Interchromosomal Recombination in *Zea mays*

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ABSTRACT

A new allele of the 27-kD zein locus in maize has been generated by interchromosomal recombination between chromosomes of two different inbred lines. A continuous patch of at least 11,817 bp of inbred W64A, containing the previously characterized *Ra* allele of the 27-kD zein gene, has been inserted into the genome of A188 by a single crossover. While both junction sequences are conserved, sequences of the two homologs between these junctions differ considerably. W64A contains the 7313-bp-long retrotransposon, *Zeon*-1. A188 contains a second copy of the 27-kD zein gene and a 2-kb repetitive element. Therefore, recombination results in a 7.3-kb insertion and a 14-kb deletion compared to the original *S*+A188 allele. If nonpairing sequences are looped out, 206 single base changes, frequently clustered, are present. The structure of this allele may explain how a recently discovered example of somatic recombination occurred in an A188/W64A hybrid. This would indicate that despite these sequence differences, pairing between these alleles could occur early during plant development. Therefore, such a somatically derived chimeric chromosome can also be heritable and give rise to new alleles.

HOMOLOGOUS recombination in higher plants plays a very important role in creating genetic diversity and at the same time genome stability. These seemingly opposing roles are accomplished by a tight regulation of where and when recombination occurs (Puchta and Hohn 1996). Homologous recombination during meiosis is obligatory to ensure proper segregation of chromosomes. Because the genomes of higher plants contain a high degree of repeated sequences, intra- and interchromosomal recombination between them could result in deficiencies and the removal of critical genes. Therefore, the assumption is that recombination occurs within unique sequences. However, most of our understanding of meiotic recombination is based on a very narrow selection of events because most studies are based on identifying them by heteroallelic loci whose normal functions are easily recognized (*e.g.*, tissue-specific pigment). Nevertheless, when a meiotic crossover was characterized in the maize genome not based on a phenotype, the exchange occurred within a homologous unique sequence that was flanked by repeated sequences (Timmermans *et al.* 1996).

Therefore, meiotic recombination appears to reflect the distribution of genes or unique sequences in the genome. Because the size of sequences between genes is variable, recombination frequencies and physical distances are quite variable throughout genomes. For instance, using long-range restriction mapping, Llaca and Messing (1998) could show that a region on maize chromosome *4*S that has exhibited a genetic distance of 3.4 cM (Chaudhuri and Messing 1995) is contained within 250 kb. This amounts to a nearly 50-fold higher frequency of recombination than the average. The molecular analysis of this region has shown that the gene density is more like that in Arabidopsis, a species with the smallest plant genome, than in the 18-fold larger maize genome. Intragenic recombination can occur at even higher frequencies and, in cases like the *bronze* locus in maize, five times higher than the intergenic example above (Dooner 1986). These results confirm that meiotic recombination avoids sequences like retroelements that are found in regions flanking genes and unique sequences.

If gene sequences are hotspots for recombination (Thuriaux 1977), one wonders what mechanism assures the proper alignment of orthologous sequences within a tandem array of gene copies. For instance, we found that the maize lines BSSS53 and W22 have maintained the same order and spacing of five 22-kD zein genes and that orthologous sequences are more conserved than the tandem copies among themselves (Llaca and Messing 1998). Since BSSS53 and W22 are separated by many generations, unequal crossing over between nonorthologous 22-kD zein gene sequences appears to be rare. However, this restriction appears be less stringent for the 27-kD zein gene locus on chromosome *7* of maize. In this report, we compare three alleles of this locus at the nucleotide sequence level. One is derived from the *S*+A188 allele, one from the *Ra*+W64A allele, and one from an *Ra*+A188 allele. The *Ra* allele

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differs from the *S* allele in two respects. First, a crossover has occurred between 189 to 220 nucleotides 3′ of the stop codon of the two 27-kD zein gene copies, resulting in the deletion of the B copy (Das *et al.* 1990). Second, an insertion of a 7313-bp-long LTR retrotransposon has occurred 1146 nucleotides upstream of the start codon of the A gene (Figure 1). Although we have previously assumed that the appearance of the *Ra* allele in stocks of A188 could be due to this two-step process, it was surprising that this would have occurred in current inbred lines. However, the same allelic variation has been observed in other maize lines like Blanco Dentado QPM, White Flint QPM, and other stocks of A188 (Geetha *et al.* 1991). In all these lines, the 27-kD zein locus appears to be heterozygous and contains both the *S* and the *Ra* alleles. This is in contrast to W22 and Oh43, which are homozygous for the *S* allele. If lines are maintained by selfing, one would expect them to be homozygous. If pollen contamination were to occur, why would it occur preferentially in some lines and not others? Furthermore, a hybrid can easily be distinguished from an A188 inbred because of the lack of the dwarf-like phenotype.

We have investigated several of these inbreds carrying *S* alleles that were believed to be unstable by PCR techniques, but never could find any new crossover products (G. Segal and J. Messing, unpublished results). Without any material that undergoes this rearrangement in a genetically controlled manner, it will be difficult to investigate this two-step process on the molecular level, particularly since it cannot be recognized by a simple pigment marker, such as the *Bz*, *A*, and *R* genes in maize. Nevertheless, because of the sequence polymorphisms between different inbred lines and between the A and B copies of the 27-kD zein genes, the genomic sequence of the *Ra*+A188 allele should be traceable to its origin. We therefore cloned and sequenced the *Ra*+A188 allele and found to our surprise that the *Ra* sequences in A188 were derived from the *Ra*+W64A allele rather than the *S*+A188 allele.

