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Evidence for short-patch mismatch repair in *Saccharomyces cerevisiae*

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Recombination events between non-identical sequences most often involve heteroduplex DNA intermediates that are subjected to mismatch repair. The well-characterized long-patch mismatch repair process, controlled in eukaryotes by bacterial MutS and MutL orthologs, is the major system involved in repair of mispaired bases. Here we present evidence for an alternative short-patch mismatch repair pathway that operates on a broad spectrum of mismatches. In *msh2* mutants lacking the long-patch repair system, sequence analysis of recombination tracts resulting from exchanges between similar but non-identical (homeologous) parental DNAs showed the occurrence of short-patch repair events that can involve <12 nucleotides. Such events were detected both in mitotic and in meiotic recombinants. Confirming the existence of a distinct short-patch repair activity, we found in a recombination assay involving homologous alleles that closely spaced mismatches are repaired independently with high efficiency in cells lacking *MSH2* or *PMS1*. We show that this activity does not depend on genes required for nucleotide excision repair and thus differs from the short-patch mismatch repair described in *Schizosaccharomyces pombe*.

Keywords: gene conversion/mismatch repair/ *Saccharomyces cerevisiae*/short-patch mismatch repair

Introduction

The long-patch mismatch repair (MMR) process is involved in elimination of replicative errors, in heteroduplex DNA repair during recombination and in prevention of recombination between divergent sequences. In *Escherichia coli*, it is controlled by MutS, MutL and MutH, whereas in eukaryotes several MutS and MutL homologs have been described (for reviews see Kolodner, 1996; Modrich and Lahue, 1996). The biological importance of this system is evidenced by the severe mutator phenotype of the mutants, and in mammals defects in MMR result in cancer predisposition (for reviews see Modrich and Lahue, 1996; Peltomaki and Vasen, 1997).

However, the long-patch system is not the only mechanism that is able to correct mispaired bases. Besides systems specialized in the repair of specific mismatches, the existence of secondary generalized MMR processes has been suggested by a number of genetic, molecular and enzymic studies (reviewed in Kolodner, 1996). The existence of an MMR process that can at least partially substitute for, or complement the long-patch mechanism should also be a factor of genetic stability. Indeed, Fleck *et al*. (1999) have recently demonstrated in the yeast *Schizosaccharomyces pombe* the presence of a short-patch MMR activity involved in recombination and mutagenesis. This system is independent of the long-patch MMR pathway and is controlled by nucleotide excision repair (NER) genes. It corrects not only C/C mismatches, which are poorly recognized by the long-patch MMR process, but also other types of mismatch.

In this study, we present molecular and genetic evidence for the existence of short-patch MMR activity in *Saccharomyces cerevisiae*. It was revealed by DNA sequence analysis of tracts generated by recombination between parental alleles differing by ~10% of silent mutations and by two non-silent mutations allowing prototroph selection. The sequence differences allow the localization of repair events that occurred on the DNA heteroduplex intermediates. In many cases, and especially in mutants deficient in long-patch MMR, we observed 'patched' sequences best explained by short-patch repair events. If this interpretation were right, we reasoned that this activity should repair independently two closely spaced mismatches. Homologous recombination assays show that this is indeed the case and that this short-patch MMR activity is not dependent on NER genes, in contrast to that described in *S.pombe*.

Results

Genetic system

To construct a genetic system allowing the study of heteroallelic recombination between homeologous alleles, the *ARG4* gene from *Saccharomyces douglasii* (*ARG4D*) was integrated into the genome of *S.cerevisiae*. The two *ARG4C* and *ARG4D* genes, shown in Figure 1A, differ by ~8% of substitutions in the open reading frame (ORF) and 20% of base substitutions and small insertions/deletions in the intergenic regions (Adjiri *et al*., 1994). A 2.1 kb *Hpa*I fragment containing *arg4D* mutated at the *Bgl*II restriction site (*arg4D-Bg*, a 4 bp insertion) was integrated into the *S.cerevisiae* genome. Two types of strains were used in this study (Figure 1B): allelic diploids and 'ectopic cells' containing the alleles in ectopic locations. In allelic diploids, both alleles are located at the natural position on chromosome VIII. One of the chromosomes carries the *Hpa*I segment of *S.douglasii* bearing *arg4D-Bg* while the other allele, from *S.cerevisiae*, is mutated at the *Eco*RV site (*arg4C-RV*, a 2 bp deletion). The cells containing the ectopic genes have the *arg4C-RV* allele at its natural position on chromosome VIII and the *arg4D-Bg* allele integrated into chromosome V. The two genes are in

