Plant Epigenetics: MEDEA's Children Take Centre Stage

Dispatch

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The *Arabidopsis* Polycomb group gene *MEDEA* is imprinted in early development and regulates cell proliferation in seeds. A recent study identifies the first direct target of MEDEA regulation. This is an important step towards the genetic manipulation of seed development, and should help clarify the role of Polycomb-group proteins in imprinting in plants.

It is increasingly recognised that epigenetic modifications — mitotically or meiotically heritable changes in gene function that do not result from alterations in DNA sequence — are important in development. In plants and animals, the Polycomb-group (Pc-G) genes mediate a wide variety of epigenetic phenomena, including X chromosome inactivation, genomic imprinting and transgene silencing [1]. They generally repress transcription of their target genes, and are thought to act at the level of chromatin structure to confer mitotically heritable repression. In *Arabidopsis*, three Pc-G genes, collectively termed the '*FERTILISATION INDEPENDENT SEED*' development (*FIS*) genes, have attracted intense interest for their role in controlling seed development [2,3]. Progress in understanding how the *FIS* genes act has been hindered because their targets were unknown. Kohler and colleagues [4] now report an important advance, the identification of a target gene that is directly regulated by the FIS proteins.

Plants reproduce sexually by producing seeds. The seed contains the diploid embryo and also a second zygotic tissue, the endosperm (Figure 1). The endosperm transmits nutrients and signals from the surrounding maternal tissue to the embryo, and it is ephemeral in *Arabidopsis*, being largely consumed by the embryo during seed maturation. The endosperm is economically important as in some plants, particularly the cereals, it is more persistent and forms the bulk of the mature seed.

The three *FIS* genes — *FIS2*, *MEDEA* (*MEA*) and *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*) — are all required maternally for seed viability. Thus, all seeds that inherit a mutant *fis* allele maternally abort, irrespective of the genotype of the paternal allele [2,3]. By contrast, seeds that inherit a wild-type maternal allele and a mutant paternal allele develop normally. One possible explanation for these parent-of-origin effects is that the *FIS* genes are imprinted, so that only the maternal alleles are active during endosperm and/or embryo development. The paternal alleles are silent and therefore can not rescue maternal mutant alleles. An alternative explanation is that the *FIS* gene

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products are required in the female gametophyte (Figure 1) before fertilisation for normal embryo and/or endosperm development. The expression of the *FIS* genes is consistent with both possibilities. All are expressed before fertilisation in the female gametophyte. After fertilisation, expression is exclusively from the maternal allele during early seed development, but later it becomes bi-allelic [5–8]. Imprinting may be most relevant for *MEA*, as the paternal allele is silenced more persistently than the paternal alleles of the other *FIS* genes, and genetic studies suggest that reactivation of the paternal *MEA* allele in seeds can rescue the *mea* phenotype [5,7].

Characterisation of the *DEMETER* (*DME*) gene has identified an important extra level in the regulatory hierarchy controlling seed development. *dme* mutations have similar parent-of-origin effects to *fis* alleles, so that only the maternal allele of *DME* is essential for seed development [9]. Unlike the *FIS* genes, *DME* is expressed before fertilisation in the female gametophyte, but not subsequently during seed development. *DME* appears to act at least partially through *MEA*, as it is required to activate the maternal *MEA* allele in the gametophyte and subsequently in the seed (Figure 2). *DME* encodes a DNA glycosylase, and a related protein has been shown to regulate gene expression by reducing DNA methylation at target gene promoters [10]. It is unlikely, however, that DME acts directly on methylation of *MEA*, because this was not altered in *dme* mutants [9].

To gain further insight into the regulatory pathway controlling seed development, Kohler *et al.* [4] conducted transcriptional profiling of *mea* and *fie* mutants. RNA samples were extracted from siliques containing very early stage *mea* and *fie* mutant seeds. This strategy minimized secondary effects, as mutant seeds at this developmental stage appear morphologically similar to wild type. One of the genes that was commonly derepressed in the mutants was found to encode a MADS box class transcription factor and named *PHERES1* (*PHE1*) after the Greek myth in which Medea murdered her sons Pheres and Meidos. MADS box genes have also been implicated as the targets of several other plant Pc-G members [11,12]. PHE1 represents a distinct and evolutionary more ancient class of MADS box protein, however, and is the first of this class to be functionally characterised in plants.

