Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7809–7813, October 1990
Genetics

A somatic gene rearrangement contributing to genetic diversity in maize

(mitotic recombination/zein genes/genome evolution/*Zea mays*)

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Communicated by C. S. Levings III, July 16, 1990 (received for review December 20, 1989)

ABSTRACT **We have discovered a somatic genomic rearrangement that occurs at high frequency at a duplicated zein locus in certain cultures of the maize inbred line A188. The rearranged allele arises from the duplication by a two-step process involving a homologous recombination and a second event, which may be a deletion, inversion, or insertion; both steps always occur together. The frequency of rearrangement is lower in homozygous states of the parental allele than in heterozygotes. In both cases, the rearrangement is shown to be mitotic. The rearranged product can be transmitted through meiosis, providing another mechanism for genome evolution in higher eukaryotes.**

Although meiotic recombination has been observed in all eukaryotes, somatic gene rearrangements are rarer and are often associated with developmentally significant events. Developmentally regulated events of this type include mating-type switching in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (1), heterocyst formation in *Anabena* (2), and antigenic variation in *Borrelia* (3). Somatic gene rearrangements involving illegitimate recombination mediated by signal sequences create diversity in the mammalian immune system (4), whereas gene conversion is the mechanism operating in the chicken immune system (5). Somatic deletions and translocations have been implicated in neoplasia and have been observed in mammalian (6) and plant (7) tissue culture. In plants, somatic sectoring has been observed as a result of transposon activity during development; since a germ line does not exist in plants, these mutations can be inherited and spread through the population (8).

Rearrangement of reptitive sequences in plants has been previously considered to occur during meiosis by unequal crossing-over (9). Dooner and Kermicle (10) have conducted an extensive genetic study of the *R* locus in maize, which consists of duplicated genes at a genetic distance of 0.16 map unit. They have demonstrated much higher frequencies of unequal crossing-over compared to intrachromosomal recombination or gene conversion in this system. The *P* locus in maize has also been shown to contain a tandemly repeated DNA sequence by molecular analysis (11); genetic studies have demonstrated stability of this duplicated allele (12). While rare instances of somatic mutations resulting in sectored tissue have been described at the *R* (13) and the *P* (14) loci, no examples of frequent somatic gene rearrangements have been described except when transposons were present at the locus.

Earlier studies identified two alleles of the 27-kDa-zein locus (15). One, which we term the *S* or "standard" allele, consisted of a duplicated 12-kilobase (kb) segment of DNA with one functional copy of a 27-kDa-zein gene in each repeat. The two genes for the 27-kDa zein in this allele were termed A and B and were distinguishable by Southern blot analysis or by endosperm RNA analysis with gene-specific oligonucleotide probes (15). The other allele, termed *Ra* for "recombinant with the A gene," contained only the A gene (15, 16). Both alleles were common among maize inbred lines; inbreds W22 and W23 were *S/S* whereas W64A, B37, and BSSS53 were *Ra/Ra* (15). Since the presence of either allele does not affect seed or plant viability, these alleles appear to be selectively neutral.

One common inbred line of maize, A188, was *S/S* in most of the cultures tested. However, certain isolates of A188 were *Ra/S* or *Ra/Ra*. We have obtained direct evidence, presented in this report, that the *S* allele can actively rearrange to the *Ra* allele in A188 and its hybrids and that this process can occur somatically.

MATERIALS AND METHODS

Maize Stocks. Most of the A188 cultures and those of other inbreds used in this study were derived from material kindly provided by Ronald Phillips (Department of Agronomy and Plant Genetics, University of Minnesota). A stock of A188 obtained from Burle Gengenbach of the same department was found to be *Ra/Ra* and another from Molecular Genetics (Minnetonka, MN) was found to be *Ra/S*. For the experiments shown in Figs. 3 and 4, segregating progeny were obtained from three self-pollinated *S/Ra* plants whose *Ra* alleles were paternally contributed by W64A and A619 and whose *S* alleles were maternally derived from A188. These F_2 populations were designated 9203 for the plant with W64A as male parent and 9204 and 9205 for two plants with A619 as male parent. Some immature kernels from these ears were harvested and frozen, while the rest were allowed to mature. These F_2 progeny were used in preference to *Ra/S* A188 because linked restriction fragment length polymorphism (RFLP) markers on chromosome 7 (17) can be employed to identify the parental chromosomes of each progeny.

