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Timing of the Maternal-to-Zygotic Transition during Early Seed Development in Maize [W]

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In animals, early embryonic development is largely dependent on maternal transcripts synthesized during gametogenesis. Recent data in plants also suggest maternal control over early seed development, but the actual timing of zygotic genome activation is unclear. Here, we analyzed the timing of the maternal-to-zygotic transition during early *Zea mays* seed development. We show that for 16 genes expressed during early seed development, only maternally inherited alleles are detected during 3 d after fertilization in both the embryo and the endosperm. Microarray analyses of precocious embryonic development in apomictic hybrids between maize and its wild relative, Tripsacum, demonstrate that early embryo development occurs without significant quantitative changes to the transcript population in the ovule before fertilization. Precocious embryo development is also correlated with a higher proportion of polyadenylated mRNA in the ovules. Our data suggest that the maternal-to-zygotic transition occurs several days after fertilization. By contrast, novel transcription accompanies early endosperm development, indicating that different mechanisms are involved in the initiation of endosperm and embryo development.

INTRODUCTION

The maternal-to-zygotic transition is the first major developmental switch of an organism's life cycle. It occurs after fertilization and entails extensive reprogramming of gene expression, leading to the establishment of an embryo-specific developmental program. In animals, including mammals, *Caenorhabditis elegans*, or Xenopus, there is a delay between fertilization and the maternal-to-zygotic transition (i.e., the zygotic genome becomes active only after several rounds of cell division) and early embryogenesis is largely dependent on maternal transcripts deposited in the egg cell before fertilization (Flach et al., 1982; Newport and Kirschner, 1982; Newman-Smith and Rothman, 1998; Pelegri, 2003). The length of this delay is species dependent, occurring at the two-cell stage in mice, the 28-cell stage in *C. elegans*, and at the mid-blastula stage in Xenopus. As a result, early events involved in cell division or pattern formation are programmed almost entirely through maternal mRNA.

The timing and mechanisms of the maternal-to-zygotic transition in higher plants are unclear. The reproductive cycles in angiosperms and animals differ in important ways (reviewed in Walbot and Evans, 2003). First, plants do not have a germline as such; whereas specialized cells are defined early during embryonic growth in animals, plants produce meiocytes after a developmental switch in somatic stem cells late in development. Moreover, gametes in plants are formed after a long haploid phase that includes the formation of multicellular gametophytes. The outcome of reproduction, the seed, contains tissues of distinct origin, maternal tissues like the seed coat, and two hybrid tissues, the embryo and the endosperm, resulting from the double fertilization process that occurs within the female gametophyte. Current genetic data show that proper embryo formation requires synchronized development and cross-communication among all three types of tissues (Ray et al., 1996; Colombo et al., 1997; Grossniklaus and Schneitz, 1998). Also, the many forms of asexual reproduction in plants illustrate some flexibility regarding the cell types that can be involved in reproductive pathways. Many cell types, for example, are apparently capable of late dedifferentiation and can be directed toward somatic embryogenesis in tissue culture. Furthermore, spontaneous occurrence of both gynogenic (development of female haploids) and androgenic (male haploids) embryos indicates that neither the male nor the female genomes are strictly necessary for embryo formation in plants.

Genetic analyses in *Arabidopsis thaliana* have suggested that the activity of many genes acting during early embryo and endosperm formation depends largely on transcription from the maternally inherited alleles (Vielle-Calzada et al., 2000). In this experiment, none of the paternally inherited alleles at 20 different loci were expressed during early seed development, leading to the conclusion that maternal transcripts might be sufficient to direct early developmental stages in both the embryo and the

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endosperm. This model is still controversial; some evidence has been presented for early-transcribed paternal alleles in Arabidopsis (Weijers et al., 2001; Kohler et al., 2005) and expression of a paternally inherited transgene driven by a viral promoter in maize (*Zea mays*) (Scholten et al., 2002). Also, the phenotypes of numerous embryo lethal mutants segregate with a typical sporophytic 3:1 ratio (data can be found at www.seedgenes.org; Tzafrir et al., 2004), suggesting no apparent maternal effects. However, paternal silencing (or a least a strong reduction in the expression of paternal alleles) has also been independently confirmed for several developmental genes or markers (Baroux et al., 2001; Sorensen et al., 2001; Golden et al., 2002).

Even assuming that most of the paternally inherited genome undergoes delayed activation, important questions remain unanswered. First, it is unclear whether paternal genome silencing could be generalized to early seed development in other plants. The analogy with animal models suggests that extensive variability is possible. Second, even for those genes where maternal control has been clearly demonstrated, it is not known whether the maternal transcripts are gametophytic (deposited before fertilization), zygotic (produced after fertilization), or both. Third, it is unclear whether the activation course of the genome is similar in the embryo and the endosperm.

To address these issues, we first analyzed the parent-of-origin expression of seed-expressed genes during early seed development in wild-type maize plants. Next, we analyzed transcription during early embryogenesis both in maize and in apomictic hybrid plants between maize and Tripsacum, where the embryo develops parthenogenetically without paternally inherited alleles (Leblanc et al., 1996). We showed that the transcriptional delay observed for some or all of the paternally inherited genome in Arabidopsis is also an attribute of early maize seed development. Our data further indicate that the early stages of embryo development occur without detectable modification to the transcript population already in the ovule but are associated with a significant increase in the amount of polyadenylated mRNA in the ovules. It appears, therefore, that the maternal-to-zygotic transition in maize occurs only after several divisions of the zygote. By contrast, a novel transcription pattern of maternal alleles accompanies early endosperm development, indicating that different mechanisms are involved in the initiation of endosperm and embryo transcriptional programs.

RESULTS

Delayed Activation of the Male Genome during Early Seed Development in Maize

Data from Arabidopsis (Vielle-Calzada et al., 2000) suggest that a large number of seed-expressed genes experience delayed activation of the paternally inherited genome after fertilization. To verify whether this model is also relevant to early maize seed development, we used allele-specific RT-PCR 3 d after pollination (DAP) to test for the presence of male and female transcripts of 16 genes expressed during early seed development.

Because it is technically difficult to extract the endosperm and the embryo from the surrounding nucellar tissues at early stages, the experiments were conducted with dissected ovaries or seeds, which consist predominantly of maternal sporophytic tissues. In the text, we will refer to the ovule whenever considering either the ovule before fertilization or the samples obtained by dissecting the developing seed (not including the pericarp and external nucellar cell layer) after fertilization of the ovule. In both cases, it consists of either the nucellus and the gametophyte (before fertilization) or the nucellus, embryo, and endosperm (after fertilization).

To demonstrate the feasibility of detecting embryo- or endosperm-specific expression in fertilized ovules with RT-PCR, we first tested several genes with known expression patterns within the early seeds. Three of them (Zm*fie2*, Zm*ocl1*, and Zm*ocl5*) are expressed in the embryo. Specifically, Zm*fie2* is first detected in the mature female gametophyte and, after fertilization, in the embryo and, at a lower level, in the endosperm (Danilevskaya et al., 2003); both Zm*ocl1* and Zm*ocl5* are expressed in the protoderm layer of the embryo during early development (Ingram et al., 2000). We similarly assayed three genes coding for storage proteins in the endosperm, a legumin homolog (AW216194), an α-globulin (AF371278), and a β-zein (AB073081). Experiments with mRNA and antibody localization in maize have shown that these genes are specific to the growing endosperm (Woo et al., 2001).

Some of these genes, particularly the storage protein genes, belong to large families. We therefore designed all primer pairs to amplify specific loci. We further sequenced the PCR products to verify that the amplifications matched a single locus. As a control against possible contaminations by samples at later stages of development, we also analyzed the expression of Zm*fie1*, which is known to be expressed exclusively in the endosperm starting 5 to 6 DAP (Danilevskaya et al., 2003). Expression of Zm*fie1* in our materials was consistent with prior reports (Danilevskaya et al., 2003) in that it was not detected before 5 DAP and strongly expressed starting at 6 DAP (data not shown).