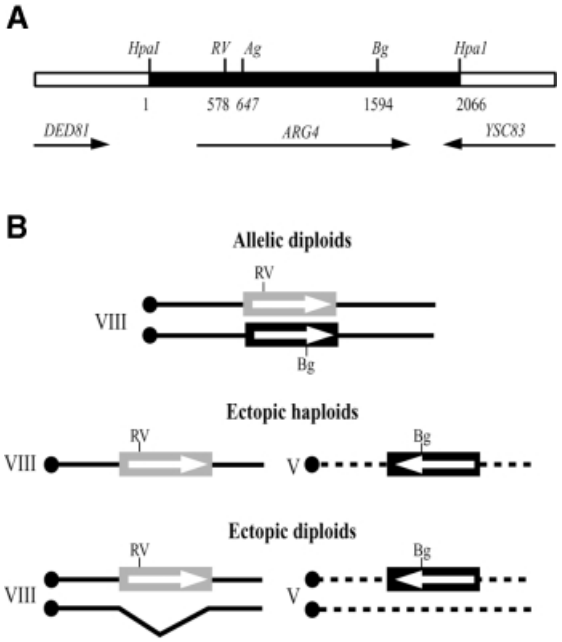

Fig. 1. Structure of the *ARG4* chromosomal region and schematic representation of the different genetic systems used. (**A**) The organization of this region is similar in *S.cerevisiae* and *S.douglasii*. The *ARG4* ORFs differ by 8% of base substitutions, and the intergenic regions by ~20% of substitutions and small insertions/deletions. The *Hpa*I segment, shown in black, is the region substituted in *S.cerevisiae* by the corresponding fragment of *S.douglasii*. Frameshift mutations were introduced at the *Eco*RV (*RV*, –2 bp), the *Age*I (*Ag*, +4 bp) or the *Bgl*II site (*Bg*, +4 bp). Coordinates are given with respect to the *S.cerevisiae* sequence, number 1 being the first nucleotide of the *Hpa*I site. (**B**) Diagram of the genetic constructs involving *RV* and *Bg*.

opposite orientation with respect to the centromeres. In this configuration, the formation of a wild-type gene by a reciprocal exchange between the mutated sites would be lethal, due to the formation of acentric and dicentric chromosomes. Ectopic diploids were obtained by crossing ectopic haploids with cells bearing a deletion of the *Hpa*I–*ARG4*–*Hpa*I segment, so that only ectopic alleles can interact to form an *ARG*$^+$ gene.

Rationale of the molecular analyses

Owing to the presence of a large number of silent polymorphic sites that differentiate both alleles, our genetic system allows the structure of recombinant tracts to be characterized precisely. It permits it to be asked whether the absence of the long-patch MMR uncovers another repair mechanism, which may confer tract profiles different from those found in wild-type cells.

We chose to study ectopic recombinants in order to be able to amplify one of the alleles specifically. Our analyses were focused on the *ARG*$^+$ alleles located on chromosome VIII and not at the ectopic position (see Materials and methods). Among the mitotic ARG$^+$ recombinants, the wild-type allele was found to be equally distributed between the two loci. After meiosis, >90% of the *ARG*$^+$ alleles were on chromosome VIII, indicating a preferential conversion of the *RV* mutated site. This difference between mitotic and meiotic recombinants probably reflects, as for allelic diploids, a polarity of meiotic gene conversion due to initiation by a double-strand break in the promotor region of *ARG4* (Nicolas *et al*., 1989). The polarity results in a much higher frequency of conversion at the *Eco*RV than at the *Bgl*II site. Because the system excludes the recovery of ARG$^+$ clones resulting from cross-overs between the mutated sites, we know that we analyzed the recipient allele converted by the ectopic donor carrying the *Eco*RV$^+$ site. Assuming that the event occurred through formation of a heteroduplex DNA, the heteroduplex region must have covered the mutated *RV* site, forming an *RV/Eco*RV$^+$ mismatch, and is expected to most often not reach the 1 kb distant *Bgl*II$^+$ site. If the *RV/Eco*RV$^+$ mismatch is repaired to *Eco*RV$^+$/*Eco*RV$^+$, both strands of the heteroduplex encode a wild-type *ARG4*$^+$ gene or its complementary information and are recovered in daughter cells on selective medium. If this mismatch remains unrepaired before replication, only one strand encodes *ARG4*$^+$ and is recovered in the colony.

