The relationship between homology length and crossing-over during the repair of a broken chromosome

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RUNNING TITLE: Gene conversion and crossing-over in DSB repair
SUMMARY

Homologous recombination can result in the transfer of genetic information from one DNA molecule to another (gene conversion); these events are often accompanied by a reciprocal exchange between the interacting molecules (crossing-over). This association suggests that the two types of events could be mechanistically related. We have analyzed the repair, by homologous recombination, of a broken chromosome in yeast. We show that gene conversion can be uncoupled from crossing-over when the length of homology of the interacting substrates is below a certain threshold. In addition, a minimal length of homology on each broken chromosomal arm is needed for crossing-over. We also show that the coupling between gene conversion and crossing-over is affected by the mismatch repair system: mutations in the \textit{MSH2} or \textit{MSH6} genes cause an increase in the crossing-over observed for short alleles. Our results provide a mechanism to explain how chromosomal recombinational repair can take place without altering the stability of the genome.
INTRODUCTION

Homologous recombination is a universal process that plays a role in generating diversity during meiosis, and is an important DNA repair mechanism in vegetative cells. Recombination results in the transfer of genetic information from one DNA molecule to a homologous one (gene conversion) and in the reciprocal exchange of DNA fragments between chromosomes (crossing-over). The association between gene conversion and crossing-over events has led to the assumption that they are mechanistically related (1-5; Figure 1). One of the characteristic features of most eukaryotic genomes is the presence of large amounts of repetitive DNA. Reciprocal recombination between dispersed repeats may result in chromosomal aberrations, such as deletions, translocations, etc. that can affect the reproductive fitness of an organism or lead to cancer. Therefore, in order to maintain the genome integrity, crossing-over must be prevented during recombinational repair of DNA lesions involving dispersed repeats.

Double-strand breaks (DSBs) in the DNA of living organisms occur as a consequence of the natural cell metabolism, or can be created by exogenous sources, such as chemical agents or radiation. If left unrepaired, DSBs result in broken chromosomes and cell death (6). Mitotic recombination plays an important role in the repair of this damage. In addition, DSBs are generated during certain developmental processes, such as meiosis (7) and mating-type switch in yeast (8). In different experimental systems, it was found that the level of association between gene conversion and crossing-over varies, from no coupling (e.g.: mating type switch (8-10) or recombination between direct repeats (11)) to a level of association of 70% (5). In two of the currently-held models of recombination, the synthesis-dependent strand annealing (SDSA) model (12) and the DSB repair model (4), recombination is initiated by the creation of a DSB in one of the two participating DNA duplexes (Figure 1). While the mechanism suggested by the SDSA model accounts mainly for gene conversion events, the DSB in the second model leads to the formation of an intermediate, which can be resolved either as a gene conversion event or as a gene conversion accompanied by a crossover (4). The dispersed repeated sequences in the genome can serve as a source of homology to repair broken chromosomes. If the
recombinational repair results in a crossover, however, a deleterious aberration may occur; hence regulating the coupling between gene conversion and crossing-over is crucial to the maintenance of genome integrity. The mechanism that determines the coupling, however, is still unknown. In this paper we dissect the rules that determine the association between gene conversion and crossing over in vegetative cells under conditions in which the initiation of the recombinational event is not limiting.

EXPERIMENTAL PROCEDURES

Strains: All the yeast strains used in this study are isogenic derivatives of strain OI27 (MATa-inc ura3-HOcs-inc ade3::GALHO ade2-1 leu2-3,112 his3-11,15 trpl-1 can1-100) (13). The ura3-HOcs alleles were created by inserting a 39 bp oligonucleotide (14) at the NcoI site of the 5.6 kb BamHI fragment containing the URA3 gene. The different alleles used were subclones of this fragment inserted at a HpaI site within LYS2 sequences in the integrative plasmid pOI5 (TRP1 LYS2 URA3). They were integrated into the yeast chromosome II by a two-step replacement method, selecting first for Trp+ transformants, and then plating on 5-FOA plates to obtain Trp− Ura− derivatives, which have replaced the LYS2 allele on chromosome II by the appropriate lys2:: ura3-HOcs allele. All the strain configurations were checked by Southern blot analysis.

