Genetic recombination in plants

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Meiotic recombination generates novel allelic arrays on chromosomes. Recent experiments have revealed an extraordinarily nonrandom distribution of recombination breakpoints along the lengths of plant chromosomes; for example, recombination breakpoints often resolve within genic sequences, and thereby generate novel alleles. The mechanism by which recombination breakpoints are determined is an area of active investigation. In addition, recent developments are providing recombination-based technologies for creating targeted alterations in the architecture of plant genomes.

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Abbreviation
DSB double-strand break

Introduction

Genetic linkage is a consequence of the large-scale organization of genetic material into chromosomes; in the absence of recombination, all genes residing on the same chromosome would segregate as a unit and thus display absolute genetic linkage. There are obvious advantages to an organism in maintaining blocks of favorable alleles as a unit. This advantage, however, must be balanced with the evolutionary requirement for genetic diversity through the generation of novel allelic arrays via crossing-over, a process which generates recombinant chromosomes.

Recombination events can result in either reciprocal recombination or the related process of gene conversion. Reciprocal recombination arises via the exchange of genetic information between two nonsister chromatids. Gene conversion represents the nonreciprocal transfer of DNA sequences from one nonsister chromatid to another. Because gene conversion and reciprocal recombination appear to share a common pathway, mechanistic models have been sought that explain both phenomena.

Currently, the most widely accepted models for meiotic recombination are based upon the double-strand break (DSB) repair model [1]. According to this model (which is based on data from bacteria, phage and fungi), recombination initiates via the formation of a DSB on one chromatid. Exonucleases resect the 5′ ends of the DSB, thereby generating two single-stranded regions that flank the position of the original DSB. Subsequently the exposed 3′ ends displace the DNA strands with the same polarities from the nonsister chromatid. After the invading strands basepair with the complementary strands of the nonsister chromatids, they are used as primers for DNA synthesis for which the complementary DNA strand is used as the template (Figure 1). Ultimately this repair mechanism generates double Holliday junctions. Depending upon how these junctions resolve, reciprocal crossovers or gene conversion events can result. Support for this model has been provided by recent experiments which demonstrate that genes required for meiotic recombination in *Saccharomyces* yeast encode proteins that catalyze reactions predicted by the DSB repair model (see, for example, a review by Haber [2•]). Indeed, some of these proteins are conserved among species, including plants [3]. One such conserved protein is that encoded by the *recA* gene which is involved in strand invasion (Figure 1).

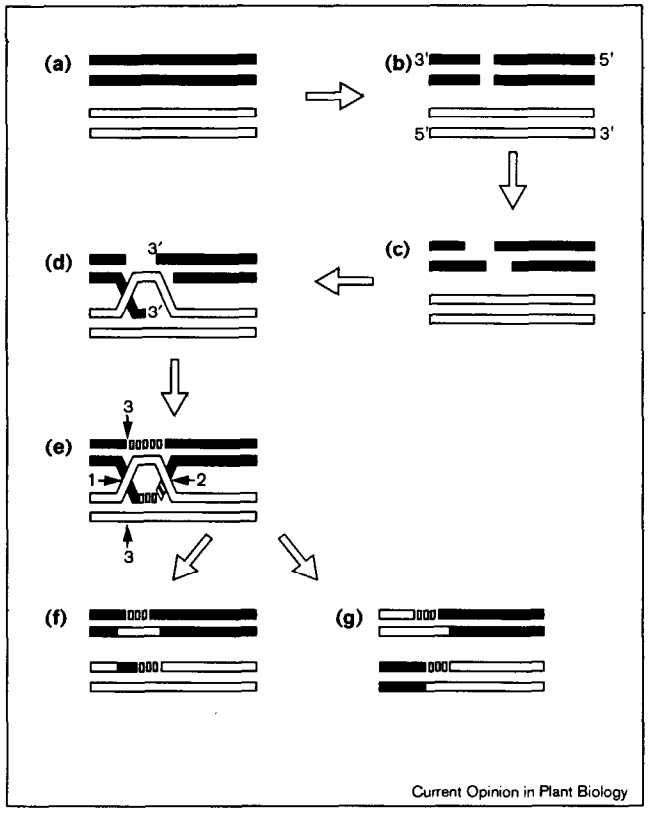
Homology-dependent DSB repair models are consistent with the data obtained from meiotic recombination experiments. Some of the data obtained from experiments using plants and other organisms and designed to study the integration of extrachromosomal DNA into chromosomes via mitotic recombination, or the repair of DSBs in mitotic cells, cannot be explained by these types of models [4,5•]. Hence, the single-strand annealing model (see [6] and Figure 2) and the one-sided invasion model (see [7] and Figure 3) have been proposed to explain recombination events that occur in the absence of significant amounts of DNA sequence homology, or when this homology is limited to a single side of a DSB, respectively.

