Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4867–4871, May 1994
Genetics

Allele-specific parental imprinting of *dzr1*, a posttranscriptional regulator of zein accumulation

(gene dosage/B–A translocation/epigenetic/*Zea mays*)

SUMITA CHAUDHURI AND JOACHIM MESSING*

Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ 08855-0759

Communicated by Oliver E. Nelson, February 14, 1994 (received for review September 20, 1993)

ABSTRACT **Parental imprinting describes the phenomenon of unequivalent gene function based on transmission from the female or male parent. We have discovered parental imprinting of an allele of the *dzr1* locus that posttranscriptionally regulates the accumulation of 10-kDa zein in the maize endosperm. The imprinted allele of MO17 inbred origin, *dzr1+MO17*, conditions low accumulation of the 10-kDa zein and is dominant when transmitted through the female but recessive when transmitted through the male. Analyzing endosperms with equal parental contributions of *dzr1+MO17* ruled out the possibility that the unequivalent phenotype of *dzr1+MO17* was due to parental dosage imbalance in the triploid endosperm. Second-generation studies show that the dominant or recessive phenotype of *dzr1+MO17* is determined at every generation based on immediate parental origin with no grandparental effect.**

Parental imprinting, with examples in both animals (1) and plants (2–6), is an exception to Mendel's first law on the equivalency of F_1 hybrids since it refers to differential gene function based on inheritance from the female or male parent. Evidence for parental imprinting in flowering plants is available for traits affecting only the maize endosperm, which is a triploid tissue formed by the union of two haploid central nuclei with a haploid sperm nucleus during double fertilization. When the standard ratio of two maternal to one paternal chromosomal set in the endosperm is altered, it results in impaired kernel development, explained by differential function of paternal and maternal chromosome sets (2). When several chromosome arms of paternal origin are replaced by identical arms of maternal origin, a significant reduction in kernel size occurs, again indicating differential gene function in the maternal and paternal chromosomes (3–5). The most direct evidence for imprinting is available for the *R* locus, certain alleles of which confer solid anthocyanin coloration to kernels when transmitted maternally but patchy coloration (mottled) when transmitted through the pollen (6).

We report here the parental imprinting of another maize locus, *dzr1* (formerly *Zpr10/22*), which is a posttranscriptional regulator for the accumulation of 10-kDa zein in the endosperm. The 10-kDa zein is encoded by a single copy gene, *zps10/22* (7), located on chromosome 9L (8). Although the transcription rates of *zps10/22* are similar in the inbreds BSSS53, W64A, and MO17 (9, 10), the 10-kDa zein and its steady-state mRNA are severalfold higher in BSSS53 than in W64A and MO17. The *dzr1* locus on chromosome 4S (8) controls this posttranscriptional regulatory step of 10-kDa zein accumulation. The *dzr1* allele in inbred BSSS53, *dzr1+BSSS53*, thus conditions severalfold higher accumulation of the 10-kDa zein compared to the alleles in W64A, *dzr1+W64A* and MO17, *dzr1+MO17*.

We have found that the imprinting of the *dzr1* locus is allele-specific. While both *dzr1+BSSS53* and *dzr1+W64A* are dose-responsive in function, the *dzr1+MO17* function is dependent on its parental origin. *dzr1+MO17* is dominant, conditioning low accumulation of 10-kDa zein, when female transmitted but recessive when male transmitted.

MATERIALS AND METHODS

Plant Materials. Maize (*Zea mays* L.) inbred lines BSSS53, MO17, and W64A were provided by R. L. Phillips (University of Minnesota, St. Paul). The TB-4Sa translocation stock of unknown genetic background was obtained from the Maize Genetics Stock Center (University of Illinois, Urbana). TB-4Sa is the reciprocal translocation between chromosome 4 and the accessory B chromosome of maize. The point of interchange in chromosome 4 is in the short arm close to the centromere and that in the B is the euchromatic segment adjacent to the centromere. The linkage map is T–*sul*–*adh2*–*dzr1*.

For the introgression of TB-4Sa into BSSS53 (BSSS53-TB4Sa), we have backcrossed the TB-4Sa line as female to BSSS53 for four generations. At each backcross, individuals carrying the translocation were selected by Southern blot analysis of leaf DNA by using a B chromosome centromere-specific probe. The MO17 line with introgressed TB-4Sa, MO17-TB4Sa (Ht), was provided by E. Coe (University of Missouri, Columbia).