The ura3 fragments present on chromosome II in the strains used are as follows: strain OI70 carries a 5.6 BamHI DNA fragment. MK187 carries a BamHI to SmaI 4.7 kb fragment. MK186 carries a SnaBI to BamHI 4.1 kb insert. OI87 has a SnaBI-HincII 2.9 kb fragment. OI86 and MK201 carry a 1.9 kb and a 1.7 kb Asel-Asp700 fragments, respectively. OI90 carries an Asel-HincII 1.2 kb allele. MK29 has a 1.2 HindIII-HindIII insert. OI92 carries an Asel-HincII 1 kb fragment, OI30 has a PstI-StuI 0.5 kb allele, OI91 carries a 4.1 kb BamHI-AccI fragment, and OI94 a 2.9 Ndel-PvuII allele. In this last strain a PCR fragment carrying 923 bp to the right of the 5.6 kb fragment was added to the cloned ura3 allele before integrating it at the LYS2 locus.
Deletion of the MMR genes were created by one- or two-step transplacement using plasmids pSR211 for \textit{PMS1}, pSR453 for \textit{MLH1}, pSR395 for \textit{MSH2}, pRK366 for \textit{MSH3}, and pRK465 for \textit{MSH6} (15,16). All the configurations were checked by Southern blot analysis.

**Media and Growth Conditions:** \textit{S. cerevisiae} strains were grown at 30\(^\circ\)C. Standard YEP medium (1\% Yeast extract, 2\% Bacto peptone) supplemented with 3\% glycerol (YPEGly), 2\% galactose (YPEGal) or 2\% Dextrose (YPED) was used for nonselective growth. 1.8\% Bacto-Agar was added for solid media.

**Recombination assay:** Single colonies were resuspended in rich YEPGly medium, grown to logarithmic phase, centrifuged and resuspended in YEPGal or YEPD medium. DNA was extracted from samples at timely intervals, and subjected to Southern blot analysis using \textit{URA3} or \textit{LYS2} sequences as probes. Two Southern blot analyses were performed: In the first one the probe can detect the presence of a parental band, or of the broken chromosome. The repaired chromosome appears as a band of the same size as the parental one (see Figure 2B). In the second Southern blot the same DNA samples were digested with \textit{BamHI} or \textit{EcoRI}. This blot allows monitoring the parental allele, which is not cut by these restriction enzymes (e.g.: Figure 3). In all the experiments presented, by 12 hs after transfer to galactose less than 5\% of the DNA retained its parental configuration (was not cut by \textit{BamHI} or \textit{EcoRI}). The blots were also hybridized to a probe carrying the \textit{LEU2} gene. This gene is located on a separate chromosome, and was there unchanged during the course of the experiments, serving as a loading control. The blots were quantified with a Fujix BAS1000 phosphorimager.

For each strain tested, the experiment was repeated at least three times. Survival was assayed by plating at different times during the experiment. Survival of all strains was usually ~90\%. Individual colonies were also subjected to PCR and restriction digestion with \textit{BamHI} or \textit{EcoRI}, to confirm the transfer of information from the donor chromosome in individual colonies. Reciprocal translocations were also monitored by Southern blot analysis from independently obtained colonies grown on galactose.
RESULTS

**Experimental system:** In order to study the way crossing-over is associated to gene conversion, we have developed an assay for DSB-initiated interchromosomal recombination (20, Figure 2A). Haploid strains of the yeast *Saccharomyces cerevisiae* bear two copies of the *URA3* gene; one of them, on chromosome II, carries the recognition site for the yeast HO site-specific endonuclease (8,17) inserted as a short oligonucleotide (*ura3-HOcs*). The second copy, located on another chromosome (V), carries a similar site containing a single-bp mutation that prevents recognition by the endonuclease (*ura3-HOcs-inc*, 17). In addition, the two *ura3* alleles differ at two restriction sites, located to the right and to the left of the *HOcs-inc* insertion; these polymorphisms are used to follow the transfer of information between the chromosomes. In these strains the HO gene is under the transcriptional control of the *GAL1* promoter (18). Upon transfer of the cells to galactose-containing medium, the HO endonuclease is produced at high levels. The enzyme creates a DSB that is repaired by recombination in essentially the whole cell population. The repair is carried out by copying the *HOcs-inc* information, together with the flanking markers, resulting in a gene conversion event. During the repair, the donor chromosome remains unchanged. When the gene conversion event is accompanied by a crossover, a reciprocal translocation between chromosomes II and V is created. The region around the break is completely homologous with the unbroken allele, so as to avoid production of nonhomologous tails, which might affect the recombinational repair (19,20). Since there is no genetic selection for recombination, repair is monitored in the entire cell population (Figure 2A). During the course of the experiment, cell viability remains high (~90%).

