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A recombination hotspot in the maize *A1* intragenic region

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Summary. We find that recombination between two alleles of the maize *A1* locus that contain transposon insertions at known molecular positions can occur at 0.04–0.08 cM per kbp (centimorgan per kilobase pair), which is two orders of magnitude higher than the recombination rate for the whole maize genome. It is however, close to the rates found within the *bronze* locus, another maize structural gene for which both genetic and molecular data are available. This observation supports the idea that the genome consists of regions that are highly recombinogenic – in some cases, at least, structural genes – interspersed with regions that are less recombinogenic.

Key words: *Zea mays* – Recombination – *A1* locus

Introduction

The correlation between genetic distance, measured by meiotic recombination frequencies, and physical distance, measured molecularly or cytologically, varies within different parts of individual genomes (Bridges 1938; Bridges and Bridges 1939; Smith et al. 1987; Dooner 1986). Chromosomal rearrangements such as inversions and transpositions can alter the local rates of recombination in higher eukaryotic genomes (Sturtevant and Beadle 1936; Rhoades 1968; Nelson 1975; Dooner and Kermicle 1986) and the rates of recombination in unlinked segments of the genome as well (Hinton 1965; Suzuki 1963). This may indicate that recombination in large genomes is normally regulated in its frequency within different parts of the genome, and that this regulation can be perturbed by sequence changes. It has been proposed that recombination in large genomes is restricted to specific small regions, which may be the structural genes (Thuriaux 1977). Cases of genomic distance that can be compared genetically and molecularly are useful for studying variation in rates of recombination (Dooner 1986; Clark et al. 1986, 1988). The *A1* locus of maize provides such a case.

A1 encodes an NADPH-dependent reductase (Reddy et al. 1987) essential for the biosynthesis of anthocyanins in the plant. Combinations of alleles that do not contribute to anthocyanin production, due to the insertion of a transposon at the gene, have been tested for intragenic meiotic recombination, which could restore *A1* function (McClintock 1965; Neuffer 1965). One combination (*a1-m2/a1*) was previously shown to produce a small but reproducible fraction of *A1* individuals among the progeny, as expected if recombination had restored a functional gene (McClintock 1965). We report on a second combination of alleles in which recombination restores *A1* expression at a low but reproducible frequency (*a1-Mum2/a1*). *A1* and the alleles of *A1* used in recombination tests have been cloned and characterized (O'Reilly et al. 1985; Masson et al. 1987; Schwarz-Sommer et al. 1987; Brown et al. 1989). We utilize genetic and molecular distances between mutations in each allele of a heteroallele pair to define recombination rates at *A1* within the gene. We compare our calculated recombination rate within *A1* to the recombination rates reported for the *Bronze1* gene and to the estimated values for the maize genome overall.

Materials and methods

Allele descriptions

A1. *A1* is a structural gene encoding dihydroquercetin reductase, whose function is required for anthocyanin biosynthesis in maize (Emerson 1918; Reddy et al. 1987).

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a1. *a1* is a null allele (Emerson 1918); it contains an *rDt* insertion and mutates to *A1* in the presence of the unlinked transposon *Dotted* (*Dt*) (Rhoades 1936). All tests described used plants that did not contain *Dt* in their genomes, in which case *a1* acts as a stable null allele.

a1-Mum2. *a1-Mum2* is an unstable allele that gives a colorless kernel background and mutates to *A1* in small somatic sectors (Robertson 1978). It does not show detectable germinal reversion (reversion frequency less than one in 10^5).

a1-m2. *a1-m2* is an unstable allele that gives variable gene expression controlled by an *Spm* insertion at *a1* (descriptions in McClintock 1967; Masson et al. 1987).

sh2. *sh2* is a stable allele closely linked (0.2 cm) to *a1*; kernels homozygous for *sh2* are severely shrunken.

Position of transposon insertions

The positions of transposon insertions are indicated in Fig. 1, and described in detail in the text (see 'Results').