Use of BSSS53-TB4Sa or MO17-TB4Sa as pollen parent alters the dosage of *dzr1+BSSS53* and *dzr1+MO17*, respectively, to the endosperm from the normal one to two or zero due to the high frequency of nondisjunction of TB-4Sa during pollen gametogenesis (11).

Immunoblot Analysis of Zeins. Total zeins from 50 mg of finely ground mature kernels were extracted with 1 ml of 70% ethanol/2% (vol/vol) 2-mercaptoethanol; 10 μl of 10% SDS was added to 100 μl of the extract, the mixture was dried and resuspended in 100 μl of distilled water. Estimation of zein concentration was by colorimetric assay (12). Total zeins were separated by SDS/PAGE on a 15% gel and transferred to a nitrocellulose membrane. Immunodetection was performed using a 1:2000 dilution of rabbit polyclonal antibody raised against isoelectric focusing-gel purified 10-kDa zein extracted from BSSS53. The secondary antibody was horseradish peroxidase-linked protein A at a 1:3000 dilution. Visualization of immunoreactive proteins was by the enhanced chemiluminescence detection kit (ECL; Amersham).

RNA and DNA Analyses. RNA and DNA were extracted from immature endosperms and seedling leaves (13). The 10-kDa zein-specific probe was a 450-bp *Nco* I–*Xba* I fragment of the clone 10 kz-1 (7). The *Adh2* probe was the 475-bp cDNA insert in clone pZmL841 provided by M. Sachs (University of Illinois, Urbana) (14). The B chromosome

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: RFLP, restriction fragment length polymorphism.
*To whom reprint requests should be addressed.

centromere-specific probe was the 250-bp *Bam*HI–*Xho* I fragment in clone pZmBs provided by J. Birchler (University of Missouri, Columbia) (27). The probes were radiolabeled by random priming. The 27-kDa zein A copy gene-specific probe was an end-labeled 18-mer oligonucleotide (15). Hybridization and washing conditions were as described (9).

RESULTS

Accumulation of 10-kDa Zein and mRNA in Inbreds and Reciprocal Hybrids. Immunoblot analysis of zeins from mature endosperms showed a severalfold higher level of 10-kDa zein in BSSS53 compared to that in MO17 and W64A (Fig. 1). Surprisingly, a maternal effect on the accumulation of 10-kDa zein was seen in the reciprocal F_1 hybrids of BSSS53 and MO17. Hybrids with BSSS53 as the female parent (Fig. 1, lanes 11 and 12) had an accumulation of the 10-kDa zein as high as that in BSSS53 (Fig. 1, lanes 9 and 10). In the reciprocal hybrid with MO17 as the female parent, the level of 10-kDa zein was as low (Fig. 1, lanes 13 and 14) as that in MO17 (Fig. 1, lanes 15 and 16).

The abundance of 10-kDa zein mRNA in immature endosperms of BSSS53, MO17, and the reciprocal F_1 hybrids was analyzed to determine whether the maternal effect was present at the mRNA level. The level of 10-kDa zein mRNA 18 days after pollination is shown in Fig. 2*A*. Consistent with the protein levels, the hybrid with BSSS53 as the female parent had a high level of 10-kDa zein mRNA that was comparable to the level in BSSS53, whereas the reciprocal hybrid had a low level comparable to the level in MO17. The 10-kDa zein mRNA abundance was quantitated at several points during endosperm development and the relative levels at the different stages of endosperm maturity are shown in Fig. 2*B*. The data indicate that the maternal effect seen in the accumulation of 10-kDa zein in the mature endosperm is also present at the mRNA steady-state level throughout endosperm development.

Interestingly, the maternal effect was absent in reciprocal crosses of BSSS53 and W64A. Instead, the 10-kDa zein level corresponded with the dosage of *dzr1+BSSS53* and *dzr1+W64A* in the endosperm. The hybrid with BSSS53 as the female parent had less accumulation of the 10-kDa zein (Fig. 1, lanes 3 and 4) than did BSSS53 (Fig. 1, lanes 1 and 2). A further decrease in accumulation was seen in the hybrid with W64A as the female parent (Fig. 1, lanes 5 and 6) and in W64A, which had the lowest level (Fig. 1, lanes 7 and 8).

