level of *FLC* expression represses flowering. *FLC* expression is promoted by the protein *FRIGIDA* (Johanson *et al.*, 2000; Michaels and Amasino, 1999; Sheldon *et al.*, 1999). In contrast, cold treatment induces a downregulation of *FLC* expression, which is maintained after transfer to normal temperature and accelerates flowering (Michaels and Amasino, 1999; Sheldon *et al.*, 1999). Screens for mutants affected in the response to vernalisation have isolated some key factors involved in the long-term repression of *FLC* after cold exposure. Mutations in *VERNALIZATION 1 (VRN1)* (Levy *et al.*, 2002) or *VRN2* (Gendall *et al.*, 2001) prevent vernalisation-induced flowering. VRN1 is a DNA-binding protein and VRN2 is a PcG protein, homologue of Su(z)12. *VRN1* and *VRN2* expression is detected in all tissues, at all developmental stages and is not induced by cold exposure. In *vrn* mutants, *FLC* down regulation is induced during cold treatment, but *FLC* expression is recovered when plants are transferred to normal growth temperature. These results suggest that *VRN1* and *VRN2* genes are involved in maintenance, but not the establishment, of *FLC* MADS box gene repression after cold exposure. VRN1 and VRN2 present fundamental characteristics of PcG proteins: they are ubiquitously expressed, are involved in the maintenance of gene repression and are regulators of development.

Recent work has partially elucidated the mechanism by which PcG mediated repression is established and maintained at the *FLC locus* (Sung and Amasino, 2004). In the *vernalization insensitive 3 (vin3)* mutation, the response to vernalisation is abolished. VIN3 protein contains a Plant Homeodomain (PHD) motif, which is characteristic of chromatin remodelling factors (Aasland *et al.*, 1995). *VIN3* is specifically and transiently ex-

pressed in shoot and root apical meristems during cold exposure, i.e. in cells where *FLC* expression must be repressed. Furthermore, *FLC* expression is never repressed in *vin3* mutants, even after long exposure to cold temperature. These data provide evidence that VIN3 is responsible for the establishment of *FLC* repression. As VIN3 is only transiently expressed, it is reasonable to hypothesize that VRN1 and VRN2 maintain the repression initiated by VIN3. There is also evidence that an epigenetic control of *FLC* expression is mediated by histone modification (Bastow *et al.*, 2004; Sung and Amasino, 2004). In the absence of vernalisation, the *FLC* promoter and first intron, both of which are necessary for vernalisation response (Sheldon *et al.*, 2002), are acetylated on histones consistent with active expression. Vernalisation induces a deacetylation of histones on these regulatory regions. Histone deacetylation establishment is dependent on VIN3 protein, whereas VRN1 and VRN2 are necessary for its maintenance. Vernalisation treatment also induces methylation of lysines 9 and 27 on histone H3 (H3K9/H3K27) and this methylation is VRN2-dependent. By homology with animals, one can speculate that VRN2 is part of a PcG complex, containing FIE, MSI1 and CLF or SWN (Fig. 2).

Control of flowering time requires also the EMF2 PRC2

CLF controls flowering time as loss-of-function *clf* alleles show precocious flowering (Fig. 2). Reduction of flowering time is increased when weak *clf* alleles are combined with the weak alleles of *emf2* (Chanvivattana *et al.*, 2004). Similarly precocious flowering is observed in plant with reduced amounts of *FIE* transcripts (Katz *et al.*, 2004b) or of *MSI1* transcripts (Hennig *et al.*, 2003). Hence, the EMF2 PcG complex also controls floral transition. The fact that strong alleles of *emf2* (Yang *et al.*, 1995; Yoshida *et al.*, 2001) and strong suppression of *FIE* (Kinoshita *et al.*, 2001) cause seedlings to flower indicate that the EMF2 complex maintains the vegetative phase from late embryogenesis onwards.

