Role of Non-Zygotic Parental Genes in Embryogenesis and Endosperm Development in Flowering Plants

Val Raghavan

*Department of Plant Cellular and Molecular Biology, The Ohio State University,
318 West 12th Avenue, Columbus, Ohio 43210, U.S.A.*

After a prolonged period of uncertainty about the precise role of maternal genes in initiating embryogenesis in flowering plants, considerable evidence for the involvement of maternal genes in embryo and endosperm development in *Arabidopsis thaliana* has accumulated in recent years. Much attention has centered on a group of mutants known as *fis*, which display an ability to initiate partial embryogenesis and endosperm development in the absence of double fertilization. This article presents a brief overview of our current understanding of the role of non-zygotic parental genes in the development of these products of double fertilization in *A. thaliana*. Evidence shows that the expression of paternal alleles of some genes is frequently delayed during embryogenesis and endosperm development, and that the silencing occurs at the transcriptional level by genomic imprinting.

Key words: *Arabidopsis thaliana*, embryogenesis, endosperm development, *fis* class mutants, genomic imprinting.

INTRODUCTION

The most defining feature of the reproductive biology of flowering plants is the process of double fertilization resulting in the development of the diploid zygote and the triploid primary endosperm nucleus. Whereas the zygote gives rise to the embryo – the progenitor of the future plant – the primary endosperm nucleus forms a nutritive tissue known as the endosperm. In contemplating how the embryo of a flowering plant with its well-defined shoot and root apical meristems has evolved from a single-celled zygote, it is hard to avoid postulating a role for gene action at successive stages of embryogenesis. The genes activated come either from parts of the parental genome or from the zygotic genome, or both. Indeed, it has been clear for a long time that genetic factors are intrinsically responsible for establishing the polarity and body plan of the early embryo and in programming the morphogenetic and tissue differentiation processes, general housekeeping chores, and seed protein accumulation at appropriate embryogenic stages; more recently, gene action has been implicated in the lapse into dormancy of embryos during their final stage of development (for review: Raghavan, 1997). This paper presents a brief overview of our current understanding of the role of non-zygotic, parental genes – maternal and paternal – during early embryogenesis,

a topic that has lain dormant for a long time but has suddenly been reawakened, yielding to insightful investigations employing genetic and molecular techniques. Although the life of general conclusions based on studies of one or two model systems may be short, we are on the threshold of gaining precise information on the role of parental genes in embryogenesis and endosperm development.

Reviews by Chaudhury and Berger (2001), Chaudhury et al. (2001) and Grossniklaus et al. (2001) have covered parts of this topic from different perspectives.

ASYMMETRY IN PARENTAL GENOME CONTRIBUTIONS

One of the established concepts in animal embryology is that the unfertilized egg cytoplasm is blessed with templates of stored mRNAs to code for the first proteins necessary to guide the initial development of the embryo. After fertilization, the influence of the embryo genome over further development generally begins to be exerted with the blastula stage of the embryo. Initial support for the presence of stored mRNAs in animal eggs came from investigations showing nearly normal development in parthenogenetically activated enucleated eggs of sea