The F_1 analysis indicates that while the accumulation of 10-kDa zein in hybrids of BSSS53 and W64A was *dzr1*-dosage-dependent, there is a maternal effect in crosses involving BSSS53 and MO17.

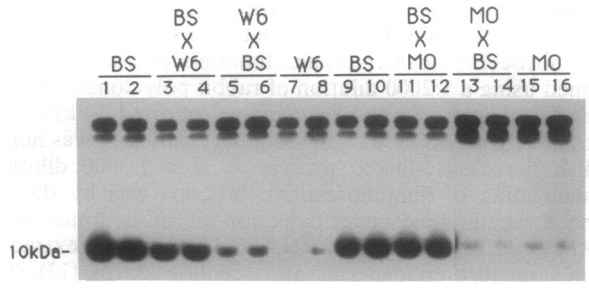

FIG. 1. Differential accumulation of 10-kDa zein in mature endosperms. Total zein (2 μg) from BSSS53 (BS), W64A (W6), MO17 (MO), and their reciprocal F_1 hybrids was analyzed on immunoblots by using the 10-kDa zein antibody. Lanes 13–16 were exposed to film twice as long as the other lanes to detect the bands specific for 10-kDa zein. The 10-kDa zein antibody cross-reacts with higher molecular mass zeins providing a control for equivalent protein loading.

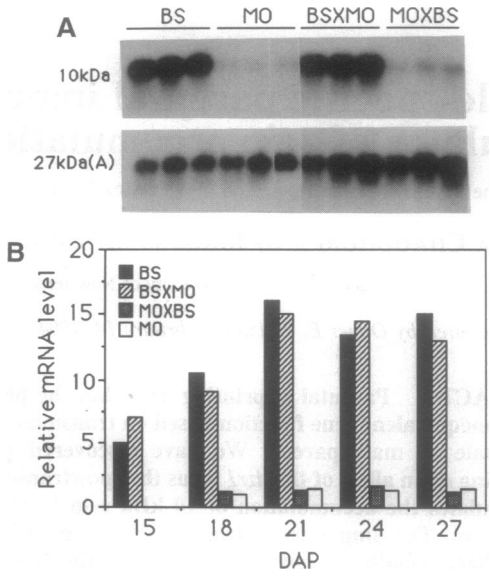

FIG. 2. Differential levels of 10-kDa zein mRNA in immature endosperms. (*A*) Abundance of 10-kDa zein mRNA at 18 days after pollination. Total RNA (2 μg) isolated from three individual endosperms of BSSS53 (BS), MO17 (MO), and their reciprocal F_1 hybrids was analyzed on a Northern blot. The blots were successively hybridized with the 10-kDa zein gene and the A copy of the 27-kDa zein gene probes. The 27-kDa zein A copy mRNA is present in equivalent levels in BSSS53 and MO17 throughout development providing a control for equal RNA loading. (*B*) Relative levels of 10-kDa zein mRNA at various days after pollination (DAP). Total RNA isolated from immature endosperms of BSSS53 (BS), MO17 (MO), and their reciprocal F_1 hybrids was analyzed on slot blots. The blots were successively probed with the 10-kDa zein gene and 27-kDa (A) zein gene probes. Hybridization intensities to each probe were determined by Ultroscan-XL densitometry (Pharmacia LKB). Ratios between hybridization intensities of the two probes (10 kDa/27 kDa) were expressed as values relative to the ratio obtained for MO17 18 days after pollination, which was arbitrarily given a value of 1. Each bar represents an average obtained using 1, 2, and 4 μg of RNA from two pools of four kernels obtained from separate ears.

Linkage Analysis of Low 10-kDa Zein in MO17. Segregation of the accumulation of high and low levels of 10-kDa zein was compared to segregation of the *Adh2* gene, which is linked to *dzr1*, by restriction fragment length polymorphism (RFLP) mapping among 67 backcross progeny of (MO17 × BSSS53) × MO17 (Table 1). Single-locus segregation and its strong linkage to *Adh2* suggest that the factor controlling the low accumulation of 10-kDa zein in MO17 is likely to be allelic to *dzr1*. This linkage was also confirmed with other RFLP markers on chromosome 4S (data not shown). The linkage of the low accumulation of 10-kDa zein with the *dzr1* locus in MO17 discounts any other nuclear, sporophytic, or cytoplasmic factor accounting for the maternal effect.