As shown in Figure 1A, all three embryo-expressed genes were detected by RT-PCR in ovules containing mature female gametophytes (0 DAP) and at 3 DAP. Similarly, we detected expression for all three storage protein genes at 3 DAP (Figure 1C). Thus, for all six genes, RT-PCR was sensitive enough to detect expression in the embryo and/or the endosperm.

We then attempted to analyze the expression of these genes in an allele-specific manner. For the three storage protein genes, we took advantage of the presence of simple sequence repeat (SSR) motifs within the transcript sequences (Figure 1B). A (GTC)n repeat was found at position 827 in the legumin transcript, a (GCA)n repeat was found at position 428 in the 15-kD β-zein transcript; and two (GGC)n repeats were found in the α-globulin transcript sequence (positions 125 and 282). We crossed two highly polymorphic maize inbred lines, CML216 and CML72, and dissected the seeds at various times after fertilization. RNA and DNA from 30 unfertilized ovules (0 DAP), 300 ovules 3 DAP, and 30 ovules 7 DAP were extracted in bulks of 30 ovules each, and the RNA reverse-transcribed. Primer pairs for an actin control, Zm*fie2*, and Zm*fie1*, all three pairs expending intron-exon boundaries, were used to control cDNA samples for possible contamination by genomic DNA or DNA from later stages of development (in the case of Zm*fie1*).

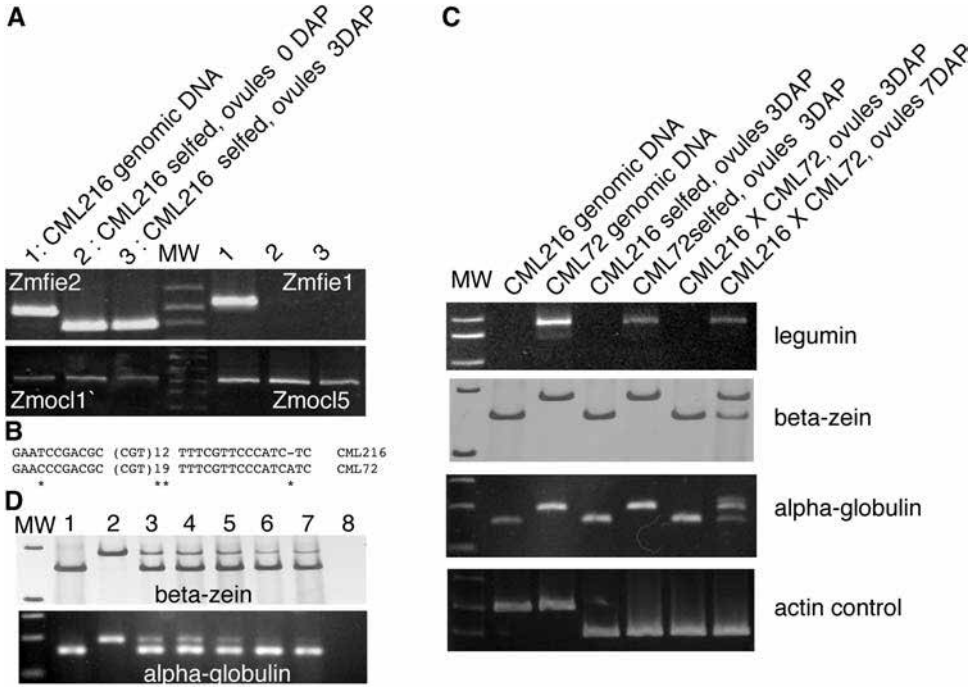

Figure 1. RT-PCR Analyses of Seed-Expressed Genes in Maize.

(A) RT-PCR assay of genes expressed during early seed development in maize. Genomic DNA was extracted from a bulk of leaf, stem, and root samples; *Zmfie1* and *Zmfie2* provide controls for genomic DNA contamination. All PCR reactions consisted of 40 cycles of amplification. MW, molecular weight.

(B) SSR-based strategy for the identification of allele-specific PCR conditions, illustrated here for the β-zein sequence. A single primer pair designed from the DNA flanking the repeats can be used to amplify both alleles.

(C) Allele-specific expression of three endosperm-expressed genes at 3 and 7 DAP. In all three cases, a paternal CML72-specific allele is detected either after amplification of genomic DNA or amplification of cDNA 3 DAP from selfed plants. Only the maternal alleles are detected at 3 DAP in a cross between both lines. Both alleles are detected 7 DAP. Note the presence at 7 DAP of an extra transcript for the α-globulin not found in fertilized ovules from selfed plants 3 DAP.

(D) Detection threshold for biallelic amplification of CML216 and CML72 alleles. Lane 1, cDNAs from ovules collected at 3 DAP from CML216; lane 2, cDNAs from ovules collected at 3 DAP from CML72; lanes 3 to 7, controlled dilutions of the same cDNAs mixed in the following proportions: 1:2, 1:10, 1:25, 1:50, and 1:100; lane 8, water negative control.

All three SSR loci were polymorphic between CML216 and CML72 when assayed either with genomic DNA or with cDNA extracted from fertilized ovules 3 DAP, as shown in Figure 1C. In the case of the legumin transcript, we detected a PCR amplification product only in the CML72 inherited allele. For the other two genes, we could detect biallelic amplification products using a single primer pair designed around the SSR motifs (Figure 1C). In all three cases, only the female transcripts were detected at 3 DAP in reciprocal crosses. At 7 DAP, by contrast, the male and female transcripts of the globulin and legumin loci were detected in each bulk (Figure 1C), as evidenced by biallelic amplification.

These results suggest either that the male alleles were not expressed at 3 DAP or that they were expressed at a lower level than the female alleles, below a detection threshold possibly induced by competitive amplification. To try to estimate this detection threshold, we performed RT-PCR with controlled dilutions of cDNA obtained from fertilized ovules at 3 DAP in CML216 and CML72. As shown in Figure 1D, dilutions up to 1:50 resulted in detectable biallelic PCR products for both loci, much below the dilution levels expected in the case of dosage-dependent gene expression in the triploid endosperm. Thus, transcripts of paternal origin, if any, were expressed at a much lower level than the female alleles. In the case of AI746088 (the β-zein transcript), for which a single CML72-specific allele was detected, the absence of amplification products at 3 DAP similarly indicated that the male allele was either not expressed or expressed below the detection threshold of RT-PCR. We did not test allele-specific expression of Zm*fie2*, which has already been reported in the literature (Danilevskaya et al., 2003) and for which only the maternal allele is expressed during the first days of seed development. The paternal Zm*fie2* allele is activated at 5 DAP. Unfortunately, we could not define satisfactory allele-specific PCR conditions for the Zm*ocl* sequences, which did not contain SSR motifs. Further screening of diverse maize genotypes would be required to obtain parental lines with appropriate polymorphisms, such as single nucleotide polymorphisms.

To further analyze the allele-specific expression of seed-expressed genes in maize, we searched the public maize EST

