Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5419–5424, May 1996
Genetics

Recombinational repair of gaps in DNA is asymmetric in *Ustilago maydis* and can be explained by a migrating D-loop model

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Communicated by Charles M. Radding, Yale University, New Haven, CT, February 9, 1996 (received for review October 6, 1995)

ABSTRACT **Recombinational repair of double-stranded DNA gaps was investigated in *Ustilago maydis*. The experimental system was designed for analysis of repair of an autonomously replicating plasmid containing a cloned gene disabled by an internal deletion. It was discovered that crossing over rarely accompanied gap repair. The strong bias against crossing over was observed in three different genes regardless of gap size. These results indicate that gap repair in *U. maydis* is unlikely to proceed by the mechanism envisioned in the double-stranded break repair model of recombination, which was developed to account for recombination in *Saccharomyces cerevisiae*. Experiments aimed at exploring processing of DNA ends were performed to gain understanding of the mechanism responsible for the observed bias. A heterologous insert placed within a gap in the coding sequence of two different marker genes strongly inhibited repair if the DNA was cleaved at the promoter-proximal junction joining the insert and coding sequence but had little effect on repair if the DNA was cleaved at the promoter-distal junction. Gene conversion of plasmid restriction fragment length polymorphism markers engineered in sequences flanking both sides of a gap accompanied repair but was directionally biased. These results are interpreted to mean that the DNA ends flanking a gap are subject to different types of processing. A model featuring a single migrating D-loop is proposed to explain the bias in gap repair outcome based on the observed asymmetry in processing the DNA ends.**

Genetic analysis of meiotic recombination has revealed a close connection between gene conversion, the nonreciprocal transfer of information, and crossing over (for a review, see ref. 1). Insight into the mechanistic basis for this connection has come from investigation of DNA double-stranded break and gap repair, and models accounting for the association have been proposed based on the homolog interaction of one or both DNA ends resulting from duplex DNA breakage (2, 3). As a test of the double-strand break repair model in *Saccharomyces cerevisiae*, the repair of a gap in plasmid DNA was measured after transformation of mitotic cells (4, 5). The observed association of plasmid integration with repair of a gap in 50% of the events was in precise agreement with the predicted distribution hypothesized in the model and has reinforced the view of universality of the double-strand break repair pathway.

Nevertheless, studies on double-stranded break or gap repair in other systems have not generally revealed a close association with crossing over. For instance, mating type interconversion in *S. cerevisiae* takes place by gene conversion following introduction of a double-stranded DNA break at the *MAT* locus, but no crossing over is found to be associated (6). Similarly, the double-stranded break resulting from P element mobilization in *Drosophila melanogaster* was found to be repaired through recombination with a homologous sequence, but no crossing over was observed to accompany the repair (7). In a recent study (8) of transformation of *S. cerevisiae* with plasmid DNA cut to the linear form by introduction of a double-stranded break that was quite similar in experimental design to the prototype system used by Orr-Weaver and Szostak (4), the overwhelming majority of double-stranded break repair events was found to occur without associated crossing over. No mechanistic explanation has been presented to reconcile the contradictory findings. It is possible that alternative pathways are in operation for repair of double-stranded breaks, which are not universal (9), or that recombination at particular genetic loci is strongly influenced by the local chromatin structure, which might not be uniform (10). It seems clear that a mechanism responsible for the strong bias against crossing over noted in examples above is operational in mitotic cells.

We have been investigating the genetic and molecular basis of recombination in *Ustilago maydis* and have initiated studies using plasmid DNA substrates. Experimental systems were designed to categorize the types of recombination events taking place between nonreplicating plasmids and chromosomal sequences (11) and to examine extrachromosomal recombination between replicating plasmids (12). Our studies indicated that recombination was strongly stimulated by introduction of double-stranded breaks and that a potent end-joining activity was present that could repair double-stranded breaks in the absence of homolog interaction (12). These observations plus the differences noted in the degree of association between double-stranded break repair and crossing over in various systems have led us to investigate the mechanism of double-stranded break repair in *U. maydis*.

