Analysis of Recombination Sites Within the Maize *waxy* Locus

Ron J. Okagaki* and Clifford F. Weil†

**Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706 and* †*Department of Biological Sciences, University of Idaho, Moscow, Idaho 83843*

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ABSTRACT

Genetic fine structure analysis of the maize *wx* locus has determined that the ratio of genetic to physical distance within *wx* was one to two orders of magnitude higher than the average for the maize genome. Similar results have been found at other maize loci. In this study, we examined several mechanisms that could account for this pattern. First, crossovers in two other maize genes resolve preferentially at specific sites. By mapping exchanges between *wx-B1* and *wx-I* relative to a polymorphic *SstI* site, we found no evidence for such a hotspot at *wx*. Second, deletion of promoter sequences from *wx* alleles had little effect on recombination frequencies, in contrast to results in yeast where promoter sequences are important for initiating recombination in some genes. Third, high levels of insertion polymorphism may suppress intergenic recombination. However, the presence of a 2-kb *Ds* element 470 bp upstream of the *wx* transcription start site did not further suppress recombination between *Ds* insertions in nearby *wx* sequences. Thus, none of these mechanisms is sufficient to explain the difference between intergenic and intragenic recombination rates at *wx*.

IN maize, several lines of evidence suggest that recombination occurs primarily within genes, or perhaps unique sequences, and rarely in intergenic regions. Within genes the rate of recombination as a function of physical distance is ≤100-fold higher than the average for the maize genome. Estimates of this relationship for the maize genome range from 1460 to 4750 kb/cM (DOONER *et al.* 1985; CIVARDI *et al.* 1994). At the *bronze1* (*bz1*) locus, 1 cM equals ~14 kb (DOONER 1986), while at the *anthocyaninless1* (*a1*) locus there are an estimated 12.5–25 kb/cM (BROWN and SUNDARESAN 1991). In contrast, recombination rates in sequences adjacent to *bz1* and *a1* are very low. In the *bz-s:2094(fAc)* allele, an *Ac* transposable element was mapped 0.05 cM proximal to *bz1* (RALSTON *et al.* 1989). Within *bz1* this genetic distance would correspond to <1 kb; however, the *Ac* element lies 25–100 kb from the locus, corresponding to ≥400 kb/cM (RALSTON *et al.* 1989; H. K. DOONER, personal communication). The distance between *a1* and the adjacent *shrunken2* (*sh2*) locus is 140 kb, or 0.09 cM, giving an estimated 1560 kb/cM (CIVARDI *et al.* 1994).

TIMMERMANS *et al.* (1996) examined the distribution of recombination sites using an approach that did not restrict their search to known genes. Instead of looking at recombination between markers within a particular gene, a crossover event was identified using a series of restriction fragment length polymorphisms (RFLP) probes. The crossover junction was cloned, sequenced and found to lie within a unique sequence flanked by repetitive polymorphic sequences. This result suggested that recombination might occur frequently in localized regions of low copy number sequence, which would include genes, and less often in the highly repetitive sequences that characterize intergenic regions (SAN MIGUEL *et al.* 1996).

High intragenic *vs.* low intergenic recombination rates suggest a physical difference(s) between these regions may affect recombination. For example, this difference could be the presence of specific sequences in or near genes at which recombination occurs preferentially. In yeast studies, recombination "hotspots" are regarded as sites where recombination is *initiated* with unusually high frequency, seen genetically as a peak in a gradient of gene conversion that spreads away from the initiation site (NICOLAS *et al.* 1989; FAN *et al.* 1995). For example, some promoter sequences in yeast also act as sites where recombination may be initiated frequently (WU and LICHTEN 1994); this mechanism, if found in maize, could directly connect recombination with transcription units. In maize studies, where gene conversion is difficult to assess, recombination hotspots have been reported as regions at which crossover junctions are found with unusually high frequency, suggesting they are preferred sites for crossover *resolution.* Hotspots have been found at the maize *a1* (XU *et al.* 1995), *b1* (PATTERSON *et al.* 1995) and *r1* (EGGLESTON *et al.* 1995) genes. Alternatively, the difference in recombination within and between genes may be that recombination is inhibited in intergenic DNA. Intergenic regions in maize appear to be composed largely of repetitive, retroelement-like sequences (SAN MIGUEL *et al.* 1996); sequence and insertional polymorphism among

Corresponding author: Ron J. Okagaki, Department of Biochemistry, University of Minnesota, 435 Delaware St. SE, Minneapolis, MN 55455–0347. E-mail: okaga002@tc.umn.edu

these repeats could make recombination more difficult (DOONER 1986; WALDMAN and LISKAY 1988; GODWIN and LISKAY 1994). Differences in chromatin structure (NICOLAS *et al.* 1989; THOMAS and ROTHSTEIN 1989) between intragenic and intergenic DNA might also affect recombination.