Possible Mechanisms of *dzr1+MO17* Maternal Effect. The maternal effect observed in crosses of BSSS53 and MO17 can be explained by *dzr1+MO17* having unequivalent phenotype

Table 1. Segregation of the level of 10-kDa zein and *Adh2* gene resulting from the cross of (MO17 × BSSS53) × MO17

Accumulation of 10-kDa zein	*Adh2* gene: BSSS53/MO17	*Adh2* gene: MO17/MO17	Total
High	24	6	30
Low	5	32	37
Total	29	38	67

χ^2 values: high/low 10-kDa zein (1:1), 0.54 ($0.50 > P > 0.30$); *Adh2* (1:1), 0.95 ($0.50 > P > 0.30$); independence, 27.2 ($P < 0.001$).

when transmitted by the female and male parent. In the hybrid where MO17 was the female parent, the accumulation of 10-kDa zein was as low as that in MO17, indicating the dominance of female-transmitted *dzr1+MO17*. In the reciprocal hybrid where MO17 was the male parent, the accumulation of 10-kDa zein was high, indicating that the male-transmitted *dzr1+MO17* is recessive.

Two explanations could account for the parental bias on *dzr1+MO17* phenotype. One is a dosage effect, as the dosage of *dzr1+MO17* contributed by the female and male parents are different in the triploid endosperm. It is therefore possible that maternally transmitted *dzr1+MO17* is dominant because a minimum of two doses are necessary to effect its dominance. The alternate explanation for the parental bias is genomic imprinting of the *dzr1+MO17* locus. Accordingly, the *dzr1+MO17* locus is subject to gametic-origin-based epigenetic modifications such that it is differentially expressed when transmitted by the female and male parents. Upon female transmission, *dzr1+MO17* functions as a dominant regulator of the expression of 10-kDa zein, whereas male transmission causes functional inactivation of the *dzr1+MO17* locus rendering it recessive.

Test of the Gene Dosage Hypothesis. The gene-dosage hypothesis was tested by analyzing endosperms with equal parental dosage of *dzr1+MO17*. As normal female *dzr1+MO17* dosage to the endosperm is two, the maize B–A translocation, TB-4Sa, which carries *dzr1*, was used to introduce two doses of *dzr1+MO17* to the endosperm through the male. This change of gene dosage through the male from the normal one to two is possible because of the very high frequency of nondisjunction of B–A translocations during pollen gametogenesis (11).

The TB-4Sa translocation was introgressed into BSSS53 and MO17 and the converted lines, designated BSSS53-TB4Sa and MO17-TB4Sa, were used as male parents in crosses indicated in Table 2. The converted heterozygous translocation lines carried the respective *dzr1* allele on both the normal chromosome 4 and on the translocation. Therefore, the converted lines contributed one paternal dose of *dzr1* to the endosperm and embryo in ≈50% of the progeny due to proper disjunction of normal chromosome 4. The remaining ≈50% of the progeny received either zero or two doses of *dzr1* in the endosperm and two or zero doses in the corresponding embryo, due to the high frequency of nondisjunction of TB-4Sa. Thus, by using the BSSS53-TB4Sa or MO17-TB4Sa as pollen parents, the male dosage of the respective *dzr1* alleles in the progeny endosperm was varied from zero to two doses. Sixty kernels from each cross were analyzed, and the frequency of each of the three dosage classes obtained is shown in Table 2.

Table 2. Kernel classes and frequencies in crosses with BSSS53-TB4Sa and MO17-TB4Sa as pollen parents

Cross		Embryo	Endosperm	Kernels,
Female	Male	genotype	genotype	no.
W64A	BSSS53-TB4Sa	W/−	WW/BB	9
		W/B	WW/B	32
		W/BB	WW/−	19
MO17	BSSS53-TB4Sa	M/−	MM/BB	8
		M/B	MM/B	36
		M/BB	MM/−	16
BSSS53	MO17-TB4Sa	B/−	BB/MM	10
		B/M	BB/M	28
		B/MM	BB/−	22

B, *dzr1+BSSS53*; M, *dzr1+MO17*; W, *dzr1+W64A*. The higher frequency of hyperploid embryo class in comparison to the hypoploid embryo class is due to preferential fertilization of the egg nucleus by the hyperploid sperm nucleus (16).