In the present work, we designed a system for studying the recombinational repair of double-stranded gaps in DNA. In particular, we were interested in determining (i) if gap repair in *U. maydis* is accompanied by crossing over and (ii) whether or not processing of the DNA ends might provide clues to the mechanism of recombination.

MATERIALS AND METHODS

Plasmids. The *U. maydis LEU1* gene used in these studies was contained on a 3.0-kbp *Hin*dIII–*Eco*RI genomic DNA fragment that fully complements the *leu1-1* mutation (13, 14). pCM216 (12) is pBluescript II SK$^+$ (Stratagene) containing this 3.0-kbp fragment and the 383-bp *U. maydis ARS* that confers autonomous replication (15). An essential 710-bp *Nco*I fragment extending from nucleotides +656 to +1366 relative to the initiating ATG of the *LEU1* gene was removed from within the coding region of the *LEU1* gene in pCM216 to yield pCM291. pCM524 was constructed from pCM216 by replacement of 195 bp residing between the unique *Sph*I and *Mlu*I sites with 785 bp of heterologous DNA inserted in by a triple ligation involving the digested plasmid, a 337-bp *Sph*I–*Eco*RV fragment from the tetracycline resistance gene of pBR322, and

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Abbreviation: RFLP, restriction fragment length polymorphisms.

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a 448-bp *Eco*RV–*Mlu*I fragment from the *lacI*^{q} gene of pET11c (Novagen). pCM521 was derived from pCM216 by an inverse polymerase chain reaction procedure such that a 35-bp block was deleted from the *LEU1* sequence and a *Sma*I site was concomitantly created after closure of the sequences flanking the deleted stretch. Two oligonucleotides complementary to *LEU1* coding and noncoding sequences were designed as primers to direct PCR DNA synthesis on pCM216 template away from each other. The 5′ ends were positioned 35 bp apart so that blunt-end ligation of PCR product would effectively create a 35-bp gap. The 5′ termini were located at nucleotides +1155 and +1190. Primer location was such that blunt-end rejoining generated the *Sma*I site. Each primer was also designed to contain a single-base change that would result in a restriction site polymorphism in the *LEU1* gene, but these were silent mutations that would not alter the amino acid sequence. Oligonucleotides were 5′-GGGCACGAGCATACAGTACAGTCCA-3′ and 5′-GGGGCTCGACAAAATCTTCCAGGCG-3′ with the restriction site mutations 13 residues from the 5′ ends in both cases. These mutations destroyed *Sph*I (GCATGC) and *Bgl*II sites (AGATCT), as underlined, in the respective oligonucleotides. The PCR reaction was performed with pCM216 DNA as template using Vent DNA polymerase (New England Biolabs). DNA sequence analysis confirmed the site-directed mutations and the 35-bp gap. The *PYR6* gene was contained on a 4.3-kbp *Pst*I fragment that was subcloned from the original 8-kbp isolate (16). pCM242 contained this *Pst*I fragment inserted into the multiple cloning site of pUC12 and the 383-bp *U. maydis ARS* inserted at the *Ssp*I site. pCM549 is pCM242 with 90 base pairs deleted from the *PYR6* gene by removal of an internal *Nru*I fragment. This gap ranged from nucleotides 592–682 relative to the putative translational start codon. pCM556 is pCM242 with a 206-bp fragment removed from *PYR6* between unique *Eco*RV and *Sph*I sites, located at nucleotides 293 and 499, respectively. The removed fragment was replaced with a 337-bp *Eco*RV–*Sph*1 fragment from the tetracycline resistance gene of pBR322. The *ADE1* gene was contained on a 3.3-kbp fragment subcloned from the original isolate (17). pCM369 is pBluescript II SK$^+$ containing this 3.3-kbp *ADE1* gene fragment inserted between the unique *Xba*I and *Apa*I sites in the polylinker and the 383-bp *ARS* inserted in place of a nonessential 127-bp *Ssp*I fragment in the plasmid. pCM523 is pCM369, with a 90-bp *Nco*I fragment removed from within the *ADE1* gene. All plasmids were amplified in *Escherichia coli* strain XL-1 Blue (Stratagene) *endA1 hsdR17 supE44 thi1 λ⁻ recA1 gyrA96 relA1 lac⁻ [F′ proAB lacI^q lacZ DM15 Tn10 et^R]*.