*e-mail: raghavan.1@osu.edu

urchins, and partial development of fertilized eggs of insects, sea urchins, and amphibians treated with inhibitors of mRNA synthesis such as actinomycin-D and α-amanitin. Following confirmation of the presence of stored templates by in vitro translation of mRNAs extracted from unfertilized eggs of model organisms, further biochemical and molecular studies of these systems have provided a detailed chronology of the changes in messenger abundance in the egg, zygote and early stage embryos, the disappearance of stored templates, and the assumption of transcriptional control by the embryo genome. The decisive experiments in this context are reviewed by Davidson (1986). As embryogenesis in flowering plants occurs within the privileged confines of the embryo sac which itself is embedded in the sporophytic tissues of the ovule, the inaccessibility of egg cells, zygotes and early-division embryos has been a hindrance to biochemical and molecular investigations in search of stored mRNAs and their disposition during development. However, there is considerable indirect evidence in favor of, and against, the involvement of the maternal genome during embryogenesis in flowering plants. Support for claims of an independent role for maternal transcripts in directing early embryo development in the absence of fertilization includes stimulation of division of the egg cell by pollen tubes from which sperm are inactivated by physical and chemical agents (Lacadena, 1974), and the purported origination of embryos from egg cells of cultured unpollinated ovules or ovaries (Yang and Zhou, 1982). In a broad sense, a role for maternal programming of embryo development independent of fertilization can be invoked to explain diplosporous and aposporous types of apomixis – plants in which the embryo arises parthenogenetically from an unreduced egg cell or from a somatic cell of the ovule – with the caveat that apomicts might be considered special cases in which the sexual processes have been changed over evolutionary time to the apomictic type (for reviews: Ramachandran and Raghavan, 1992; Koltunow, 1993; Koltunow and Grossniklaus, 2003). Although endosperm development in most apomicts follows pollination and/or fertilization by a process known as pseudogamy, there are reports of division of the polar nuclei unencumbered by fusion with a second sperm cell to generate the endosperm in certain apomicts (Koltunow, 1993; Koltunow and Grossniklaus, 2003). In the absence of a full elucidation of the mechanisms involved, the maternal genome might be considered to trigger autonomous endosperm development in the small number of apomicts, and as well as endosperm formation observed in cultured unfertilized ovules of certain plants (Wijowska et al., 1999). A substantial contribution of the maternal genome in promoting embryogenesis and plant formation is seen in interspecific hybrids of *Hordeum vulgare* × *Hordeum bulbosum*. The appearance of *H. vulgare*-like haploid progenies in the cross, coupled with cytological analysis of embryos, led to the conclusion that the chromosomes of *H. bulbosum* are lost following fertilization, allowing the zygote to progress through embryogenesis using the maternal genome (Kasha and Kao, 1970). Some of the above findings generally support the view that the unfertilized angiosperm egg cell has the potential to initiate the developmental program of the embryo using maternal transcripts. The suggestion that maternal transcripts might not be necessary for early zygotic divisions (Russinova and de Vries, 2000) is supported by the finding that embryo-like structures can be produced in the absence of the maternal environment, namely, somatic embryogenesis (embryogenic pathway followed by somatic cells in tissue culture; Raghavan, 2005), particularly when taken together with work on pollen embryogenesis (the transformation of pollen grains of cultured anthers into embryo-like structures; Reynolds, 1997). That both parental alleles of a large number of genes strewn through the embryo genome control embryo development is suggested, however, by the isolation of many recessive embryo-defective and embryo-lethal mutations from *Arabidopsis thaliana* (Meinke, 1994) and *Zea mays* (Clark and Sheridan, 1991).

EVIDENCE FOR MATERNAL-EFFECT GENES

An appreciation of the classical view – that following double fertilization, formation of viable seeds in flowering plants depends upon the coordinated parallel development of the embryo and endosperm within the haploid female gametophyte and of the diploid sporophytic ovular tissues surrounding the female gametophyte – has considerably strengthened the evidence for maternal programming of embryogenesis in angiosperms by the so-called "maternal-effect" genes, but not without a few twists. Recognition of the importance of cellular interactions between the embryo, endosperm, and the maternal gametophyte and sporophyte has led to the interpretation that seed development entails possible non-zygotic influences in the form of gene action from sporophytic and gametophytic parts of the ovule. This means that any mutations on seed development affecting the signature of maternal genes can be caused by either gametophytic or sporophytic genes of maternal origin. The involvement of a maternal gene controlling embryo development in flowering plants first came to light from genetic analysis of embryogenesis in the recessive *short integuments1* (*sin1*) mutant of *A. thaliana*. The mutant ovules, which have abnormal integuments, also fail to form a normal embryo sac due to aberrant megasporocyte meiosis and are thus female-steriles (Robinson-Beers et al., 1992; Schauer et al., 2002). A series of crosses between flowers either homozygous or heterozygous for the wild-type *SIN1* allele as the female parent (+/+, +/*SIN1*) and either wild-type or *sin1* mutant plants as pollen donors showed that embryos of homozygous mutants are normal in every respect when they develop within the embryo sac of a heterozygous mutant maternal sporo-

phyte; however, when the maternal sporophyte is a homozygous mutant (*sin1/sin1*), defects confined mainly to the cotyledons are observed in the surviving embryos. These results led to the conclusion that the *sin1* mutation displaying a maternal effect on embryogenesis is sporophytic in nature and that the *SIN1* gene product might influence embryo development by the production of a signaling molecule from tissues of the ovule lining the embryo sac (Ray et al., 1996). Another study has shown that in transgenic *Petunia hybrida* the down-regulated expression of two MADS-box genes, *FLORAL BINDING PROTEIN7* (*FBP7*) and *FBP11*, leads to the production of shrunken ovules with partially or totally disintegrated endosperm and slow-growing, occasionally arrested embryos. Here also, genetic analysis has established that the shrunken ovule phenotype is a maternal sporophytic effect which indirectly causes a major lesion in endosperm development (Colombo et al., 1997). The fact that the integuments of the ovule are affected in both *Arabidopsis* and *Petunia* mutants might, however, indicate a secondary effect of the abnormal integuments on the development of the embryo.