Floral transition and flower development are closely related in *Arabidopsis* and controlled by the EMF2 PcG complex

LEAFY (LFY) and *APETALA1 (AP1)* genes are essential transcription factors for flower initiation (Mandel *et al.*, 1992; Weigel *et al.*, 1992). When developmental and environmental conditions are favourable, *LFY* expression is induced. Activation of *AP1* expression by *LFY* provokes SAM transition from a vegetative to a reproductive state, producing inflorescences (Fig. 2). Constitutive expression of *LFY* (Weigel and Nilsson, 1995) or *AP1* (Mandel and Yanofsky, 1995) accelerates floral transition, showing that their expression must be repressed as long as a flowering decision has not been made. *LFY* is necessary later for activation of floral organ identity gene expression. Loss-of-function in EMF2 leads to early activation of LFY and precocious transition from a vegetative to an inflorescence meristem. Furthermore, *emf2* mutations lead to derepression of homeotic genes that control flower development (Moon *et al.*, 2003), leading to abnormal flower development. A similar deregulation of floral homeotic genes patterns with corresponding flower abnormalities is observed in *clf* and *clf swn* double mutants (Chanvivattana *et al.*, 2004) and in plants with reduced levels of *FIE* or *MSI1* transcripts (Katz *et al.*, 2004; Hennig *et al.*, 2003; Kinoshita *et al.*, 2001). These data indicate that the EMF2 complex regulates both floral transition and flower development (Fig. 2). However the PcG complex has not been formally isolated. Another question remains as to how repression is broken when flowering time arises? Expression data of PcG genes show that the process is not transcriptionally regulated, but no alternative model has been proposed.

The control of floral transition is an example in which a parallel between *Arabidopsis* and animals PcG proteins can be drawn. Inflorescence meristems in *Arabidopsis* produce flower buds as long as the plant is alive. Interestingly, when *MSI1* or *FIE* are silenced a terminal flower is produced after the production of only a few flowers and a similar phenotype is observed in *emf2* (Chen *et al.*, 1997; Hennig *et al.*, 2003; Katz *et al.*, 2004a; Yang *et al.*, 1995). Such an early termination of flower production by the inflorescence meristem can be interpreted as a loss of maintenance of stem cell identity. This phenotype shows that the EMF2 complex is required for the maintenance of stem cells identity, preventing their differentiation. A similar link between PcG function and stem cell identity has been demonstrated in humans (Lessard and Sauvageau, 2003a; Ohta *et al.*, 2002). Also target genes of PcG proteins are also well conserved, as main targets of PcG proteins in plants are homeotic genes as is the case in *Drosophila* and mammals. Histone H3 lysine 27 methylation involvement in *FLC* regulation further supports the evolutionary conservation of PcG pathways between plants and animals.

The FIE-MEA complex controls double fertilisation and seed development

After meiosis development of the female gametophyte involves three successive mitoses. After cellularisation of the syncytial gametophyte containing eight nuclei, seven cells form the embryo sac (Fig. 3). The central cell inherits two nuclei and is therefore homodiploid. Once the embryo sac is differentiated, cell division arrests. Double fertilisation is the signal by which development will be re-initiated leading to endosperm and embryo development.

fertilization independent seed (fis) mutants are characterised by autonomous seed formation in absence of fertilisation (Chaudhury *et al.*, 1997, Ohad *et al.*, 1996, Peacock *et al.*, 1995; Kohler *et al.*, 2003a; Guitton *et al.*,2004). The *fis* mutants comprise *mea*, *fis2*, *fie*, *msi1* and *borgia*. To the exception of the unidentified *BORGIA* gene, all *fis* mutants participate in the genetic pathway controlled by the FIE-MEA complex (Grossniklaus *et al.*, 1998; Luo *et al.*, 1999; Ohad *et al.*, 1999; Guitton *et al.*,2004). Autonomous seeds in *mea*, *fis2*, *fie* contain only endosperm, resulting from central cell division and no embryo development was reported (Chaudhury *et al.*, 1997; Kiyosue *et al.*, 1999; Ohad *et al.*, 1996). These data confer to the *FIS* class PcG proteins an essential role in repressing central cell development in the absence of fertilisation. Autonomous seeds produced in *msi1* mutant alleles also contain an embryo-like structure, which expresses early embryo markers and acquire an apical-basal polarity typical of early embryogenesis in higher plants (Guitton and Berger, 2005). Parthenogenetic development of *msi1* haploid embryos, arrests after a few cell divisions. This