Isolation and Analysis of Genomic DNA. Frozen immature kernels harvested 25–30 days after pollination were peeled of pericarp tissue after brief thawing and separated into embryos and endosperms, and DNA was isolated as described (18). To identify chimeric plants, seeds were incubated with 0.05% sodium hypochlorite for 15 min, washed, and germinated on moist filter paper in the dark. After germination, the seedlings were transferred to light and harvested into root and shoot samples when the shoot was about 1 cm long and the taproot (radicle) was 7–8 cm. In some cases, the taproot was excised and the seedling was transplanted to soil. Genomic DNA was isolated from these tissues as described (18). The yield of DNA from these tissues varied between 5 and 10 μg.

DNA from each sample was digested with the appropriate restriction enzyme, separated in 0.8% agarose gels, and

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blotted to nitrocellulose filters. For Figs. 2 *Upper*, 3, and 4, the blots were hybridized to the 3.7-kb *Sal* I fragment (see Fig. 1) as described (18) and washed at high stringency (15 mM NaCl/1.5 mM sodium citrate at pH 7, 65°C, 60 min). For Fig. 2 *Lower*, the insertion at the 3′ end of the B duplication was cloned as a *Pvu* I–*Xba* I fragment, isolated, and nick-translated.

RESULTS

Earlier studies of the maize storage protein (zein) genes (19) identified a tandem duplication of 12 kb of DNA at the 27-kDa-zein locus in three maize inbred lines (W22, W23, A188) (15). A restriction map of this region split in two is shown in Fig. 1 *Upper*, with the duplicated regions aligned to indicate homology. Each copy of the duplication, designated A and B, contains a transcribed coding sequence (open boxes) for the 27-kDa zein; the B copy contains a unique inserted sequence (striped box) at the 3′ flanking region. In other inbred lines (W64A, BSSS53, B37), a rearranged version of this locus is found (15), whose restriction map is shown below that of the duplicated allele.

Though most of the A188 cultures tested were *S/S* at this locus, some were found that contained the *Ra* allele (*Materials and Methods*). To test whether these *Ra* alleles were similar to those previously identified in other inbreds, gene-specific oligonucleotide probes that distinguish between transcripts of the A and B genes (15) were used to probe RNA samples from individual endosperms (18) from a self-pollinated *Ra/S* A188 plant; DNA from the same endosperms (18) was analyzed for genotype (data not shown). In this F_2 population, complete correspondence was demonstrated between genotype and phenotype. *S/S* and *S/Ra* individuals contained transcripts from both the A and the B gene, whereas *Ra/Ra* individuals contained transcripts from the A gene only. Further, the expression of the B gene was observed to correspond to dosage of the *S* allele; in triploid endosperm tissue, 0, 1, 2, and 3 copies of the *S* allele occur in an F_2 population. Such dosage-dependent expression had been demonstrated in reciprocal crosses of W23 (*S/S*) and BSSS53 (*Ra/Ra*) (15). Therefore, the *Ra* allele in A188 was similar to those analyzed previously in terms of the DNA sequence at the oligonucleotide binding site and gene expression.

The structure of the rearranged *Ra* locus was further analyzed at the restriction-site level. DNA from an *Ra/Ra* BSSS53 plant was compared to DNA from an *Ra/Ra* A188 plant and from an *S/S* A188 plant on Southern blots after digestion with various restriction enzymes. The blot was sequentially probed with the 27-kDa-zein gene (Fig. 2 *Upper*) and with the unique sequence present 3′ of the B gene in the duplicated allele (Fig. 2 *Lower*; see Fig. 1 for the location of the fragment). With *Sca* I, *Bgl* I, and *Sst* I, the bands that hybridized to the 27-kDa-zein probe were the same for A188 *Ra* (lanes 1) and for BSSS53 *Ra* (lanes 2) but different from A188 *S* (lanes 3). Similar results were obtained with over 20 other enzymes (data not shown). This demonstrated that the *Ra* allele in A188 was the same as that found in other maize populations and that it was distinct from the *S* allele. *Eco*RI digestion, however, revealed a difference; A188 *Ra* gave a 5-kb band while BSSS53 (and other inbreds; data not shown) gave a 10-kb band. This was the only identified restriction site polymorphism of the *Ra* allele between A188 and other lines.

