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Chromatin Silencing and Arabidopsis Development: A Role for Polycomb Proteins

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All organisms undergo dramatic shifts in gene expression patterns during early development, particularly at fertilization when the zygotic program is initiated. In flowering plants, fertilization launches four distinct developmental programs that lead to the formation of the embryo, endosperm, seed coat, and mature fruit. Coordination of all four programs most likely requires the exchange of multiple developmental signals and precise control of gene expression. Now, recent investigations by three laboratories reveal that in Arabidopsis, the dramatic developmental shifts that accompany seed formation require proteins of the polycomb group—chromatin silencing factors known to regulate early development in mammals, insects, and nematodes.

Each Arabidopsis ovule contains a haploid female gametophyte, consisting of an egg, two synergid cells, three antipodal cells, and a diploid central cell (reviewed in Drews et al., 1998). These cells are genetically identical, arising from mitotic divisions of a single haploid cell. Yet, after fertilization, the egg and central cell undergo distinct developmental programs: fusion of a sperm and egg yields a zygote, and fusion of a sperm with the central cell forms the endosperm. Embryo development proceeds through a highly ordered series of cell divisions that first form a globular embryo and ultimately a mature seedling (Goldberg et al., 1994). The endosperm instead undergoes a series of mitotic divisions without cytokinesis, forming a syncytium of nuclei that fill the central cell. Subsequently, the endosperm cellularizes, produces starch, proteins, and lipids and is then absorbed by the developing embryo (Lopes and Larkins, 1993).

Recently, mutations that shift the balance between embryo and endosperm development have been described. Mutations in *FIE* (*FERTILIZATION-INDEPENDENT ENDOSPERM*; Ohad et al., 1996) or *FIS* (*FERTILIZATION-INDEPENDENT SEED*; Chaudhury et al., 1997) genes can activate endosperm development, seed coat formation, fruit elongation, and even partial embryo development in the absence of fertilization. These defects are particularly exciting because they resemble developmental patterns normally found in plants that undergo apomixis, a fertilization-independent form of reproduction. In a separate screen, mutations in the *MEDEA* gene were identified that were reported to have the opposite effect, causing proliferation of the embryo at the expense of the endosperm (Grossniklaus et al., 1998). All of these mutations display a second property—they cause aberrant development and embryo lethality only when a mutant allele is contributed by the female parent and thus resemble classic "maternal-effect" defects. These maternal-effect mutations differ from those commonly studied in animals in one important respect: they affect the haploid stage of female development. Consequently, plants that are heterozygous for *fie*, *fis*, or *medea* produce both wild-type and mutant female gametophytes. The inability to transmit these mutations through the female parent precluded standard complementation tests; consequently, molecular analysis was required to reveal the relationships between the genes.

In a recent set of papers, cloning of *FIE* (Ohad et al., 1999), *FIS2* (Luo et al., 1999), and several new *medea* alleles, *fis1* (Luo et al., 1999), *f644*, and *emb173* (Kiyosue et al., 1999), revealed that each of the gene products most likely acts in the same regulatory complex. *FIE* is 40% identical to polycomb group genes that encode a WD domain, including *extra sex combs* (*esc*) from Drosophila and *embryonic ectoderm development* (*Eed*) from mammals (Ohad et al., 1999). *MEDEA* contains a SET domain with 55% identity to another member of the polycomb group, *enhancer of zeste* (*E(z)*) (Grossniklaus et al., 1998). *FIS2* encodes a protein predicted to contain a zinc finger and nuclear localization signal, suggesting that it is involved in transcriptional control (Luo et al., 1999). In vitro and in vivo binding assays indicate that the Drosophila esc and E(z) proteins interact (Jones et al., 1998; Tie et al., 1998); thus, the products of the Arabidopsis *FIE* and *MEDEA* genes are likely to interact directly and to perform a similar role.

Polycomb group proteins form a complex that regulates gene expression through epigenetic silencing (Pirrotta, 1998). Their roles are best understood in Drosophila; after early patterns of homeotic gene expression are established, the polycomb proteins contribute to the maintenance of those patterns by

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silencing homeotic genes in appropriate regions. A complex of polycomb proteins interacts with DNA sequences (polycomb response elements, or PREs) that are scattered throughout the genome (Pirrotta, 1998). These interactions can silence the expression of genes that contain PREs as well as the expression of nearby genes. It is unclear if polycomb proteins directly interact with promoters and enhancers or if instead they remodel chromatin into less accessible forms. In any case, the absence of methylation in the Drosophila genome indicates that polycomb-mediated gene silencing, at least in flies, acts through methylation-independent mechanisms.

The discovery of polycomb proteins in Arabidopsis provides an exciting opportunity to investigate the function of these complexes in plants. A large collection of *FIE*, *FIS2*, and *MEDEA* alleles has been cloned, making it possible to discern the loss-of-function phenotype. Of five *fie* alleles, three alter splice junctions and two result in early stop codons (Ohad et al., 1999). Similarly, alleles of *fis2* include an early stop codon, a defect at a splice junction, and a transposon insertion that likely alters gene expression (Luo et al., 1999). In addition, expression of the cloned wild-type genes in mutant female gametophytes confirmed that the *fie* and *fis2* alleles are recessive. All of these mutations result in a similar phenotype—excessive proliferation of the endosperm and an early defect in embryo development. In contrast, *medea* mutations initially appeared to have the opposite effect, resulting in excessive proliferation of the embryo (Grossniklaus et al., 1998). However, this may be a gain-of-function phenotype because it was found only with alleles that eliminate the C-terminal SET domain. More recent investigations show that early stop codons in the *MEDEA* coding sequence (*fis1*, *f644*, and *emb173*) instead cause the same phenotype as *fie* or *fis2*, with excessive endosperm proliferation, even in the absence of fertilization (Kiyosue et al., 1999; Luo et al., 1999).