Using alleles of the maize *waxy* (*wx*) locus, we have tested several mechanisms that could promote recombination within genes or inhibit intergenic recombination. We find no evidence for a preferred site of crossover resolution within the *wx* locus or a dependence on an intact promoter for initiation of recombination. In addition, we present evidence that the effects of insertion polymorphisms on recombination may be limited to relatively small (≤1 kb) regions around those polymorphisms; the implications for intergenic recombination rates are discussed.

MATERIALS AND METHODS

Analysis of *Wx* recombinants from *wx-B1/wx-I*: Heterozygous *wx-B1/wx-I* plants were crossed as females by *wx-C34*, and nonmutant *Wx* kernels were recovered and propagated. Putative *Wx* alleles arising from recombination between *wx-B1* and *wx-I* were checked by Southern blot analysis to exclude contaminants. DNA isolation and Southern blotting procedures have been described previously (WEIL *et al.* 1992). The *wx-B1* allele has a 1-kb deletion beginning in intron 1 that extends 5′ into promoter sequences (WESSLER *et al.* 1990); *wx-I* has a large insertion at the 3′ end of the gene (S. E. WHITE, personal communication). *wx-C34* lacks detectable homology with *wx* sequences (WESSLER and VARAGONA 1985). Any nonmutant *Wx* alleles arising from pollen contamination could be identified because such kernels would have fragments characteristic of the nonmutant *Wx* allele as well as additional bands specific to either *wx-B1* or *wx-I*. Sequences 5′ and 3′ of the *wx* locus were also marked by RFLPs: *wx-B1* has a *Sal*I site 9 kb 5′ and an *Eco*RI site 5 kb 3′ of the *wx* locus while *wx-I* has a *Sal*I site 3 kb 5′ and an *Eco*RI site 15.5 kb 3′ of the locus (WESSLER and VARAGONA 1985; R. J. OKAGAKI, unpublished results). Last, a polymorphic *Sst*I site within the *wx* locus allowed placement of exchange sites within the gene. Figure 1 depicts *wx-B1*, *wx-I*, and recombination events that could be distinguished.

Pollen analysis of *wx-m5* derivatives: A series of *Wx-m5* derivative alleles were used to study the effect of insertion/deletion polymorphism on recombination in nearby sequences (WEIL *et al.* 1992). *Wx-m5* contains a 2013-bp *Ds* transposable element inserted 474 bp upstream of the transcription start site. This insertion reduces enzymatic activity to between 25 and 50% of nonmutant in *Wx-m5* endosperm, but the visible phenotype is indistinguishable from wild-type *Wx*. Alleles derived from *Wx-m5* have the *Ds* element inserted into the *wx* transcription unit, and these alleles have a mutant phenotype. In derivatives *wx-m5:CS-8313* and *wx-m5:CS-13*, the upstream *Ds* insertion was retained, while in *wx-m5:CS-18* and *wx-m5:CS-22* the original *Ds* insertion is absent. Hereafter, these alleles are referred to as *CS-8313*, *CS-13*, *CS-18*, and *CS-22*. After isolation, alleles were outcrossed with inbred line Missouri 20 to improve vigor and subsequently self-pollinated to identify homozygous mutant kernels.

Procedures for isolating and staining pollen were adapted from NELSON (1968). Central spikes of mature tassels were collected and stored in 70% ethanol after the first anthers began shedding; samples from two or more plants were taken each year. Approximately 25 spikelets were removed and rinsed in 70% ethanol to minimize pollen contamination. Pollen was isolated by cutting open spikelets in microfuge tubes containing 70% ethanol and vortexing. Debris was removed, and pollen pelletized by a brief centrifugation; ethanol was replaced by 750 ml of stain. Stain was prepared according to NELSON (1968) except that gelatin was not added, and the stain was not heated. Pollen was stained for 2–5 min and pelletized, and the stain was replaced by 50% glycerol. Resuspended pollen was spread onto filter paper using a filter