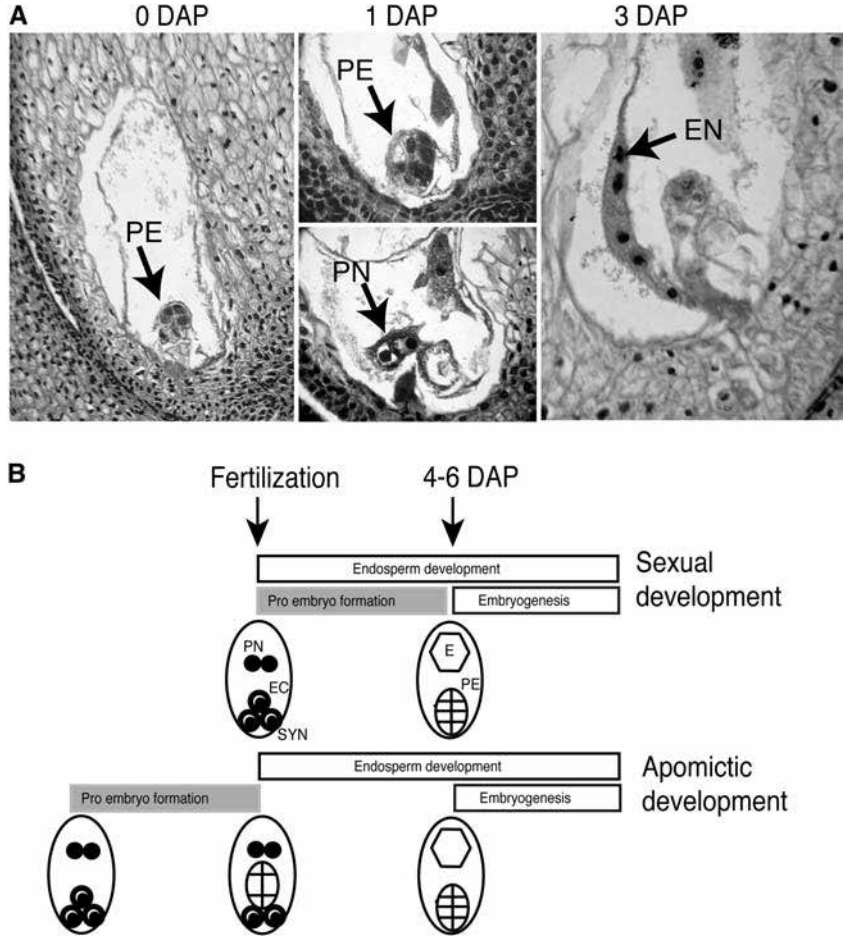

Figure 2. Strategy for Microarray Analysis of Early Seed Development.

In apomictic hybrid plants between maize and Tripsacum, as in Tripsacum apomicts, the developmental courses of the embryo and the endosperm are desynchronized. Unfertilized mature gametophytes in apomicts (0 DAP) contain a proembryo (PE) that is arrested after four to five divisions **(A)**. Micrographs of two consecutive sections of the same ovule at 1 DAP show the simultaneous occurrence of a proembryo (top section) and unfused polar nuclei (PN; bottom section). At 3 to 4 DAP, the development of the endosperm (EN) after fertilization occurs in the absence of further embryo growth, which resumes only later during development, after endosperm cellularization. Therefore, profiling mRNA from mature sexual gametophytes against mRNA from mature apomictic gametophytes **(B)** captures transcriptional differences related to early embryogenesis. Similarly, transcription specific to early endosperm development is revealed by profiling unfertilized ovules of maize against fertilized ovules in apomicts collected at 3 DAP. E, endosperm; EC, egg cell; SYN, synergids.

databases for coding sequences expressed in early seed development and containing SSR motifs in their transcript sequence as a source of allele-specific PCR products. Out of 100 sequences, we identified 43 genes with appropriate polymorphisms between CML216 and CML72 (see Supplemental Table 1 online). A key technical issue with allele-specific RT-PCR analysis is whether the relative proportion of maternal and paternal transcripts influences the detection of allele specific products (i.e., whether an overrepresentation of the maternal transcripts might dilute paternal transcripts below a biallelic expression threshold). This would be particularly relevant for genes that are also expressed somatically in the maternal tissues of the dissected ovules. For all 43 transcripts, we thus tested the detection threshold of biallelic amplification on genomic DNA of fertilized ovules sampled at 3 DAP, where the male alleles are strongly underrepresented (being present only in the embryonic cells and the endosperm nuclei). We assumed that if PCR conditions are stringent enough to detect both the male and female genomic alleles, we should also be able to detect their transcripts, whenever the genes are expressed. The male and female genomic alleles could be detected by PCR at 3 DAP in all bulks for 16 of the 43 ESTs (including the three storage proteins described earlier) in a cross between CML216 and CML72. In addition, we also used controlled dilutions to estimate a detection threshold for biallelic amplification (data not shown). For all 16 sequences, the threshold was lower than 1:100. The 16 loci were also evaluated in the reciprocal cross (CML72 by CML216). Satisfactory RT-PCR conditions were obtained for four of them (AW066244, AI833700, AI746088, and AI745997). The other 12 either failed to detect paternal genomic DNA in seed samples

at 3 DAP or showed a relatively high detection threshold. We performed RT-PCR analysis of the 16 loci with diverse maize tissues. The expression pattern of the storage proteins was consistent with the published literature (data not shown). Of the 13 sequences selected from the EST database, nine were widely expressed in plant tissues, and four showed distinctive patterns in ovules and seeds (see Supplemental Figure 1 online).

Using allele-specific RT-PCR in a cross between CML216 and CML72, only the female (CML216) transcripts were detected at 3 DAP. At 7 DAP, by contrast, the male and female transcripts were detected in each bulk for all 16 sequences. In the reciprocal cross, similar results were obtained for the four sequences tested. Thus, we assume that only the female alleles for all 16 genes were expressed at detectable levels during early seed development.

Delayed Maternal-to-Zygotic Transition in the Maize Embryo

To further characterize transcription during early seed development, we used maize microarrays to compare the transcript population present in the female gametophyte before fertilization with the transcript population present specifically in the embryo or the endosperm during their first divisions. In most sexual plants, studying embryo-specific transcription is made difficult by the process of double fertilization. The fusion of one sperm nucleus with the egg cell nucleus is concomitant with the fertilization of the central cell in the female gametophyte by a second male sperm cell, which gives rise to the endosperm. Thus, profiling of early seed tissues does not discriminate between the transcriptional patterns within the fast growing endosperm and the slower embryo.

To get around this problem, we took advantage of a specific attribute of apomictic Tripsacum, a wild relative of maize. In apomictic maize-Tripsacum hybrids, which reproduce asexually through seeds, embryo and endosperm development is uncoupled for several days (Grimanelli et al., 2003). As illustrated in Figure 2, the embryo performs up to five divisions before fertilization. After fertilization, endosperm development initiates while the embryo remains arrested developmentally, showing signs of neither cell division nor developmental differentiation throughout a 5- to 6-DAP period (i.e., once cellularization in the endosperm is complete). Thus, mature, nonfertilized ovules from apomictic maize-Tripsacum hybrids and sexual maize (or sexual maize-Tripsacum hybrids) differ only in the presence of a proembryo. Differences in expression profiles between both samples would therefore represent either proembryo-specific expression or maternal transcripts produced in somatic tissues of the ovule (nucellus and integuments) after the initiation of embryo development.

We used maize microarrays to compare the expression profile of 5534 unique sequences between unfertilized ovules from apomictic maize-Tripsacum hybrid plants (genotype 38C; Leblanc et al., 1996) with a developing proembryo (but no endosperm) and ovules from sexual maize plants with neither endosperm nor embryo. As a control, we also profiled the same set of genes with RNA extracted from fertilized ovules at 3 and 7 DAP from sexual maize, against the same nonpollinated sexual maize materials. Although maize and Tripsacum are closely related, a possible bias might have been introduced in our experiment by the interspecific nature of the apomictic maize- Tripsacum hybrids. To discard the possibility that Tripsacum cDNA would not hybridize on maize sequences spotted on the microarrays, we performed DNA gel blot analysis for a random set of 225 maize cDNA on both maize and Tripsacum genomic DNA. We quantified the intensity of the hybridization signal obtained on Tripsacum DNA gel blots and compared it to maize DNA gel blots used as a control. All maize cDNAs (data not shown) provided hybridization signals of equivalent intensity between maize and Tripsacum DNA. We therefore assumed that most, if not all, Tripsacum transcripts would cross-hybridize to the maize cDNA with significant intensity; our results therefore reflect the expression profiles of both the maize and Tripsacum transcripts during early embryo development.

The profiles obtained at 3 and 7 DAP for sexual maize indicate significant changes in transcriptional activity when compared with unfertilized ovules (Table 1). This indicates that our detection methods are sensitive enough to detect transcriptional changes during early seed development. By contrast, we could not detect significant differences in transcriptional profiles (with $P < 0.001$) between apomictic and sexual plants before fertilization. Thus, within the limits of sensitivity of microarray-based detection, proembryo development (i.e., early embryogenesis) does not induce detectable changes to the transcript population already prevalent in both the gametophyte and the ovule for the 5534 genes sampled on the array.