MATERIALS AND METHODS

Plant material: Maize inbred lines A188 and W64A were derived from stocks kindly provided by Dr. Burle Gengenbach and Dr. Ronald Phillips (University of Minnesota, St. Paul, MN). Probes bnl 15.40 and umc 116 were obtained from the stock center.

DNA gel blot analysis: Genomic DNA from leaf tissues was isolated as described (Das *et al.* 1990). DNA samples were digested with the appropriate restriction enzymes in (5- to 10-fold) excess and fractionated on 1% agarose gels. Gels were blotted onto Nytran membranes (Schleicher & Schuell, Keene, NH). Southern blots were prepared by standard protocols and prehybridized for 4 hr at 42° in a buffer containing 50% formamide, 5× SSC, 5× Denhardt's solution, 1% sodium dodecyl sulfate (SDS), 0.05 mg/ml denatured salmon sperm DNA, and 0.05% sodium pyrophosphate (pH 8.0). Membranes were hybridized to the appropriate probes in the same buffer as the prehybridization buffer at 42°. Membranes were washed three times with 1× SSC, 0.1% SDS at room temperature and twice with 0.3× SSC, 0.5% SDS at 65° for 20 min each. Probes were isolated with the Gene-Clean kit (BIO 101, Inc., Vista, CA) and labeled with [α-^{32}P]dCTP by random priming. All membranes were exposed to Kodak (Rochester, NY) XAR-5 film at −80° for 4 to 48 hr with intensifying screens.

Construction and screening of subgenomic and genomic libraries: For the subgenomic libraries, genomic leaf DNA from the *S*+A188 allele, the *Ra*+A188 allele, and the *Ra*+W64A allele were digested with *Bam*HI, separated on 0.9% agarose gels, and transferred onto NA-45 membranes (Schleicher & Schuell) inserted alongside the lanes by rotating the gels by 90°. These membranes were cut into sections and the size-fractionated DNA eluted. DNA from a 14- to 20-kb fraction of each genomic DNA was ligated with the *Bam*HI-digested EMBL-3 lambda DNA. For the complete genomic library, genomic leaf DNA from the *S*+A188 was partially digested with *Sau*3A and ligated to the *Bam*HI-digested EMBL-3 lambda DNA. Packaging and transfection of lambda DNA followed the manufacturer's protocol (Stratagene, La Jolla, CA). Recombinant phages were plated on *Escherichia coli* strain XL1-blue and screened with probe zpB 36, and positive plaques were plaque-purified twice.

Subcloning and DNA sequence analysis: Four positive lambda clones were isolated and purified from the *S*+A188, the *Ra*+A188, and the *Ra*+W64A libraries. Lambda DNA was isolated by the QIAGEN Lambda Kit (QIAGEN Inc., Chatsworth, CA). The 5SP lambda DNA has a 12-kb *Sau*3A insert containing the A gene of the 27-kD zein of the *S*+A188 allele. The SP3 lambda DNA has a 14-kb *Bam*HI insert containing the B gene of the 27-kD zein gene of the *S*+A188 allele. The LP3 lambda DNA has a 17.5-kb *Bam*HI insert containing the *Zeon*-1 element and the A gene of the 27-kD zein gene of the *Ra*+A188 allele. The WP3 lambda DNA has a 17.5-kb *Bam*HI insert containing the *Zeon*-1 element and the A gene of 27-kD zein gene of the *Ra*+W64A allele. The restriction maps of these lambda inserts were constructed and several fragments from their inserts were subcloned into pUC118. Plasmid DNA was prepared by the QIAprep Spin Plasmid Miniprep Kit (QIAGEN). The resulting plasmids were sequenced by double-stranded sequencing (Vieira and Messing 1982) and their sequence comparisons were made using the LASERGENE software (DNASTAR, Inc., Madison, WI).

RESULTS

Recombination between A188 and W64A at the 27-kD zein gene locus: The two maize inbred lines A188 and W64A are easily distinguished because A188 has a dwarf-like phenotype. Hybrids lack the dwarf-like phenotype as well; therefore, A188 inbreds are readily recognized at maturity. A188 chromosomes can easily be distinguished by a set of standard RFLP markers (Timmermans *et al.* 1996). The 27-kD locus on chromosome *7* can also be used to distinguish the two inbreds. A188 has a 12-kb duplication, each containing a copy of the 27-kD zein gene, while W64A compared to A188 has only one repeat, the one that contains the 27-kD A gene (Figure 1). Furthermore, the sequence homology of the repeat is interrupted by the 7.3-kb-long LTR-retrotransposon *Zeon*-1 in the W64A allele (Hu *et al.* 1995). As we reported earlier, the F_2 population of an A188/W64A hybrid exhibited a bias toward the *Ra* allele (Das *et al.* 1990). If this reflects increased recombination be-