This review summarizes recent data that expand our understanding of the extraordinarily nonrandom distribution of recombination breakpoints within plant genomes. It explores those features of the recombination machinery (and/or the genome structure) that may account for these observations, and it highlights the recent development of recombination-based tools that will facilitate the remodelling of plant genomes. The reader is additionally directed to other recent reviews on recombination [4,8–11].

Distribution of recombination breakpoints

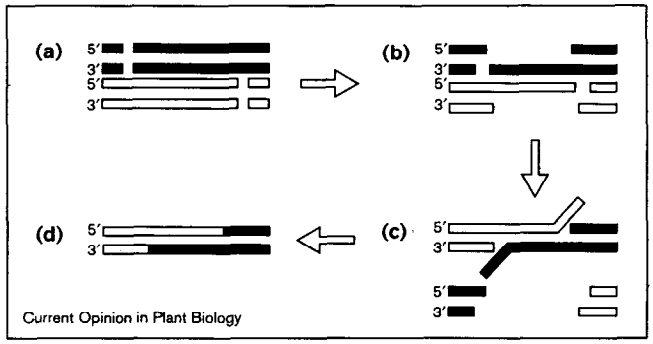
It has long been known that recombination does not occur randomly across the lengths of chromosomes. The development of detailed genetic maps and major advances in the physical mapping of plant genomes are making it possible to directly correlate physical and genetic distances over large intervals. For example, yeast and bacterial artificial chromosome (YAC and BAC, respec-

Figure 1



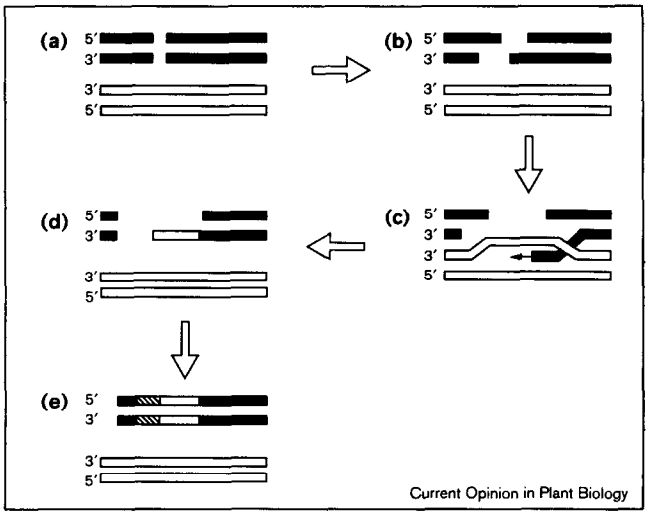
The double-strand DNA break repair model. **(a)** Two DNA duplexes. **(b)** A double-strand break occurs in one duplex. **(c)** A 5′ to 3′ exonuclease attacks the exposed 5′ ends. **(d)** Strand invasion. The free 3′ ends are used as primers for DNA synthesis and the uncut homologue (white) acts as the template. **(e)** DNA synthesis (dashed lines) results in a four stranded intermediate with two Holliday junctions. **(f)** Gene conversion in the absence of reciprocal recombination occurs if cuts and religation occur at positions 1 and 2 respectively as indicated in (e). **(g)** Gene conversion in combination with reciprocal recombination would result if cuts and religation occur at positions 2 and 3 respectively as indicated in (e).