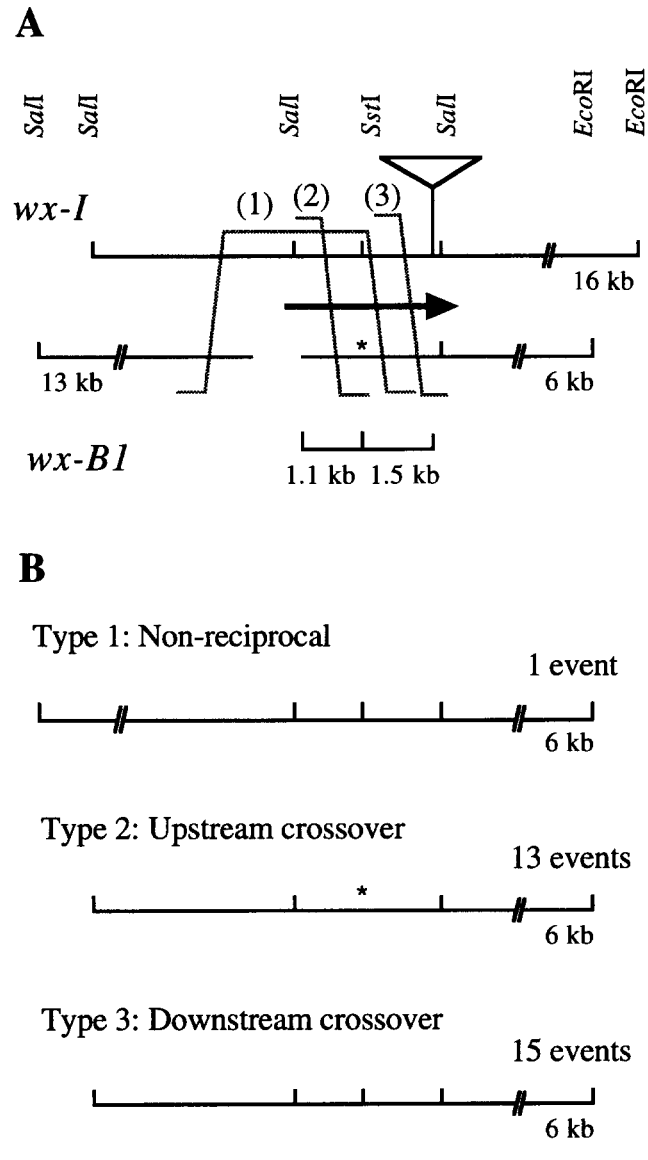

FIGURE 1.—Recombination between *wx-B1* and *wx-I*. (A) The diagrams of *wx-I* and *wx-B1* show only those restriction enzyme sites relevant for Southern blot analysis, the deletion in *wx-B1*, the insertion in *wx-I*, and the recombination events recovered. The location of the *wx* transcription unit is shown (→); *, the deleted *Sst*I site in *wx-B1*. Stippled lines represent the three types of recombination events described below, and the numbers identify the events. (B) Three classes of recombinant *Wx* alleles were identified. The type 1 allele was either a product of a double crossover or gene conversion. Type 2 alleles had an exchange in the 1.1-kb upstream *Sal*I-*Sst*I region, and type 3 alleles had an exchange in the 1.5-kb *Sst*I-*Sal*I region. Note that alleles produced by gene conversion events with conversion tracts extending beyond flanking RFLPs could be misclassified as crossover alleles.

funnel. Samples were examined under a dissecting microscope to identify *Wx* pollen grains, and the total number of pollen grains was estimated by counting 10 1-mm squares.