In wild-type cells, the *RV/Eco*RV$^+$ mismatch is expected to be repaired, leading to the rescue of both strands of the heteroduplex. On the contrary, in *msh2* mutants, this mismatch is expected often to remain unrepaired, and the progeny of only one strand will be recovered. However, data in the literature indicate that in *msh2* cells, both strands of a heteroduplex appeared to be recovered in a significant proportion of cases. Notably, an analysis of meiotic segregation of the *arg4-RV* heterozygous mutation in *msh2* cells showed that non-Mendelian events include 30–50% of *Eco*RV$^+$/*Eco*RV$^+$ or *RV/RV* convertants (Alani *et al*., 1994). These could be generated either through double-strand gap repair (Orr-Weaver *et al*., 1981; Szostak *et al*., 1983), or by the action of an alternative MMR process. We reasoned that in the latter case, repair of the *RV/Eco*RV$^+$ mismatch would not necessarily be concerted with repair of the many mismatches present on the heteroduplex DNA. Mismatches left unrepaired would segregate upon replication and the ARG$^+$ colony should in fact be sectored for silent mutations. The sequence profile of *ARG4* amplified from the whole colony should then indicate the presence of two different DNAs, uncovered by superimposed bases corresponding to the sites of unrepaired mismatches. The finding of such a sequence profile would demonstrate the involvement of an intermediary heteroduplex and would allow detection of an alternative MMR process.

We found such cases and an example is shown in Figure 2. This recombinant was selected as an ARG$^+$ mitotic colony from *msh2* ectopic cells. On the first line is shown the sequence of a 40 bp tract of *ARG4* amplified from the whole original clone. The presence of overlapping bases (red arrows) at two sites reveals an intermediary heteroduplex containing two unrepaired mismatches that were resolved by replication, as evidenced by the two different sequences found in subclones (middle and lower sequences). In between these two unrepaired mismatches, the two adjacent C/T and A/C mismatches (or G/A and T/G, green arrow) were co-repaired and the excision tract was at most 20 nucleotides long, indicating a short-patch repair event. Another mismatch, T/C (or A/G) at the right, was also repaired. Examination of the whole sequence allows the organization of the patches on each strand to be determined and the original recombinant molecule to be visualized.