arrest probably results from the absence of *msi1* function, shown to be necessary for division in diploid embryos homozygous for *msi1* produced by fertilisation in *msi1* /+ plants (Guitton *et al.*, 2004). This role of plant PcG proteins in controlling cell cycle progression may be mediated via MSI1, which also likely binds the Retinoblastoma homologue RBR1 in *Arabidopsis* (Ach *et al.*, 1997). Mutants for *RBR1* show limited autonomous seed development and abnormal proliferation of the female gametophyte (Ebel *et al.*, 2004). Such a phenotype has also been observed with a low penetrance in *msi1* female gametophytes. (Berger, personal communication). Two main scenarios can be envisaged to account for autonomous seed development in *fis* mutants (Fig. 3). Autonomous seed development results from the absence of cell cycle arrest of the female gametes, which could be mediated directly by loss of MSI1-RBR1 regulation of the transition from G1 to S phase. This control would be modulated by the FIE-MEA complex, notably through direct interaction between MSI1, RBR1 (Mosquna *et al.*, 2004) and FIE (Kohler *et al.*, 2003a). According to an alternative hypothesis, the developmental program of female gametophyte is perturbed earlier by abnormal function of the PcG complex or by another MSI1 containing complex and never achieves proper maturation. Hence the signals required for cell cycle arrest are not executed and the central cell keeps dividing into an endosperm-like structure. Similarly the egg cell, in the case of the most penetrant mutations in *msi1*, also keeps dividing and initiates limited parthenogenetic development.

When *fis* ovules are fertilised a maternal effect leads to abnormal seed development. Endosperm development is deeply affected (Kiyosue *et al.*, 1999; Sorensen *et al.*, 2001; Guitton *et al.*, 2004; Ingouff *et al.*, 2005a). Wild type endosperm development undergoes a series of four major developmental phases timed by successive synchronous nuclei divisions (Boisnard-Lorig *et al.*, 2001). The first three phases describe endosperm syncytial development (Fig. 3). After phase 1 consisting of three synchronous nuclei divisions, mitotic domains are defined in phase 2 (relative to stage V). Phase 3 starts with the onset of nuclei migration towards the posterior pole at stage VIII (Guitton *et al.*, 2004). At stage IX cellularisation marks the end of the syncytial phase (phase 4) (Sorensen *et al.*, 2002). In *fis* endosperm the transition from phase 1 to phase 2 is absent and mitotic domains are either not present of ill-defined (Ingouff *et al.*, 2005a). Accordingly, the expression of seven markers of phase 1 and phase 2 is temporally extended to a later phase of development when endosperm acquires a cellular state in the wild type. In *fis* seeds, endosperm does not cellularise (Grossniklaus *et al.*, 1998; Kiyosue *et al.*, 1999). Continuance of the initial syncytial state leads to overproliferation after stage IX.

In spite of such changes, specific sequences of developmental events still take place as in the wild type. The pace of cell division is not altered during the early syncytial phase and some antero-posterior pattern elements are normally expressed (Kiyosue *et al.*, 1999; Ingouff *et al.*, 2005a). Overall, *fis* mutations cause a temporal deregulation in ontogenic sequence of endosperm development and can be defined as heterochronic, leading to a complex pleiotropic phenotype (Ingouff *et al.*, 2005a). The molecular origin of the various aspects of the *fis* phenotype is not understood. Köhler *et al.* identified *PHERES1 (PHE1)* as a direct target of MEA-FIE complex by using microarray analysis (Kohler *et al.*, 2003b). *PHE1* belongs to the type I-MADS box transcription factor family and is expressed during early endosperm development but its role is not understood. Another recently identified potential target of the FIE-MEA PcG complex is the actin nucleator FORMIN HOMOLOGY PROTEIN 5 (AtFH5) (Ingouff *et al.*, 2005b). The gene *AtFH5* was identified in the enhancer trap line KS117 with an endosperm-specific expression. The GFP marker associated to KS117 is overexpressed in *fis* mutants. As AtFH5 loss-of-function impairs cellularisation and the development of the posterior pole, both parts of the *fis* phenotype, it is possible