For kernel classification, parental dosage of *dzr1* in the kernels was determined by Southern blot analysis of seedling DNA with the *Adh2* gene probe, since the *Adh2* gene is included in the translocated segment of chromosome 4 in TB-4Sa. By using an RFLP between the parents, the male *Adh2* dosage in individual progeny was determined by the hybridization intensity of the RFLP band corresponding to the male parent relative to that in the female parent. Two individual embryos representative of each dosage class arising from the crosses are shown in the upper panels of Fig. 3.

For every embryo genotyped, the level of 10-kDa zein in the corresponding endosperm was determined by immunoblot analysis. The accumulation of 10-kDa zein in individual endosperms corresponding to the embryos represented in the upper panels, is shown in the lower panels of Fig. 3. In the progeny of W64A × BSSS53-TB4Sa, with increasing doses of *dzr1+BSSS53* in the endosperm, there was a corresponding increase in the accumulation of 10-kDa zein. Endosperms receiving two paternal doses of *dzr1+BSSS53* (represented as WW/BB in Fig. 3*A Lower*, lanes 3 and 4) had higher accumulation of 10-kDa zein than the ones receiving one dose of *dzr1+BSSS53* (represented as WW/B in Fig. 3*A Lower*, lanes 5 and 6). Endosperms receiving no dose of *dzr1+BSSS53* (represented as WW/− in Fig. 3*A Lower*, lanes 7 and 8) had the lowest accumulation of 10-kDa zein. These results indicate that *dzr1+BSSS53* has the normal dosage phenotype when carried on TB-4Sa.

However, despite increasing doses of *dzr1+BSSS53* in the endosperms of the progeny arising from MO17 × BSSS53-TB4Sa, all three dosage classes (represented as MM/BB, MM/B and MM/− in Fig. 3*B Lower*, lanes 11–16) had uniformly low levels of 10-kDa zein as in the MO17 parent. This indicates that *dzr1+MO17* is dominant to *dzr1+BSSS53* when present in 2:1 (in MM/B) and 2:2 (in MM/BB) allelic ratios.

The progeny arising from BSSS53 × MO17-TB4Sa varied in male dosage of *dzr1+MO17* to the endosperm. However, the 10-kDa zein level in all three dosage classes (represented as BB/MM, BB/M, and BB/− in Fig. 3*C Lower*, lanes 19–24) were uniformly high as in BSSS53 parent. In hyperploid endosperms arising from this cross, which had a *dzr1+BSSS53* to *dzr1+MO17* ratio of 2:2 (BB/MM), *dzr1+MO17* was recessive despite its presence in two doses. This result clearly demonstrates that the differential phenotype of *dzr1+MO17* cannot be explained by the gene dosage hypothesis, which supposes that two doses of *dzr1+MO17* are sufficient for its dominance.

Most significantly, although hyperploid endosperms arising from the two crosses, MO17 × BSSS53-TB4Sa and BSSS53 × MO17-TB4Sa, were identical in *dzr1* allelic composition with two doses each of *dzr1+BSSS53* and *dzr1+MO17* (MM/BB and BB/MM in Fig. 3 *Lower*, lanes 11 and 12 and lanes 19 and 20, respectively), their phenotypes were different. Two doses of *dzr1+MO17* when maternally transmitted (as in MM/BB) were dominant, whereas two doses of *dzr1+MO17* when paternally transmitted (as in BB/MM) were recessive. This strongly suggests that the parental bias on *dzr1+MO17* function is due to genomic imprinting of the locus.

Effect of Alteration of *dzr1+MO17* Parental Origin in Second Generation. Analysis of (BSSS53 × MO17) × BSSS53 progeny showed a 1:1 segregation of high- and low-level accumulation of 10-kDa zein (Fig. 4). This indicates that while paternally transmitted *dzr1+MO17* is recessive in the (BSSS53 × MO17)F_1 hybrid (Fig. 1, lanes 11 and 12), the same allele is dominant in the next generation when maternally transmitted. In a second cross, BSSS53 × (MO17 × BSSS53), all the progeny had a high level of 10-kDa zein that was similar to the level in BSSS53 (Fig. 4). Thus, the dominant phenotype of maternally transmitted *dzr1+MO17*