RESULTS

Resolution of crossovers between *wx-B1* and *wx-I*: From test crosses of the *wx-B1/wx-I* double mutant, 36 nonmutant *Wx* alleles were recovered and characterized by Southern blot analysis. Seven alleles contained bands characteristic of *wx-B1* or *wx-I* in addition to bands from a nonmutant allele and were classified as contaminants. Of the remaining 29 alleles, examination of a polymorphic *Sal*I site 3.3 kb 5′ and a polymorphic *Eco*RI site 5 kb 3′ of *wx* classified 28 as crossover events occurring between *wx-B1* and *wx-I*. Crossover alleles had 5′ flanking markers from *wx-I* and 3′ flanking markers from *wx-B1*; the single noncrossover allele had *wx-B1* flanking markers (Figure 1). Tests for the presence or absence of a polymorphic *Sst*I site between *wx-B1* and *wx-I* allowed placement of crossover junctions among revertants. This *Sst*I site lies 1.1 kb downstream of *wx-B1* and 1.5 kb upstream of the *wx-I* insertion. Thirteen alleles lacked the polymorphic *Sst*I site, indicating that exchanges occurred in the 1.1-kb region between *wx-B1* and the *Sst*I site. The remaining alleles retained the *Sst*I site, indicating that exchange occurred downstream of this site in the one noncrossover allele and remaining 15 crossover alleles (Figure 1B). If the probability of exchange depended solely on sequence length, then we would expect 13 exchanges in the region 5′ of the *Sst*I site, in good agreement with the 12 observed. These results suggest there is no hotspot for resolution of exchanges within the *wx* gene as is seen at *a1* and *b1*. These data do not address where recombination is initiated; however, if there is a hotspot for initiation, then there appears to be no polarity across the gene for where those recombination events are resolved (see DISCUSSION). Our results are consistent with resolution of exchanges being a function of physical distance in this region of the *wx* gene.

The fine structure mapping data of NELSON (1968) examined recombination within smaller subdivisions of *wx*. A combined physical-genetic map was made to compare the relationship between physical and genetic distances within the *wx* locus. In Figure 2, eight alleles were selected for which the physical positions are known and which span most of the locus: *wx-C4, wx-B1, wx-B* (WESSLER *et al.* 1990), *wx-C* (OKAGAKI *et al.* 1991), *wx-C31* (K. POPPLETON and C. F. WEIL, unpublished results), *wx-m1* (WESSLER *et al.* 1986), *wx-B2* (BUREAU and WESSLER 1992), *wx-m8* (KLOSGEN *et al.* 1986) and *wx-I* (WHITE *et al.* 1994). As *wx-I* and *wx-K* have the same mutation (S. E. WHITE, personal communication) recombination data for these alleles have been combined. Together these alleles, and the ratio of physical distance to genetic distance was calculated for each interval. Estimates ranged from 10 to 49 kb/cM. While these data suggest that recombination frequencies may vary across the

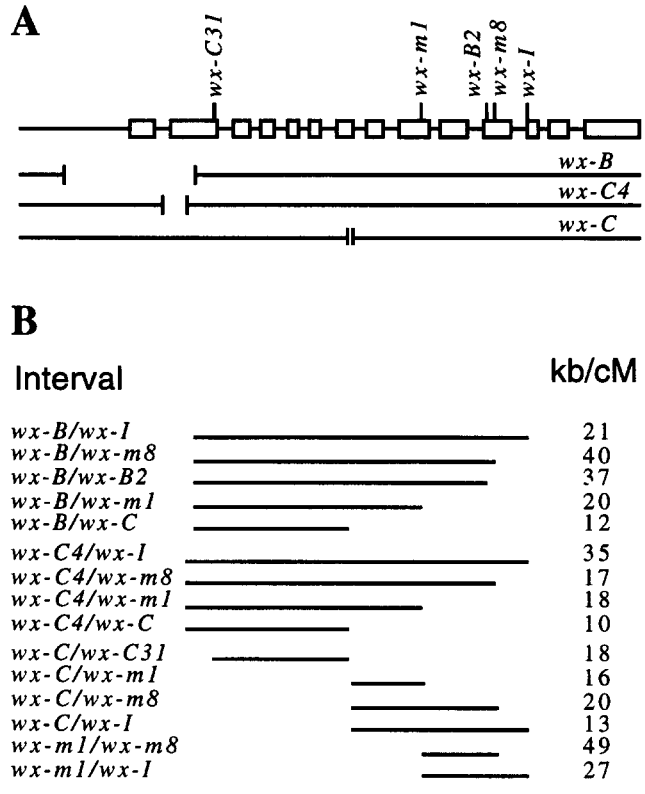

FIGURE 2.—Recombination rates across *wx*. (A) Location of mutations within the *wx* gene. The top line shows the *wx* locus; □, exons. *wx-C31* is a missense mutation, while *wx-m1*, *wx-B2*, *wx-m8*, *wx-I*, and *wx-B4* are insertions. Deletion alleles are shown below. (B) Recombination between *wx* alleles. Shown are the heterozygous combinations, the intervals defined by pairs of alleles, and the recombination frequencies expressed as kilobases/centimorgan. Data was taken from NELSON (1968) where data from more than two plants from the same year was available.

gene, these discrepancies may also be due to variation inherent in measuring *wx* recombination frequencies. Frequencies of nonmutant *Wx* pollen grains from heterozygous combinations may vary twofold between two different growing seasons (NELSON 1968). In any event, our analysis does not reveal a strong hotspot at *wx* that could account for the 100-fold increase in intragenic *vs.* intergenic recombination rates.