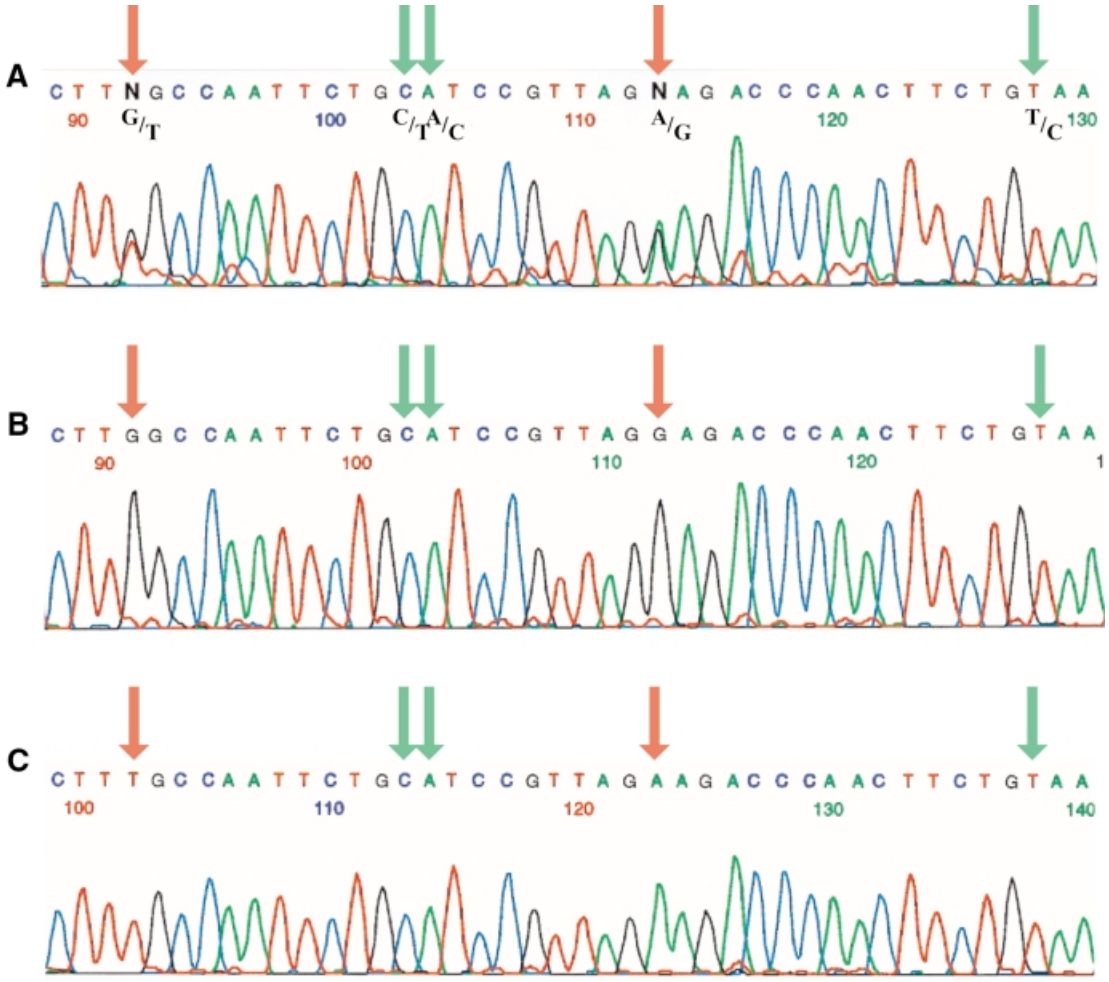

Fig. 2. Sequence profile uncovering short-patch repair. (**A**) A partial sequence of *ARG4* amplified from a single recombinant. The arrows point to sequence differences between the parental alleles, which are indicated underneath. Red arrows correspond to sites where the two parental bases are superimposed indicating an absence of MMR and uncovering a mixed clone. Green arrows indicate sites where only one of the parental bases is found. (**B** and **C**) The sequence of two subclones derived from the initial mixed clone.

Molecular evidence for the existence of a short-patch MMR process

Evidence for short-patch repair tracts was first obtained from mitotic cells. To investigate whether similar results are found after meiosis, we sequenced a few meiotic recombinants as well, which also revealed a short-patch repair activity. However, due to the small number of meiotic tracts analyzed in *msh2* cells, this sample should not be considered as representative of the recombinant cell population.

In Figure 3 are shown the length and position of the mitotic and meiotic recombinant tracts, as indicated in the legend. Their exact size is unknown since their borders may be located anywhere between two polymorphic sites. Therefore, we have indicated the minimal and maximal size of the entire rearranged region and that of the intervening tracts.

For wild-type cells (Figure 3A), out of nine recombination tracts from spontaneous mitotic events (cases 1–9), one is discontinuous, with a short intervening tract of *S.cerevisiae* covering four polymorphic sites. Among the five meiotic tracts (nos 10–15), two are discontinuous (nos 14 and 15) and include five and one polymorphic sites, respectively.

In the *msh2* context (Figure 3B), among the 12 mitotic tracts (cases 1–12), five are discontinuous (nos 8–12), and among the three meiotic ones, two are discontinuous (nos 14 and 15). Case 15 was screened as a monosporic sectored ARG^+/arg^- colony derived from allelic diploids. Allelic diploids, rather than ectopic ones, were used to screen ARG^+/arg^- sectors because the rates of recombination between the homeologous genes are much higher in allelic cells (our unpublished results), allowing easier screening of sectors by replica-plating of unselected monosporic clones.

The sequence of both strands of the intermediary recombination structure could be determined in two mitotic (nos 11 and 12) and two meiotic (nos 14 and 15) cases. As expected, recombinants 11, 12 and 14, selected as ARG^+, contained the *Eco*RV^+ site on both strands of the intermediary heteroduplex DNA, while in case 15 a mismatch at this site persisted. These four sectored clones are not due to an artifact where two independent colonies would have been analyzed. Indeed, for three of them, the