Early Endosperm Development Is Accompanied by Novel Transcriptional Patterns

Our data indicate that, unlike the early embryo, early endosperm development correlates with significant novel transcription in the ovule. Using maize microarrays (UG 1.02) containing 7300 unique sequences (derived among others from endosperm libraries) we compared the transcript populations present in the apomictic hybrid materials at 0 DAP (presence of a proembryo but no endosperm) and at 3 DAP (where the early endosperm grows in absence of further embryo formation). Computational analysis, summarized in Table 2 and Figure 3, identified 111

Table 1. Microarray Analysis of Gene Expression in Dissected Ovules from Unfertilized Apomictic Genotype 38C and the Sexual Maize Line CML216 at 3 and 7 DAP[a]

Tissues	Expressed Genes	Differentially Expressed Genes ($P < 0.001$)
38C, 0 DAP	5275 (91.60%)[b]	0
Maize, 3 DAP	5313 (92.24%)	129 (2.24%)
Maize, 7 DAP	5365 (93.14%)	213 (3.70%)

[a] The table summarizes the results for four independent hybridizations using Unigene 1.01 chips, and only signals consistent across all repetitions were used for statistical analyses. mRNA from unfertilized ovules of CML216 was used as a reference for all comparisons.

[b] Percentage of the total number of unique sequences printed on the arrays.

Table 2. Number and Distribution by Ratio of Genes with Significant Differential Expression during Early Endosperm Formation[a]

Expression 3 DAP	Class of Ratios[b]	Number of Genes
Downregulated	Repressed	4
	0.00–0.05	4
	0.50–0.67	9
	0.67–0.83	26
Upregulated	1.25–1.50	17
	1.50–2.0	16
	2.0–3.20	5
	Induced	30

[a] The table summarizes the data obtained by comparing RNA profiles in the ovules of the apomictic phenotype 38C at 0 DAP (presence of an arrested proembryo but before the initiation of endosperm formation) and 3 DAP (presence of growing endosperm and an arrested proembryo).
[b] Ratio = 1, no variation in gene expression; ratio > 1, upregulation in the endosperm after fertilization; ratio < 1, downregulation in the endosperm after fertilization.

genes with differential expression (with $P < 0.005$, corresponding to false discovery rate [FDR] = 0.032). Of these, 77 corresponded to genes expressed in both samples but with significantly different intensities, and 34 corresponded to genes whose expression was significantly different from null expression in only one of the two samples.

To validate these data, we performed semiquantitative RT-PCR of different tissues before and after fertilization for a sample of 27 out of the 111 coding sequences with contrasting expression. Although semiquantitative RT-PCR cannot be used to validate the absolute fold change estimates provided by the much more quantitative microarray experiment, the results (Table 3) indicate that 20 out of the 27 genes showed detectable changes in expression patterns after fertilization, in the direction suggested by the microarray data. Seven of the 27 genes identified on the microarrays were either experimental artifacts or real differences not detected with semiquantitative RT-PCR. Based on this sample, we assume that at least 75% of the genes identified by microarray analysis correspond to real transcriptional differences. In our sample, 14 corresponded to downregulated genes (expressed in the mature gametophyte, but absent or expressed at significantly lower levels in the fertilized ovules 3 DAP) and six to upregulated genes. Illustrative examples are provided in Figure 4. They offer evidence for novel transcriptional activity concomitant to early endosperm development.

Early Embryo Development in Apomictic Plants Is Associated with a Higher Proportion of Polyadenylated mRNA

Although the array data showed that the global transcript population present in the ovules after the initiation of embryo formation is not statistically different from the population found in ovules containing mature gametophytes, more subtle qualitative changes in the organization of the mRNA population might exist. An important technicality with mRNA labeling in our microarray experiments is that reverse transcription is initiated both at random sites in the transcripts and at the poly(A) tails, using a mix of both random primers and oligo(dT) primers. This labeling method therefore captures the total mRNA population regardless of possible differences in RNA maturation. To verify whether our samples were equivalent in terms of RNA maturation, we compared the relative proportion of polyadenylated mRNA and nonpolyadenylated mRNA among unfertilized maize ovules (no embryo), fertilized ovules from maize plants sampled 3 DAP

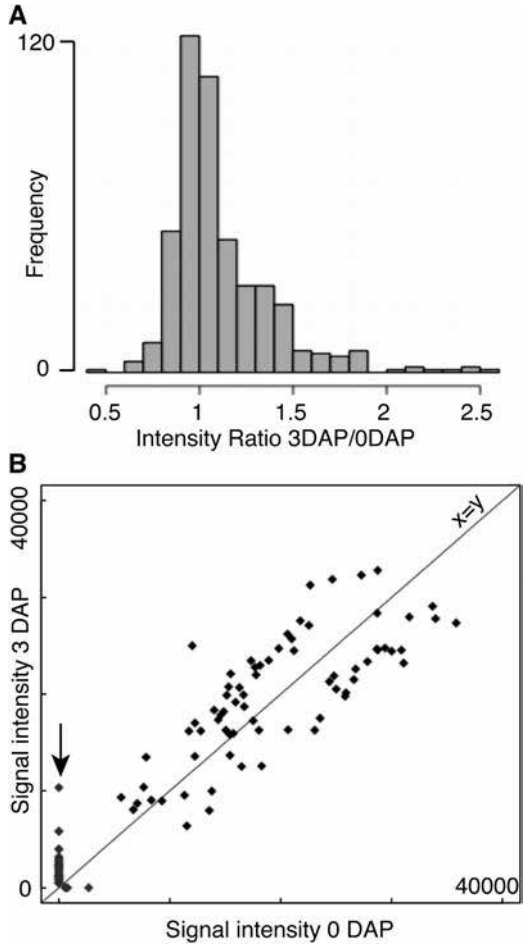

Figure 3. Microarray Analysis of Early Endosperm Development.

(A) Frequency distribution of intensity ratio between samples at 3 and 0 DAP. Each gene is represented by the mean of the adjusted intensity values of all replicates.

(B) Scatter plot of spatially adjusted fluorescence intensities between RNA extracted 3 DAP (*y* axis) and RNA from nonfertilized ovules from the apomictic genotype 38C (*x* axis). The spots indicate the localization of genes with significantly different expression at $P < 0.001$. Each gene is represented by the mean of the adjusted intensity values of all replicates. The group of spots indicated by the arrow indicates those genes where either x value was not significantly different from 0 but y value was not equal to 0 (and thus likely repressed after fertilization), or y value was not significantly different from 0, but x value was not equal to 0 (and thus likely induced after fertilization).

Table 3. Validation of Genes with Significant Differential Expression during Early Endosperm Formation[a]

GenBank Entry	P Values[b]	Ratio[c]	Log U.lim[d]	Log L.lim[e]	Predicted Function	RT-PCR[f]
AI881293	0.0015	1.63	1.281	0.135	ATPase inhibitor	−
AI649910	0.0001	3.03	2.170	1.024	Aconitate hydratase	+
AI665004	0.0001	0.42	−0.671	−1.839	F-box protein	+
AI861494	0.0004	1.72	1.355	0.209	Galactosyltransferase	+
AI438413	0.0003	1.76	1.400	0.233	Helicase	+
AI629534	0.0020	0.63	−0.087	−1.233	Importin	−
AI820392	0.0007	1.70	1.352	0.184	γ-Zein mRNA	+
AI770450	0.0008	0.60	−0.160	−1.327	Membrane protein	+
AI820386	0.0001	3.04	2.177	1.031	Metal binding protein	+
AI734543	0.0001	0.55	−0.281	−1.449	GTP binding protein	−
AI881878	0.0001	2.01	1.594	0.427	Receptor kinase	−
AI622733	0.0003	1.75	1.379	0.233	Replication protein	+
AI714918	0.0001	2.63	1.979	0.811	Ribosomal protein	+
AI461564	0.0006	0.59	−0.190	−1.336	RNA binding protein	+
AI820302	0.0011	1.66	1.302	0.156	*sup1*	+
AI677072	0.0001	0.19	−1.794	−2.962	Tumor protein	+
AI461566	0.0041	0.64	−0.066	−1.234	Unknown protein	−
AI619247	0.0010	0.61	−0.139	−1.307	Unknown protein	+
AI665151	0.0001	2.63	1.979	0.812	Unknown protein	−
AI714900	0.0001	1.62	1.109	0.290	Unknown protein	+
AI715072	0.0012	1.67	1.319	0.152	Unknown protein	+
AI737979	0.0040	0.64	−0.061	−1.207	Unknown protein	−
AI770943	0.0030	1.57	1.221	0.075	Unknown protein	+
AI833477	0.0042	0.64	−0.064	−1.210	Unknown protein	+
AI834598	0.0001	0.32	−1.242	−2.061	Unknown protein	+
AI855049	0.0040	1.57	1.234	0.066	Unknown protein	+
AI861472	0.0040	1.54	1.197	0.051	Unknown protein	+