Deletion of *wx* promoter sequences: In yeast, some recombination hotspots are associated with promoter sequences. Disruption of the *HIS4, GAL10* and *ARG4* promoter regions inhibit recombination and gene conversion within these loci (NICOLAS *et al.* 1989; THOMAS and ROTHSTEIN 1989; PETES *et al.* 1991). It is thought that the susceptibility of promoter sequences to double-strand DNA breaks may account for their involvement in recombination (CAO *et al.* 1990; WU and LICHTEN 1994). Interestingly, hotspot activity at these promoters requires the binding of transcription factors but not necessarily transcription (WHITE *et al.* 1993). If maize promoter sequences have a similar role in initiating recombination, then deletions within the *wx* promoter might inhibit recombination rates. In our system, it was

not possible to look at recombination between two alleles containing promoter deletions. Instead, the frequencies of nonmutant *Wx* pollen grains from heterozygous plants were used to compare recombination rates between an allele with a 5′ end deletion and a second allele whose mutation lay 3′ of the deletion. The *wx-B* allele has a deletion from positions −459 to +505, thus removing a significant part of the promoter, while *wx-C4* has a deletion only from positions +257 to +424 (Figure 2A; WESSLER *et al.* 1990). Physical distances between the 3′ breakpoints in these deletion alleles and mutations further 3′ in the gene are therefore similar, and recombination frequencies between these deletions and mutations located 3′ should also be similar if the *wx* promoter region does not initiate recombination. In contrast, if the *wx* promoter region is important for initiating recombination, *wx-B* should recombine less often while *wx-C4* should recombine more efficiently with other alleles.

Frequencies of nonmutant *Wx* alleles produced by recombination between alleles with 5′ end deletions and alleles located 3′ in the gene were reported by NELSON (1968). We compared recombination between the 5′ deletion alleles *wx-B* and *wx-C4* and the 3′ alleles *wx-C*, *wx-m1*, *wx-m8*, and *wx-I* (Figure 2B). These data did not indicate any consistent, major role for the *wx* promoter in stimulating recombination.

Effect of multiple insertions on recombination: One hypothesis for why recombination between genes is lower in maize is that the high degree of insertion polymorphism in intergenic DNA suppresses recombination. DOONER (1986) had previously shown that the presence of a single insertion polymorphism can decrease recombination at the *bz1* locus. These data suggested that the decrease was more severe nearer the polymorphism, but did not address whether multiple, nearby, insertion polymorphisms, the situation more likely to occur in intergenic regions, have any combined effect on recombination. Two *Wx-m5* derivative alleles, *CS-8313* and *CS-22*, provided an opportunity to study the effect of neighboring insertion polymorphisms on recombination. These alleles originated from the same progenitor by *Ds* transposition into the *wx* coding region; the transposon insertion sites are 11 bp apart, and the *Ds* elements are in the same orientation (WEIL *et al.* 1992). In recombination experiments, the only significant difference between these alleles should be limited to the presence of a *Ds* element in *CS-8313 vs.* a transposon footprint at approximately -470 in *CS-22* (Figure 3A). The *Ds* upstream of *wx* does not affect the wild-type phenotype of the kernels or the pollen (WEIL *et al.* 1992).

Alleles *CS-8313* and *CS-22* were crossed with two other *Ds* alleles of *wx* derived from *Wx-m5*, *CS-18* and *CS-13* to compare recombination rates. In the *CS-8313/CS-18* heterozygotes, there was an upstream insertion polymorphism not present in *CS-22/CS-18*. If increasing