Loss-of-function mutations in a cluster of genes of *Arabidopsis thaliana* now known as *FERTILIZATION-INDEPENDENT SEED2* (*FIS2*) (Chaudhury et al. 1997), *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*, allelic to *FIS3*) (Ohad et al., 1996, 1999; Luo et al. 1999), *MEDEA* (*MEA*, allelic to *FIS1, F644*) (Ohad et al., 1996, 1999; Grossniklaus et al., 1998; Kiyosue et al., 1999; Luo et al., 1999), *MEDICIS* and *BORGIA* (Guitton et al., 2004) have been shown to condition a program of seed development resulting in very little or no embryo development and some endosperm proliferation in the absence of fertilization. Of these mutants, those that have received the most attention are *fis2*, *fie*, and *mea* (referred to as *fis* mutants; Grossniklaus et al., 2001) which display a maternal-effect seed abortion phenotype, in addition to their ability to initiate a partial embryo and endosperm developmental program in the absence of fertilization. According to Grossniklaus et al. (1998), the wild-type *MEA* gene is expressed in the female gametophyte of *A. thaliana* before fertilization, and is required for normal post-fertilization seed development, especially of the endosperm. Genetic analysis of the effect of the mutant gene on seed formation has given tantalizing glimpses of its activity: the constellation of developmental defects observed, such as delayed morphogenesis, excessive cell proliferation in the embryo, and reduced free nuclear divisions in the endosperm caused by the mutation, has been attributed to disruption of action of the gene transmitted through the female gametophyte. The basis for this conclusion is the observation by a traditional genetic approach that when the mutant heterozygote (*mea/+*) is self-fertilized, nearly 50% of the seeds house defective embryos. Since half of the haploid female gametes generated in the cross carry the mutant allele, maternal gametophytic control of embryogenesis is apparent here. Normal seed set occurs when wild-type females are pollinated with *mea/+* pollen, but nearly 50% of seeds derived from mutant eggs in the reciprocal cross collapse late in ontogeny by suffering significant embryo and endosperm developmental defects. As the oversized embryos derived from mutant eggs succumb irrespective of the nature and dosage of the paternal contribution, completion of embryogenesis and the formation of viable seeds appear to depend upon the presence of a wild-type *MEA* allele in the female gametophyte – specifically, to reduce cell proliferation in the embryo. A possible endosperm effect in the causation of embryo lethality in the *mea* mutant was ruled out by showing that two paternal copies of the *MEA* gene in the endosperm, generated by crossing *mea/+* females with pollen from a tetraploid line, could not overcome the 50% abortion rate in seeds (Grossniklaus et al., 1998). Whether the *MEA* gene functions to restrict embryo growth in wild-type *Arabidopsis* has been questioned, however, the suggestion being that the gene might act principally to inhibit endosperm proliferation (Scott et al., 1998b). By genetic analysis of the inheritance pattern of two mutant *mea* alleles, Kiyosue et al. (1999) have independently confirmed a requirement for only the maternal, gametophyte-specific wild-type *MEA* allele, and the dispensability of the paternal allele, for normal embryo and endosperm development in *A. thaliana*. Like the *mea* mutant, phenotypes of *fie* and *fis1* mutants cause partial endosperm development and inflict embryo lethality only when the mutant alleles are inherited from the female parent, and thus resemble maternal-effect defects. In addition to the seed abortion phenotype, the three mutants display, at a low frequency, precocious endosperm proliferation before fertilization (Ohad et al., 1996; Chaudhury et al., 1997; Kiyosue et al., 1999). Also, subsequently it has been shown that in addition to the maternal sporophytic effect described earlier, the pattern of inheritance of post-zygotic expression of the *SIN1* gene in *A. thaliana* is suggestive of a maternal gametophytic effect (Golden et al., 2002).

