Unequal contribution of the parental genomes to the developing embryo can occur by a variety of mechanisms. In Drosophila, for example, maternally produced transcripts determine the embryonic formation of body axes (St. Johnston and Nüsslein-Volhardt, 1992). Inequality in parental contribution of a different nature is achieved by genetic imprinting, in which either the maternal or paternal allele of a given gene pair is silenced. In Drosophila, studies with compound chromosomes consisting of either two maternally inherited or two paternally inherited arms established that no imprinting occurs (Strommen, 1982), even among early patterning genes. In mammals, imprinting can involve either the paternal or maternal copy of a gene and is thought to reflect an additional level of control over fetal growth (Tilghman, 1999). Indeed, many of the genes found to be imprinted appear to affect the growth and development of the fetus. Several recent papers have addressed the importance of maternal contributions to embryonic development in plants and have also provided evidence for the existence in plants of parental silencing during embryogenesis.

In plants, the gametophytic (haploid) phase begins with the formation of the megaspore mother cell embedded deep in the ovule primordium and with formation of microspores in the developing anther. The sporophytic phase begins after two sperm cells reach the female gametophyte and fertilize the egg cell and the binucleate central cell, giving rise to the diploid embryo and the triploid endosperm, respectively. The embryo develops within the haploid female gametophyte, itself embedded in diploid maternal tissue. On the basis of the finding that somatic cells are able to deviate from their normal fate and embark upon an embryo pathway in the absence of a maternal environment (Martienssen, 1998), it is often argued that maternal transcripts do not contribute to the early zygotic embryo. Thus, zygotic plant embryos are thought to rely exclusively on the activation of zygotic genes immediately after fertilization. However, the coordinated interplay between maternal and gametophytic tissues is required to deliver a fully differentiated and developed embryo within the seed. In tissue culture, such interplay can be mimicked by "nurse cells" that take over the role of maternal tissues (McCabe et al., 1997; Mordhorst et al., 1997).

Genes have been described that have either a maternal gametophytic effect (Ohad et al., 1996; Chaudhury et al., 1997; Grossniklaus et al., 1998) or a maternal sporophytic effect (Ray et al., 1996; Colombo et al., 1997) on seed development. One of the genes with a maternal contribution to seed development, the Arabidopsis MEDEA (MEA) gene, encodes a SET domain protein of the Polycomb group (Grossniklaus et al., 1998). Genes belonging to the Polycomb group of genes are involved in maintenance of higher order chromatin structure. Loss-of-function alleles of such genes can result in failure to maintain active transcription or to maintain active repression of other genes during development. The mea phenotype is complex, and mutants with a defective allele in the embryo sac show excess cell division in the embryo and reduction of free nuclear divisions in the endosperm. In addition, they exhibit a fertilization-independent seed (fis) phenotype at low frequency (Grossniklaus and Vielle-Calzada, 1998). No gametophytic phenotype has been reported, although the gene is expressed both before and after fertilization in embryo sac, embryo, and endosperm cells.

The first clue for paternal silencing of the MEA gene during early endosperm development came from in situ hybridization data that showed only two transcribed mea loci in the triploid central cell nuclei after fertilization. Analysis by reverse transcription-polymerase chain reaction (RT-PCR) revealed that paternally inherited mea alleles are silenced in endosperm and in midglobular stage embryos (Vielle-Calzada et al., 1999). Further genetic analysis showed that the transcriptional regulation of the paternal MEA gene is allele-independent, although some effect of ecotype variation on the rescue of mutant mea alleles was noted. The partial rescue of the mea seed abortion phenotype by a ddm1 mutant that shows a 30% reduction in overall genomic DNA methylation suggested a link between chromatin organization and genomic imprinting of the mea locus. The observed gametophytic maternal effect on development of the embryo is thus concluded to be due to the prevention of expression of the paternal mea allele by genomic imprinting (Vielle-Calzada et al., 1999).

A second paper dealing with the
same mea mutation confirms these studies and argues that the silencing of the paternal allele occurs only in the endosperm and not in the embryo itself (Kinoshita et al., 1999). Employing allele-specific RT-PCR, the authors looked at maternal and paternal MEA expression in torpedo-stage embryos and in the endosperm. Only in the endosperm was the maternal copy detected, whereas the embryo expressed both parental genes. These results suggested that the paternal silencing was a transient effect occurring in the embryo at least up to late globular stage. These results underscore the speculation of Grossniklaus et al. (1998) that an early and important interaction occurs between the two tissues, because defective mea activity in the endosperm results in an embryo mutant phenotype. No further molecular details are available to indicate whether this interaction involves a single gene or several genes. Nevertheless, a picture is emerging in which the MEA gene, first expressed in the female gametophyte, controls the proper balance of cell division in the embryo and in the endosperm, and in which only maternal copies of the gene are required for the completion of seed development.