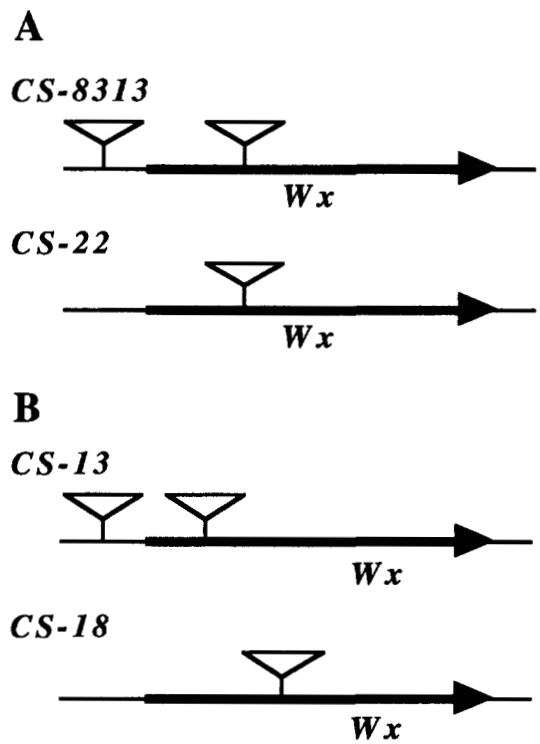

FIGURE 3.—*Wx-m5* derivative alleles. (A) Derivatives *CS-8313* and *CS-22* were used to examine the effect of an insertion on recombination in adjacent homologous sequences. Two-kilobase *Ds* insertions are indicated (▽). (B) Derivatives *CS-13* and *CS-18*, each crossed with both *CS-8313* and *CS-22* to make heterozygotes in which recombination rates were compared.

polymorphisms suppresses recombination in nearby sequences, then recombination rates in *CS-8313/CS-18* plants should be lower than in *CS-22/CS-18* plants. Data were collected during 2 years, and the recombination rates in the two heterozygotes were nearly identical (Table 1). In contrast to *CS-18*, *CS-13* not only has a *Ds* insertion in the *wx* coding region, but retains a *Ds* insertion at −470 as well (WEIL *et al.* 1992). In *CS-8313/CS-13* heterozygotes, both alleles had the upstream *Ds* insertion, while in the *CS-22/CS-13* heterozygote, the absence of the upstream *Ds* insertion defines an insertion polymorphism. Recombination rates in this pair of heterozygotes were also nearly identical (Table 1). Together, these results indicate that the addition of a second insertion polymorphism did not have a strong effect on recombination within *wx*.

RALSTON and coworkers (1987) noted that nonmutant pollen was present in samples from crosses between overlapping deletions where recombination could not produce a functional *Wx* allele. Therefore, as a negative control, pollen from the *CS-22/CS-8313* heterozygote was tested. Eleven basepairs separate these two mutations, making it unlikely that recombination between them could produce a functional *Wx* allele. Estimated recombination frequencies were 20-fold lower than for other allelic combinations, suggesting that false positives were not a significant source of error.

TABLE 1

Nearby insertion polymorphism and recombination

CS-13/CS-8313	29.1 ± 8.5[a]
CS-13/CS-22	30.6 ± 4.1
CS-18/CS-8313	25.2 ± 4.7
CS-18/CS-22	24.6 ± 7.0
CS-8313/CS-22	1.2 ± 0.03

[a] Wx pollen per 100,000 grains.

DISCUSSION

The high recombination rates within the maize *a1* (BROWN and SUNDARESAN 1991) and *bz1* (DOONER 1986) loci relative to sequences adjacent to these loci (RALSTON *et al.* 1989; CIVARDI *et al.* 1994) argues that recombination occurs primarily within genes and not within intergenic regions in maize. High intragenic recombination rates measured at several other maize loci including *adh1* (FREELING 1976), *ae1* (MOORE and CREECH 1972), *b1* (PATTERSON *et al.* 1995), and *wx* (NELSON 1968), support such a conclusion. This phenomenon could be caused by specific sequences promoting recombination within genes, by specific inhibition of recombination between genes or by some combination of the two. We have tested whether recombination hotspots, such as those observed in maize at *a1* and *b1* (PATTERSON *et al.* 1995; XU *et al.* 1995) or in yeast at *ARG4*, *HIS4* and *GAL10* (NICOLAS *et al.* 1989; THOMAS and ROTHSTEIN 1989; PETES *et al.* 1991) can explain the prevalence of recombination within the maize *wx* gene. Alternatively, physical differences between intergenic and intragenic regions may determine the distribution of crossover sites, and we have examined the effects of multiple insertion polymorphisms on recombination.