**Kinetics of DSB repair:** The kinetics of recombination of a strain carrying 5.6 kb of shared homology between the two *ura3* alleles (OI70) was followed. DNA samples taken at intervals were subjected to two different restriction digestions followed by Southern blot analysis. In the first blot (Figure 2B) we monitored the appearance and disappearance of the DSB. Induction of the HO endonuclease created a chromosomal break, which could be followed by the appearance of two new bands, and the concomitant decrease in intensity of...
the parental band. Repair of the broken chromosome led to the disappearance of the small DSB bands and the creation of a band of the same size as the parental. In order to distinguish between the parental band and the recombinant products the same DNA samples were subjected to a second Southern blot analysis. In this blot the conversion products were recognized by the presence of EcoRI or BamHI sites transferred during the repair of the DSB (data not shown and Figure 3A). Only unbroken chromosomes that have not been repaired by gene conversion give a parental band. Figure 3A shows that most of the population has undergone a gene conversion event by time 10 hr.

The results of the two blots can be integrated in a quantitative way (Figure 2C). As a reference point in each lane we used the intensity measured for either the band created by hybridization to the unbroken chromosome V or to the LEU2 gene on chromosome III, which does not participate in the repair event (the results were identical). Within thirty minutes after transfer, two new bands were seen, representing the broken chromosome arms. One hour after transfer a broken chromosome II could be detected in almost all the cell population. Most of the cells remained with unrepaired chromosomes for another hour, and then the break was repaired by gene conversion in a process that lasted ~2.5 hs. Repair of the broken chromosome led to the disappearance of the small DSB bands and the creation of a band of the same size as the parental. The repair involved the transfer of the BamHI and EcoRI restriction site polymorphism from chromosome V to chromosome II. This was confirmed by a second Southern blot analysis (using these restriction enzymes) of both whole cell populations (Figure 3A), and of 24 individual cells that undergone the repair process. Concomitantly with the gene conversion, there was an accumulation of the bands created by the associated crossing-over, which reached a maximal level of 12.4% (Figure 2B, C), in agreement to the level of association seen in previous mitotic studies (1,5,21,22). Analysis of individual recombinant colonies confirmed the presence of reciprocal translocations between chromosomes II and V (data not shown).

When a strain carrying only 1.2 kb of shared homology between the two *ura3* alleles (OI29) was analyzed, the DSB was repaired in all the cell population by gene conversion (Figure 2B). Surprisingly, however, no crossing-over products could be detected in
Southern blot analyses. By quantitative PCR we have estimated that crossing-over in OI29 occurred only in 0.09% of the cells; this level of crossing-over is lower by two orders-of-magnitude than that seen in the strain with the longer homologous substrates (12.4%). Hence, in both strains the DSB was efficiently repaired by gene conversion, but an uncoupling of crossing-over from gene conversion occurred during recombination in the strain with shorter substrates.

**The relationship between homology size and crossing-over** To further study how homology size affects the coupling between gene conversion and crossing-over we tested a series of yeast strains carrying *ura3-HOcs* alleles of different lengths (Figure 4). Gene conversion was very efficient (>90% of the cells) independently of the length of the shared homology. Even short alleles (e.g.: OI30, 496 bp) were efficiently repaired by gene conversion; in contrast, crossing-over could not be detected by Southern analysis for alleles shorter than 1.7 kb (Figure 4). These results are consistent with the existence of a homology length threshold. In strains that bear alleles sharing homology longer than the threshold, the coupling of crossing-over to gene conversion increased with the overall length of homology between the recombining DNA molecules.

Figure 4 shows that the coupling does not depend only on overall homology length: a minimal region of homology in each broken arm is necessary to obtain crossing-over. For example, strains OI91 and MK186 each bear 4.1 kb of shared homology between the *ura3* alleles. Whereas the first strain, carrying 139 bp of homology on one arm, failed to show any crossing-over (below the level of detection of Southern blot analysis, ~0.5% of the DNA), MK186, with 2.5 kb and 1.6 kb of shared homology on the broken arms, showed 4.5% crossing-over (Figure 4).