Figure 2



The single-stranded annealing (SSA) model of recombination. **(a)** Double-stranded breaks are induced in both recombination partners. **(b)** 5′ to 3′ exonuclease attacks the exposed 5′ ends of both duplexes. **(c)** Free 3′ ends anneal forming a chimeric molecule. **(d)** Only the chimeric molecule is recovered. Unpaired overhangs are removed and single-stranded gaps are repaired by DNA synthesis.

Figure 3



The one-sided invasion (OSI) model of recombination. **(a)** A double-strand break is generated in one DNA duplex. **(b)** A 5′ to 3′ exonuclease attacks the exposed 5′ overhangs. **(c)** D-loop formation. A free 3′ end is used as a primer for DNA synthesis and the uncut homologue (white) acts as a template. **(d)** The resulting intermediate. **(e)** The gap is filled and the break repaired via illegitimate recombination which introduces new genetic information (hatched region).

tively) genomic libraries are now available or becoming available for many plant species, for example *Arabidopsis*, maize, rice, tomato, soybean, wheat, and barley. In some cases, most notably *Arabidopsis* [12–15], tomato [16–19] and rice [20], these YAC and BAC clones are being organized into large contigs. These contigs represent a resource for investigating the relationship between the physical nature of chromosomes and the dynamics of recombination. For example, it is now clear that in many plant species rates of recombination can vary up to an order of magnitude over relatively large intervals. Typically the regions surrounding the centromeres and, in at least some species, the nucleolus organizer region exhibit very low rates of recombination per physical length [21•,22,23•].

Two hypotheses can be advanced to explain the causal basis of these diversities in recombination rates. The first hypothesis suggests that at least some of the recombinationally hyperactive regions of chromosomes may have been identified as such because they contain a high density of genes. In contrast, the second hypothesis suggests that genes merely have a tendency to reside in recombinationally hyperactive regions. Both of these hypotheses depend on the facts that gene density varies across chromosomal regions and that plant genes are

recombination hot spots. In fact, genes exhibit rates of recombination per kilobase that are 10 to 100-fold higher than the genome average (reviewed by Lichten and Goldman [11]). For example, one-fifth of all recombination events that occurred across the 140 kb *a1–sh2* interval in maize resolved within a 377 bp region of the *a1* gene [24]. The *a1* (*anthocyaninless1*) and *sh2* (*shrunken2*) genes encode for dihydroquercetin reductase (EC 1.1.1.219) and ADP-glucose pyrophosphorylase (E.C. 2.7.7.27), respectively.

To understand the nature of the recombinationally hyperactive regions of chromosomes it is important to determine firstly whether genes reside in recombinationally active intervals larger than single genes, and secondly whether all recombination hot spots are genes. The *a1–sh2* interval of maize is being used as a model to address the first question, that is, do genes define discrete recombinationally active units embedded in larger recombinationally inert intervals. Analyses to date support the interpretation that genes *per se* are recombination hot spots [24,25].

An important cautionary note, however, is that, because most fine-scale studies of recombination have assayed recombination rates only within genes, it is not possible to conclude that all hot spots are genes. One way to settle this question would be to identify a collection of recombination hot spots and then determine whether these hot spots are genes. This process, however, is greatly complicated by the difficulty in distinguishing between genic and non-genic regions in complex genomes which are rich in retrotransposons and other repetitive sequences [26••,27]. The first step in testing the hypothesis that not all hot spots are genes comes from the identification of a single meiotic crossover that occurred in an apparently nongenic region of the maize genome [28•]. This crossover occurred in a DNA segment that exhibits a high degree of sequence identity between the two parental homologies. In addition this sequence is present at low copy number in the maize genome. This finding suggests that recombination events may resolve in low-copy, highly conserved regions of chromosomes.