To confirm that *PHE1* is regulated by the *FIS* genes, Kohler *et al.* [4] compared the spatial and temporal pattern of *PHE1* transcription in wild type and fis class mutants using *in situ* hybridisation and *PHE1 PROMOTOR::GUS* reporter genes. *PHE1* is not expressed in the female gametophyte, but is detectable 1–2 days after pollination in the embryo and endosperm in both *fis* mutants and wild type. Later in seed development, expression of *PHE1* becomes restricted to the chalazal region of the endosperm in wild type, whereas in
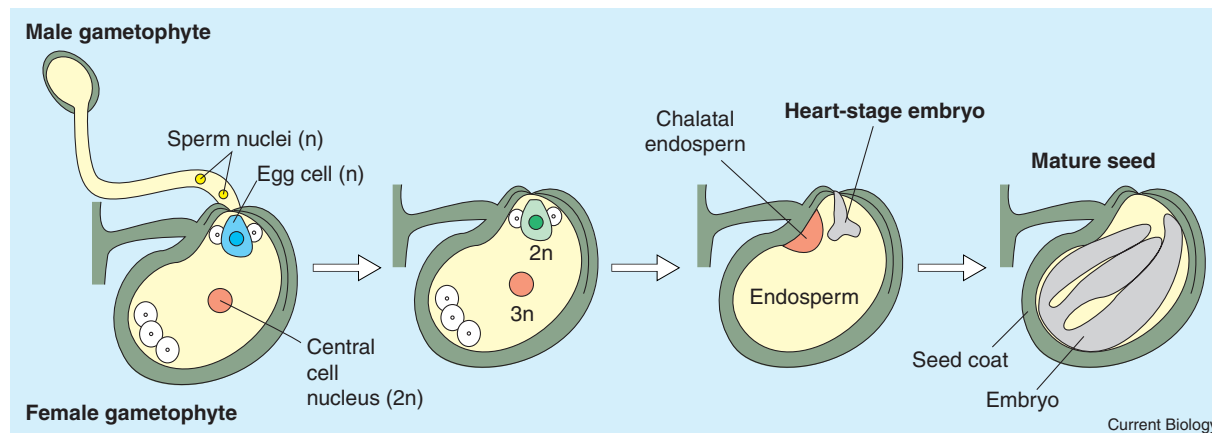
Figure 1.
In plants, gametes are produced in multicellular haploid structures termed gametophytes, within which gametes are produced. In *Arabidopsis* the male gametophytes, the pollen grains, contain two cells, one of which divides mitotically to produce two sperm cells. The female gametophyte originates from one haploid spore that divides three times, producing eight nuclei. The central cell retains two nuclei that fuse (orange) and therefore the mature gametophyte consists of seven cells. Both the egg and the central cells participate in a double fertilization event which is unique to higher plants. One sperm cell fuses with the egg cell to produce the diploid zygote, while the second fertilises the diploid central cell to produce a triploid cell which gives rise to the endosperm. As in animals, the diploid zygote is the progenitor of the embryo.

mea and *fie* mutants *PHE1* is expressed both more strongly and more extensively.

This is strikingly reminiscent of Pc-G mutant phenotypes in *Drosophila*, where target genes are initially expressed normally but subsequently become activated ectopically [1]. Ectopic *PHE1* expression may also contribute to another aspect of the *fis* mutant phenotypes: autonomous endosperm development. In all three mutants, unlike wild type, the central cell (Figure 1) undergoes limited proliferation to form endosperm in the absence of fertilisation [2]. This is correlated with precocious *PHE1* expression in the central cell of emasculated *fie* mutants. It will be interesting to discover if this is causal, for example by testing whether mis-expressing *PHE1* can confer autonomous endosperm (or embryo) development. An intriguing possibility is that fertilisation normally initiates seed development by introducing an active paternal *PHE1* allele.

In order to discover whether *PHE1* is a direct target of MEA and FIE, Kohler *et al.* [4] conducted chromatin immunoprecipitation using antibodies to FIE or MEA proteins. This showed that the FIE and MEA proteins are associated with the proximal part of the *PHE1* promoter and beginning of the coding region. Thus, *PHE1* is a direct target of FIE and MEA, and this is the first rigorous demonstration of a direct Pc-G target in plants. MEA shares homology with the *Drosophila* protein Enhancer of Zeste (E(Z)), which sets an epigenetic mark by methylating lysine 27 on the histone H3 amino-tail (mK27 H3) [13–15]. It is not known however if MEA has similar histone methyltransferase activity. Now that *PHE1* has been identified as a direct target of MEA, the use of chromatin immunoprecipitation to compare the mK27 H3 methylation pattern at the *PHE1* locus in wild type and *mea* mutant plants provides an exciting opportunity to answer this question.