The similarity of the mutant phenotypes, along with the resemblance to polycomb group proteins, implies that wild-type *FIE*, *MEDEA*, and possibly *FIS2* silence gene expression programs during plant development. These genes are clearly required to restrict the growth of endosperm cells until after fertilization; their roles in other tissues could be direct or indirect. For example, these wild-type polycomb genes might restrict seed coat or fruit development until after fertilization; alternatively, development of those tissues may initiate in response to endosperm growth. Similarly, defects in these polycomb genes cause embryo lethality, but that lethality could be a consequence of aberrant partitioning of resources between the embryo and the hypertrophic endosperm. Such issues can be resolved by investigating the cell autonomy of the *FIE*, *FIS2*, and *MEDEA* genes.

One of the most intriguing aspects of *FIE*, *FIS*, and *MEDEA* is their apparent requirement only in reproductive development. This was clearly established for *medea* (Kiyosue et al., 1999) and *fis* (Chaudhury et al., 1997), which can display incomplete penetrance. This property allowed the recovery of rare, homozygous mutant plants with aberrant endosperm development in each ovule but remarkably normal vegetative development. Intriguingly, fertilization of mutant female gametophytes by wild-type sperm leads to embryo lethality, yet reciprocal crosses develop normally. Recent investigations have ruled out one possible explanation—although endosperm is formed from one male and two female genomes, differences in dosage of the wild-type and mutant alleles cannot account for the observations. Three separate studies confirm that additional paternal copies of wild-type *MEDEA* or *FIE* cannot rescue mutant female gametophytes (Grossniklaus et al., 1998; Kiyosue et al., 1999; Ohad et al., 1999).

How do endosperm and embryo development depend on the inheritance of a wild-type allele from a female but not a male gametophyte? One explanation is that the *FIE*, *FIS*, and *MEDEA* alleles may be silenced in the paternal genome, or that sperm cells contain inhibitors of these genes. Alternatively, the activity of *FIE*, *FIS*, and *MEDEA* could be required before fertilization or before alleles contributed by the sperm cells can be expressed. Interestingly, Drosophila *E(z)* mutants and *Caenorhabditis elegans MES-2* (similar to *MEDEA*) and *MES-6* (similar to *FIE*) mutants all display maternal-effect phenotypes (Korf et al., 1998). Further, Drosophila *esc* expression is limited to oogenesis and early embryogenesis; it is required for the establishment of silencing but not for its maintenance (Tie et al., 1998).

Although 10 polycomb proteins have been identified to date in Drosophila, the total size of the polycomb group is estimated at 30 (Tie et al., 1998). In Arabidopsis, the polycomb group includes *FIE* and *MEDEA*, as well as *CURLY LEAF*, which is required to regulate floral homeotic genes (Goodrich et al., 1997), and *EZA1*, which has an unknown function (GenBank accession number AF100163). Although genomic hybridization has revealed no close homologs of *FIE* or *FIS2*, the sequence of the Arabidopsis genome will likely reveal additional polycomb members, and some of these may provide essential roles in vegetative development. At present, it is not clear whether there are only a few plant polycomb proteins that are required in specific tissues at specific times, or whether a very large set of polycomb proteins interacts in different combinations and regulates development throughout the plant. Thus, despite the normal vegetative phenotype of *fie* and *medea* mutants, it is possible that these genes do function in vegetative tissues and that other

polycomb genes play a redundant role. Screens for mutants with altered vegetative patterning in a *fie*, *fis*, or *medea* background could reveal other polycomb members.

An understanding of the polycomb complex is only the first step toward understanding other components required for polycomb-mediated silencing. Like that of Drosophila, the Arabidopsis genome probably contains binding sites for the polycomb complex; these PREs likely reside near genes that are regulated by *FIE* and *MEDEA.* A large-scale comparison of messages expressed in wild-type and mutant ovules may reveal these target genes and a comparison of their upstream regions may reveal plant PREs. Presumably, after binding to PREs, the Arabidopsis polycomb complex modulates gene expression; whether this occurs in a methylation-independent manner, like that observed in Drosophila, remains to be tested. If methylation is required for polycomb silencing in plants, *fie*, *fis*, or *medea* phenotypes may be revealed by examining mutants defective in DNA methylation. Finally, polycomb complexes have been shown in Drosophila to interact with each other and to stabilize interactions between homologous transgenes, resulting in gene silencing (Pirrotta, 1998). This is reminiscent of cosuppression in plants, where the addition of a transgene often results in silencing of an endogenous locus. Conceivably, *FIE*, *MEDEA*, or other Arabidopsis polycomb components directly mediate cosuppression; if so, polycomb-deficient mutants may display cosuppression defects. As the function of plant polycombs is unraveled, the implications are likely to be wide ranging, informing our view of apomixis, chromatin structure, and the modulation of transgene expression.

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