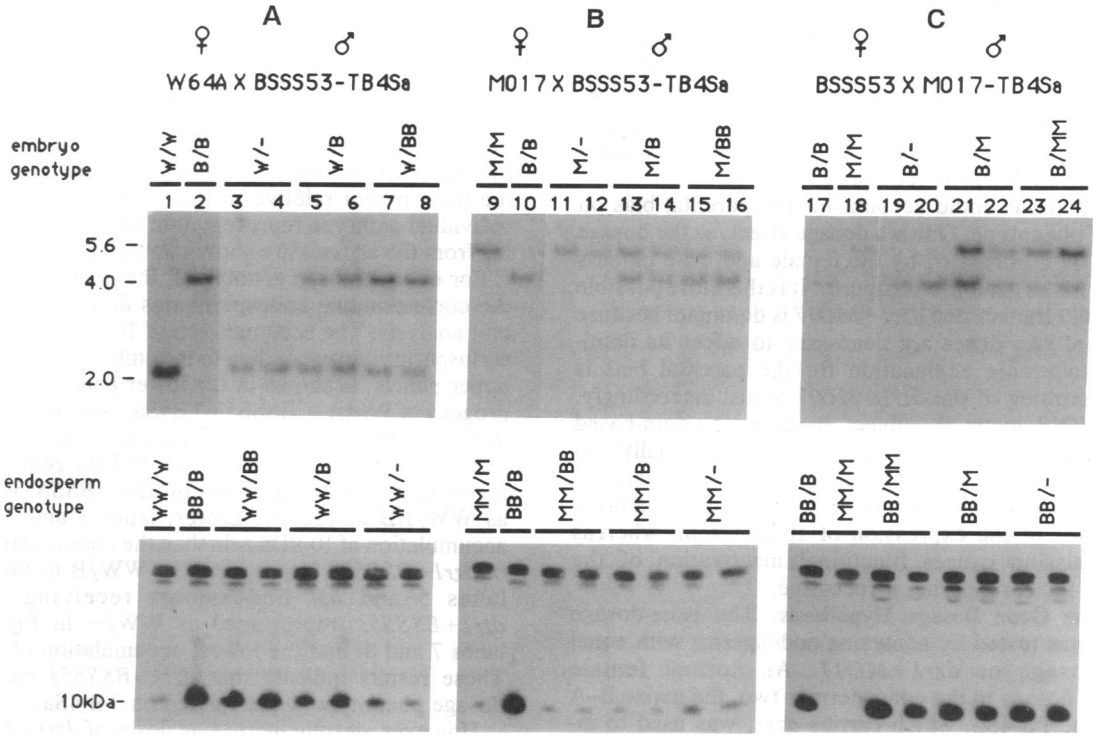

FIG. 3. Effect of *dzr1* dosage changes on the accumulation of 10-kDa zein in the endosperm. Dosage of *dzr1* in the embryos and the accumulation of 10-kDa zein in the corresponding endosperms were analyzed in the progeny of W64A × BSSS53-TB4Sa (*A*), MO17 × BSSS53-TB4Sa (*B*), and BSSS53 × MO17-TB4Sa (*C*). (*Upper*) Southern blot analysis of two individual seedlings representing each dosage class. Seedling DNAs were digested with *Nde* I, electrophoresed in 0.9% agarose gels, blotted, and probed with *Adh2* cDNA. (*Lower*) Immunoblot analysis of zeins (2 μg) from endosperms with the 10-kDa zein antibody. The two left lanes in *A*, *B*, and *C* are the parents for each cross. Each lane represents seedling DNA analysis (*Upper*) and the endosperm level of 10-kDa zein (*Lower*) from the same kernel. The genotype of each dosage class is identified above the lanes with W, B, and M, representing *dzr1+W64A*, *dzr1+BSSS53*, and *dzr1+MO17*, respectively. To analyze individual kernels, portions of the endosperm were excised and used to estimate the level of 10-kDa zein by immunoblot analysis. The kernels were then planted and seedling leaf DNA was analyzed on a Southern blot. The approximate molecular size in kilobases of each hybridizing band is indicated in the extreme left (*Upper*).

in (MO17 × BSSS53)F_1 hybrid (Fig. 1, lanes 13 and 14) is lost when paternally transmitted to the next generation. These results demonstrate that the phenotype of *dzr1+MO17*, dominant or recessive, is determined at every generation based on immediate parental origin and is not influenced by grandparentage. Furthermore, the lack of recovery of any individual with a low level of 10-kDa zein among the 50 progeny of BSSS53 × (MO17 × BSSS53) analyzed indicates that no other segregating factor unlinked to the *dzr1+MO17* locus is required for rendering the locus recessive when male transmitted.