***U. maydis* Procedures.** Preparation of media, procedures for growth and transformation of *U. maydis*, and methods for DNA preparation and Southern analysis have been described previously (11, 17). *U. maydis* strains used in this study include UCM5 (*leu1–1 ade1–1 a2b2*) and UCM163 (*pyr6–7 inos1–3 nic1–2 a2b2*) in which *leu, ade, pyr, inos*, and *nic* indicate requirements for leucine, adenine, uracil, inositol, and nicotinic acid, respectively, and *a2b2* indicates the genotype at the mating type loci. Protoplasts used in individual transformations were prepared from 10^7 cells. DNA used in analysis of repair of gaps was prepared by cleavage of the appropriate plasmid followed by thermal inactivation of the added restriction endonuclease(s). Recombination frequencies were standardized for a particular batch of protoplasts by comparison with the transformation frequency obtained with the appropriate autonomously replicating plasmid containing the intact gene of interest (17). The plasmids used were pCM216 for standardization at *LEU1*, pCM242 for *PYR6*, and pCM369 for *ADE1*. The recombination frequency is the number of prototrophs arising from the gapped plasmid divided by the number of transformants per microgram of appropriate transformation control. The specific recombination frequency is the number of prototrophs arising from the gapped plasmid per microgram divided by the number of transformants per microgram of the appropriate autonomously replicating standard. In general, transformations with the control plasmid standards were carried out using 30 ng of DNA.

RESULTS

Gap Repair Strategy. Recombinational repair of double-stranded DNA gaps was investigated by analyzing the frequency and mode of recombination between a cloned, selectable gene lacking an essential internal sequence and a corresponding homologous allele inactivated by a mutation outside of the sequence comprising the gap (Fig. 1 *A* and *B*). The gapped gene was on a plasmid that contained an autonomously replicating sequence enabling propagation when episomal, while the homologous allele resided within the *U. maydis* genome. Recombination events were identified by generation of prototrophs following introduction of the plasmid into cells. Removal of essential internal segments from the selectable marker genes assured that the potent end-joining capability of *U. maydis* could not result in generation of prototrophs by simple religation of double-stranded breaks. The potent end-joining activity also gave us reason to design the plasmids with only one selectable marker. Analysis of gap repair in the absence of selection for correct repair would not have been feasible because the number of recombinants would have been small and probably could not have been detected among the large number of transformants arising from simple religation of the linear plasmid. The reversion frequency of host mutants

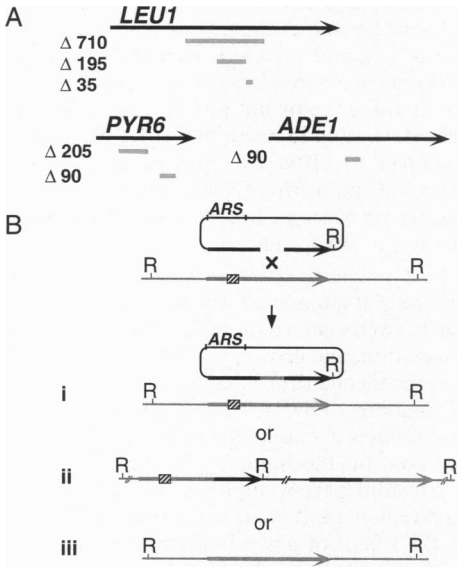

FIG. 1. Repair of gaps in plasmid borne alleles. (*A*) Diagram illustrating the location and sizes of gaps in *LEU1*, *PYR6*, and *ADE1*. The thick black lines depict the open reading frames, while the gray bars below each open reading frame represents the internal sequences that were removed to create gaps of lengths indicated on the left by the symbol Δ. (*B*) Schematic representation of the three modes of recombination between a plasmid containing an allele of a cloned gene with an essential internal portion of its sequence removed and a chromosomal allele. (*i*) Gap repair of plasmid DNA with no associated cross-over, (*ii*) gap repair of plasmid DNA accompanied by a cross-over, (*iii*) replacement of the chromosomal mutation with wild-type sequence from the plasmid DNA flanking the gap. The example shown is for repair of a 195-bp gap in *LEU1*. Digestion of plasmid DNA with the appropriate restriction endonuclease prior to transformation into *U. maydis* generates double-stranded DNA ends flanking the gap (solid arrow). The lesion responsible for the chromosomal *leu1*-1 allele (hatched box) is located outside of the region corresponding to the gap and has been mapped to the first 655 bp of the open reading frame. The sites of the lesion responsible for the *pyr6–7* and *ade1–1* mutations are not known. R, restriction endonuclease site.