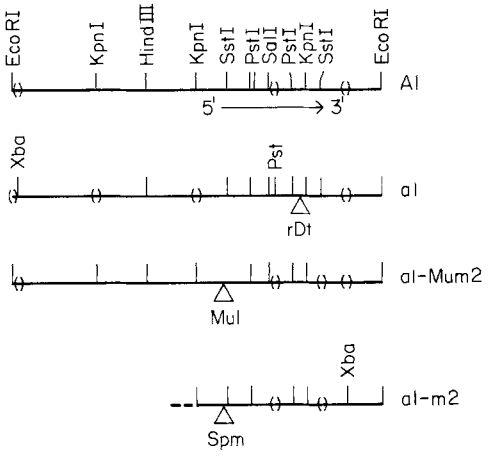

Fig. 1. Comparison of *A1* alleles restriction enzyme maps and transposon insertion sites. The *A1* map is shown at the *top*, with the direction and extent of transcription indicated by a *horizontal arrow*. Restriction sites in common between alleles are indicated in the top map, and indicated by *vertical lines* in the other maps, while additional sites are separately indicated in each map. Absence of a site is denoted by parenthesis at that position. The transposon insertion site in each allele is indicated by a triangle containing the name of the transposon. See 'Materials and methods' for allele phenotypes and text for references

Recombination tests

The cross $\frac{a1\text{-}Mum2\ Sh2}{a1\ sh2} \times \frac{a1\ sh2}{a1\ sh2}$ was scored for *A1* progeny.

No *Dt* was present in the genomes of tested plants. Neither *a1-Mum2* nor *a1* give revertant *A1* progeny when homozygous.

Results

The frequency with which transposon-containing mutant alleles of *A1* recombine has been compared to the mutation position in each allele, in order to determine a recombination rate at *A1*. Molecular maps of alleles used in recombination studies, and a wild type allele, are diagrammed in Fig. 1. The positions of transposon insertions in each allele are indicated.

We tested *a1-Mum2* (*a1 Mutator-induced mutable 2*) for intragenic recombination with *a1*. *a1-Mum2* contains a 1.4 kb *Mutator* (*Mu*) 1 inserted upstream of the *A1* transcription start site, at nucleotide-97 (Shepherd et al. 1988). *a1* contains an 0.7 kb *rDt* (*response to Dotted*) inserted in the fourth exon of *A1*, at nucleotide +1,083 (Fig. 1, Brown et al. 1989). *rDt* is a defective transposon that is activated *in trans* by the transposon *Dotted* (Rhoades 1936). The positions of the *Mu* and *rDt* insertions are 1.19 kb apart. *A1* individuals among the progeny of *a1-Mum2*/*a1* heterozygotes, which may result from crossing over between *a1-Mum2* and *a1* during meiosis, are found at a frequency of 0.045% (Table 1). The *A1* progeny were always *sh2*. Neither *a1-Mum2* nor *a1* give *A1* progeny when either is homozygous. Doubling the frequency of *A1 sh2* cases to account for undetected reciprocal products of recombination gives 0.09% (i.e., 0.09 cM) within *A1* (Table 1). This gives a rate of recombination of 0.08 cM/kb within the gene, in sharp contrast to the estimate of $2.5–5 \times 10^{-4}$ cM/kb, on average, for the maize genome overall [based on 1,200 cM (Coe et al. 1984) and $2.8–5.7 \times 10^9$ bp (Galbraith et al. 1983)].

Previously, *a1-m2* (*a1-mutable 2*) and several alleles derived from *a1-m2* had also been tested for recombination with *a1* (McClintock 1965). *a1-m2* and the states of *a1-m2* that have been analyzed molecularly each contain

Table 1. Intragenic recombination rates at *A1*

Parental genotype	No. of *A1sh2* kernels[a]	Total progeny	Distance between insertions		Rate of recombination in cM/kb[b]
			(A) genetic, in cM	(B) physical, in kb	
a1sh2/*a1sh2*	0	[c]	0	0	–
a1-Mum2Sh2/*a1sh2*	11	23,470	0.09	1.19	8×10^{-2}
a1-m2Sh2/*a1sh2*	17[d]	70,039	0.05	1.19	4×10^{-2}