**Role of the mismatch repair system:** The mismatch repair (MMR) system has been implicated in the prevention of recombination between diverged sequences in many organisms (15,16,23-25). The MMR gene products thus are good candidates for proteins that may affect the association between gene conversion and crossing-over. We inactivated the MMR system by introducing mutations in the *MSH2*, *MSH3*, *MSH6* and *PMS1* genes in strain OI29 (1.2 kb of shared homology). Whereas repair by gene conversion was not
affected in the quadruple mutant, crossing-over was elevated 24-fold in comparison to the wild type strain: 2.2% of the mutant cells showed a crossover associated with the gene conversion (Figure 5a). Thus MMR proteins play a role in uncoupling crossing-over from gene conversion. Analysis of strains bearing mutations in individual MMR genes revealed that the increased crossing-over can be seen in the absence of the Msh2 or Msh6 proteins, but not when the MSH3 or PMS1 genes are deleted (Figure 5b). Although in the figure the double mutant msh2 msh6 shows slightly higher levels of crossing-over than the individual msh2 or msh6 mutants (2.5% vs. 1.8% and 1.7% of the total DNA), this difference does not seem to be significant, as it was not seen in other Southern blots. We conclude that the MSH2 and MSH6 genes operate in the same pathway. The Msh2 protein was shown to form heterodimers with both Msh3p and Msh6p (26,27). The Msh2-Msh6 heterodimer shows affinity for both base pair mismatches and single nucleotide insertion/deletions (26,28,29). Lately, the Msh2/Msh6 protein complex has been shown to bind directly to Holliday junctions, providing a possible mechanism that can link the mismatch repair system to recombination (30).

DISCUSSION

When a chromosome is broken, homologous sequences present in the genome can be used as templates for its repair by recombination. The transfer of genetic information to the broken DNA molecule during the recombinational repair prevents mutagenesis by restoring information that may have been eliminated at the site of the DNA damage. In addition, repair by recombination ensures the restoration of the original chromosomal structure, when a chromosome is fragmented. On the other hand, recombinational repair of DNA damage using repetitive sequences as templates may result in deleterious chromosomal aberrations, when it results in crossing-over of the interacting molecules. Repetitive DNA sequences are abundant in the genomes of most eukaryotes. Hence, it is important to understand how crossing-over involving dispersed repeats is prevented.
The study of the mechanism of homologous recombination is hampered by the fact that it usually requires the selection of rare genetic products. This selection introduces biases in the type of events analyzed, and precludes an understanding of the processes that take place in the majority of the cells. In order to overcome these problems, we have developed a recombination system that allows to monitor the fate of a cell population that has suffered a chromosomal break, without the need to select for recombinants. The repair of the broken chromosome is carried out in the whole cell population by a gene conversion event. This event may or may not be associated with a crossover. We have systematically investigated the rules that govern this coupling.

We have found that gene conversion can take place efficiently between sequences that share a short stretch of homology (250 bp on each broken arm, OI30). The coupling to crossing-over, in contrast, requires a minimal homology length of about 1.7 kb (MK201). Below this threshold, the level of association decreases by two orders of magnitude. In agreement to these results, the repair of a chromosomal DSB by gene conversion in mouse ES cells carrying alleles that share 1.1 kb of homology was not associated with crossing-over (31). The coupling between gene conversion and crossing-over does not depend only on overall homology length: in order to obtain a crossover, the template allele must share a minimal amount of homology with both broken chromosomal arms. When one of the arms shares only a short stretch of homology, no crossing-over is observed, even when a large region of the other arm is homologous (Figure 4). Among the strains that exhibit crossing-over, we found a correlation between the total homology length and the level of associated crossing-over ($r^2 = 0.75$). This linear relationship, however, is not necessarily the best function to describe the relationship between homology length and associated crossover (see for example (32)). Previously, a similar correlation between the length of the homologous partners and the level of associated crossing-over was seen in a system that monitored spontaneous interchromosomai recombination (5). In a different study the level of crossing-over showed a correlation with the length of the conversion tract (33). These results were interpreted to mean that crossing over requires the formation of a long heteroduplex intermediate; alleles sharing short homology length would be unable to create such an
intermediate and therefore to produce crossovers. Our results agree with those obtained for spontaneous recombination. When monitoring spontaneous recombination, however, the nature and location of the initiating lesion is unknown. In our strains the initiating lesion is of a defined nature (a DSB) and is always at the same location. The results we have obtained thus provide direct evidence that under conditions in which the initiation is not limiting, efficient gene conversion takes place even between very short homologous regions, whereas crossing-over requires a larger minimal length.