utilized in this study was $<10^{-7}$, which was so low that no revertants appeared during the course of transformation. Therefore generation of prototrophy under the conditions of these experiments was a true measure of recombination.

Three modes of recombination were possible (Fig. 1*B*). These were (i) gap repair of the missing information in the plasmid allele using the sequence of the chromosomal allele as template, but no integration of the plasmid; (ii) gap repair of the plasmid allele accompanied by integration of the plasmid into the genome; and (iii) replacement of the chromosomal mutation by the corresponding wild-type sequence present on the plasmid, without gap repair. Recombinants in this third mode could arise by gene conversion or by a double cross-over spanning the region but will be referred to as gene replacements for simplicity. The three modes of recombination were easily distinguishable by Southern hybridization analysis. This was accomplished by digestion of genomic DNA with a restriction enzyme that cut the transforming plasmid DNA at a unique site within the vector sequence, but not within the sequence of the cloned marker, and followed by hybridization with a probe corresponding to the DNA sequence within the gap. Gap repair without associated crossing over was recognized as the appearance of a plasmid-length band of multicopy intensity along with a band representing the endogenous allele. Gap repair accompanied by crossing over was recognized by the absence of the band representing the endogenous allele, but the appearance of two new bands whose sizes were the sum of the endogenous band plus the plasmid. Gene replacements were recognized by a single band representing the endogenous allele.

Crossing Over Accompanies Gap Repair Infrequently. Recombinants were readily obtained when plasmid DNA with a gap was introduced into the appropriate host strain. Transformation of *leu1-1* with plasmid DNA containing a gap in *LEU1* of 35 or 710 bp (Fig. 1*A*) resulted in an approximately linear response in Leu^+ recombinants with increasing transforming DNA up to an input of 10 μg (data not shown), yielding several hundred colonies per microgram of input DNA. To control for variability in cellular competence and DNA uptake, parallel transformations were conducted using pCM216, an autonomously replicating plasmid containing the intact *LEU1* gene. Transformation in this case does not rely on recombination to confer leucine prototrophy, and therefore is a measure of transformation competence. Furthermore, since linear pCM216 containing the entire *LEU1* gene confers the same transformation frequency as does the circular form (12), it can be concluded that conformation of DNA makes no difference in uptake. The frequency of transformation was about 2-fold higher when plasmid DNA with the 35-bp gap was utilized compared with the plasmids with the 710- or 195-bp gap (Fig. 1 and Table 1).

Sets of recombinants obtained with plasmids containing gaps in *LEU1* of 35, 195, and 710 bp were examined by Southern hybridization to determine the mode of recombination as described above. The majority of recombinants in each set arose through gap repair, with gene replacement comprising only 10% of the total (Table 1). Examination of gap repair events revealed a pronounced asymmetry in outcome indicated by a bias against plasmid crossing over. Regardless of the gap size, plasmids were repaired without crossing over several times more frequently than with crossing over. χ^2 contingency tests confirmed that there was indeed a bias against crossing over associated with gap repair ($P < 0.01$) but did not support the notion that the strength of the bias was related to the size of the gap ($0.05 < P < 0.1$).

Recombinants obtained after transformation with plasmids containing gaps in two other genes were also examined (Table 1). Gaps of 90 and 206 bp were generated in the *PYR6* gene, and a gap of 90 bp was generated in the *ADE1* gene. The two gaps in the case of *PYR6* were not overlapping, unlike those in *LEU1* in which the largest gap spans the smaller two (Fig. 1). Gap repair of *PYR6* occurred almost exclusively without associated crossing over. Furthermore, none of the recombinants obtained in the analysis of *PYR6* was due to gene replacement. Repair of the 90-bp gap in the *ADE1* gene also occurred with little associated crossing over, although the majority of Ade^+ recombinants was found to have arisen from gene replacement. These results indicate that recombinational repair of gaps in plasmid DNA takes place with a strong bias against crossing over. It is of interest to note that the frequency of recombination at *pyr6* and at *ade1* was considerably higher than that observed at *leu1*. The reason for these differences is unclear, but the occurrence of marker specific effects in recombination is well known.