To test whether deregulated *PHE1* expression contributes to the *mea* phenotype, Kohler *et al.* [4] reduced *PHE1* activity in a *mea* mutant background. Because *phe1* mutants have not yet been isolated, the approach involved expressing an antisense *PHE1* gene (*PHE1a/s*) under the control of the *MEA* promoter. Several transgenic lines showed reduced seed abortion and this was correlated with *PHE1* downregulation, suggesting that seed death in *mea* mutants is caused by misexpression of *PHE1*. The rescued seeds were not normal, however: they were enlarged, remained green for longer than normal, and showed reduced tolerance of dessication [4].

Intriguingly, this phenotype is reminiscent of mutants, such as *abscisic acid independent 3* (*abi3*), in which embryos do not respond to the hormone abscisic acid, and show delayed maturation and desiccation intolerance [16]. One possibility is that the partial rescue of *mea* by *PHE1a/s* reveals other, later acting targets that impinge on the abscisic acid pathway. Alternatively, the abnormal seeds may be a result of the residual *PHE1* expression observed in the *PHE1a/s* background [4]. The isolation of *phe1* mutants may help resolve this issue, though because the *Arabidopsis* genome contains a closely related gene — *PHE2* — which also shows mis-regulation in a *mea* background [4], *phe1 phe2* double mutants may be required.

mea mutant seeds can also be rescued by mutations in the *DECREASE IN DNA METHYLATION1* (*DDM1*) gene, which encodes a putative chromatin remodelling factor of the SWI2/SNF2 class [17]. In *ddm1* inbred lines, rescue of seed abortion does not depend on a wild-type *MEA* allele, suggesting that targets of *MEA* repression might be down-regulated as a result of reduced *DDM1* activity [3,8]. Indeed, Kohler *et al.* [4] found significantly lower *PHE1* expression in *ddm1 mea* double mutants than in *mea* mutants, in accordance with restored seed viability [4].
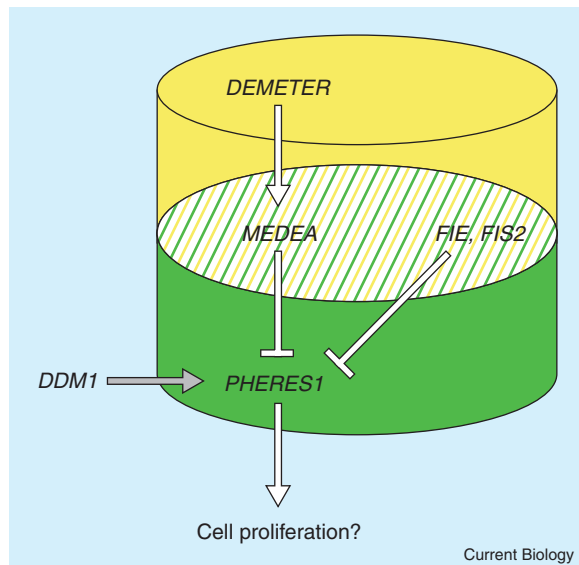
Figure 2.
PHERES1 (*PHE1*) adds another level of regulation to the pathway controlling seed development in *Arabidopsis*. *DEMETER* (*DME*) activates maternal *MEDEA* (*MEA*) which represses *PHE1*. The paternal *MEA* allele is silent early in development. *FIE* and *FIS2* also repress *PHE1*, but are not regulated by DME. In *ddm1* inbred lines, *PHE1* is downregulated independent of a functional *MEA* allele. Yellow, expression before fertilization; green, expression after fertilization. The expression pattern of *DDM1* is not taken into account.

Thus, MEA and DDM1 act antagonistically on *PHE* (Figure 2).

The elegant new work of Kohler *et al.* [4] raises many interesting questions which can be addressed in the near future. *MEA* is unusual amongst imprinted genes in that it encodes a chromatin modifying enzyme. It is therefore possible that MEA may itself imprint its target genes. Indeed, the *mea* phenotype has been interpreted as resembling those of seeds in which imprinting is disturbed [18]. *PHE1*, which acts antagonistically to *MEA* to promote cell proliferation in endosperm and/or embryos, might therefore be imprinted by MEA such that it is normally paternally active and maternally silenced in seed development. MEA might set the maternal imprint on *PHE1* by methylating H3 histones at the *PHE1* locus. If so, this would represent a remarkable instance of convergent evolution in plants and animals. Imprinting occurs late in evolution in both kingdoms, and has therefore evolved independently. Remarkably, the mouse homologues of *FIE* and *MEA* were recently shown to be involved in X chromosomal and some cases of autosomal genomic imprinting [19,20]. In contrast to Greek mythology, it seems that PHERES, the son of MEDEA can expect a bright future.

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