Location of recombination exchanges in *wx*: Recombination intermediates resolve within small localized hotspots in *a1* and *b1* (PATTERSON *et al.* 1995; XU *et al.* 1995); it remains unclear whether recombination also initiates in these regions. If most crossovers in maize are resolved at such hotspots and if these hotspots are located within genes, then this mechanism would explain the observed high rates of intragenic recombination. This model predicts that most exchanges at a locus will be clustered in the hotspot and not distributed across the locus. Mapping the location of crossover resolutions in recombinant alleles derived from *wx-B1* and *wx-I* relative to a polymorphic *Sst*I site did not support this prediction (Figure 1). The possibility of two recombination hotspots, one on each side of the *Sst*I site, was not excluded by our *wx-B1/wx-I* data. However, it was excluded by examining recombination data from eight alleles that together span most of the locus. High recombination rates were found in all intervals defined by the alleles (Figure 2). These results argue against the idea that crossovers tend to resolve at a specific sequence in the *wx* locus.

Our data support the idea that more than one pattern of intragenic recombination is possible within maize genes. At *a1* and *b1*, exchanges resolve at recombination hotspots and few crossover junctions were found outside these distinct regions (PATTERSON *et al.* 1995; XU *et al.* 1995). The genetic fine structure map of the *wx* locus (NELSON 1968), along with the *ae1* (MOORE and CREECH 1972), *adh1* (FREELING 1976), and *bz1* (DOONER 1986) loci, suggest a second pattern where recombination junctions resolve frequently at different sites across the entire locus with no preferential hotspot. Fine structure genetic maps placed mutant alleles relatively evenly across these loci. If there was a strong recombination hotspot, mutations in these genes would appear to cluster on either side of the hotspot on the genetic map. Finally, the *r1* locus may represent an intermediate pattern where most crossovers resolve in the 3′ half of the locus (ROBBINS *et al.* 1991; EGGLESTON *et al.* 1995).

Recombination and promoter sequences: In several yeast genes, the double-strand breaks that initiate recombination often occur within the promoter but not within the gene itself (CAO *et al.* 1990; PETES *et al.* 1991; WU and LICHTEN 1994; FAN *et al* 1995), and disruption of these promoters can inhibit recombination in these genes (NICOLAS *et al.* 1989; THOMAS and ROTHSTEIN 1989; WHITE *et al.* 1992). Locations where these breaks occur also exhibit DNAaseI hypersensitivity; open chromatin structure and transcription factor binding may also make these regions more susceptible to double-strand breaks (WHITE *et al.* 1993; WU and LICHTEN 1994). We have tested whether the *wx* promoter stimulates recombination in maize by comparing recombination rates within *wx* between alleles that either have, *wx-C4*, or lack, *wx-B*, promoter regions (Figure 2). Under this model, the promoter deletion in *wx-B* should permit fewer double-strand breaks to occur in this allele, and therefore *wx-B* would initiate recombination less often than alleles with intact promoters, such as *wx-C4*. While a small decrease was observed in heteroalleles using *wx-B* as opposed to *wx-C4* in some cases, this decrease was inconsistent (see Figure 2B data for *wx-I*) and was not significant in all cases. The small differences observed might be accounted for by repair of gaps created from double-strand breaks in the *wx-C4* promoter using sequence information from the 5′ ends of *wx-C*, *wx-m1*, *wx-m8* or *wx-I* (Figure 2A). Resolution of the intermediate structure could result in either a noncrossover or a crossover *Wx* revertant allele. Repair of double-strand breaks in the reverse direction, *i.e.*, promoter sequences from *wx-C* (or the other more 3′ alleles tested) mediated by sequences from *wx-C4*, are unlikely to produce revertant *Wx* alleles.

We cannot exclude the possibility that double-strand breaks upstream of the *wx-B* deletion have a role in recombination, particularly because the 5′ limits of the *wx* promoter region are not clearly defined. However, there are lines of evidence that suggest there is not a strong preference for initiating recombination in the *wx* promoter. First, recombination between *wx-B1*, an

allele deleting 196 bp further 5′ than *wx-B* (WESSLER *et al.* 1990), and 3′ allele *wx-C* occurs at similar rates as with *wx-B* and *wx-C4* (OKAGAKI *et al.* 1991). Second, recombination might initiate within regulatory sequences further 5′ even than the *wx-B1* deletion, however, ~4 kb upstream of these *wx* alleles are highly repetitive sequences that characterize maize intergenic DNA (T. RINEHART, C. GEIDT and C. F. WEIL, unpublished results). Initiation beyond this point would be inconsistent with the observed lack of recombination in intergenic regions (RALSTON *et al.* 1989; CIVARDI *et al.* 1994). Finally, even partial deletion of the yeast *ARG4* and *HIS4* promoter regions can decrease recombination (NICOLAS *et al.* 1989; WHITE *et al.* 1992), although plant and yeast promoter regions may differ in organization and size.