Heterologous DNA Blocks Gap Repair Only from One End. To gain insight into the mechanism responsible for the bias against crossing over, we investigated recombination of gapped genes under conditions in which homologous sequence was present at only one side of the gap. For these experiments, a heterologous DNA sequence was inserted into the gap. Transformation was then performed using plasmid DNA cut on one side of the heterologous block or the other, thereby generating a gap, but with a heterologous stretch at the side either proximal or distal to the promoter (Fig. 2). When recombination was measured at *LEU1* and *PYR6*, the results indicated that there was no appreciable effect on recombination fre-

Table 1. Analysis of recombinants

		Southern analysis			
				Gap repair (Crossing over)	
Gap size	Specific recombination frequency, 10^{-2}*	Total analyzed	Gene replacement	−	+
LEU1					
710 bp	3.0	49	5	41	3
195 bp	2.2	36	5	28	2
35 bp†	7.1	54	4	39	11
PYR6					
90 bp	24	35	0	34	1
206 bp	29	35	0	34	1
ADE1					
90 bp	20	75	50	22	3

*Frequencies calculated from 2–4 independent transformations, each of which yielded 200–800 colonies. Southern analysis was performed on a subset of each transformation.
†pCM521 was used which contains RFLP point mutation markers flanking the gap sequence.

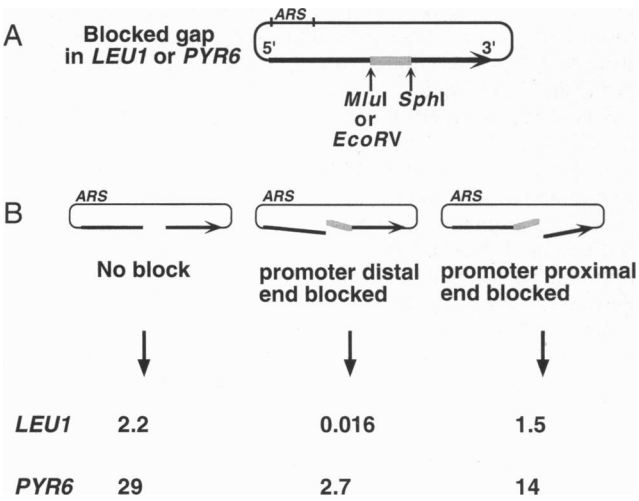

FIG. 2. Repair of gaps blocked with heterologous DNA. (*A*) Gap repair was examined at *LEU1* and *PYR6* (solid arrow) in which gaps were filled with heterologous DNA (gray bar). By cutting the plasmid with the appropriate restriction endonuclease prior to transformation, one of the two homologous DNA ends adjacent to the gap was exposed, while the other remained blocked by the heterologous insert. Digestion with both restriction endonucleases whose sites flank the heterologous insert allowed both ends to be exposed prior to transformation. Gap repair of *LEU1* was performed using pCM524, which contains 825 bp of heterologous DNA in place of an internal 195-bp *Mlu*I–*Sph*I fragment, while gap repair of *PYR6* was performed using pCM556, which contains 337 bp of heterologous DNA in place of an internal 206-bp *Eco*RV-*Sph*I fragment. (*B*) Recombination frequencies obtained at *leu1–1* and *pyr6–7* after transformation with the appropriate plasmid. The left column shows the frequencies observed when plasmid DNA with both ends exposed was transformed. The middle column shows frequencies obtained after leaving the promoter distal side blocked, while the third column shows frequencies obtained after leaving the promoter proximal side blocked. The numbers above are the average of three independent transformations of 1 μg of input DNA. Recombination frequencies were calculated as described.

quency when the promoter-proximal end was blocked. In contrast, there was a precipitous drop in recombination at both loci when the promoter-distal end was blocked. This overall inhibition of recombination by heterologous DNA blocking one side of a gap but not the other is indicative of asymmetry in the processing mechanism leading to gap repair. However, based upon these findings alone, no definitive conclusion can be drawn as to whether the heterologous sequence blocks a step in initiation or a later step in resolution.