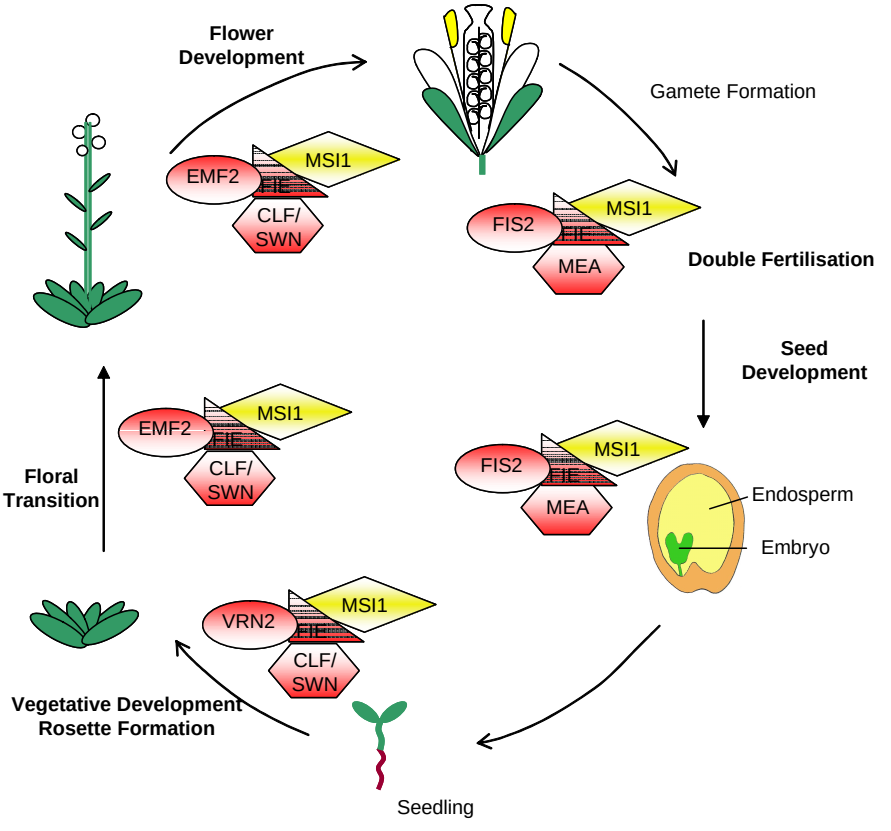

Fig. 2. Control of the *Arabidopsis* life cycle by PRC2 complexes. *Upon germination, the two embryonic cotyledons and primary root emerge. Further development leads to rosette formation and floral transition initiates the inflorescence. The hermaphrodite flower is composed of four whorls: from the outside to the inside, green sepals, white petals, male organs anthers and female organ pistil, which contains ovules. Meiosis takes place in ovules and in developping pollen in anthers and forms the female and the male gametes respectively. Double fertilisation leads to formation of two organisms: the embryo and the surrounding endosperm. Embryo germinates and is at the origin of the next generation. At least three types of PRC2 complexes control several transition from the vegetative to the reproductive phase of* Arabidopsis *development.*

that AtFH5 mediates some action of the FIE-MEA complex, which could directly control its expression.