DISCUSSION

We have demonstrated that an allele of the regulatory locus *dzr1* that posttranscriptionally controls the accumulation of 10-kDa zein in the maize endosperm is parentally imprinted.

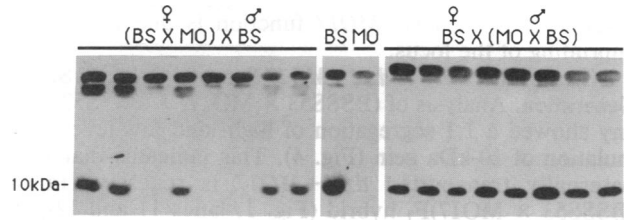

FIG. 4. Accumulation of the 10-kDa zein in backcross progeny. Total zeins (2 μg) from mature endosperms of BSSS53 (BS), MO17 (MO), (BSSS53 × MO17) × BSSS53, and BSSS53 × (MO17 × BSSS53) were analyzed on an immunoblot by using the 10-kDa zein antibody.

The imprinted allele, *dzr1+MO17*, is dominant, conditioning low expression of 10-kDa zein, when transmitted through the female but recessive when transmitted through the male. We have tested the possibility of the *dzr1+MO17* unequivalent phenotype being due to the difference in parental gene dosage to the endosperm. By analyzing endosperms with equal *dzr1+MO17* dosage from the parents, we conclude that the unequivalent phenotype of *dzr1+MO17* is due to differences in its parental origin and not dosage.

The key feature of imprinting is that the mechanism must be reversible at every generation (17–20). That is, for example, the maternal imprinting marking of a gene must be erased and reset to the paternal form in the gamete of the male offspring. While reversibility is a strict requirement for imprinted genes in mammals where the germ cells arise from the single fertilized egg, this is not the case for genes that are imprinted in the endosperm, as the endosperm is distinct from the embryo and is a terminally differentiated tissue that does not contribute to the generation of germ cells. Thus, if the marking differentially modifies only the central nuclei and not the egg during female gametogenesis, the marking need not be reversed since the unmarked gene in the embryo gives rise to the germ cells. However, if the marking occurs during female gametogenesis with both the egg and central nuclei being marked, it would require erasure and resetting of the imprint in the gametes. Marking during male gametogenesis also needs to be reversible since it would involve modification of both sperm nuclei, which are indistinguishable. Thus, the requirement for reversibility of imprinted genes in the endosperm depends at which gametogenesis stage the marking is set. For the *R* locus in maize, although *R* conditions

pigmentation in both embryo and endosperm, imprinting was observed only in the endosperm (21), indicating that *R* is imprinted in the female gametophyte and is staged after the division that gives rise to the egg and the central nuclei. As zeins are exclusively expressed in the endosperm, the stage at which *dzr1+MO17* is imprinted cannot be determined, as is possible for *R*.

Imprinting of *R* and *dzr1* differs from the imprinted genes in mouse in one important respect. Only certain alleles of *R* and *dzr1* are imprinted, in contrast to the absence of such allele-specific imprinting in the mouse. The allele specificity could suggest that the imprinted alleles of *R* and *dzr1* possess a unique DNA sequence, an "imprinting box" (22), that imposes some form of gametic-origin-based epigenetic modifications. The epigenetic modifications may involve chromatin structure alterations and/or changes in DNA methylation (23, 24). However, understanding the molecular mechanism underlying allele-specific imprinting of *dzr1* awaits the cloning of the various alleles of this locus.

The loss of a dosage effect of *dzr1+BSSS53* in heterozygotes when *dzr1+MO17* is silenced through imprinting provides clues to the mechanism of *dzr1* function as a posttranscriptional regulator. One model is that the allelic forms of the posttranscriptional factor encoded by *dzr1* in the heterozygotes are in competition for the target that is likely be the 10-kDa zein mRNA. Silencing of male-transmitted *dzr1+MO17* by imprinting eliminates such competition, as in the BSSS53 × MO17 hybrid, and consequently, the phenotype of the hybrid is that of BSSS53. This model also explains the dosage effect of *dzr1+BSSS53* when present in the heterozygote with an unimprinted allele such as *dzr1+W64A*, by the competition of the allelic products that are present in proportion to their gene dosage for the target 10-kDa zein mRNA.