When the same blot was probed with the unique 3′ sequence, the results were as expected; when the enzyme cut between the gene and the insertion, all three alleles were identical (*Sst* I), whereas when it did not, the same fragment that hybridized to the gene probe was detected (*Eco*RI, *Sca*

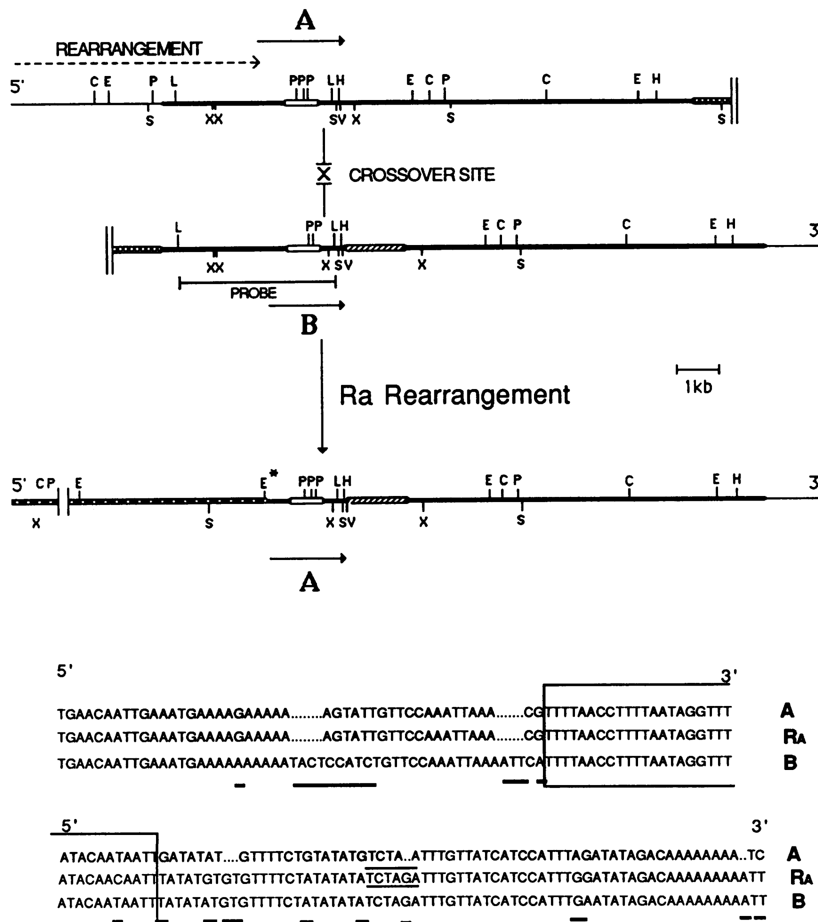

FIG. 1. Restriction maps of the *S* and *Ra* alleles of the 27-kDa-zein locus. (*Upper*) The first two maps represent a continuous stretch of DNA containing the tandem duplication units of the *S* allele with the A and B genes (15, 18). The duplicated regions are shown by heavy lines, the coding region by open boxes, and the insertion sequence 3′ of the B gene by a striped box. The region between the two duplication units is represented by a cross-hatched box broken in two with vertical lines, and horizontal arrows indicate transcription direction. The region deleted by the complex rearrangement at the 5′ end is indicated by a dashed line above the upper map. The third map shows the *Ra* arrangement; the unidentified region brought proximal to the 5′ flanking end of the A gene in the *Ra* arrangement is denoted by a white-dotted heavy bar. Restriction sites: C, *Sca* I; E, *Eco*RI; H, *Xho* I; L, *Sal* I; P, *Pst* I; S, *Sst* I; V, *Pvu* I; X, *Xba* I. (*Lower*) Comparison of the sequences (a continuous stretch shown split in two) of the A and B genes (top and bottom lines, respectively; ref. 19) of the *S* allele with the sequence of the *Ra* allele (middle line; ref. 16). Differences between *Ra* and either A or B are indicated by underlining. A unique *Xba* I site present in *Ra* and the B gene but not the A gene is shown by double lines. The presumed site of crossover is represented by a box covering both sets of sequences.