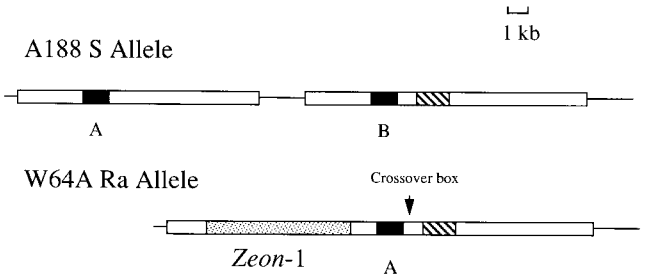

Figure 1.—Schematic representation of the 27-kD zein locus in A188 and W64A. The A188 *S* allele has two copies of the 27-kD zein gene, called A and B genes that are part of a 12-kb duplication. The W64A *Ra* allele has lost one duplication containing the B gene and has acquired the *Zeon*-1 LTR-retrotransposon upstream of the A gene. Open box, the 12-kb duplication separated by a single line indicating a repetitive sequence element in maize; black box, the coding region of the 27-kD zein gene; striped box, an 1.8-kb insertion unique to the 3′ copy of the 27-kD zein genes or the B gene; dotted box, the *Zeon*-1 LTR-retrotransposon. A size marker of 1 kb is given as a reference.

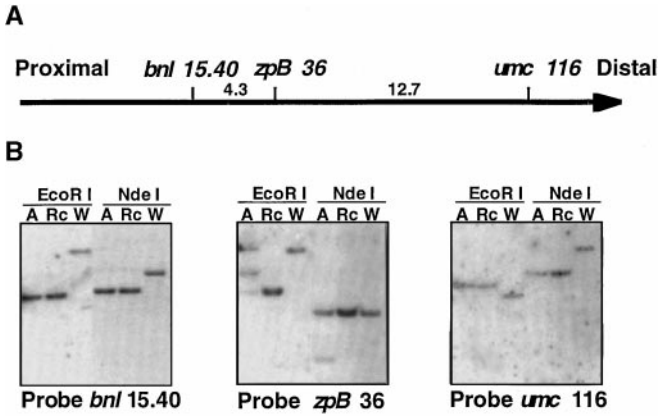

Figure 2.—Analysis of three closely linked RFLP chromosomal loci: *bnl 15.40*, *zpB 36*, and *umc 116* on chromosome *7* long arm. (A) Genetic map of chromosome *7*L. The chromosomal locations of markers proximal and distal to *zpB* 36 are shown; *zpB 36* represents the 27-kD zein gene locus. Map distances between each pair of RFLP loci are in centimorgans. (B) Southern blot analysis of the 27-kD zein gene alleles. A single blot was sequentially hybridized with probe bnl 15.40 (left), probe zpB 36 (middle), and probe umc 116 (right). The restriction enzymes are also indicated. Lanes marked A are *S*+A188 DNA from leaf tissue, those marked Rc are *Ra*+A188 DNA from leaf tissue, and those marked W are *Ra*+W64A DNA from leaf tissue.

tween the two zein genes of the A188 homolog, it could have arisen by an intrachromosomal recombination event similar to the events described at the *P* and the *knotted* 1 loci (Athma and Peterson 1991; Lowe *et al.* 1992). Because we previously found by DNA sequence analysis that recombination between the A and B copies of the 27-kD zein genes occurred within the *Ra* crossover box, between 189 to 220 nucleotides 3′ of the stop codon, such an intrachromosomal event could be analyzed by sequencing the crossover box from an A188 plant. Therefore, a plant homozygous for the *Ra* allele, derived from selfing a heterozygous A188 × W64A hybrid, was used to clone and sequence the 27-kD zein gene 3′ region. However, we found that a 476-bp DNA sequence 3′ of the stop codon was identical to W64A rather than to the A188 27-kD A gene (data not shown) previously sequenced (Das *et al.* 1991). Because within this short sequence A188 and W64A differ in 48 positions, it became clear that the *Ra* allele contained W64A DNA.

Crossover between A188 and W64A: DNA sequencing analysis was repeated with an *Ra* allele derived from a different A188 stock and the same result was obtained. Rather than finding that the deletion of the B gene was due to an intrachromosomal recombination event, we found again that it contained the *Ra* crossover box of W64A. This plant, which was homozygous for the *Ra* allele, was used for further analysis. Since the 27-kD locus is located on chromosome *7*, we can use a set of flanking RFLP markers, *bnl* 15.40 and *umc* 116, to determine which chromosome *7* this plant has, the one from A188 or the one from W64A. As can be seen in Figure 2B, these flanking markers clearly indicate that the genomic DNA is derived from A188 and differs from W64A. However, when these blots were reprobed with zpB 36, a 27-kD cDNA (Burr *et al.* 1982), the *Eco*RI-restricted genomic DNAs exhibited a polymorphism of the progeny allele different from either parent. The plant homozygous for the *Ra* allele was also homozygous for both the *bnl* 15.40+A188 and the *umc* 116+A188 alleles, (Figure 2A). Therefore, this allele was designated *Ra*+A188. This assortment of flanking marker alleles and the presence of the *Ra*+W64A polymorphisms at the 27-kD zein locus are consistent with the *Ra*+A188 allele, having arisen either via a double crossover or gene conversion-like event (Figure 2B). Indeed, the *Eco*RI pattern indicated that resolution of a crossover may have occurred within the 5.7-kb *Eco*RI fragment, which extends 0.6 kb upstream of the start codon and 4.5 kb downstream of the coding region.