Both animal (1) and plant (2–5) studies establish the involvement of imprinting in the expression of genes critical for normal development and viability. This has led to speculation about the role of imprinting in the evolution of reproductive strategies, viviparity, and speciation (18, 23). In plants, the confinement of imprinting only to the endosperm has been postulated to account for hybrid vigor or "epihybridity" of the endosperm (3, 25, 26). This could explain the rapid initial growth of the endosperm necessary for it to fulfill its role as a nutritive tissue for the relatively slowly developing embryo (26). Imprinting of nonessential genes, such as *R* and *dzr1*, and the allele-specific nature of their imprinting illustrate that the process can also affect functions not strictly required for normal development and viability. In addition, this feature allows the study of *R* and *dzr1* imprinting by regular Mendelian genetics.

We gratefully acknowledge the helpful comments on this manuscript from J. Kermicle, M. Benner, P. Maliga, S. Swarup, and two anonymous reviewers. This work was supported by U.S. Department of Energy Grant 84ER1367 to J.M.

1. Surani, M. A., Sasaki, H., Ferguson-Smith, A. C., Allen, N. D., Barton, S. C., Jones, P. A. & Reik, W. (1993) *Phil. Trans. R. Soc. London B* **338**, 165–172.
2. Lin, B. Y. (1984) *Genetics* **107**, 103–115.
3. Kermicle, J. L. & Alleman, M. (1990) in *Genomic Imprinting Development 1990 Supplement*, eds. Monk, M. & Surani, A. (Company of Biologists, Cambridge, U.K.), pp. 9–14.
4. Lin, B. Y. (1982) *Genetics* **100**, 475–486.
5. Birchler, J. A. & Hart, J. R. (1987) *Genetics* **117**, 309–317.
6. Kermicle, J. L. (1970) *Genetics* **66**, 69–85.
7. Kirihara, J. A., Hunsperger, J. P., Mahoney, W. C. & Messing, J. W. (1988) *Mol. Gen. Genet.* **211**, 477–484.
8. Benner, M. S., Phillips, R. L., Kirihara, J. A. & Messing, J. W. (1989) *Theor. Appl. Genet.* **78**, 761–767.
9. Cruz-Alvarez, M., Kirihara, J. A. & Messing, J. W. (1991) *Mol. Gen. Genet.* **225**, 331–339.
10. Schickler, H., Benner, M. S. & Messing, J. W. (1993) *Plant J.* **3**, 221–229.
11. Roman, H. (1947) *Genetics* **32**, 391–409.
12. Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356.
13. Das, O. P., Cruz-Alvarez, M., Chaudhuri, S. & Messing, J. W. (1990) *Methods Mol. Cell. Biol.* **1**, 213–222.
14. Dennis, E. S., Sachs, M. M., Gerlach, W. L., Finnegan, E. J. & Peacock, W. J. (1985) *Nucleic Acids Res.* **13**, 727–743.
15. Das, O. P. & Messing, J. W. (1987) *Mol. Cell. Biol.* **7**, 4490–4497.
16. Carlson, W. R. (1986) *CRC Crit. Rev. Plant Sci.* **3**, 201–226.
17. Monk, M. (1988) *Genes Dev.* **2**, 189–192.
18. Solter, D. (1988) *Annu. Rev. Genet.* **22**, 127–146.
19. Sapienza, C. (1990) *Sci. Am.* **263**, 52–60.
20. Jorgensen, R. (1993) *Philos. Trans. R. Soc. London B* **338**, 173–181.
21. Brink, R. A., Kermicle, J. L. & Ziebur, N. K. (1970) *Genetics* **66**, 87–96.
22. DeChiara, T. M., Robertson, E. J. & Efstratiadis, A. (1991) *Cell* **64**, 849–859.
23. Surani, M. A., Allen, N. D., Barton, S. C., Fundele, R., Howlett, S. K., Norris, M. L. & Reik, W. (1990) *Phil. Trans. R. Soc. London B* **326**, 313–327.
24. Barlow, D. P. (1993) *Science* **260**, 309–310.
25. Kermicle, J. L. (1978) in *Maize Breeding and Genetics*, ed. Walden, D. B. (Wiley, New York), pp. 357–371.
26. Brink, R. A. & Cooper, D. C. (1947) *Bot. Rev.* **13**, 423–541.
27. Alfenito, M. R. & Birchler, J. A. (1993) *Genetics* **135**, 589–597.