Gene Conversion of Restriction Fragment Length Polymorphisms (RFLPs) in Sequences Flanking a Gap. A second approach addressing the mechanism of biased gap repair was to examine gene conversion of RFLP markers in the sequences adjacent to a gap. To this end, a 35-bp gap was engineered in *LEU1* with point mutations at sites 13 bp from either side of the gap. The mutations were silent in terms of codon usage, but each eliminated a restriction enzyme site. The *Sph*I site upstream of the gap and proximal to the 5′ end of the *LEU1* gene was altered, and the *Bgl*II site downstream of the gap, or distal, was likewise altered. The disposition of these restriction enzyme sites was determined in recombinants that arose through gap repair unassociated with crossing over. Analysis of integrated plasmids was not undertaken due to the small sample size obtained and increased difficulty in interpreting the nature of the events by restriction analysis.

Analysis of DNA from 24 recombinants containing gap-repaired but unintegrated plasmids indicated that in all 24 cases there was gene conversion directed by the chromosomal *leu1–1* allele of at least one of the plasmid RFLP markers flanking the gap (Fig. 3). In 19 instances, all of the plasmid DNA present in a particular recombinant was sensitive to cleavage by *Sph*I, indicating that the mutated *Sph*I site had been converted to the recognition sequence (two-stranded conversion). These events could have resulted either from enlargement of the gap beyond the engineered restriction site mutation or from mismatch repair of heteroduplex DNA immediately flanking the gap. In the remaining five samples, there was a mixed population of gap-repaired plasmids in which about half of the DNA was resistant to cleavage by *Sph*I and half was sensitive, indicating that both the mutated *Sph*I sequence and the normal sequence were present. These latter events presumably arose from postdivision segregation of heteroduplex DNA formed on the gap-repaired plasmid (one-strand conversion). While no example was found of a gap-repaired recombinant plasmid without gene conversion at the proximal RFLP marker (*Sph*I site), in a significant fraction of the gap repair events there was no conversion of the distal RFLP marker (*Bgl*II site). In eight recombinants, the mutated *Bgl*II site remained unconverted, while in the other 16 there was an even distribution of one- and two-strand conversions. The apparent predominance of conversion events on the proximal side of the gap could indicate a polarity or end preference in processing. These results could mean that the gap is enlarged more readily toward the proximal side or that the mismatch repair system operates more efficiently on that side. Not a single example was found in which there was conversion of the chromosomal RFLP markers directed by the mutated sequences on the plasmid, implying that if the observed RFLP conversions arose by mismatch repair, the mechanism would have to be strongly biased in favor of correction to the sequence of the invaded duplex (18).

DISCUSSION

There are two principal findings in this study. First, double-stranded DNA gaps in a cloned gene on a plasmid can be repaired through recombinational transfer of information from homologous sequences within the genome but with little