We propose the following model to explain the mechanism that determines the coupling between gene conversion and crossing-over in recombination (Figure 6). It incorporates features from both currently held recombination models: the DSB-repair (4), and the SDSA (12,34-37) models (Figure 1). The first model assumes that both broken arms invade the donor DNA molecule, whereas the different versions of the SDSA model propose that recombination is initiated by the invasion by only one of the DNA ends. We have observed that during repair, genetic information is always transferred to both broken arms of chromosome II. In 22/24 cases analyzed, the restriction site polymorphisms located to the right and to the left of the DSB were co-transferred. This observation could be explained by one-ended models, but it would require to make several assumptions. A simpler and more parsimonious model assumes that recombination takes place by the invasion of the two broken arms, either concomitantly or sequentially. We present the following model, which incorporates features from the two types of models discussed, to explain the mechanism that determines the coupling between gene conversion and crossing-over in recombination (Figure 6).

After the formation of the break, a 5’ to 3’ resection of ssDNA exposes 3’ ends (Figure 6a), that can invade the homologous DNA (Figure 6b). At this stage the mismatches present in the short DNA duplex created by the invasion are repaired. This repair is directional: the invading DNA strand is always corrected using the information present in the invaded DNA (Figure 6c). Evidence for a fast, directional repair was obtained for recombination at the MAT locus (38). The repair could be carried out by the 3’ to 5’ proofreading activity of the DNA polymerase itself (19,39) which would then proceed with
DNA synthesis. Recombination can then continue by two different pathways: in one of them, the invading DNA strands complete DNA synthesis, resulting in the formation of a double-Holliday junction (Figure 6d) which can now be resolved, to give either a gene conversion event, or a gene conversion event accompanied by a crossover (Figure 6e,f) (4). In the second pathway, the intermediate shown in Figure 6c is disassembled, and the newly synthesized DNA molecules re-anneal to each other resulting in a gene conversion event that is never accompanied by a crossover (Figure 6e) (12, 34-37). The choice of pathway determines whether there is a coupling between gene conversion and crossing-over (9,11). We suggest that the choice is dependent on the stability of the key intermediate depicted in Figure 6c. Hence, the correlation between homology length and crossing-over association reflects increasing stability of this intermediate, in agreement with results obtained when spontaneous recombination was monitored (5, 33).

The choice between the pathways could be regulated in an active way to allow the uncoupling to crossing-over in response to specific conditions, such as limited homology on either of the invading DNA ends (Figure 4). Specific proteins may recognize the recombination intermediate, and influence its stability. Good candidates for such proteins are the members of the MMR system, which have been shown to play an antirecombinational role in many systems (23). We have shown that the MMR proteins play a role in uncoupling crossing-over from gene conversion: mutations in the MSH2 or MSH6 genes allow the detection of crossing-over in strains bearing short homologous alleles. Our results suggest that the Msh2-Msh6 heterodimer may be responsible for preventing crossing-over after strand invasion, either by recognizing mismatches in the newly formed heteroduplex, or by destabilizing the Holliday junction. Consistent with our results, the conversion tracts were found in a recent study to be larger in MMR- mutants, compared to the wild type (25). The presence of bound MMR proteins may destabilize the intermediate depicted in Figure 6C, for example, by affecting the direction of branch migration, leading to the resolution of the intermediate (15). The annealing of the newly synthesized strands would result in a gene conversion event not associated with crossing-over (Figure 6E). In the absence of the Msh2 or Msh6 proteins, the intermediate is not destabilized, resulting in an increased level of
crossing-over (Figure 5). It should be noted that in contrast to their effect on crossing-over, our results imply that the MMR proteins are not required in order to carry out gene conversion events during mitotic recombinational repair of DSBs (40). Msh2p and Msh3p, however, do seem to play a role in DSB-induced gene conversion, when nonhomologous genetic information has to be removed from the broken chromosomal ends (13,19,20).

The ability of yeast cells to find homology and use it to repair the break is essential for their survival. The presence of repetitive sequences in the genomes of most eukaryotes presents a challenge: if a chromosome breaks close to a member of a repetitive sequence family, recombinational repair may produce chromosomal aberrations. The mechanism that allows the cells to deal with this challenge involves uncoupling gene conversion from crossing-over when repeated sequences are identified through their limited homology length and/or sequence divergence.