The size of the recombinant *Eco*RI fragment did not allow us to determine whether this interchromosomal recombination event occurred intragenically or in flanking regions. Therefore, additional Southern blot analysis of genomic DNA of homozygous *Ra*+A188 and *Ra*+W64A alleles was performed. This required the cloning of unique sequences, F 0610 and F 0510 (Figure 3A), flanking the 5′ and 3′ regions of the 27-kD zein gene sequences, respectively. Figure 3B shows that the 5′ junction must be closer than the *Xba*I site present in W64A, but absent in A188, potentially still far away from the gene sequences of the 27-kD zein gene. The *Bgl*II site in the 3′ region, present only in A188, is also located outside of the 27-kD zein gene. Again, these data confirm that the flanking sequences of the *Ra*+A188 allele were homozygous for A188 and that the *Ra*+W64A sequences are likely to have arisen from a crossover event

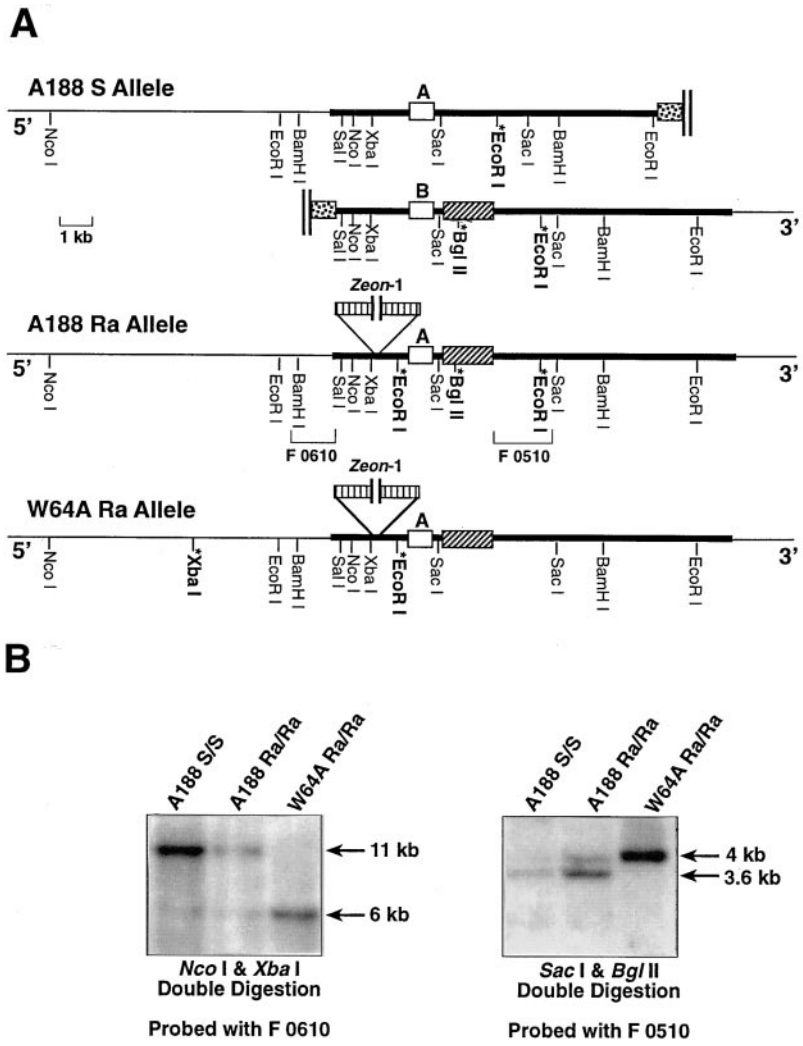

Figure 3.—Analysis of flanking markers of the *S*+A188 allele, the *Ra*+A188 allele, and the *Ra*+W64A allele. (A) Restriction maps of all three alleles. The A and B genes of the duplication characteristic of the *S* allele are drawn one below the other to highlight the sequence homology of the 12-kb repeat. Heavy line, homologous region; open box, the coding sequence; striped box, an insertion unique to the B copy. The *Ra*+A188 allele and the *Ra*+W64A allele have the A gene and the *Zeon*-1, a 7313-bp LTR-retrotransposon inserted 1146 bp upstream of the ATG of the A gene. *Eco*RI, *Bgl*II, and *Xba*I polymorphisms among the three alleles are marked with asterisks. The *Ra*+A188 allele has two *Eco*RI sites with asterisks located at the 5′ and 3′ flanking regions of the A gene, giving a unique 5.7-kb *Eco*RI fragment that hybridized with probe zpB 36. The locations of probe F 0610 and probe F 0510 are indicated below the *Ra*+A188 allele. Both probes can hybridize to the homologous regions in the *S*+A188 allele and the *Ra*+W64A allele. (B) Southern blot analysis of flanking regions with probe F0610 and probe F0510. Genomic DNA samples from leaf tissues of *S*+A188 DNA, of *Ra*+A188 DNA, and of *Ra*+W64A DNA are double-digested with two restriction enzymes *Nco*I and *Xba*I, and then hybridized with probe F 0610 (left). Genomic DNA from leaf tissues of *S*+A188 DNA, *Ra*+A188 DNA, and *Ra*+W64A DNA are double-digested with two restriction enzymes *Sac*I and *Bgl*II, and then hybridized with probe F 0510 (right). The molecular weight of these hybridization bands is marked at the right side of each blot.