Consistent with this hypothesis, in wheat the *Ph1* locus appears to enhance recombination between homologous intervals at the expense of homeologous intervals, possibly via a sequence similarity searching mechanism [29]. In addition, because recombination between repetitive sequences in complex genomes would generate deleterious chromosomal rearrangements, there must be mechanisms that suppress recombination between duplicated sequences on nonhomologous chromosomes; however, although the ribosomal RNA (rRNA) spacers exhibit sequence dissimilarity among species, the members of the tandem arrays of rRNA genes within a given species are virtually identical. Hence, there must be mechanisms that promote homogenization of these sequences during evolution, possibly via gene conversion. The observation that variants associated with the *Arabidopsis* rRNA genes can spread throughout a nucleolus organizer region supports the existence of such a process [30].

If recombination preferentially occurs within genes, one should be able to find evidence for its occurrence by analyzing the DNA sequences of multiple alleles of a given locus. Indeed, the structures of haplotypes obtained from such studies often suggest an evolutionary origin dependent upon gene conversion, or closely linked reciprocal crossovers, perhaps separated by one or more generations [31–34,35•]. Given that intragenic recombination is occurring, it represents a mechanism that could generate novel chimeric alleles, that is to say, genetic variability. This mechanism would have the capacity to combine the evolutionary advantageous domains from distinct alleles into a single novel allele upon which selection could act. Indeed, recombination at the *Rp1* locus has generated novel resistance alleles to the maize pathogen *Puccinia sorghi* [36]. In contrast, it has been proposed that structural polymorphisms at the self-incompatibility (*S*) locus in the *Brassica* genome have evolved specifically to repress recombination and thereby facilitate the maintenance of co-evolved genes within given haplotypes [37•].

Recent investigations have begun to explore the molecular nature of intragenic recombination events. These experiments have been conducted by isolating and analyzing large numbers of intragenic recombinants from five maize loci that are themselves recombination hot spots. In several instances, these analyses mapped recombination breakpoints to a resolution of tens of base pairs. Such analyses of the bronze (*bz1*) [38••] and waxy (*wx1*) [39•] loci have revealed that recombination breakpoints are randomly distributed across each of these genes. In contrast, similar analyses have revealed significantly nonrandom distributions of recombination breakpoints within the *a1* [24], *b1* (*booster*) [40], and *r1* (*red1*) [41] loci (*b1* and *r1* code for myc-like transcriptional activators. Specifically, most recombinants map to recombination hot spots at the 5′ end of the *a1* and *b1* loci and at the 3′ end of the *r1* locus.

The basis for these differences in the distribution of recombination breakpoints has not yet been determined, but at least three hypotheses can be considered. First, because these experiments were conducted in different genetic backgrounds, differences in *trans*-acting factors may have influenced the spectrum of recombination outcomes. Indeed, mutations that affect recombination rates have been identified in *Arabidopsis* [42•]. But of even more significance relative to this hypothesis, putative *trans*-acting factors have been identified in maize that appear to influence rates of recombination within specific genetic intervals [43•].

Second, different genes may have different patterns of recombination. This could be a consequence of

gene-specific factors or may be influenced by structural features larger than genes. Indeed, the genes included in these studies exhibit rates of recombination that differ by an order of magnitude. The reasons for these differences in rates are as yet unknown. In fact, the reason genes themselves are recombination hotspots has not yet been determined. Deletions of the *wx1* gene promoter of maize, however, do not affect recombination rates in this genic recombination hotspot, so in this case promoter activity must not be a requirement for high levels of recombination [39•].

Third, structural features of the allele combinations used in these experiments may have influenced the spectrum of recombination products that were obtained. Specifically, it has been suggested [38••] that the transposon insertions present in some of the allele combinations used in certain of these experiments may be responsible for the nonrandom distribution of recombination hot spots. Indeed, it is known that transposon insertions can influence the rates at which recombination occurs in the *bz1* and *a1* genes [24,44]. In addition, *Ds* (*Dissociator*) transposons appear to affect the distribution of recombination breakpoints in the *bz1* locus. In contrast, during experiments involving *a1* alleles that are sequence identical but for the presence or absence of the *Mu1* (*Mutator1*) transposon, no differences in the distribution of recombination breakpoints were observed [24].