It could be argued at this point that imprinting in plants is restricted to the mea locus only and is not a general phenomenon of the genome as is the case in mammals (Tilghman, 1999). The question of the extent of genomic imprinting in plants is the subject of the most recent paper by Vielle-Calzada et al. (2000), in which two lines of evidence are presented to suggest that paternal silencing during early embryogenesis involves many loci. The first piece of evidence in favor of general paternal silencing is the fact that none of 20 genes inspected during early seed development were expressed from the paternal copy. The second argument is more complex and involves a mutation in the EM30/GNOM gene. This gene encodes a guanine nucleotide exchange factor (GEF) that acts on ADP ribosylation factor (ARF)-type G proteins and is involved in vesicular trafficking (Steinmann et al., 1999). The function of ARF GEF proteins is to aid in assembly of the coatamer complex essential for vesicle budding. gnom mutant embryos appeared in several screens for early embryo mutants and are believed defective in establishing the apical-basal embryo axis that arises as the normal asymmetric, first division of the zygote is replaced by a symmetric one. Genetic analyses of the gnom mutation showed that it acts zygotically and segregates in a normal mendelian manner; no gametophytic or maternal defects have been reported. On the basis of mislocalization of the auxin efflux carrier PIN protein in gnom mutant embryos, a role for the GNOM protein in polar auxin transport was proposed (Steinmann et al., 1999). These findings also provided the first clear evidence that an auxin gradient established in the early globular Arabidopsis embryo is instrumental in proper axis formation. It is not clear to what extent the phenotype of gnom in the zygote relates to the mislocalized expression of the PIN protein. Employing interaction screening, Grebe et al. (2000) recently identified cyclophilin 5, a peptidylprolyl cis/trans isomerase, as another member of the GNOM-containing vesicle coatamer complex.

What is the connection between the studies of paternal silencing during early embryogenesis in Arabidopsis and the GNOM protein? To detect allele-specific GNOM expression in developing seeds, Vielle-Calzada et al. (2000) combined an allele-specific restriction site in the gnom locus (Columbia ecotype) with RT-PCR. The results were that no paternal transcript could be detected up to 24 hr after pollination, suggesting that the GNOM gene undergoes paternal silencing and that the zygotic embryo depends exclusively on the maternal copy to perform early globular patterning. Of course, such a conclusion would be weakened if the ratio of transcripts in maternal tissues to transcripts in early embryo and endosperm is too low to allow detection of the paternal transcript.

The second piece of evidence from Vielle-Calzada et al. (2000) that suggests paternal silencing of GNOM comes from the pollination of plants that are heterozygous for the gnom mutation. If paternal imprinting of GNOM occurs, the expectation is that in selfed heterozygous plants, 50% (rather than 25% if the paternal allele is not silenced) of embryos would show the early mutant phenotype; pollination by wild-type pollen would similarly result in embryos affected at a rate of 50%. Vielle-Calzada et al. found in fact a frequency of 12% for early aborted embryos, regardless of the paternal genotype. All individuals derived from the heterozygote pollinated with wild-type pollen, moreover, behaved as wild type, which would be predicted from activation of the paternal gene later in embryogenesis. The observation that only 12% of embryos from these crosses show the gnom phenotype is attributed by Vielle-Calzada et al. (2000) to low expressivity. The authors suggest that gnom is a paternally rescuable maternal-effect phenotype, scored as a zygotic lethal (which would give the observed 25% lethality). Although this is a highly exciting finding, it needs to be further verified by looking at mutants with a higher penetrance during early embryogenesis.

In any event, it is clear that genomic imprinting is involved in the regulation of the MEA gene. Whether the fascinating suggestion that a general silencing of the paternal genome occurs in early embryo development holds true in all cases may require analysis of other early-acting genes. The experiments reported above also fuel questions relating to the ways that microspore embryos overcome paternal silencing and to the functional significance of paternal silencing in self-fertilizing plant species.
REFERENCES