located close to the gene locus, but not necessarily within the 27-kD zein gene sequence. Moreover, it appears that the *Zeon*-1 LTR-retrotransposon is contained within the sequence patch derived from W64A, while the 3′ insertion element is at least in part derived from A188. Therefore, pairing between these alleles must have allowed the looping out of more than 7 kb of sequences of W64A and 14 kb of A188, and the alignment of many sequence polymorphisms.

The 5′ and 3′ junctions of the chimeric A188 genomic DNA: If a single crossover has occurred, then resolution or initiation must have occurred at a rather far distance. To determine the length of the tract of W64A sequence within the A188 chromosome *7*, it became necessary to identify and sequence the junction points. Genomic libraries were prepared from homozygous *Ra*+A188, *S*+A188, and *Ra*+W64A DNA and subcloned for sequence analysis as described in materials and methods. Indeed, sequences between the retrotransposon insertion and the start site of the zein gene were identical to the W64A sequence, confirming that the resolution or initiation of the crossover occurred farther upstream of the retrotransposon outside of the 27-kD zein gene sequence. *Sal*I/*Xba*I subclones from all three alleles adjacent to the previously sequenced 1.3-kb *Bam*HI/*Sal*I fragment (Figure 3A) were sequenced and their DNA sequences compared. Figure 4A highlights the comparison results within a 500-bp region from 1830 bp to 1331 bp upstream of the *Zeon*-1 insertion site. The *Ra*+A188 allele and the A gene in the *S*+A188 allele have 36 nucleotide mismatches in a 215-bp region from 1545 bp to 1331 bp upstream of the *Zeon*-1 insertion site while the *Ra*+A188 allele is identical to the *Ra*+W64A allele within this region. The 1.7-kb regions 5′ beyond 1545 bp upstream of the *Zeon*-1 insertion site are identical among all alleles. Therefore, the 5′ junction site could be anywhere between the 5′ *Xba*I polymorphism site and 1545 bp upstream of the *Zeon*-1 insertion site or 10,015 bp upstream of the ATG of the 27-kD zein gene of the *Ra*+A188 allele.

Similar to identifying the 5′ junction, three 2.5-kb *Sac*I-*Sac*I fragments, including the *Bgl*II and *Eco*RI polymorphic sites (Figure 3A), were subcloned and sequenced. Their nucleotide sequences were compared

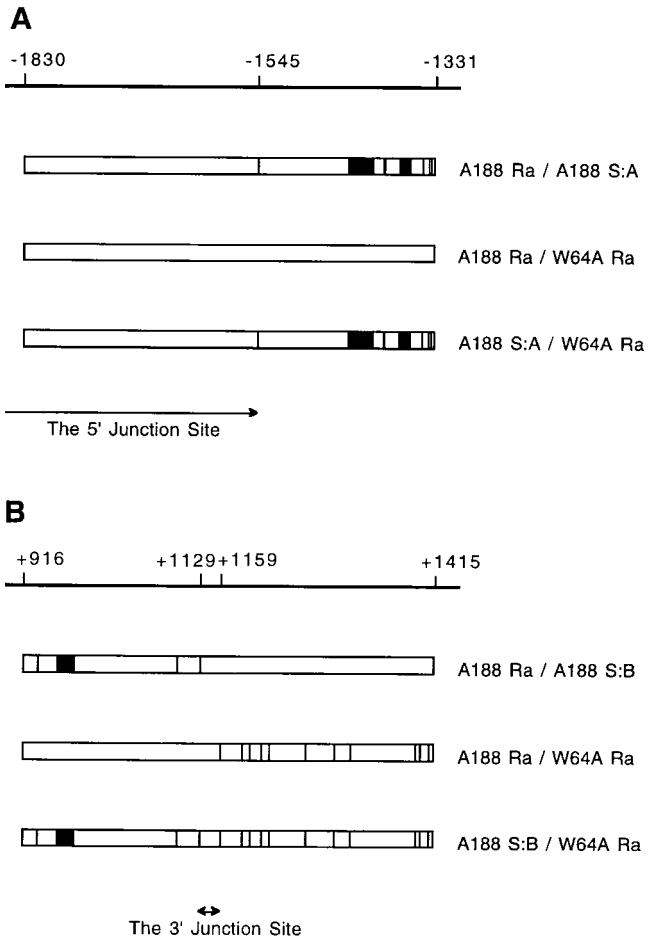

Figure 4.—Pairwise sequence comparisons between the *S*+A188 allele, the *Ra*+A188 allele, and the *Ra*+W64A allele. Schematic representations of the sequence analyses of the 5′ and 3′ junction sites are shown. The vertical lines represent sequence polymorphisms between the compared alleles. The width of the lines reflects the extent of the polymorphic region, but no distinction is made between nucleotide deletions, insertions, or substitutions. (A) Pairwise sequence comparisons in a 500-bp region from 1830 bp to 1331 bp upstream of the *Zeon*-1 insertion site. A large region with a potential location of the 5′ junction site of the chimeric region is indicated by a line with one arrow. (B) Pairwise sequence comparisons in a 500-bp region from 916 bp to 1415 bp downstream of the 27-kD zein coding region. A 29-bp region encompassing the 3′ junction site is indicated by the line between the two arrows.