Another structural feature that may affect the distribution of recombination breakpoints is the presence of small base pair heterologies between allelic combinations. For example, fewer than expected numbers of recombination events resolve in regions of the *bz1* locus that have high densities of heterologies [38••]. The impact of heterologies, however, on recombination in plants appears to be significantly less than is observed in *Saccharomyces* [45] and other fungi. For example, in *Ascobolus*, heterozygotes at the *b2* locus that exhibit 10% DNA sequence polymorphism in their 5′ ends experience gene conversion rates one to two orders of magnitude lower than heterozygotes with fewer sequence polymorphisms [46].

There are other differences in the outcomes of recombination between plants and other taxa. For example, polarity of gene conversion is commonly observed in yeast, fungi and *Drosophila*. Polarity can be defined as the gradient across a gene in the frequencies at which sequence polymorphisms are converted via recombination. In addition, conversions of polymorphisms at the end with the lower conversion rate are often accompanied by co-conversions of polymorphisms at the other end of the same gene. Polarity is thought to be a consequence of the fact that the DSBs that initiate recombination occur at discrete positions [11,47]. As discussed above, the distribution of recombination breakpoints is consistent with polarity in only some experiments conducted in maize.

In addition, although apparent gene conversions have been observed in maize [24,44,48], the ratio of gene conversions to crossovers appears to be low relative to that found in *Saccharomyces*. On the basis of a small sample of maize conversion events [38••], however, the sizes of conversion tracts appear to be similar in maize, *Saccharomyces* and *Drosophila* (i.e. 1 kb).

Recombination-based tools

An efficient and reliable system to target transgenes to defined locations within plant genomes would have great utility not only in conducting gene replacement procedures on endogenous genes (for example, gene knockouts and allele substitutions), but also in ensuring the stable expression of novel transgenes. Current transformation systems rely on the apparently random integration of foreign DNA. This leads to several problems, including the inability to inactivate endogenous genes and the unwanted silencing of transgenes due to position effects. These disadvantages can be overcome via the use of a homologous recombination system in which an endogenous copy of a given DNA sequence recombines with an ectopic donor copy with a similar sequence. Depending upon the nature of the donor sequence, such a recombination event can result in either a gene knockout or replacement.

Homologous recombination occurs readily in bacteria and yeast, where it is used for gene replacement experiments. More recently it has been developed as a tool for gene replacement in mammals. For example, the introduction of DSBs in yeast and mammalian genomes has been used to increase recombination rates. This approach usually involves the introduction into the genome of a target sequence containing a rare-cutting restriction enzyme recognition site (for a review, see [8]). Enzymes that have been used for this purpose are I-*Sce*I (the intron-encoded endonuclease I from the intron homing system of *Saccharomyces*) and the HO (homothallic switching) endonuclease (which is involved in mating-type gene rearrangements in *Saccharomyces*). Subsequently, the restriction enzyme and a donor sequence that shares homology with the target sequence are introduced into the cell. Because these enzymes have recognition sequences of 18 and 24 bp, respectively, their recognition sites occur infrequently in a genome. Hence, in most cases, the introduced restriction enzyme creates a DSB only at the target sequence, which can then be repaired via a homologous recombination event between the donor and target sequences. Such a strategy has proven successful in plants. For example, in tobacco protoplasts, the I-*Sce*I system enhances recombination rates between extrachromosomal donor sequences and target sequences that are either extrachromosomal or chromosomal [5•,49]. Similarly, the HO endonuclease increases by 10-fold the rate of intrachromosomal recombination in somatic cells of *Arabidopsis* [50]. To be most useful as a tool for genome rearrangements, it would be desirable to recover germinal

recombination events. Although no such events were recovered in this experiment, subsequent modifications may lead to this capacity.