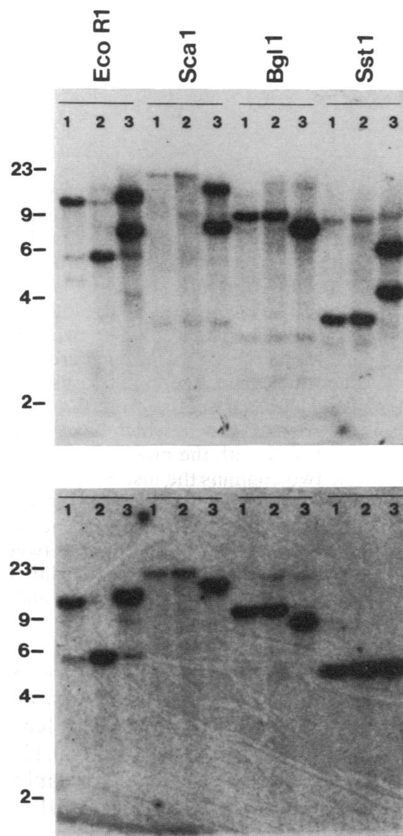

FIG. 2. Comparison of 27-kDa-zein alleles. (*Upper*) DNA from *Ra/Ra* BSSS53, *Ra/Ra* A188, and *S/S* A188 plants (lanes 1, 2, and 3, respectively) was digested with the indicated enzyme, blotted, and probed with a mixture of the 3.7-kb *Sal* I fragments (see Fig. 1 *Upper*) of the A and B genes. (*Lower*) The blot was stripped and reprobed with the insertion element shown by the striped box in Fig. 1 *Upper*. Hybridization and washing conditions were as described (18). Note that in all lanes probed with the 3.7-kb *Sal* I fragments (*Upper*), the same weakly hybridizing band is found for all three alleles with a given enzyme; this band derives from the 16-kDa-zein gene, which has sequence homology to the 27-kDa-zein gene. The faint bands in the *Eco*RI lanes arise from partial and "star" activity of the enzyme in this experiment. Numbers at left are size markers in kilobases.

I, and *Bgl* I). This indicated that the insertion fragment that was linked to the 3′ end of the B gene in the *S* arrangement was linked to the single A gene in the rearranged allele.

Based on restriction data and published sequences of the *S* and *Ra* alleles (16, 19), we have arrived at a model accounting for the origin of the *Ra* allele. Two steps are required; one is a homologous crossover at the 3′ site indicated in Fig. 1. Sequences from the *Ra/Ra* inbred W64A (16) and the A and B genes of the *S* allele (19) at this crossover site are shown in Fig. 1 *Lower*. Bases that are different in *Ra* from either A or B are underlined; it is clear that 5′ of the boxed region, the *Ra* sequence is identical to the A gene sequence whereas 3′ regions are similar to the B sequence. Therefore, the crossover site should be within the boxed region; the *Xba* I site (TCTAGA) shown by the double line in the sequence is present in *Ra* (data not shown), as expected. Note that the crossing-over is exact, with no site duplication or footprints. To account for the loss of preexisting sites at the 5′ region of the A gene and for the presence of sites such as the *Sst* I site in the new 5′ region, a second rearrangement, which may be a deletion, inversion, or insertion, must occur at the 5′ end. The new *Eco*RI site (E*) allows us to map the loss to within ≈800 bases 5′ of the translation start site of the A gene.

We have obtained several types of evidence that point to an active rearrangement of the *S* allele to the *Ra* allele in A188 and its progeny. Double fertilization in flowering plants gives rise to a triploid endosperm and a diploid embryo of identical genotypes in each seed, with the exception that the endosperm contains two copies of the maternally derived genes. However, in some F_2 populations involving A188 segregating for the *S* and *Ra* alleles, we have observed violations of genotype concordance. Fig. 3 compares embryo and endosperm genotypes of kernels from an F_2 of (A188 × W64A). The endosperm samples shown in lanes 5 and 20 were *Ra/Ra/S* whereas the embryos were *Ra/Ra*. The endosperm sample in lane 13 was *Ra/S/S* whereas the embryo was *S/S*. These allele compositions were confirmed by digestion with *Bam*HI (data not shown). Nonconcordance of embryo and endosperm may result from somatic rearrangement of the *S* allele to the *Ra* allele. An alternative explanation is a rare phenomenon termed heterofertilization (20), in which two pollen grains fertilize one egg such that the embryo and endosperm are nonidentical.