to each other, and Figure 4B highlights the sequence differences within a 500-bp region from 916 bp to 1415 bp downstream of the coding region. The *Ra*+A188 allele and the B gene of the *S*+A188 allele have 17 nucleotide mismatches in a 214-bp region from 916 bp to 1129 bp downstream of the coding region. The *Ra*+A188 and the *Ra*+W64A allele have 11 nucleotide mismatches in a 257-bp region from 1159 bp to 1415 bp downstream of the coding region. Therefore, the 3′ junction of *Ra*+A188 allele maps to a 29-bp region between 1130 bp and 1158 bp downstream of the 27-kD zein gene, also outside of the gene region. Based on the positions of the 5′ and 3′ junctions of the *Ra*+A188 allele, the distance between the two junctions is at least 11,817 bp (Figure 5).

Continuous tract of W64A DNA in A188: To determine the continuity of the sequence between the two junctions, we compared three regions from all three alleles, 672 bp of the coding region of the 27-kD zein gene, 1130 bp of the 3′ flanking region, and 2103 bp of the 5′ flanking region including the *Zeon*-1 insertion site. Their nucleotide sequences represent >87% between the crossover points except the *Zeon*-1. The nucleotide sequences from the *Ra*+A188 and the *Ra*+W64A allele were identical, suggesting that the conversion tract is likely to be continuous with no additional crossovers. Therefore, it appears that the *Ra*+A188 allele has resulted from a gene conversion-like event with an unusually long tract that in addition contains an insertion of a 7313 bp LTR-retrotransposon.

DISCUSSION

The structure of the recently arisen *Ra*+A188 allele of the 27-kD zein gene locus in maize is best explained by an interchromosomal recombination event from an A188/W64A hybrid. Resolution of this event involves a single crossover yielding a conversion tract of over 11 kb. Because the 27-kD zein genes from A188 and W64A differ by nucleotide sequence polymorphism and insertions and deletions, the A188 zein gene appears to be converted by the W64A gene. Gene conversion and other homologous recombination events play important roles in generating genomic diversity. Gene conversion, defined as a nonreciprocal transfer of genetic markers, has been studied in a broad range of organisms including yeast (Klein and Petes 1981), trypanosomes (Pays *et al.* 1983), silkworm (Xiong *et al.* 1988), chickens (Reynaud *et al.* 1987), and mammals (Baltimore 1981).

Meiotic gene conversion gives rise to a 3:1 aberrant segregation pattern in yeast (Petes *et al.* 1991). In yeast, it is observed at every locus and every allele that has been examined in detail at frequencies that vary from about 0.5% (for *trp1*) to 30% (for *his4*; Fogel *et al.* 1988; Nag *et al.* 1989). Conversion events may include point mutation, small (1–4 bp) and large insertions and deletions (Fink and Styles 1974; Fogel *et al.* 1988; McKnight *et al.* 1981; Pukkila *et al.* 1986; Borts and Haber 1987; Symington and Petes 1988). Meiotic gene conversion tracts often exceed several hundred base pairs in length; ~20% of the tracts in one study were in excess of 5 kb (Symington and Petes 1988). In studies involving strains with heterozygous restriction sites, the average tract lengths were 1.5 kb (Borts and Haber 1987), 3.7 kb (Symington and Petes 1988), and 2.3 kb (Judd and Petes 1988) due to the yeast strains or the positions in the genome. Gene conversion in plant and mammalian genomes is difficult to study because only one meiotic product is recovered. Identi-