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FIGURE LEGENDS

Figure 1: Schematic representation of two of the currently held models of recombination. 

A: The Double-strand break repair (DSBR) model (4). Both broken arms are resected (a) and invade the homologous sequence (b). After DNA synthesis (c) and ligation (d), a double Holliday Junction is formed, which can be resolved to give noncrossover (e) or crossover (f) products. Repair of heteroduplex DNA (so that now both strands carry gray information) creates a gene conversion event in both cases. 

B: The simplest version of the Synthesis-Dependent Strand Annealing (SDSA) model (12,34) proposes that after resection (a), one arm invades the homologous sequence (b), and copies its content through a “migrating bubble” (c). Annealing back to the other arm of the broken chromosome creates heteroduplex DNA that can lead to gene conversion (transfer of gray information in this case). This mechanism cannot generate reciprocal crossing-over events. Variations of this model that allow some crossing-over also exist (see ref. 19).

Figure 2: A: Schematic representation of the experimental system used in this study. Open rectangles represent the ura3 alleles on chromosomes II and V. Striped rectangles represent LYS2 sequences on chromosome II. A stippled box represents the HOcs; black boxes represent the inactive HOcs-inc, which also shows two lines, representing polymorphic sites recognized by the EcoRI and BamHI restriction enzymes. Transfer of the cells to galactose-containing medium induces the production of HO endonuclease, which recognizes the HOcs and creates a DSB. This break is repaired by a gene conversion event that transfers the HOcs-inc to chromosome II thus preventing further recognition by the HO endonuclease. An associated reciprocal exchange creates a translocation between chromosomes II and V. 

B: Southern blot analysis of DNA extracted from strain OI70 at intervals. Single colonies were resuspended in rich YEPGly medium, grown to logarithmic phase, centrifuged and resuspended in YEPGal or YEPD medium. DNA was extracted from samples at various times, and subjected to Southern blot analysis. The DNA was digested with SphI and XbaI and probed with a fragment of chromosome V carrying the URA3 gene. The reference band is the unbroken chromosome V, which is used to estimate the amount of DNA in each lane.
A schematic map of the bands seen in the Southern blot is given below. An additional Southern blot analysis demonstrates the transfer of the BamHI and EcoRI sites from chromosome V. **C:** Kinetics of double-strand-break repair. The Southern blot shown in **B** was scanned; the intensity of each band is presented after normalization.

**Figure 3:** The broken chromosome is repaired by gene conversion. **A:** Strain OI70. Lanes 1-3 show DNA from cells taken at 0, 2, or 10 hs after transfer to galactose-containing medium. The DNA was digested with SphI and XbaI and probed with a fragment of chromosome V carrying the URA3 gene. The reference band comes from the unbroken chromosome V, which is used to estimate the amount of DNA in each lane. Two hours after transfer most cells show broken chromosomes. Ten hours after transfer the DSB bands disappeared, and the parental band reappeared (together with the products of translocation). Lanes 4,5 show the same DNA samples digested with NcoI and BamHI. The presence of the BamHI transferred during the gene conversion can be monitored (a similar analysis was carried out to detect the transfer of the EcoRI site). The parental band seen ten hours after transfer accounts for 1.2% of the DNA and represents the maximal estimate of repair events that did not involve the transfer of the BamHI site. Thus, ~99% of the cells have repaired the DSB by gene conversion 10 hs after transfer to galactose. **B:** Strain OI29. Similar to **A,** except that the probe is a fragment from the LYS2 gene on chromosome II. The DNA was digested with BglII (lanes 1-3) or BglII and BamHI (lanes 4,5). The reference bands represent adjacent fragments on chromosome II. The parental band left 10 hs after transfer represent 0.9% of the DNA, implying, again, that 99% of the cells of OI29 repaired the broken chromosome by gene conversion. No bands indicative of reciprocal recombination could be detected (asterisks).

**Figure 4:** Dependence of crossing-over on homology length. A schematic representation of the ure3-HOcs alleles inserted at chromosome II is given. For each strain, the length of the allele is depicted as a rectangle, with a black box representing the HOcs.; the numbers at both sides of the box give the length (in bp) of the homologous region at each broken arm. b. d.: below detection. The lower detection limit of our Southern blot analyses is approximately 0.5% of the population. Evidence that the repair was carried out by gene conversion was obtained by monitoring the presence of EcoRI and BamHI sites transferred from
chromosome V. This was done either by Southern blot analysis or by PCR of 24 individual colonies followed by restriction digestion, or by direct sequencing. According to this analysis, the repair of the DSB was carried out by gene conversion in >95% of the population in all the strains analyzed.