The product of the *MEA* gene is a member of a subgroup of the polycomb group of proteins of *Drosophila melanogaster*; the hallmark of proteins of this subgroup is a 130-amino-acid motif known as the SET domain – a family of regulatory proteins encoded by genes such as *SUPPRESSOR OF VARIEGATION*, *ENHANCER OF ZESTE*, and *TRITHORAX*. Polycomb proteins are a structurally disparate set of proteins which have the intriguing ability to function as gene silencers by controlling the normal one-way traffic of transcription factors to DNA. The protein products of *MEA*, *FIE*, and *FIS1* genes have close affinities, thus reinforcing the view that they are part of a complex that determines the expression of regulatory genes during seed development. The *FIE* gene product shares strong similarities with a second subgroup of polycomb proteins whose defining characteristic is the presence of a WD domain – a

40–60-amino-acid repeat unit which usually ends with the tryptophan-aspartic acid pair. Proteins in this subgroup are encoded by *Drosophila EXTRA SEX COMBS* (*ESC*) and mouse and human *EMBRYONIC EC-TODERM DEVELOPMENT* (*EED*) genes (Ohad et al., 1999). The polycomb protein with the SET domain has resurfaced in *FIS1* (considered allelic to *MEA*), *F644* (a *FIE*-like gene), and *EMB173*, a previously reported gene that causes defects in embryo development, reassigned as an *MEA* gene allele (Castle et al., 1993; Kiyosue et al., 1999; Luo et al., 1999). The only holdout from the polycomb net seems to be the *FIS2* gene, whose protein is predicted to contain a zinc finger motif and three nuclear localization signals, suggesting that it is linked to the transcriptional machinery (Luo et al., 1999).

In general terms, the polycomb proteins may be thought to regulate, by hitherto unknown mechanisms, target genes involved in cell proliferation in the embryo and endosperm of developing seeds of *A. thaliana*. Köhler et al. (2003) have assigned the MADS-box gene *PHERES1* (*PHE1*) the key role of a downstream target for transcriptional repression by the FIS-class protein products. This is based on the transient expression of the PHE1 gene in seeds of wild-type plants containing preglobular-stage embryos, and its considerably high level of expression in the seeds of *fis*-class mutants. With its focus on the *mea* mutant, this work also showed that high levels of expression of the *PHE1* gene in the mutant are causally linked with seed abortion, and that it is possible to rescue the seed abortion phenotype in the mutant by a reduction in the *PHE1* expression level. These results fit well with the proposed role of polycomb proteins in embryo development of *A. thaliana*.

SILENCING OF PATERNAL GENES

In the work on *mea* mutant described earlier, indicative of maternal transcription of the *MEA* gene in the female gametophyte of *A. thaliana*, in situ hybridization showed the presence of *MEA* mRNA in the synergids, egg and central cell before fertilization. There was a persistent presence of transcripts in the cells of the embryo and endosperm after fertilization, probably due to zygotic transcription. An improved in situ hybridization procedure which allowed quantitation and detection of nuclear dots associated with nascent gene transcripts also revealed that nuclear dots present in the triploid primary endosperm nucleus are of the maternally inherited *MEA* allele, and not of the paternal allele. This raises a question as to when the paternal genome becomes active during the post-fertilization interlude. That the paternally inherited *MEA* alleles are silenced during the development of the embryo and endosperm was shown by examining *MEA* gene expression by reverse-transcription polymerase chain reaction (RT-PCR) of RNA prepared from embryo-bearing siliques of reciprocal crosses between wild-type and *mea* mutant