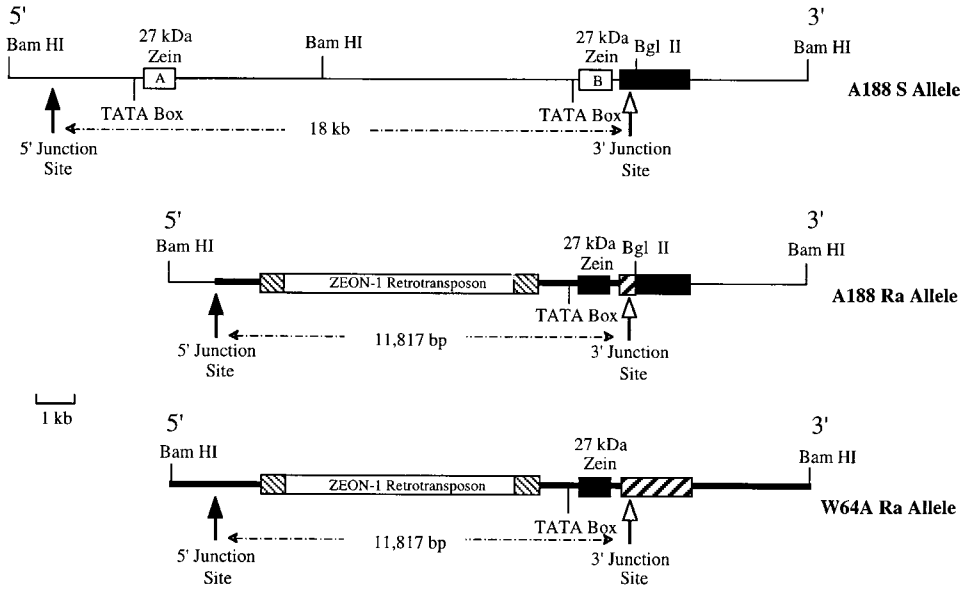

Figure 5.—Physical map of the *S*+A188 allele, the *Ra*+A188 allele, and the *Ra*+W64A allele with the 5′ and the 3′ junction sites of the chimeric region. The top thin line shows the *Bam*HI restriction map of the *S*+A188 allele. The 12 kb between the first two *Bam*HI sites contains the A gene, which has been cloned into the *Bam*HI site of phage lambda (5SP). The 14 kb between the next *Bam*HI sites contains the B gene and the unique 1.8-kb insertion (black box). The phage lambda clone of this *Bam*HI fragment is called SP3. The *Ra*+A188 allele has a single 17.5-kb *Bam*HI fragment that has been cloned into the *Bam*HI site of phage lambda (LP3). The *Ra*+W64A allele shown as a heavy line also has one 17.5-kb *Bam*HI fragment, and its lambda clone has been designated WP3. The closest possible positions of the 5′ junction sites in all three alleles are indicated by solid arrows and the 29-bp patches at the 3′ junction site are indicated by open arrows. The region between the solid arrow and the open arrow in the *Ra*+A188 allele represents the conversion tract. The positions of the *Zeon*-1 LTR-retrotransposons and TATA boxes are also marked.

fication of these products is therefore dependent on flanking polymorphic markers. Using this approach, Dooner and Martinez-Ferez (1997) have identified several such events between *Bz*-W22 and *Bz*-McC and determined conversion tracts between 1 to 1.5 kb, consistent with conversion tracts described above. They also noted that recombination frequencies were reduced with increased sequence polymorphism and in particular insertions. Compared to their observation, the *Ra*+A188 conversion tract appears to be unusually large.

We do not know the recombination frequency between the *S*+A188 and the *Ra*+W64A alleles, but we could make a prediction on the basis of data obtained for intragenic recombination frequencies of the *Bz*, the *A1*, the *B*, and the *R* loci (Dooner 1986; Eggleston *et al.* 1995; Patterson *et al.* 1995; Xu *et al.* 1995). Although meiotic intragenic recombination may range between 0.05 and 0.1% (Dooner 1986; Brown and Sundaresan 1991), these frequencies can be lowered by sequence polymorphisms (Dooner and Martinez-Ferez 1997). In particular, if one of the heteroalleles contains an insertion, recombination can be reduced 10-fold. Because the *S* allele and the *Ra* allele differ by two insertions of 7.3 and 14 kb, recombination between the two end points of the sequence homology of these two alleles could be <0.01%. Furthermore, in contrast to the 27-kD zein gene, the phenotype of some of these loci can easily be scored because they pigment the aleurone layer of the kernel, allowing the detection of rare meiotic events (10^{5-}). Recombination of 27-kD zein alleles, however, would have to occur at a frequency higher than 1% to be detected by Southern blot analysis or PCR-based strategies. Therefore, interchromosomal meiotic recombination frequencies between these two alleles is likely too low to be recovered from a population as small as 100 progeny plants as described previously (Das *et al.* 1990).

Gene conversion also occurs in mitotic cells, *e.g.*, yeast mating-type interconversion (Herskowitz *et al.* 1992). Spontaneous mitotic recombination between homologous chromosomes occurs at a rate about three to four orders of magnitude lower than the meiotic rate (Esposito and Wagstaff 1988). They are more difficult to analyze for two reasons. First, spontaneous events are usually too infrequent to be analyzed by nonselective techniques. Second, because spontaneous events can occur at any time during growth of the organism, the frequency of cells containing recombinant products can be very different. The rates of spontaneous mitotic conversion are similar at different positions within a gene, in contrast to the situation for meiotic conversion. Mitotic gene conversion tracts might be on average considerably longer than meiotic tracts. Studying the meiotic and mitotic gene conversions at the yeast *URA3* locus reveals that the largest mitotic conversion tracts are between 4 and 10 kb, compared to 200 bp to 3 kb for meiotic conversions in the same strains (Judd and Petes 1988). In line with these results, the *Ra*+A188 tract has a greater resemblance to a mitotic conversion tract.