[a] Twenty-seven genes identified from the microarray data were validated with semiquantitative RT-PCR.
[b] Individual P values obtained from the microarray data with the mixed linear model.
[c] Ratio calculated using the mean of the adjusted signal intensity values.
[d] Log value of the upper limit of the confidence interval.
[e] Log value of the lower limit of the confidence interval.
[f] The plus sign indicates data confirmed by semiquantitative RT-PCR (i.e., for which the microarray data and the RT-PCR indicate a change in the same direction when comparing mature unfertilized ovules and ovules 3 DAP); the minus sign indicates no significant difference detected by RT-PCR.

(presence of both an embryo and a coenocytic endosperm), and nonfertilized ovules from nonpollinated apomictic 38C genotype (presence of a proembryo but no endosperm). Using the incorporation of digoxigenin-dUTP (dig-dUTP) molecules during reverse transcription as a marker, the amount of dig-dUTP incorporated by reverse transcription with random primers was compared with the amount of dig-dUTP incorporated with oligo(dT) primers. Random primers should transcribe RNA regardless of the presence of a poly(A) tail, whereas oligo-dT primers should capture only mRNA with a poly(A) tail.

The data summarized in Figure 5 indicate significant differences in the relative proportions of polyadenylated RNA: the ratio of the amount of dig-dUTP incorporated using oligo(dT) primers to the amount of dig-dUTP incorporated using random primers was 0.72 in the nonpollinated ovules from apomictic 38C materials and 0.58 in the nonpollinated maize ovules (significantly different, χ^2 test, $P < 0.02$). The same measurement with pollinated maize ovules 3 DAP resulted in a ratio of 0.69, not significantly different from the nonpollinated 38C genotype. This indicates that the early events taking place in the ovule after fertilization are accompanied by a significant increase in the proportion of polyadenylated to immature mRNA. The same increase occurs when only the proembryo initiates development, as shown with the apomictic materials.

DISCUSSION

Several mutant screens in Arabidopsis have clearly established that seed development requires gene activity in the gametophyte and, thus, that maternal control plays a crucial role in early seed development (Grossniklaus et al., 1998; Springer et al., 2000; Choi et al., 2002; Grini et al., 2002; Pagnussat et al., 2005). In some cases, such as mutants from the FERTILIZATION INDEPENDENT SEED class of genes from Arabidopsis or for the *no-apical-meristem related protein 1* gene in maize (Guo et al., 2003), it has been demonstrated that the maternal and paternal contributions to seed development are not equivalent (at least in the endosperm) because of genomic imprinting (Kinoshita et al., 1999; Vielle-Calzada et al., 1999; Luo et al., 2000; Yadegari et al., 2000). Another remarkable demonstration of nonequivalence of the paternal and maternal genomes was offered by Vielle-Calzada et al. (2000), who showed that the paternally inherited

copies of 20 seed-expressed genes are silent during the early steps of seed development, while the maternally inherited copies are active. Here, we obtained similar results in maize for 16 genes expressed during early seed development. Three of them had been previously characterized as endosperm specific. In our experiment, only the maternal alleles were detectable by RT-PCR during the early days of seed formation. We also obtained similar results for 13 randomly chosen seed-expressed genes. Further experiments using mRNA in situ hybridization would strengthen the analysis of those 13 random sequences. However, both the dilution series used in this experiment and the genomic PCR controls indicate that the male alleles of all 13 genes are either silent or expressed at a much lower level than the female alleles.

Although we used only a limited number of genes that were chosen for purely experimental reasons, such as the presence of repeats within their EST sequences, they represent unrelated biological functions and are evenly distributed across the genome. Thus, our results, together with those for two other maize genes that show maternal expression during early stages of endosperm development and biallelic expression at later stages (Zm*fie* and *maternally expressed gene1*; Danilevskaya et al., 2003; Gutierrez-Marcos et al., 2004), offer a growing body of evidence that in maize, as in Arabidopsis, a high proportion of the male alleles are either not expressed or expressed at much lower levels than the female alleles until at least 3 DAP.

Our data further indicate that the establishment of an embryo-specific developmental program is delayed by several days after fertilization. Using cDNA microarrays, we could detect no significant difference between mRNA populations from ovules

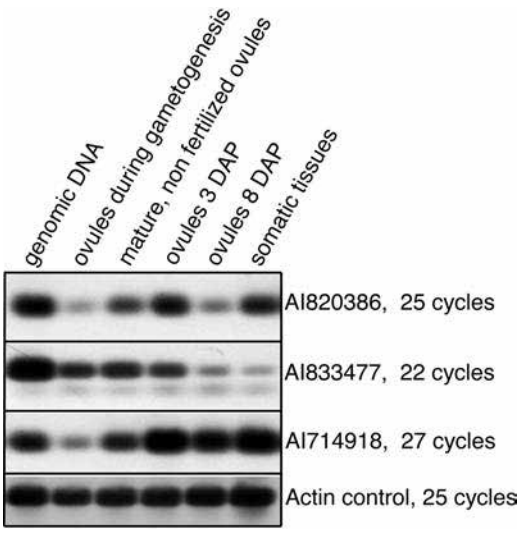

Figure 4. Validation of Microarray Data Using RT-PCR.

Somatic tissues are represented by a bulk containing RNA from leaves, stem, husk, and roots. The examples illustrate three situations of down-regulation (AI833477) or upregulation (AI820386 and AI714918) of genes after fertilization. AI820386 illustrates a case of more complex regulation. The optimum number of PCR cycles was chosen for each gene so that the signal would fall within the log phase of PCR amplification.

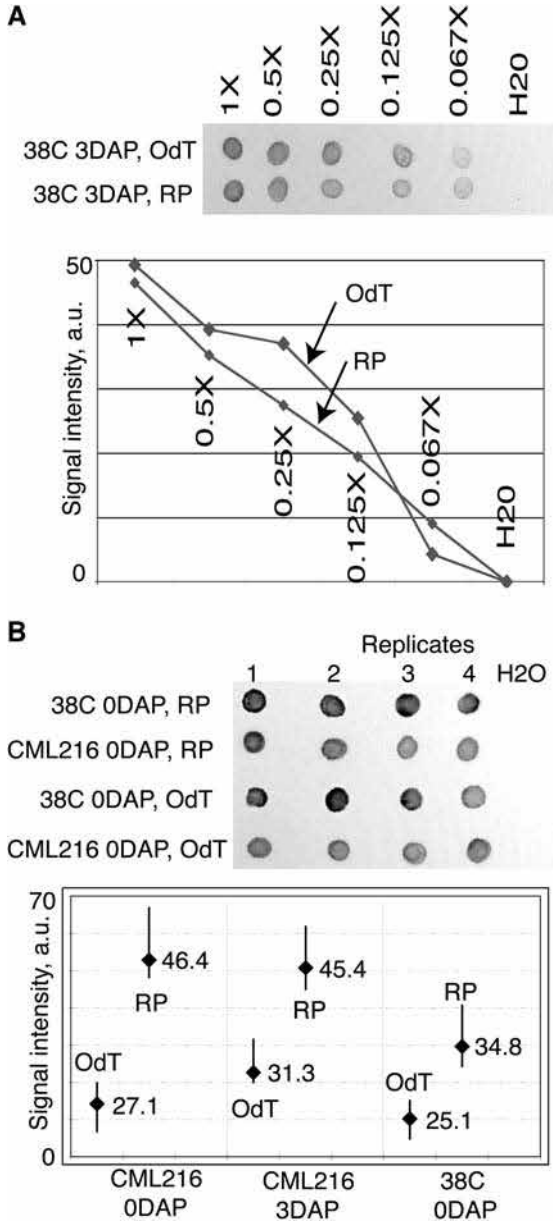

Figure 5. Relative Amounts of Polyadenylated mRNA in Ovules of Unfertilized Apomictic and Sexual Plants.