Site-specific recombinases (e.g. *FLP/FRT*, Cre/*lox* and R-*RS*s; for a review, see [9]) are also being tested in plants. The *FLP/FRT*, Cre/*lox* and R-*RS*s systems are derived from the 2μ circular plasmid of *Saccharomyces cerevisiae*, the bacteriophage P1, and *Zygosaccharomyces rouxii*, respectively. These enzymes catalyze reversible recombination reactions between pairs of recognition sequences. If the two recognition sequences reside on a single DNA molecule, these enzymes can generate deletions or inversions, depending upon the relative orientations of the two recognition sequences. Alternatively if the two recognition sites are on different molecules, recombination can generate targeted integrations or translocations. Because the recognition site for each of these enzymes is large (34 bp in the case of *FRT* and *lox* and 31 bp in the case of *RS*s), they are unlikely to occur by chance in a genome.

Both somatic and germinal recombination events have been recovered using the R/*RS*s system in *Arabidopsis* [51]. Similarly, the *FLP/FRT* system is functional in a number of plant species, including maize, tobacco and *Arabidopsis* [52–55]. It has recently been demonstrated that *FLP*-mediated recombination can be regulated via the use of an inducible promoter [54]. In addition, as demonstrated in *Drosophila* [56], *FLP*-mediated recombination can facilitate large-scale chromosome manipulations. A similar approach, but based on the Cre/*lox* system, has been used to generate large-scale chromosomal rearrangements in tomato, tobacco and *Arabidopsis* [57–59,60•]. As a first step in two of the more recent experiments, *Ds* transposons that carried a *lox* sequence were introduced into *Arabidopsis* and tomato genomes via T-DNA mediated transformation. Transposition events then moved the *lox* sequences from the T-DNA insertion sites to multiple positions along chromosomes. Subsequently, Cre-mediated recombination generated inversions between pairs of *lox* sites in both somatic and germinal tissues [58,60•]. In one of these experiments [60•], *in vitro* Cre-mediated recombination was used to release large chromosomal fragments which were sized using pulse-field electrophoresis. This technology could, therefore, be used to generate detailed physical and genetic maps as well as to facilitate the functional analysis of chromosomal segments via the isolation and phenotypic characterization of deletions [60•]. In addition, this technology may ultimately be used for site-specific gene targeting in plants, as has already been done in a murine cell line [61].

Transposon-induced DSBs have been used in gene targeting experiments in *Drosophila* [62]. In this species, DSBs that occur in the vicinity of a transposon can be repaired using a nonallelic donor template for gap repair. Depending upon the nature of the donor template this repair process can result in gene knockouts or allele substitutions at the target site. Because gap repair occurs in plants carrying active *Mutator* and *Ac* (*Activator*) transposon systems [63•,64•], similar transposon-based approaches to gene targeting are being tested in plants.

Other gene targeting technologies are also under development. For example, it has been reported that DNA–RNA hybrid molecules can be stably integrated into a plant genome following protoplast transfection [65], as occurs in mammalian cells [66]. In addition, homologous recombination events between donor sequences introduced via *Agrobacterium*-mediated transformation and endogenous genes in *Arabidopsis* and tobacco have resulted in successful gene disruptions [67,68] that in the most recent experiments have yielded germinally transmitted mutants [69••].

Conclusions

Meiotic recombination has been best characterized in *Saccharomyces*. The on-going plant expressed sequence tagged projects will provide important tools to ascertain whether the recombination machinery is well conserved structurally between plants and *Saccharomyces*. It is becoming clear, however, that recombination outcomes (at least) differ between plants and *Saccharomyces*. Hence, it will be important to develop a mechanistic understanding of recombination in plants to enhance the utility of the developing repertoire of recombination-based tools, which have the promise of being able to facilitate efforts to restructure plant genomes.

Two of the important questions that remain to be resolved are the nature of recombination hot spots (i.e. are all hot spots genes?) and the mechanisms by which the recombination machinery selects recombination sites within plant genomes. One significant difference between the genomes of plants and *Saccharomyces* is that the former contain large amounts of repetitive and polymorphic sequences in their intergenic regions. Hence, one possibility is that recombination is restricted to regions that exhibit relatively lower levels of DNA sequence polymorphism and/or have fewer large structural polymorphisms. Clearly, genes would meet this criterion, but so might other regions of the genome. A further explanation for why genes are recombinationally hyperactive, which has not been discussed in this review, might be that the recombination machinery operates most efficiently on open chromatin.

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