Conversions have been explained by two types of models. One type proposes heteroduplex DNA (hDNA) intermediates with conversion resulting from the correction of mismatched bases in hDNA (Holliday 1964; Meselson and Radding 1975; Radding 1982). The other type is double-strand break (DSB), or gap repair

models (Szostak *et al.* 1983; Thaler and Stahl 1988; Sun *et al.* 1989, 1991; White and Haber 1990; Sugawara and Haber 1992). DSBs are followed by the exonucleolytic degradation of their 5′ ends to yield 3′ single-strand tails (Sun *et al.* 1991). Invasion of the homologous chromatid by the broken strands occurs subsequently and leads to the formation of double Holliday junctions (Schwacha and Kleckner 1995). DNA-repair synthesis from the 3′ ends, together with mismatch correction of the heteroduplex DNA generated by the double Holliday junctions, will lead to gene conversion. Resolution of these junctions will lead to recombinant products that are either noncrossover or crossover (Szostak *et al.* 1983). Somatic recombination can be viewed as a mere byproduct of DNA repair and occurs at a low frequency that depends on DNA damages. An exception is the mating type switch in yeast, where mitotic gene conversion results from a sequence-specific DSB of the HO gene product (Kostriken *et al.* 1983).

While homologous recombination occurs during meiosis, it is rarely found during plant development. Gene replacement studies have shown that illegitimate recombination occurs at a frequency of 10^4 to 10^6 times higher than homologous recombination in plants (Miao and Lam 1995; Morton and Hooykaas 1995). While the main pathway of repairing a DSB in yeast is homologous recombination, indirect evidence suggests that a DSB in plants is repaired by illegitimate recombination (Morton and Hooykaas 1995). An exception may be a DSB at a tandem repeat, where intrachromosomal recombination can readily align homologous sequences. For instance, it has been shown that excision of transposable elements can induce intrachromosomal recombination of the 5-kb direct repeats flanking the *P* gene and the 17-kb tandem repeat containing the *knotted* 1 gene in maize (Athma and Peterson 1991; Lowe *et al.* 1992).

Given the enormous variability of maize lines, one may wonder, however, whether genetic backgrounds exist that permit the detection of higher levels of recombination. In this respect, we have found variability among particular inbred lines of maize A188. Although this is the line that has been used to develop regeneration of maize from tissue culture (Green and Phillips 1975), our material has not been derived from regenerated plants. Still, we have found recombination frequencies that deviate from other inbred lines (Timmermans *et al.* 1997). When A188 was crossed with W64A, we were able to detect plants that were chimeric for the 27-kD zein gene locus (Das *et al.* 1990). Selfed F_1 progeny of the A188/W64A hybrid (101 total) shows an unsual bias toward the *Ra* allele 17:73:31, rather than the expected 25:51:25 ratio for the segregating *S*/*S*:*S*/*Ra*:*Ra*/*Ra* alleles. When individual progeny were investigated by Southern blot analysis, heterozygous plants frequently exhibited a higher dose of the *Ra* allele as if tissues were chimeric for heterozygous and homozygous genotypes. In one plant, this chimerism was very clear because root and shoot tissues differed (Das *et al.* 1990). This chimerism can be explained if we assume that the *Ra*+W64A and the *S*+A188 alleles have paired early in development before the separation of shoot and root apical meristem has occurred. For this to occur at such a high frequency and during plant development, one would have to assume that a double-strand break has been introduced into the *S*+A188 allele. In this respect, it is interesting that McClintock (1978) has also observed a high-frequency instance where chromosome breakage leads to DNA rearrangement. Moreover, in this case, it did not involve mechanisms like the Ac-Ds system, but involved cuts in nonrandom chromosomal locations in specific genetic backgrounds that contain the *X* component.

The mechanism of intragenic meiotic recombination in plants has also been proposed to occur by the DSB model of the initiation of recombination (Dooner and Martinez-Ferez 1997). However, it has been reported that during repair of mitotically induced breaks in yeast, DNA sequence changes can occur (Strathern *et al.* 1995). In our case, the 5′ junction and the 3′ junctions of the *Ra*+A188 allele are completely homologous. Although the homology is only 29 bp in the 3′ region (Figure 5), sequences on either side are faithfully reproduced from the two parental alleles. Moreover, we could not find any evidence for nucleotide changes within the conversion tract itself. Pairing of heteroduplex sequences appears to offer no hindrance to strand invasion and resolution of the Holliday structure. This is consistent with the fidelity of recombination products observed at other loci in maize (Xu *et al.* 1995; Timmermans *et al.* 1996; Dooner and Martinez-Ferez 1997). An important aspect of the fidelity of somatic conversion-like events in plants is the pairing of homologs with different chromatin structure that may be absent during meiosis. For instance, we have shown recently that an epiallele of the *P* locus differs from the normal *P* gene by a hypermethylated site in the upstream promoter region that has also acquired resistance to DNaseI-hyposensitivity (Lund *et al.* 1995). Interestingly, the chromatin alteration remains unchanged during meiosis, but changes during plant development. It might be feasible in a conversion event that the chimeric DNA patch also retains the methylation imprint of the donor chromosome. Gene conversion in Ascobolus, for instance, has shown transference of DNA methylation interchromosomally and continuously within 7.5 kb of the *b2* gene (Colot *et al.* 1996). If this is true for methylation imprints, it might also extend to chromatin structure in general and explain some cases of gene silencing in plants (Matzke and Matzke 1998). Because there is no clear separation of the germ line in plants during early embryonic development, genome alterations including methylation imprints resulting from somatic recombination can be transmitted to subsequent gener-

ations and provide epialleles that can be studied by Mendelian genetics.

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