(A) Dilution series of total RNA extracted from ovules of apomictic genotype 38C shows that dig-dUTP incorporation is linearly proportional to the amount of total RNA, with both random-priming and oligo(dT) reverse transcription. The actual dot blots of the dilution series [RP, random primers; OdT, oligo(dT) primers] are shown. The graphical representation of the same information shows the linearity of the signal intensity. The numbers represent arbitrary intensity units (a.u.).

(B) Various independent reverse transcription reactions (replicates) were performed for both materials to estimate relative polyadenylated RNA; the graph summarizes the results for eight repetitions each. The diamonds indicate the mean value of the signal units; the bars indicate the minimum–maximum range. Note that the signal values fall within the linear part of the figure in **(A)**, suggesting that the differences are not due to a possible saturation of the reverse transcription reaction.

containing an egg cell (sexual plants) or a proembryo (apomictic plants). Given the relative importance of somatic and reproductive cells in our samples, most of the expression profiles detected by microarrays probably reflect somatically expressed genes. This suggests that the formation of a proembryo in apomictic plants before fertilization does not require extensive modifications to the transcript population already prevalent in the gametophyte and the somatic tissues of the ovule. We cannot discard the possibility that the sample of genes represented on the microarrays might miss essential genes with zygote-specific expression profiles. However, this would imply that such genes have no pleiotropic effect on the expression of the ~6000 genes represented on the arrays. Similarly, it is possible that cDNA microarrays were not sensitive enough to detect relatively small changes specific to the gametophyte and the proembryo. However, we also showed that transcriptional changes after the fertilization of the central cell are readily detected under our experimental conditions, indicating a relatively low detection threshold. Altogether, we therefore propose that the first divisions in the proembryo in maize likely occur before the onset of global embryo genome activation.

This does not rule out the possibility that specific genes might be activated early in development. In Arabidopsis, for example, the *WOX9* is detected at the two-cell stage by in situ hybridization experiments, but not in the egg cell or the zygote (Haecker et al., 2004). Such examples, however, remain infrequent in the literature. This does not rule out the possibility of unequal distribution of mRNAs and the establishment of transcriptional domains within the early embryo. *WOX2* mRNA in Arabidopsis, for example, is found by in situ hybridization in the egg cell and the zygote; it becomes restricted to the basal cell of the two-cell embryo after asymmetric division of the zygote, thus defining a region of specific transcriptional profile within the very early embryo (Haecker et al., 2004). Finally, novel transcripts of paternal origin might also play a role in early seed formation. At least two genes with early paternal expression have been identified in Arabidopsis (Weijers et al., 2001; Kohler et al., 2005), one of which, the *PHERES* gene, has been shown to be paternally imprinted by MEDEA (Kohler et al., 2005). Because our profiling experiment was conducted in the absence of paternal contribution, we might have missed paternal contributions to early embryo growth.

Nonetheless, there is very limited evidence suggesting important novel maternal transcription in the zygote and the proembryo. By contrast, our data also suggest that the initiation of endosperm development is accompanied by significant novel transcription in the maize ovule. Here again, we cannot determine whether this novel transcription is specific to the growing endosperm. It might also include, or be restricted to, changes from the surrounding maternal tissues, such as the integuments, which also undergo morphological changes concomitant to endosperm growth. However, such differences were not observed with seeds containing only a proembryo, indicating that transcriptional changes in maternal tissues in maize ovules 3 DAP would be dependent upon a growing endosperm. Further analysis of in situ expression of the candidate sequences identified on the cDNA arrays will help discriminate between these possibilities.

Based on the above, it is unclear whether the maternal transcripts in the embryo are gametophytic (deposited before fertilization), zygotic (produced after fertilization), or both. We showed here that plants containing a proembryo and fertilized maize ovules 3 DAP are also characterized by a higher proportion of polyadenylated mRNA than that of unfertilized ovules. This is reminiscent of observations made in animals, mainly Xenopus and mice, where cytoplasmic elongation of poly(A) tails plays an essential role in the translational control of early embryo development (Seydoux, 1996; Johnstone and Lasko, 2001; Mendez and Richter, 2001). Examples of genes for which deposited maternal transcripts are essential in development abound in animals (Seydoux, 1996) but are limited in plants. This might reflect the inherent difficulty of analyzing transcription in plant ovules, rather than a genuine physiological difference between plant and animal development. Furthermore, this explanation has been proposed for the maternal effect phenotype of the *PROLIFERA* gene in Arabidopsis, a DNA replication factor (Springer et al., 2000). It has also been shown in maize (Dresselhaus et al., 1999) that transcripts for a translation initiation factor, *eIF-5A*, are stored in unfertilized egg cells, possibly as a way to allow rapid triggering of mRNA translation after fertilization. The higher proportion of polyadenylated mRNA found in the ovules after fertilization suggests that mRNA recruitment might also be a mechanism of importance to control early seed formation in plants.

Taken together, our data indicate that the maternal-to-zygotic transition in maize does not occur until at least 3 d after the initiation of embryo development. The mechanisms controlling this transition in plants remain unknown. However, this report suggests that plants and animals share at least a delayed maternal-to-zygotic transition. Further work might reveal more similarities in other aspects of early embryo development.

METHODS

Plant Materials

Maize (*Zea mays*) inbred lines (CML72 and CML216) were obtained from the CIMMYT gene bank (www.cimmyt.org). The apomictic maize-Tripsacum hybrid plants labeled 38C have been described elsewhere (Leblanc et al., 1996; Grimanelli et al., 2003).

Allele-Specific RT-PCR Analysis during Early Embryo Development

Primer sequences for *Zmfie2*, *Zmocl1*, and *Zmocl5* have been described elsewhere (Ingram et al., 2000; Danilevskaya et al., 2003) The public maize EST databases were searched for genes expressed in the embryo or during early seed development (http://www.zmdb.iastate.edu). We selected 100 expressed sequences from the databases that also contained SSRs within their cDNA sequence as a source of potential allele-specific PCR products. Using primer pairs already designed for the SSRs (available at http://www.agron.missouri.edu), we amplified the corresponding loci from parental genomic DNA and identified 43 pairs with polymorphisms between CML216 and CML72. Crosses in both directions were made and ovules collected at 0, 3, and 7 DAP. Embryo and endosperm tissues were also separately sampled at 8 and 12 DAP. RNA and DNA samples were then extracted from the various crosses using TRIZOL (Invitrogen, Carlsbad, CA). All RNA samples consisted of bulks of 30 dissected ovules. Absence of contaminant genomic DNA in our

samples was tested by RT-PCR using primer pairs designed for the *actin1* gene (accession number J01238). To validate the sensitivity of our RT-PCR conditions, we first verified our ability to detect genomic DNA in the bulks. We thus made the assumption that if PCR conditions were stringent enough to detect both the male and female alleles at the various stages, we should also be able to detect their transcripts; the contrary would imply that there is less than one paternal transcript per genomic copy of the allele, hence a negligible expression level. RT-PCR reactions were performed using the SUPERSCRIPT kit (Invitrogen) following the manufacturer's instructions. The accession numbers for the ESTs are as follows: AI677212, AI677270, AI670662, AI833700, AI854929, AI746192, AW216025, AW216004, AW181192, AW066927, AW066244, AW224179, AW216194, AB073081, AI746088, and AI745997. Expression of all 16 genes in the embryo was also verified using RT-PCR with the tissues collected at 8 and 12 DAP. RT-PCR data were confirmed with at least three replicates corresponding to independent biological samples.