Consequently, we tested self-pollinated *S/S* A188 plants for rearrangement to the *Ra* allele, since any instances of rearrangement in these progeny should not be subject to the caveat of heterofertilization. Two examples of rearranged alleles were found in over 300 immature kernels from 25 ears of several different pedigrees. In Fig. 4*A*, the genotype of these two embryos can be visualized, with the corresponding endosperms in Fig. 4*B*. Two unusual features of these data are (*i*) complete conversion, with both genome copies in the embryo of lane 1 and all three copies in the endosperm of lane 2 being affected, and (*ii*) rearrangement in only embryo and not endosperm (lanes 1) or vice versa (lanes 2). This demonstrates that the *S* allele in A188 can spontaneously rearrange to the *Ra* allele. The data cannot be explained by heterofertilization or pollen contamination, and they indicate a postfertilization origin for the rearrangement; if the *Ra* allele arose from meiotic recombination, heterozygous progeny would be expected in greater frequency than the homozygous cases observed. Each of these kernels was found on self-pollinated ears of sibling plants; 20 additional kernels from each of these ears were all homozygous *S* in embryo and endosperm (data not shown).

Postfertilization rearrangement may conceivably occur after a few divisions of the primary fertilized cell; in such cases, sectored tissue would be expected. Transposition in maize is a precedent for such somatic events (21). Sectoring in endosperm is difficult to test in our system, which relies on molecular probes for detection. However, rearrangement during early embryo development can be potentially detected by Southern blot comparisons of different tissues. We chose to test for differences between the taproot (radicle) and shoot or leaf tissue, since they originate from diametrically opposite ends of the embryo (Fig. 4*C*). Fig. 4*D* shows results from taproot tissue of 9 F_2 seedlings that were allowed to grow to maturity after removal of the taproot. Though most taproot samples were homozygous *S/S* (Fig. 4*D*, lane 5) or *Ra/Ra* (lane 9) or heterozygous *Ra/S* (lanes 1, 4, 6, and 8) as expected from segregation, we detected some cases of increased *Ra* relative to *S* (e.g., lanes 2 and 7). Such inconsistent allele ratios were obtained for seedlings of the F_2 cultures 9203, 9204, and 9205 in 5 of 100 taproot samples and 3 of 20 shoot samples. Similar results were also observed for immature whole embryos (data not shown). Digestion artifacts, lane contaminations, and methylation differences have been ruled out by repeating the experiments with different restriction enzymes (data not shown). Similar analysis of more than 100 embryos and 45 taproots of similar F_2 progenies of recombinationally inactive A188 revealed no cases of inconsistent allele ratios (O.P.D. and J.M., unpublished observations).

These aberrant allele ratios most probably reflect chimeric tissues, which contain sectors derived from progenitor cells

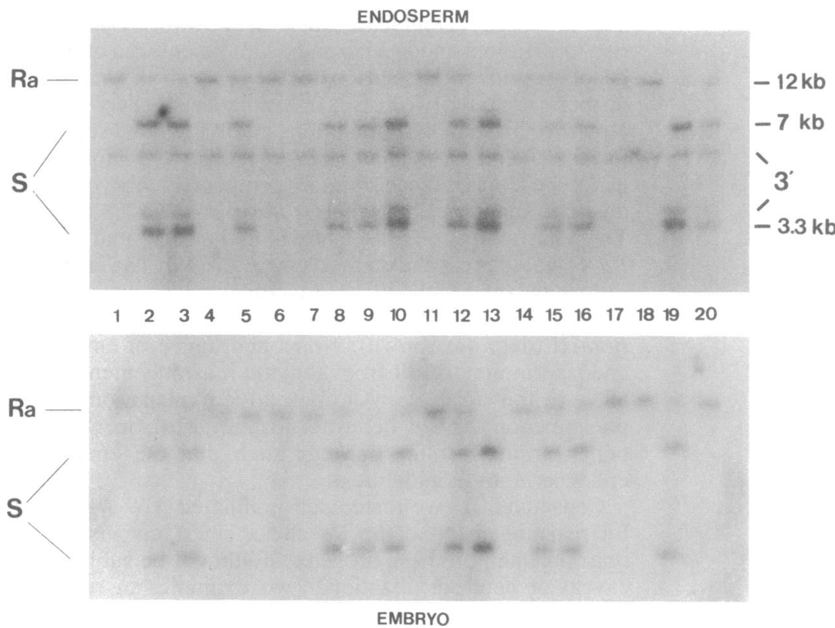

FIG. 3. Progeny analysis of a self-pollinated *Ra/S* ear. DNA from corresponding endosperms (*Upper*) and embryos (*Lower*) of the progeny of a self-pollinated *Ra/S* ear (culture 9203; see *Materials and Methods*) was digested with *Pst* I, electrophoresed, blotted, and probed as in Fig. 2 *Upper* (18). The two bands of the *S* allele and the single *Ra* band are indicated with their molecular size in kilobases. The two faint bands labeled 3′ arise from the 3′ fragments of the A and B genes, which have about 500 bp of homology with the probe (Fig. 1); the upper of the two contains the insertion sequence (Fig. 1) and occurs in both *Ra* and *S* alleles whereas the lower one is found only for *S*. The differences in intensity of these 3′ bands between the endosperm and embryo blots result from small differences in the hybridization and wash conditions for the two blots.