Endosperm and placenta development are regulated by imprinting, which involves PRC2 genes

Promoter fusion, *in situ* hybridisations and RT-PCR analyses showed that *MEA*, *FIS2* and *FIE* genes are expressed in the embryo sac, mainly in the central cell and in the endosperm after fertilisation (Grossniklaus *et al.*, 1998; Kinoshita *et al.*, 1999, Kiyosue *et al.*, 1999; Luo *et al.*, 2000; Ohad *et al.*, 1999; Spillane *et al.*, 2000; Vielle-Calzada *et al.*, 1999; Yadegari *et al.*, 2000). *FIE* is also expressed in the embryo (Luo *et al.*, 2000; Spillane *et al.*, 2000; Yadegari *et al.*, 2000) and some studies report *MEA* expression in the embryo (Kinoshita *et al.*, 1999; Vielle-Calzada *et al.*, 1999). Whereas *FIS2* and *MEA* seem to be specific for reproductive development, *FIE* has a wide pattern of expression including sporophytic expression in cauline leaves, stem and roots (Luo *et al.*, 2000; Ohad *et al.*, 1999). Expression of some *FIS* genes shows a remarkable property as during syncytial endosperm development only the maternal allele is expressed (Berger, 2004). The paternal allele remains silenced. Parental allele dependent gene expression is defined as imprinting. In *Arabidopsis* parental imprinting has been directly demonstrated only for *MEDEA* (Kinoshita *et al.*, 1999), for the endosperm specific gene *FWA* (Kinoshita *et al.*, 2004) and recently for *PHERES* (Kohler *et al.*, 2005). As expression reporters for *FIE* and *FIS2* show paternal silencing (Yadegari *et al.*, 2000; Luo *et al.*, 2000), it is assumed that corresponding genes are imprinted and only maternally expressed. *PHERES* is only paternally expressed (Fig. 3). Imprinting of *MEA* and of *FWA* involves the maintenance methyltransferase MET1 and the DNA glycosylase DEMETER (Xiao *et al.*, 2003; Kinoshita *et al.*, 2004). MET1 methylates cytosine residues present in CpG clusters in the