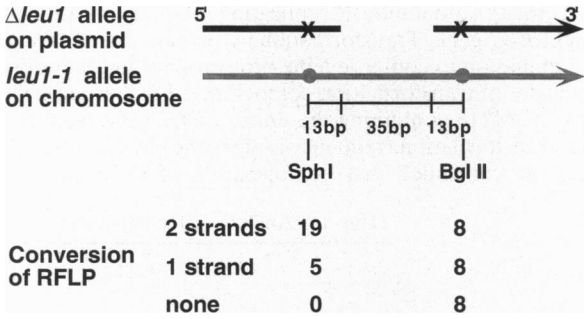

FIG. 3. Gene conversion of RFLP markers flanking a gap. (*A*) pCM521 was digested with *Sma*I to expose a 35-bp gap in the *LEU1* gene. The solid black arrow represents the portion of the *leu1* allele in the proximity of the gap; the gray arrow represents the corresponding chromosomal *leu1–1* sequence. The black Xs on the plasmid sequence represent RFLP markers 13 base pairs from the DNA ends that eliminate the *Sph*I and *Bgl*II restriction sites. These sites are present on the chromosomal allele and are represented by the gray circles. After transformation, the mode of recombination was determined by Southern hybridization analysis following digestion with *Bam*HI. Gene conversion of the point mutations flanking the gap was analyzed by RFLP analysis. Two categories of gene conversion were defined. Two-strand conversion regenerates the restriction endonuclease target site. One-strand conversion results from heteroduplex formation spanning the RFLP site followed by postreplication segregation of daughter sequences, one of which contains the mutated restriction site and one of which contains the normal sequence. The results of this analysis are shown aligned with the appropriate RFLP marker for 24 recombinants resulting from gap repair without crossing over.

associated crossing over. Second, there is unequal processing of the DNA ends flanking the gap.

Gap repair as envisioned by the double-strand break repair model features the formation of an intermediate with Holliday junctions flanking both sides of the gap (3). The proposed means for resolution of the intermediate to yield cross-over or noncross-over products invoked symmetrical cleavage of the Holliday structures and provided a theoretical mechanism in accordance with the close association between gene conversion and crossing over of genetic markers that has been observed during meiosis and plasmid transformation in yeast. By contrast, in *U. maydis* little crossing over with the genomic sequence was found to accompany recombinational repair of gapped plasmids. Examples of gap repair unassociated with crossing over have been noted in other systems, but are often considered to be special situations. These include P element-induced gap repair in *D. melanogaster* (7) and mating type switching at the *MAT* locus in *S. cerevisiae* (6), both of which are even more extreme in terms of infrequent crossing over. The bias against crossing over observed here, however, is consistent with studies on heteroallelic recombination in *U. maydis* in which the association of crossing over with gene conversion is weak (19), and seems sensible biologically. Frequent crossing over in mitotic cells could lead to chromosome imbalance as a consequence of pairing through repeated sequences and could result in disaster for the cell. Thus, it might be expected that systems are in place to prevent mitotic crossing over. These findings raise the issue that if gap repair proceeds through an intermediate featuring Holliday structures, as in the double-strand break-repair model, then there must be some restraint on the mechanism of resolution of the structures to explain the observed bias. This might arise as a result of sequence specificity of a Holliday structure resolving enzyme such as RuvC (20) and/or through constraints in resolution imposed by the tertiary conformation of the Holliday structure (21). Alternatively, symmetric Holliday junctions could be nullified by the action of a topoisomerase (22), or mechanisms could be in operation that circumvent the Holliday intermediates (22, 23).

Two experimental approaches revealed that the DNA ends adjacent to the gaps were processed asymmetrically. The strategy taken in the first approach was to ask if gap repair could be completed if one DNA terminus contained a heterologous block of DNA. In two different genes it was found that proficient repair of gaps filled with heterologous DNA proceeded, but only when the configuration of DNA was arranged so that the junction was cut between the heterologous insert and the marker gene sequence at the promoter-distal end of the gap, not at the promoter-proximal end. The strategy taken in the second approach was to follow the fate of restriction site polymorphisms in the plasmid *LEU1* sequence placed 13 base pairs away from a gap, thereby enabling a higher resolution view of processing of the ends. There was gene conversion of the RFLP marker at the proximal end sequence in all of the gap repair events examined, while the RFLP marker at the distal end escaped gene conversion one-third of the time. The results from the two approaches are consistent and could be interpreted to mean that both DNA strands at the promoter-proximal end of the gap are removed by nucleolytic digestion at an initial step or at a later step in gap repair, while at least one strand at the distal end is relatively protected throughout the process. Asymmetric enlargement of DNA gaps has been noted in other experimental systems (24, 25).