that did or did not undergo rearrangement. Since leaf tissue of the plants in lanes 2 and 7 of Fig. 4*D* was *Ra/Ra* (data not shown), these plants were mosaics consisting of a large sector of rearranged *Ra/Ra* tissue and a small sector containing the *S* allele in the taproot. Because the large sector included the reproductive structures of both plants, the rearranged allele should be transmitted normally through pollen and egg. This has been confirmed by genotype analysis of progeny (data not shown).

In a rare instance, when dissection of a seedling coincided with the boundary of such sectors, complete genotype separation was observed (Fig. 4*E*). The samples from a control plant (three left lanes) showed identical genotypes for all tissues, but the shoot DNA of a chimeric plant (two right lanes, marked with asterisks) was heterozygous *Ra/S*, whereas the taproot was homozygous *Ra*.

Chimeric plants can be detected by our assay only when rearrangement occurs differentially in the progenitor cells of root and shoot and when dissection separates zones of chimeric tissue. Since this underestimates the true rearrangement frequency, we compiled 27-kDa-zein allele compositions of an F_2 population, culture 9203, to test whether rearrangement is frequent enough to distort segregation ratios. Fig. 3 displays data from embryos and endosperms of 20 immature kernels of this population; the F_1 parent was a true *S/Ra* heterozygote, as tested by analysis of leaf DNA (data not shown). The segregation ratios were 0:12:8 (*S/S*:*Ra/S*:*Ra/Ra*) for endosperms and 1:9:10 for embryos, rather than the 5:10:5 expected for each. A total of 101 mature plants from this ear were also analyzed; when all progeny (embryos and mature plants) are considered, the segregation numbers are 17:73:31. This deviates from normal F_2 segregation to a statistically significant degree as tested by χ^2 analysis ($0.025 > P > 0.01$).

DISCUSSION

In homozygous *S/S* A188, we were able to detect two instances of complete rearrangement to the *Ra* allele, one in embryo and one in endosperm. As mentioned above, 20 other kernels from these two ears were *S/S*. This indicates that the activity is relatively infrequent but explains the presence of the rearranged allele in cultures of inbred A188. We have not detected any cases of rearrangement in ≈100 embryos and endosperms of F_1 progeny, indicating that activity is lower or comparable to that in inbred progeny. In F_2 populations the