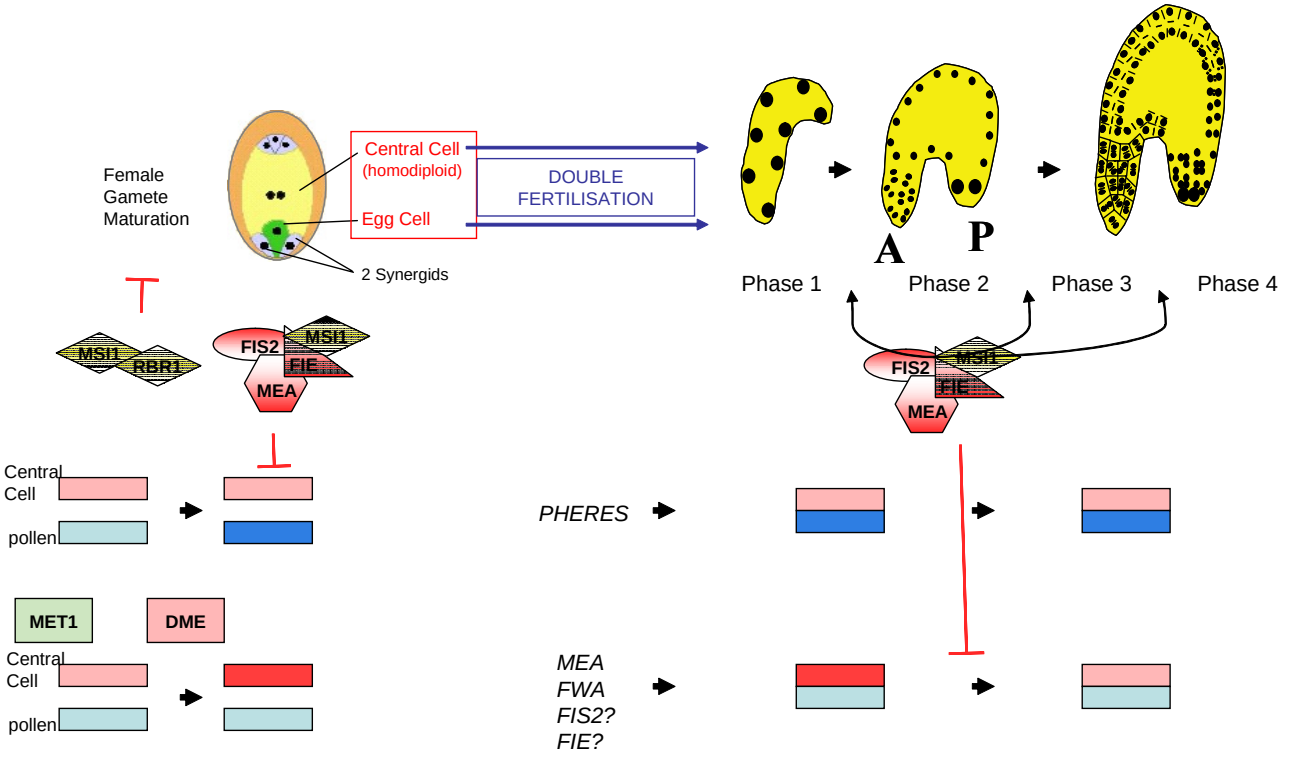

Fig. 3. Control of female gametogenesis and endosperm development by the FIE-MEA complex. *Proliferation during female gametogenesis is controlled by the MSI1-RBR1 pathway. Together with the FIE-MEA complex, MSI1 and RBR1 may also control female gametophyte sexual maturation marked by cell cycle arrest in the central cell and in the egg cell. Double fertilisation of the egg cell and the central cell give rise to the embryo and the endosperm respectively. Endosperm developmental phases are delimited by a series of cellular events: the transition from a synchronous mode of syncytial division (Phase 1) to partition of the syncytium in three mitotic domains (Phase 2), the onset of nuclei migration to the posterior pole (P) (Phase 3) located opposite to the anterior pole where the embryo develops (not shown), cellularisation leading to Phase 4 when cellular endosperm differentiate different cell types. The FIE-MEA complex is a positive regulator of the transtition between the different phases. At the molecular level, the FIE-MEA complex regulates the expression of several imprinted genes including genes encoding members of the complex (MEA and most likely FIS2 and FIE). This regulation takes place at two levels. The FIE-MEA complex may act during central cell maturation and maintains the silenced state of* PHERES, *whereas* PHERES *expression in initiated during pollen development. Hence after fertilisation,* PHERES *is imprinted with only expression of the paternal copy. The FIE-MEA complex may further maintain silencing of* PHERES *maternal allele during endosperm development.* MEA *and* FWA *represent another class of imprinted genes with silencing of the paternal allele. Both parental copies are silenced during vegetative development by maintenance of DNA methylation by the methyltransferase MET1. Activation of the maternal allele takes place during central cell maturation through the action of the DNA glycosylase activity of DME. During endosperm development, the expression of imprinted genes becomes repressed after Phase 3 under the action of the FIE-MEA complex.*

promoter and the coding sequence of imprinted genes and participate in maintenance of the silenced state of these genes through the vegetative life cycle. Only in endosperm development, is the promoter of *FWA* demethylated and the gene become expressed. Demethylation affects only the maternal copy and likely results from the action of DEMETER during female gametogenesis (Fig. 3). DEMETER is expressed specifically in the central cell and is presumed to cause single stranded DNA cuts after cytosin residues, which once repaired with non methylated cytosine residues, would lead to demethylation of the DNA (Choi *et al.*, 2002). Such a mechanism remains to be demonstrated for *FIS2* and *FIE*. In the case of *PHERES*, maintenance of repression of the maternal allele during endosperm development is at least under the control of FIE-MEA activity (Kohler *et al.*, 2005).