alleles (Vielle-Calzada et al., 1999). One question raised by the differential functioning of the maternal and paternal genes is how the development of the endosperm and embryo can proceed by inheritance of a wild-type allele from the female but not from the male gametophyte. Additional experiments have provided clear evidence to show that the expression of paternal alleles is frequently delayed during embryogenesis and seed development, and that the silencing occurs at the transcriptional level by genomic imprinting. This process, almost universally relevant in animal systems but much less so in plants, represents a situation in which the two parental alleles of a gene may show differential activity during development of the zygote, leading in extreme cases to some genes being expressed predominantly from one of the parental chromosomes only; the genome of the other parent is kept transcriptionally inert by the silencing mechanism that presumably blocks the normal flow of transcription factors. Obviously, genomic imprinting contravenes the expectation of equal participation of the genome inherited from both parents in development, and in the absence of paternally inherited alleles, early divisions of the zygote are presumed to be programmed by their maternal copies. One critical piece of evidence for genomic imprinting has come from analysis of parental chromosome-specific expression of a cluster of 20 genes during embryogenesis and seed development in reciprocal crosses between wild-type and transposants of *A. thaliana* that harbor a reporter gene construct, to monitor gene expression. It was found that from a state of initial transcriptional inactivity, paternal copies of the genes became active only after seed development progressed for more than three or four days after fertilization when the embryo produced 32–64 cells. The protein products of some of the genes showing delayed paternal expression have been found to be associated with important cellular functions such as cell cycle regulation, transcription, and the assembly of protein secondary structure, all of which makes a case for global paternal gene silencing during embryogenesis. These observations make a compelling case that the molecular effect on the embryo inheriting maternal alleles of *MEA* and other genes is the silencing of paternally inherited alleles by genomic imprinting (Vielle-Calzada et al., 2000). Another work showed that in progenies of crosses between two *A. thaliana* ecotypes the maternal *MEA* allele is detected only in the endosperm of seeds harboring torpedo-shaped and older embryos, but that both parental alleles are expressed in embryos of the same ages. The implication of these results is that in seed development, genomic imprinting directly affects the endosperm but not the embryo, whose abortion in the *mea* mutant is probably engineered by some defective endosperm function (Kinoshita et al., 1999; see also: Scott et al., 1998b). Using a reporter gene construct to monitor gene expression, in addition to the *MEA* gene, *FIS2* and *FIE* (*FIS3*) genes have also been shown to be imprinted in the embryo and endosperm nurtured in the

same embryo sac (Luo et al., 2000). Since DNA methylation associated with transcriptional repression is involved in genomic imprinting observed in animals, evidence for its involvement in parent-of-origin effects (namely, seed viability depending upon the presence of a wild-type maternal allele of a critical gene) during seed development in *A. thaliana* has been obtained by some investigators using interploidy crosses and hypomethylated plants (Scott et al., 1998a; Vielle-Calzada et al., 1999; Adams et al., 2000; Vinkenoog et al., 2000). According to Yadegari et al. (2000), *FIE* and *MEA* genes interact directly in wild-type *A. thaliana* to control development of the female gametophyte, endosperm and embryo; these authors have marshaled impressive evidence to provide a different perspective on the role of these genes by suggesting that they inflict parent-of-origin effects on seed development by different mechanisms. One such mechanism is probably through activation of the maternal copy of the *MEA* gene by a DNA glycosylase-encoding *DEMETER* (*DME*) gene (Choi et al., 2002).

Nevertheless, the precise mechanism by which an imprint is conferred on the paternal genes continues to be elusive. Moreover, the existence of differential genome-wide parental effects on the early development of the embryo and endosperm as a global or as an all-or-none phenomenon has been questioned, and evidence has been presented for an early but low paternal effect in embryos of *A. thaliana* (Baroux et al., 2001; Vielle-Calzada et al., 2001; Weijers et al., 2001) and in *Zea mays* zygotes obtained by in vitro fertilization (Scholten et al., 2002). The *PROLIFERA* (*PRL*) gene of *A. thaliana* encodes a protein that regulates DNA replication in dividing cells and which on the basis of genetic evidence appears to be preferentially transcribed from the maternally contributed genome; expression of the gene from both paternally and maternally supplied alleles in the developing embryo and endosperm, demonstrated by in situ hybridization using a reporter gene, has nevertheless ruled out imprinting of this gene (Springer et al., 2000). Two newly described *capulet* (*cap1* and *cap2*) mutants of *A. thaliana* have been found to be female-gametophytic, displaying maternal effects on embryo and endosperm development. Unambiguous evidence to support imprinting of the CAP genes is wanting, however; rather, several genetic and molecular criteria have led to the suggestion that these genes represent true female-gametophytic genes required to initiate divisions in the products of double fertilization (Grini et al., 2002).

CONCLUDING REMARKS

Evidence has accumulated to show that, far from being under the control of both paternal and maternal genomes, the first few rounds of division of the zygote and the endosperm nucleus during seed formation are under the overriding control of the maternal genome (Reyes and Grossniklaus, 2003). In most cases the ab-

sence of prefertilization expression of maternal alleles has not been demonstrated convincingly enough to conclude whether gene activity observed in the zygote or in the primary endosperm nucleus is caused by newly transcribed maternal mRNAs or by transcripts present in the cells of the prefertilization embryo sac. Undoubtedly, understanding the role of maternal transcripts in initiating development in flowering plants has become more complex than in animal systems, in part because of the occurrence of double fertilization and the genetically intractable nature of the plant life cycle, alternating between dominant sporophytic and relatively inconspicuous gametophytic generations.

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