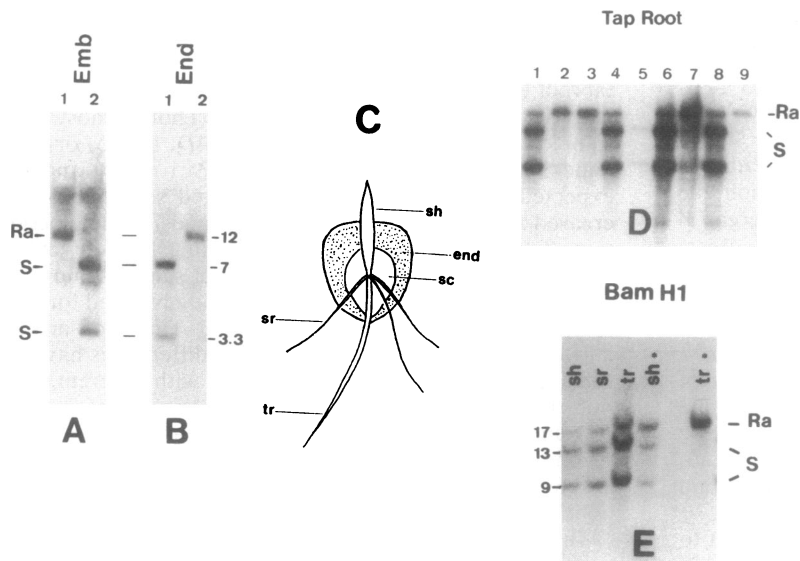

FIG. 4. Evidence for somatic gene rearrangement. (*A* and *B*) Southern blot of *Pst* I-digested DNA from corresponding embryos (Emb) and endosperms (End) of two kernels (indicated by lane numbers) from two self-pollinated *S/S* ears. Fragment sizes (kb) are at right. (*C*) Diagram to illustrate the tissues and developmental stages of the material used for *D* and *E*. end, Endosperm; sc, scutellum (cotyledon); sh, shoot; sr, side root; tr, taproot. (*D*) *Bam*HI digests of taproot (radicle) DNA samples of nine seedlings probed as before. (*E*) *Bam*HI digests of DNA from two seedlings; the first three lanes are from a normal, nonchimeric plant included for comparison; the next two lanes, marked by asterisks, contain DNA from a chimeric plant. Fragment sizes (kb) are at left. Note that in *A*, *B*, *D*, and *E*, as well as in Fig. 3, the larger size and reduced region of identity to the probe render hybridization to the *Ra* band less intense than that to the *S* bands.

activity must be considerably higher, since segregation ratios were distorted; the relatively high frequency of chimeric plants is consistent with this interpretation. Biased F_2 populations suggest gametophyte factors (22), but the frequency of heterozygotes in the population analyzed is higher than would be expected if such factors were present. Though gametophyte factors can distort F_2 segregation ratios to favor either homozygote, heterozygote frequency should remain 50% (23). The presence of chimeric plants also argues against the involvement of gametophyte factors.

The higher frequency of rearrangement in heterozygous plants is analogous to the activity of the maize transposon *Ac*, which displays an inverse relationship of activity to dosage (24). However, the homologous crossover at the 3′ end of the two genes was exact and contained no transposon footprints (Fig. 1). Although the *Ra* rearrangement involves two steps, we have never observed a case of one step occurring without the other, either in homozygous or in heterozygous A188 cultures. The products of such separate events would have been readily detected in our Southern blot analysis. In all cases, it is always the B gene that is deleted and the same new sites are present at the 5′ end. These observations imply a programmed mechanism that is site-specific.

However, another allele of this locus was identified in A188 cultures (O.P.D. and J.M., unpublished observations); this arises from homologous recombination at the 5′ region of the duplicated sequence, which results in the deletion of the A gene. In terms of the structure of the product, this rearrangement is different from the *Ra* rearrangement. It is possible that the two products result either from different modes of resolution of the same recombination intermediate or from different site-specific crossovers stimulated by the same signal. Extensive previous genetic studies on maize have demonstrated stability of duplications (10, 12), and the frequency of homologous recombination has been measured for many organisms, such as yeast (25). Therefore, the instability we observe is unusual and is probably specific for the genetic stocks we used. This is consistent with our observation that other inbred lines and other cultures of A188 are stable for the duplicated allele.

Our data provide strong evidence for the mitotic nature of the *Ra* rearrangement. Aside from the developmentally significant cases mentioned in the Introduction, we know of only one prior example of such a process in higher eukaryotes (26), where a tissue-specific amplification of one of the long interspersed repetitive element (LINE) sequences was observed in rat brain. Unlike this case, the product of the *Ra* rearrangement is heritable. The presence of inbred lines of maize containing the same *Ra* allele indicates that this process can contribute to genome evolution. While it is formally possible that *Ra* can also arise during meiosis, it is less likely that the same two steps of the *Ra* rearrangement, both of which are site-specific, also occur in meiosis. Gene conversion and asymmetric crossing-over during meiosis have been considered to be the major factors generating genetic diversity in higher eukaryotes (9). Our results indicate that similar processes can operate in mitosis as well and that they can contribute to the evolution of the genome, at least in plants where such events can be inherited. Pairing of homologous chromosomes provides a convenient developmental stage at which such events can be envisioned during meiosis; the lack of observed pairing in mitosis indicates that other mechanisms may operate to create the appropriate conditions for recombinational processes to occur. Studies on yeast indicate that double-strand breaks occurring *in vivo* trigger the mating-type switch (27) and may also stimulate ectopic recombination during mitotic divisions (28). One could speculate that a similar process operates in our system as well.

We thank Drs. Daniel Klessig and Pal Maliga for critical readings of the manuscript. This work was supported by grants from the Department of Energy (DE-FG05-85ER13667) and the National Institutes of Health (GM43261) to J.M.

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