**Figure 5:** **A.** Southern blot analysis of DNA extracted from the MMR+ strain OI29 (1.2 kb \textit{ura3} alleles) and a quadruple MMR- isogenic strain, at various times. The DNA was digested with \textit{Hind}III and \textit{Xba}I and probed with a fragment of chromosome \textit{II} carrying the \textit{LYS2} gene. The reference band is a neighboring invariant DNA restriction fragment, which is used to estimate the amount of DNA in each lane. The two bands representing crossing-over (2.2% of the DNA in the last timepoint) appear of different intensity due to different level of hybridization to the probe. The \textit{LYS2} probe is indicated as a black box with homology only to chromosome \textit{II}. **B.** Crossing over is elevated in \textit{msh2} and \textit{msh6} mutants. DNA of OI29 and different MMR- derivatives grown overnight on YEPGal were digested with \textit{Hind}III and \textit{Xba}I and probed with a fragment of the \textit{URA3} gene. The reference band is the uncut donor allele. (similar results were seen when a \textit{LEU2} probe was used).

**Figure 6:** A model for DSB-initiated recombination: After the creation of a DSB the ends are resected (\textbf{A}) and invade the homologous DNA sequence (\textbf{B}). The invading sequences are subjected to a directed mismatch repair immediately with the invasion, and DNA synthesis proceeds (\textbf{C}). If this intermediate is stable, DNA synthesis is completed, and a double Holliday Junction is created (\textbf{D}). If the intermediate is not stable the invading ends re-anneal to each other giving rise to a gene conversion event (12,34-37) (\textbf{E}). The double Holliday Junction can also be resolved to give either a gene conversion event or a gene conversion accompanied by a crossover (4).
Figure 1

Figure 2A
Figure 2B
Figure 2C

Figure 3
| Strain | Total Homology Length | % Associated crossing-over |
|--------|-----------------------|--------------------------|
| OI70   | 4,020                 | 5,653 bp                 | 12.4 ± 1.1 |
| MK187  | 4,020                 | 4,714 bp                 | 4.7 ± 0.4 |
| MK186  | 2,499                 | 4,132 bp                 | 4.5 ± 0.5 |
| OI87   | 2,499                 | 2,954 bp                 | 3.2 ± 0.8 |
| OI86   | 973                   | 1,894 bp                 | 1.6 ± 0.9 |
| MK201  | 763                   | 1,684 bp                 | 1.8 ± 0.4 |
| OI90   | 763                   | 1,218 bp                 | b. d.      |
| OI29   | 763                   | 1,202 bp                 | b. d.      |
| OI92   | 763                   | 1,082 bp                 | b. d.      |
| OI30   | 247                   | 496 bp                   | b. d.      |
| OI91   | 4,020                 | 4,159 bp                 | b. d.      |
| OI94   | 313                   | 2,869 bp                 | b. d.      |

Figure 4
Figure 5A
Figure 5B

Figure 6
Transfer to galactose → HO endonuclease → Double-strand break → Gene conversion → Gene conversion with crossing over
Fraction of DNA molecules vs. time (hs)

- Parental
- DSB
- Gene Conversion
- GC + Associated Crossing-Over
| Strain | Total Homology Length | % Associated crossing-over |
|--------|----------------------|---------------------------|
| OI70   | 5,653 bp             | 12.4 ± 1.1                |
| MK187  | 4,714 bp             | 4.7 ± 0.4                 |
| MK186  | 4,132 bp             | 4.5 ± 0.5                 |
| OI87   | 2,954 bp             | 3.2 ± 0.8                 |
| OI86   | 1,894 bp             | 1.6 ± 0.9                 |
| MK201  | 1,684 bp             | 1.8 ± 0.4                 |
| OI90   | 1,218 bp             | b. d.                     |
| OI29   | 1,202 bp             | b. d.                     |
| OI92   | 1,082 bp             | b. d.                     |
| OI30   | 496 bp               | b. d.                     |
| OI91   | 4,159 bp             | b. d.                     |
| OI94   | 2,869 bp             | b. d.                     |
The relationship between homology length and crossing-over during the repair of a broken chromosome
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