[a] No *A1Sh2* kernels were recovered
[b] Values rounded off to nearest integer
[c] Never seen in the absence of *Dt* in the genome (i.e., <1 in 10^6)
[d] Data from McClintock 1965

a complete or partial *Spm* (*Suppressor-Mutator*) transposon sequence upstream of the *A1* transcription start site, at nucleotide-99 (Masson et al. 1987; Schwarz-Sommer et al. 1987). This is very close to the site of the *Mu1* insertion in the *a1-Mum2* allele (i.e., two nucleotides upstream). The *Spm* insertion site of *a1-m2* and the *rDt* insertion site of *a1* are separated by 1.19 kb. *A1 sh2* progeny are found at 0.024% (McClintock 1965; see Table 1). Doubling this frequency to account for undetected reciprocal products of recombination gives 0.05% (or 0.05 cM) within *A1* (Table 1). This predicts a recombination rate of 0.04 cM/kb within the gene, which is similar to the recombination rate determined for *a1-Mum2/a1* of 0.08 cM/kb.

The gene *Shrunken-2* (*Sh2*) is 0.2 cM distal to *A1* and genetically marks the parental alleles. Null (*sh2*) alleles give a distinctive shrunken kernel, while *Sh2* gives a plump (normal) kernel. The recombinant *A1* alleles arising from *a1-Mum2 Sh2/a1 sh2* are linked to *sh2* in each of 11 cases. *A1* alleles arising from *a1-m2 Sh2/a1 sh2* are also linked to *sh2* in each of 17 cases (McClintock 1965). These data are consistent with crossover events between the *rDt* and *Mu1* (or *dSpm*) insertions, with the *rDt* insertion being proximal to *Mu1*, i.e., the direction of *A1* transcription is away from *sh2* and towards the centromere. However, we cannot rule out that some or all of these events may be conversion events; in that case, one type of conversion is preferred over the other so that all of the recombinants are *sh2*. We note that both crossover and conversion events have been reported to occur in intragenic recombination at other maize loci (Dooner and Kermicle 1986).

Discussion

Instances in which the frequency of recombination over a defined molecular distance have been determined are useful in considering recombination rates within the genome. For certain allele combinations, the recombination rate within *A1* is $4–8 \times 10^{-2}$ cM/kb. This is close to the rate reported within the maize *Bronze1* (*Bz1*) gene of 7×10^{-2} cM/kb for certain alleles (Dooner 1986). In both cases, the rate of intragenic recombination is two orders of magnitude higher than that estimated for the maize genome overall, $2.5–5 \times 10^{-4}$ cM/kb. The rates of recombination within the maize *A1* and *Bz1* genes are even higher than the average rate of recombination in the very small *Arabidopsis* genome (6.2×10^{-3} cM/kb, Pang and Meyerowitz 1987). When estimating molecular distance from genetic distance, it is apparent that the average value, based on the genetic map size of an organism and the length of a haploid genome in base pairs, is not predictive for intragenic regions. Variability within the genome in the correlation between genetic and molecular distances should be considered in strategies for molecular cloning that rely on a known genetic distance (but unknown molecular distance) of a gene from a cloned DNA marker. For example, if using a "chromosome walk" strategy to clone maize genes, a recombination distance of 0.05–0.1 cM between a cloned DNA sequence and the gene of interest need not indicate that the gene is at a physical distance of 100–400 kb (estimated using the whole genome value); in view of the data for *A1* and *Bz1*, the possibility that the cloned sequence may be only a few kilobases away from the gene of interest, or even within the gene, should be considered seriously.

It has been noted that the variation in genetic map distances contained in different genomes is less than the variation in genome size (Thuriaux 1977). For example, *Arabidopsis* at 7×10^7 bp contains 460 map units, while maize at 3×10^9 bp contains only 1,200 map units. This may indicate that recombination is restricted to certain smaller regions of the large maize genome. Perhaps the high rates of recombination observed between certain pairs of alleles of *A1* and *Bz* are characteristic of recombination rates within structural genes of larger genomes. Structural genes and the other small regions of the maize genome that are accessible to recombination machinery may be interspersed with large, less recombinogenic regions – possibly blocks of heterochromatin or repetitive DNA.

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