Implication of both DNA maintenance methyltransferases and of PRC2 complexes have been demonstrated in imprinting in mammals. In mammals, where imprinting was originally described, at least eighty genes are affected by this mechanism (Delaval and Feil, 2004). Several imprinted genes are particularly important for placental development (Reik *et al.*, 2003). Most imprinted genes are located in clusters around imprinting control regions (ICR) enriched in CpG islands and subjected to methylation (Reik and Walter, 2001). In contrast to plants, in which imprints are acquired by demethylation, mammalian imprint are mostly acquired by differential methylation of the maternal copy in the egg or of the paternal copy during spermatogenesis (Berger, 2004; Delaval and Feil, 2004; Reik and Walter, 2001). This involves a specific *de novo* DNA methyltransferase Dnmt3a (Hata *et al.*, 2002; Kaneda *et al.*, 2004; Okano *et al.*, 1999; Okano and Li, 2002; Suetake *et al.*, 2004). The imprint is later maintained in somatic cells as in plants by the methyltransferase Dnmt1. As was recently shown in plants, DNA methylation is not the only mechanism at the origins of imprints in mammals (Umlauf *et al.*, 2004); (Lewis *et al.*, 2004). Several genes at the *Kcnq1* and at the *Igf2r* domains show imprinting in the placenta, which does not depend on DNA methylation. It has been shown recently that imprinting status of the parental allele is linked directly to differential methylation of H3 with trimethylation at H3-Lys27 and dimethylation at H3-Lys9. Chromatin Immunopurification has shown that Ezh2 and Eed associate with the paternally silenced allele of several imprinted genes in the placenta (Umlauf *et al.*, 2004). It is yet not clear how the PRC2 is differentially recruited to the paternal allele during early embryogenesis, not how the imprinted status is conserved only in the placenta. In mice with loss-of-function of *Eed* paternal silencing is partially relieved only in some genes of the *Kcnq1* domain, indicating that other mechanisms redundant with histone methylation might be involved (Mager *et al.*, 2003). The parallel between imprinting in *Arabidopsis* endosperm and in mammalian placenta is striking by the conservation of molecular mechanisms involved. Imprinting of *MEA* involving DME and MET1 activity may also be associated with the function of the FIE-MEA itself as *MEA* is largely overexpressed in a *fie* background (Ingouff *et al.,* 2005a), though it is not yet clear whether the change in expression affects only one of the parental alleles.

Imprinting has evolved independently in mammals and in flowering plants and has targeted specifically the interface controlling maternal nutritive supply of the embryo. In both groups, this interface shares identical genetic material with the embryo, yet follows a completely divergent developmental program. It can be hypothesised that chromatin remodelling mechanisms are primarily crucial to keep the embryo lineage apart from the gametophytic lineage in plants and from the placenta lineage in mammals. Imprinting might have secondarily used such chromatin remodelling mechanisms as a result of differential selective evolutive pressure on the parents. In both mammals and plants, imbalance between the maternal and paternal genome dosage causes similar effects (Scott *et al.*, 1998; Barton *et al.*, 1985; Barton *et al.*, 1984; Surani *et al.*, 1984). An increase in paternal dosage leads to increase in placental or endosperm growth, whereas increase in maternal dosage has the opposite effect. Potential effector genes of the parental genome imbalance dosage are not known in plants. The *HAIKU* class genes controls endosperm growth in *Arabidopsis* but their molecular nature remains to be identified (Garcia *et al.*, 2005; Garcia *et al.*, 2003). Imprinted *FIS* genes do not have a direct role in the control of endosperm growth or proliferation (Kiyosue *et al.*, 1999; Ingouff *et al.*, 2005a). Overproliferation observed in *fis* mutant endosperm occurs only late during development as the likely consequence of preservation of the syncytial state rather than as a direct effect of the mutation. Only MSI1 directly controls endosperm growth and proliferation during the syncytial phase but its imprinted status is not known (Guitton *et al.*, 2004). In mammals, placental growth and proliferation are controlled by the Insulin growth receptor-like 2 factor (Ong *et al.*, 2000), but the role of most genes imprinted in the placenta remains unknown. Besides identification of the cellular and molecular pathways involved in the function of imprinted genes in placenta and in endosperm development, several question remains to be solved. Is the FIE-MEA complex involved in the regulation of paternally imprinted FIS genes? Is imprinting in plants confined to endosperm development? Are other PRC2 active during the vegetative development involved in maintenance of silencing of the endosperm imprinted genes? Are PRC2 genes imprinted in mammals? How are the imprints propagated in endosperm and in the placenta where rapid division take place? To which extent imprinting is linked with parental differentiation or to the stable and drastic isolation of lineages with different fates amongst which are gametes?

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