The asymmetry in both processing and outcome observed in these studies can be accounted for by a recombination model featuring a single migrating D-loop (Fig. 4). A single-stranded 3′ tail generated by exonucleolytic processing of a broken DNA end at one side of the gap is imagined to invade the homologous duplex forming a D-loop and a primer for a repair polymerase. The D-loop does not become enlarged, but is instead driven to migrate into the gap in a manner similar to what was observed for UvsX-dependent DNA synthesis *in vitro* (26). The newly synthesized strand is continuously extruded from the D-loop and eventually spans the entire gap. It is then free to make contact with the distal broken DNA end, and complementary base pairing is effected. After second-strand synthesis, single-stranded endonucleolytic and exonucleolytic digestion of the newly synthesized strand trailing behind the migrating D-loop will resolve the intermediate resulting in repair of the gap with no crossing over. Although rare, crossing over was observed in association with gap repair in *U. maydis*. A cross-over could result from the asymmetric mechanism described above if on occasion the endonuclease cleaved the single strand of the D-loop rather than the displaced nascent strand (Fig. 4) or if the D-loop simply collapsed releasing the newly synthesized strand. The new free single-stranded end could then pair with the homolog to yield a Holliday structure in a manner similar to the mechanism proposed in the Meselson–Radding model (27). The migrating D-loop model differs from the most common depiction of the double-strand break-repair model in that only one side of the gap interacts with the homolog and the resulting D-loop is forced to move instead of being enlarged. The initiation of gap repair on only one side is similar to an early model of double-strand break repair proposed by Resnick (2).

It remains an important question in the migrating D-loop model as to whether the protected distal side or the unprotected proximal side of the gap invades the homolog to initiate

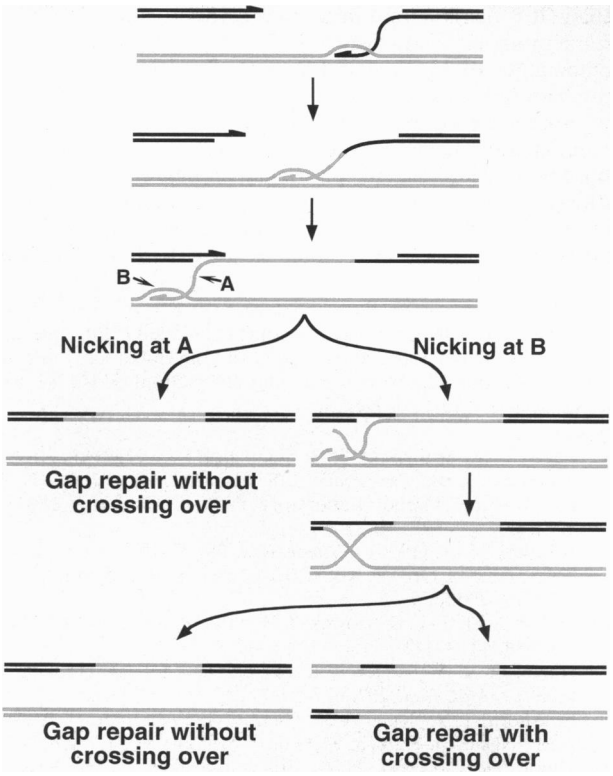

FIG. 4. The migrating D-loop model of recombination. Repair synthesis at the 3′ terminus of the invading strand drives migration of the D-loop into the gap and pushes it past the DNA end on the opposite side. The nascent single strand is displaced during D-loop migration and can thus pair with complementary sequences on the other side of the gap. After second-strand synthesis, an endonuclease cleaves the nascent single strand (arrow A), which results in gap repair without crossing over, or the single-strand of the D-loop (arrow B), which leads to formation of a Holliday junction adjacent to the repaired gap. Resolution of the junction by a Holliday endonuclease leads to crossing over in half of these instances, and thus at most 25% of the total.

repair. It is possible that in *U. maydis* the 3′ end of the DNA on the promoter distal side of the gap is protected, perhaps by components of the recombination machinery, during the search for homology and D-loop formation. It follows then that the promoter-proximal end is not as good a substrate for the recombination machinery and is thus susceptible to degradation and possibly also the mismatch repair apparatus. The difference in the action of the recombination machinery between the two sides could be due to interference by, or interaction with, local directional processes such as replication or transcription.

We thank Dr. Sally Leong for providing the cloned *PYR6* gene. and Drs. Lorraine Symington, Hannah Klein, and Jim Haber for comments on the manuscript. This work was supported by Grant GM42482 from the National Institutes of Health.

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