Sequence polymorphism and recombination: Disruption of homologous sequences by insertion or nucleotide polymorphisms can significantly reduce recombination frequencies (BRENNER *et al.* 1985; WALDMAN and LISKAY 1988; GODWIN and LISKAY 1994). In comparison with recombination between alleles lacking large insertion polymorphisms, recombination within the maize *bz1* gene is inhibited when one allele with a transposon insertion is paired with a second allele lacking an insertion (DOONER 1986). Along similar lines, XU *et al* (1995) described a 50% decrease in recombination between two alleles of the *a1* locus where both carry transposon insertions as compared with two alleles where one had a transposon insertion and one did not; furthermore, the presence of an insertion on both homologues did not affect the distribution of crossover resolution points. Even higher degrees of polymorphism within intergenic relative to intragenic regions in maize might explain the low intergenic recombination rates observed (JOHNS *et al.* 1984; SHATTUCK-EIDENS *et al.* 1990; SAN MIGUEL *et al.* 1996). However, there is an interesting paradox between this idea and the mapping of genes in inbred lines as compared with hybrid genetic backgrounds. Within inbreds there should be fewer polymorphisms between homologous chromosomes, an increase in intergenic recombination and an expansion of the genetic maps in these lines; such map expansion has not been reported.

We have examined whether addition of further insertion polymorphisms, a situation that might be expected in intergenic regions, decreases recombination between two polymorphic alleles or has no additional effect. Our data suggest that adding insertion polymorphisms has, at most, a minor role in further inhibiting recombination within the *wx* gene (Table 1; Figure 3). The insertion polymorphism that distinguishes *CS-8313* from *CS-22* is 1 kb upstream of the molecular lesion disrupting *wx* expression in *CS-8313* and *CS-22*. Recombination rates in *CS-8313/CS-13 vs. CS-22/CS-13* or in *CS-8313/CS-18 vs. CS-22/CS-18* were similar. Our results are supported by two other recent observations. First, sequence similarity between the *B-I* and *B-Peru* alleles breaks off due to an insertion 160 bp 5′ of the *DdeI* site marking the recombination hotspot (PATTERSON *et al.* 1995). Second, characterization of a recombination event originally identified by RFLP markers determined that a crossover took place within a small region of unique sequence flanked by insertion polymorphisms (TIMMERMANS *et al.* 1996). Taken together these data indicate that insertions within genes suppress intragenic recombination but that recombination is able to occur between short homologous regions even when flanked by unrelated sequences. Therefore, if insertion polymorphisms are to explain the paucity of intergenic recombination, then homologous sequences, even short ones, are rare in intergenic DNA.

Recent evidence may suggest that the density of nucleotide polymorphisms in intergenic DNA is also higher than it is within genes (SHATTUCK-EIDENS *et al.* 1990; SAN MIGUEL *et al.* 1996). Individual nucleotide substitutions have been shown to affect recombination in yeast (BORTS and HABER 1987; SELVA *et al.* 1995), and introducing two nucleotide substitutions within a 232-bp stretch of sequence identity reduced recombination in the region 20-fold in a mammalian cell culture system (WALDMAN and LISKAY 1988). In contrast, the many nucleotide differences between *bz1* alleles in maize [few stretches of sequence identity >100 bp exist between any of the four alleles that have been sequenced (FURTEK *et al.* 1988; RALSTON *et al.* 1988)] do not appear to prevent recombination from occurring all across the gene. Despite its divergence, *bz1* intragenic recombination rates are high both between alleles with a common progenitor and between alleles whose sequences are more diverged (DOONER 1986).

The idea that recombination is suppressed in regions between genes must be considered along with the idea that recombination is stimulated within genes. Our inability to associate promoter sequences or strong recombination hotspots with intragenic recombination at *wx* suggests that some other structural feature may be responsible for the difference in recombination frequencies between *wx* and intergenic DNA. Crossovers may be dispersed across a locus, as found in the *wx* locus, associated with a distinct hotspot, *a1* and *b1* (*a1*, XU *et al.* 1995; *b1*, PATTERSON *et al.* 1995), or associated with a region of a gene, *r1* (ROBBINS *et al.* 1991; EGGLESTON *et al.* 1995). There may be two or more mechanisms contributing to the high intragenic recombination rates found in these loci, and understanding structural differences between these loci should be informative in better understanding mechanisms that stimulate intragenic recombination.

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