Microarray Data Production

The rationale for the microarray experiments is summarized in Figure 1. The apomictic 38C maize-Tripsacum hybrid used in this report has been described elsewhere (Leblanc et al., 1996). These plants contain two genomes of maize ($2x = 20$) and one genome of Tripsacum ($x = 18$) and reproduce through gametophytic apomixis. We dissected ovules from the following types of materials: sexual maize plants (CML216) before fertilization, at 3 and 7 DAP; 38C hybrids before fertilization and at 3 DAP. RNA samples were obtained using TRIZOL. Maize microarrays were obtained from the Galbraith lab at the University of Arizona. We used the UNIGENE 1.01.02 microarrays (complete description available from www.zmdb.iastate.edu). Array design included ~5700 cDNAs spotted in triplicates. Slide preparation, RNA labeling, hybridization, and washes were done following Galbraith lab protocols (www.maizegdb.org/microarray.php), except for labeling reactions. Those were performed using 50 μg of total RNA and the CySCRIBE labeling kit (Amersham, Piscataway, NJ) following the recommended procedures. In this experiment, a single source of RNA (unfertilized maize ovules) was used as the control RNA sample, compared with all other samples used at treatments. Each comparison was repeated twice, each with dye inversions. For endosperm growth profiling, we extracted RNA from dissected ovules from genotype 38C at 0, 2, and 3 DAP. Hybridizations of microarrays 1.02.03 were used to compare profiles at 0 DAP with the bulk of sample from 2 and 3 DAP. Array 1.02.03 contains ~7300 cDNAs spotted in duplicates. Hybridization conditions were as described above.

Microarray Data Analysis

The same analysis procedures were used for all array data sets. Data extractions from the raw images were performed using the ARRAYPRO software (Media Cybernetics, Carlsbad, CA). We normalized the raw data sets using spatial analysis models as described by Baird et al. (2004). Briefly, spatial analysis sequentially fits a class of autoregressive-integrated-moving-average models (ARIMA) to the array data both in rows and columns. The ARIMA models not only allow data to be normalized within and between slides, but also model and thus correct the experimental spatial variability on each slide. This spatial variability is modeled as the direct product of an autoregressive correlation structure for both columns and rows. In our experiments, the spatial adjustment of the raw density and background measurements was based on blanks (spots without deposited samples), in such a way that density and background values of genes near a blank spot with high background values were adjusted downward, whereas genes with low expression values near a blank with low background values were adjusted upward. After adjusting the density, background, and, thus, signal of each spot based on the spatial model, a mixed linear model was used to test the significance of gene expression, following Wolfinger et al. (2001) and Kerr and Churchill (2001). In our model, the variables "slides" and "slide × gene" interactions were considered as random effects, whereas "genes" were considered as fixed effects. Candidate genes with treatment effects among genes with significant expression (arbitrarily set at density > local background + 2 SD) were then selected with a general linear mixed model. The estimation of the variance components in the model used the residual maximum likelihood approach. The equation for the general mixed linear model is $Y = Xl + Zu + r$, where Y is the response vector, X is the design matrix for fixed effects (l: genes, repetitions, and dyes), Z is the design matrix for random effects (u: slide, slide × gene interaction), and r is the residual. We therefore identified candidate genes based on the significance of the "treatment" variable effect on the variance–covariance structure, irrespective of the actual ratio of signal intensity estimates. For all genes with a significant "treatment" effect ($P < 0.005$), we then computed the ratios using the mean of the ARIMA-adjusted estimates of signal intensity. Estimates of FDR following Benjamini and Hochberg (1995) were further calculated for the genes selected under $P < 0.005$. The ASREML system (Gilmour et al., 2002) was used for computing the spatial analysis of the microarray experiments. Determination of the FDR was computed using the R multitest package (available from http://www.bioconductor.org/repository/release1.3/package/html/index.html). We computed the mixed model using the SAS PROCMIXED procedure. The data sets are available in Supplemental Tables 2 to 9 online.

Microarray Data Validation

We used semiquantitative RT-PCR to confirm a subset of the candidate genes identified by microarray. Because the subset of genes was randomly chosen, we assume that the frequency of type 1 errors reflects the overall level of false positives (or alternatively of differences not detected with our protocols). The semiquantitative RT-PCR protocols were as follows. We extracted ovules from staged maize ear samples during meiosis, during gametogenesis, at ovule maturity before fertilization, and at 3 and at 7 DAP. RNA extractions and reverse transcription were as described earlier. The cDNA samples were diluted 1:50 and used for PCR. PCR reactions consisted of 1 μL of cDNA, 2 μL each of 2 μM forward and reverse primers, 0.1 μL of 1 mM dig-dUTP, and 5 μL of a 2× reaction mix (REDTAQ reaction mix; Sigma-Aldrich, St. Louis, MO) containing all other necessary reagents, including deoxynucleotide triphosphates. For all genes (including an actin control), we used the same PCR cycling conditions (30 s at 94°C, 20 s at 56°C, and 1 min at 72°C) with 17, 20, 25, and 30 PCR cycles. PCR products were resolved on 1.5% agarose gels and transferred on uncharged nylon membranes. Incorporated DIG was detected using standard chemiluminescence protocols (available from www.cimmyt.org/ABC/protocol/contents.htm).

RNA Polyadenylation Measurements

We used a modification of the SUPERSCRIPT reverse transcription kit protocol (Invitrogen) to measure the relative amount of polyadenylated mRNA in unfertilized ovules of CML216, fertilized ovules from CML216 3 DAP, and the apomictic genotype 38C. Briefly, reverse transcription was as follows: a reaction containing 2 μL of 0.3 μg/μL total RNA, 1 μL of 10 mM deoxynucleotide triphosphate mix, and 1 μL of either 50 nM random hexamers or 50 nM oligo(dT) primers was heated at 65°C for 10 min and placed on ice for 5 min. We added 10 μL of a reaction mix containing dig-dUTP (2 μL of 10× reaction buffer, 4 μL of 25 mM $MgCl_2$, 2 μL of 0.1 M DTT, 1 μL of 40 units/μL RNaseOut enzyme (Invitrogen), 1 μL of 200 units/μL Superscript reverse transcriptase, and 0.1 nM dig-dUTP) and incubated it for 10 min at 25°C, followed by 50 min at 50°C. The reaction product containing the cDNAs was then precipitated with 2.5 volumes of

ethanol and 0.3 M NaOAc and resuspended in 5 μL of water. Dot blots of 2 μL were deposited on uncharged nylon membranes and fixed to the membrane by UV cross-linking, and the incorporated dig-dUTP molecules were detected on x-ray films using standard chemiluminescence detection protocols. The films were digitalized, and we used the Kodak EADS imaging system (Rochester, NY) to score each dot individually. Those were first fit to an ellipse of variable size. Intensity and background data were then recorded automatically. The dig-dUTP signal for each spot was calculated as the intensity minus the local background. The RNA pools used were the same as those used for the microarray experiment.

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REFERENCES

Baird, D., Johnstone, P., and Wilson, T. (2004). Normalization of microarray data using a spatial mixed model analysis which includes splines. Bioinformatics **22,** 3196–3205.

Baroux, C., Blanvillain, R., and Gallois, P. (2001). Paternally inherited transgenes are down-regulated but retain low activity during early embryogenesis in Arabidopsis. FEBS Lett. **509,** 11–16.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate—A practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B **57,** 289–300.

Choi, Y., Gehring, M., Johnson, L., Hannon, M., Harada, J.J., Goldberg, R.B., Jacobsen, S.E., and Fischer, R.L. (2002). DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in Arabidopsis. Cell **110,** 33–42.

Colombo, L., Franken, J., Van der Krol, A.R., Wittich, P.E., Dons, H.J., and Angenent, G.C. (1997). Downregulation of ovule-specific MADS box genes from petunia results in maternally controlled defects in seed development. Plant Cell **9,** 703–715.

Danilevskaya, O.N., Hermon, P., Hantke, S., Muszynski, M.G., Kollipara, K., and Ananiev, E.V. (2003). Duplicated fie genes in maize: Expression pattern and imprinting suggest distinct functions. Plant Cell **15,** 425–438.

Dresselhaus, T., Cordts, S., and Lorz, H. (1999). A transcript encoding translation initiation factor eIF-5A is stored in unfertilized egg cells of maize. Plant Mol. Biol. **39,** 1063–1071.

Flach, G., Johnson, M.H., Braude, P.R., Taylor, R.A., and Bolton, V.N. (1982). The transition from maternal to embryonic control in the 2-cell mouse embryo. EMBO J. **1,** 681–686.

Gilmour, A.R., Cullis, B.R., Welham, S.J., and Thompson, R. (2002). ASREML Reference Manual. (Orange, Australia: Orange Agricultural Institute).

Golden, T.A., Schauer, S.E., Lang, J.D., Pien, S., Mushegian, A.R., Grossniklaus, U., Meinke, D.W., and Ray, A. (2002). SHORT INTEGUMENTS1/SUSPENSOR1/CARPEL FACTORY, a Dicer homolog, is a maternal effect gene required for embryo development in Arabidopsis. Plant Physiol. **130,** 808–822.

Grimanelli, D., Garcia, M., Kaszas, E., Perotti, E., and Leblanc, O. (2003). Heterochronic expression of sexual reproductive programs during apomictic development in tripsacum. Genetics **165,** 1521–1531.

Grini, P.E., Jurgens, G., and Hulskamp, M. (2002). Embryo and endosperm development is disrupted in the female gametophytic capulet mutants of Arabidopsis. Genetics **162,** 1911–1925.

Grossniklaus, U., and Schneitz, K. (1998). The molecular and genetic basis of ovule and megagametophyte development. Semin. Cell Dev. Biol. **9,** 227–238.

Grossniklaus, U., Vielle-Calzada, J.P., Hoeppner, M.A., and Gagliano, W.B. (1998). Maternal control of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. Science **280,** 446–450.

Guo, M., Rupe, M.A., Danilevskaya, O.N., Yang, X., and Hu, Z. (2003). Genome-wide mRNA profiling reveals heterochronic allelic variation and a new imprinted gene in hybrid maize endosperm. Plant J. **36,** 30–44.

Gutierrez-Marcos, J.F., Costa, L.M., Biderre-Petit, C., Khbaya, B., O'Sullivan, D.M., Wormald, M., Perez, P., and Dickinson, H.G. (2004). maternally expressed gene1 is a novel maize endosperm transfer cell–specific gene with a maternal parent-of-origin pattern of expression. Plant Cell **16,** 1288–1301.

Haecker, A., Gross-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M., and Laux, T. (2004). Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development **131,** 657–668.

Ingram, G.C., Boisnard-Lorig, C., Dumas, C., and Rogowsky, P.M. (2000). Expression patterns of genes encoding HD-ZipIV homeo domain proteins define specific domains in maize embryos and meristems. Plant J. **22,** 401–414.

Johnstone, O., and Lasko, P. (2001). Translational regulation and RNA localization in Drosophila oocytes and embryos. Annu. Rev. Genet. **35,** 365–406.

Kerr, M.K., and Churchill, G.A. (2001). Experimental design for gene expression microarrays. Biostatistics **2,** 183–201.

Kinoshita, T., Yadegari, R., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (1999). Imprinting of the MEDEA polycomb gene in the Arabidopsis endosperm. Plant Cell **11,** 1945–1952.

Kohler, C., Page, D.R., Gagliardini, V., and Grossniklaus, U. (2005). The *Arabidopsis thaliana* MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. Nat. Genet. **37,** 28–30.

Leblanc, O., Grimanelli, D., Islam-Faridi, N., Berthaud, J., and Savidan, Y. (1996). Reproductive behavior in maize-*Tripsacum* polyhaploid plants: Implications for the transfer of apomixis into maize. J. Hered. **87,** 108–111.

Luo, M., Bilodeau, P., Dennis, E.S., Peacock, W.J., and Chaudhury, A. (2000). Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing Arabidopsis seeds. Proc. Natl. Acad. Sci. USA **97,** 10637–10642.

Mendez, R., and Richter, J.D. (2001). Translational control by CPEB: A means to the end. Nat. Rev. Mol. Cell Biol. **2,** 521–529.

Newman-Smith, E.D., and Rothman, J.H. (1998). The maternal-to-zygotic transition in embryonic patterning of *Caenorhabditis elegans*. Curr. Opin. Genet. Dev. **8,** 472–480.

Newport, J., and Kirschner, M. (1982). A major developmental transition in early Xenopus embryos: I. Characterization and timing of cellular changes at the midblastula stage. Cell **30,** 675–686.

Pagnussat, G.C., Yu, H.J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S., Capron, A., Xie, L.F., Ye, D., and Sundaresan, V. (2005). Genetic and molecular identification of genes required for

female gametophyte development and function in Arabidopsis. Development **132,** 603–614.

Pelegri, F. (2003). Maternal factors in zebrafish development. Dev. Dyn. **228,** 535–554.

Ray, S., Golden, T., and Ray, A. (1996). Maternal effects of the short integument mutation on embryo development in Arabidopsis. Dev. Biol. **180,** 365–369.

Scholten, S., Lorz, H., and Kranz, E. (2002). Paternal mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. Plant J. **32,** 221–231.

Seydoux, G. (1996). Mechanisms of translational control in early development. Curr. Opin. Genet. Dev. **6,** 555–561.

Sorensen, M.B., Chaudhury, A.M., Robert, H., Bancharel, E., and Berger, F. (2001). Polycomb group genes control pattern formation in plant seed. Curr. Biol. **11,** 277–281.

Springer, P.S., Holding, D.R., Groover, A., Yordan, C., and Martienssen, R.A. (2000). The essential Mcm7 protein PROLIFERA is localized to the nucleus of dividing cells during the G(1) phase and is required maternally for early Arabidopsis development. Development **127,** 1815–1822.

Tzafrir, I., Pena-Muralla, R., Dickerman, A., Berg, M., Rogers, R., Hutchens, S., Sweeney, T.C., McElver, J., Aux, G., Patton, D., and Meinke, D. (2004). Identification of genes required for embryo development in Arabidopsis. Plant Physiol. **135,** 1206–1220.

Vielle-Calzada, J.P., Baskar, R., and Grossniklaus, U. (2000). Delayed activation of the paternal genome during seed development. Nature **404,** 91–94.

Vielle-Calzada, J.-P., Thomas, J., Spillane, C., Coluccio, A., Hoeppner, M.A., and Grossniklaus, U. (1999). Maintenance of genomic imprinting at the *Arabidopsis medea* locus requires zygotic *DDM1* activity. Genes Dev. **13,** 2971–2982.

Walbot, V., and Evans, M.M. (2003). Unique features of the plant life cycle and their consequences. Nat. Rev. Genet. **4,** 369–379.

Weijers, D., Geldner, N., Offringa, R., and Jurgens, G. (2001). Seed development: Early paternal gene activity in Arabidopsis. Nature **414,** 709–710.

Wolfinger, R.D., Gibson, G., Wolfinger, E.D., Bennett, L., Hamadeh, H., Bushel, P., Afshari, C., and Paules, R.S. (2001). Assessing gene significance from cDNA microarray expression data via mixed models. J. Comput. Biol. **8,** 625–637.

Woo, Y.M., Hu, D.W., Larkins, B.A., and Jung, R. (2001). Genomics analysis of genes expressed in maize endosperm identifies novel seed proteins and clarifies patterns of zein gene expression. Plant Cell **13,** 2297–2317.

Yadegari, R., Kinoshita, T., Lotan, O., Cohen, G., Katz, A., Choi, Y., Nakashima, K., Harada, J.J., Goldberg, R.B., Fischer, R.L., and Ohad, N. (2000). Mutations in the FIE and MEA genes that encode interacting polycomb proteins cause parent-of-origin effects on seed development by distinct mechanisms. Plant Cell **12,** 2367–2382.