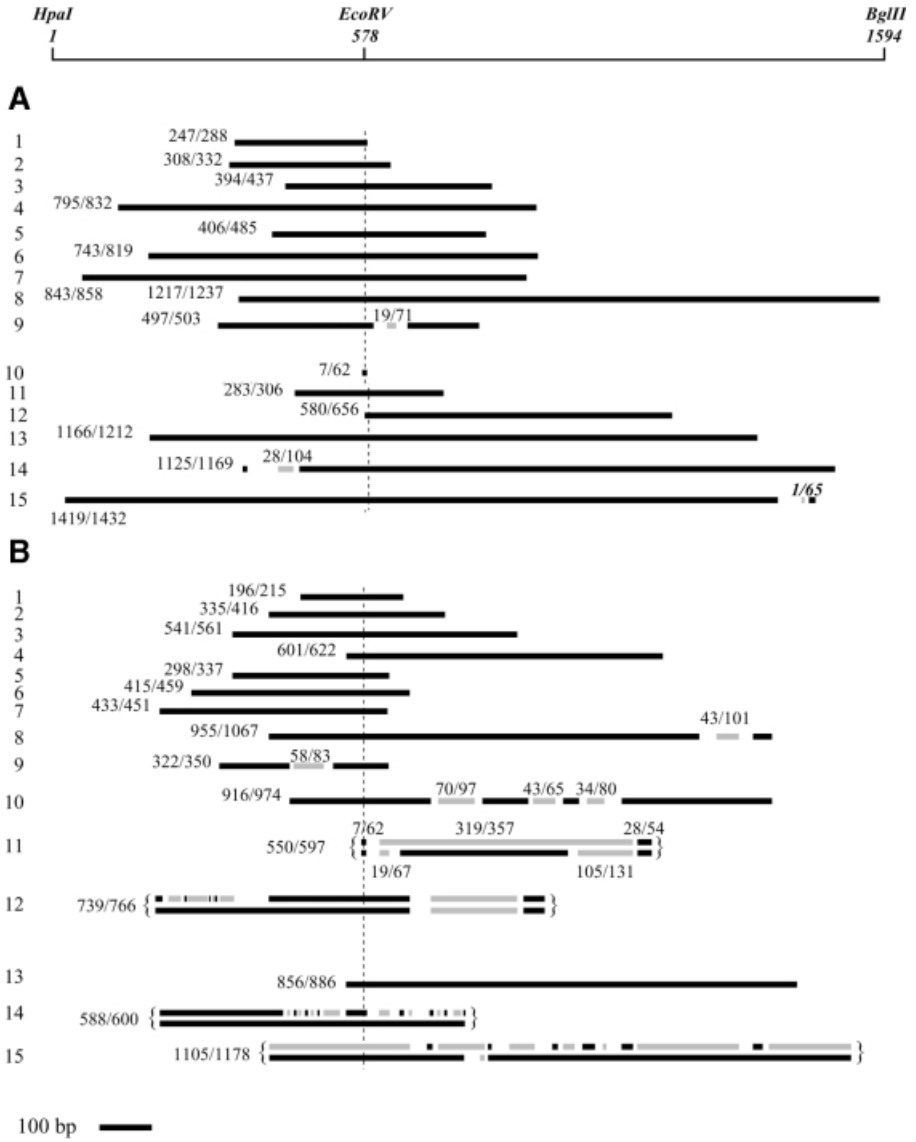

Fig. 3. Length, position and structure of recombinant tracts. Donor DNA is in black and recipient DNA in gray. The maximal and minimal size (in base pairs) of the whole rearranged region is indicated on the left, and that of the internal rearrangements above the sequence. Gaps are regions of identity between the alternate *S.cerevisiae* and *S.douglasii* tracts. (**A**) Wild-type cells. Tracts nos 1–9 were obtained from mitotic events in ectopic haploids (FF18248). Tracts nos 10–15 were obtained from meiotic events in ectopic diploids (FF181387). (**B**) *msh2* cells. The 12 first cases correspond to mitotic events in ectopic haploids (FF181378); the three others are meiotic events in ectopic cells (nos 13 and 14, FF181396) and in allelic cells (no. 15, Ec182). For recombinants 11, 12, 14 and 15, both strands of the original recombinant were recovered and sequenced. No. 15 was selected as an ARG^+/arg^- sectored colony. Cases nos 12, 14 and 15 are detailed in Figure 4.

recombinant tract on each strand starts and ends at the same position. In the fourth case (no. 15), both terminal regions of the intermediary heteroduplex DNA appear as unrepaired. However, five unlinked heterozygous markers segregated in this cross and an identical genotype was found for all ARG^+ and arg^- subclones tested. The probability for independent events to yield the same reassortment of markers is very low (3%), which strongly argues that the initial colony derived from a single spore.

For these four recombinants, the recovery and sequencing of both strands of the original duplex allow localization of PMS tracts (unprocessed heteroduplex DNA detected as a post-meiotic or post-mitotic segregant), conversion tracts (donor sequence on both strands) and restoration tracts (recipient sequence on both strands). In several cases, the patched structure of the tract would be very difficult to interpret differently than by short-patch MMR, at least for regions consisting of short alternate patches of PMS, conversion or restoration. Recombinants 12, 14 and 15 are detailed in Figure 4. They display regions of alternate short-patch PMS and conversion tracts, presumably formed by independent MMR events with preferential repair of the recipient DNA strand. Indeed, in these three recombinants a single restoration event was detected (no. 15), and mismatches were most often repaired by excision on the recipient strand.

In 14 repair events, a single mismatch was involved (11 cases shown in Figure 4 and three additional cases from Figure 3). Three transversions and 11 transitions were generated: two T→A and one C→A; three G→A, five A→G, two C→T and one T→C. Only G→C and C→G transversions generated by repair of G/G or C/C mis-

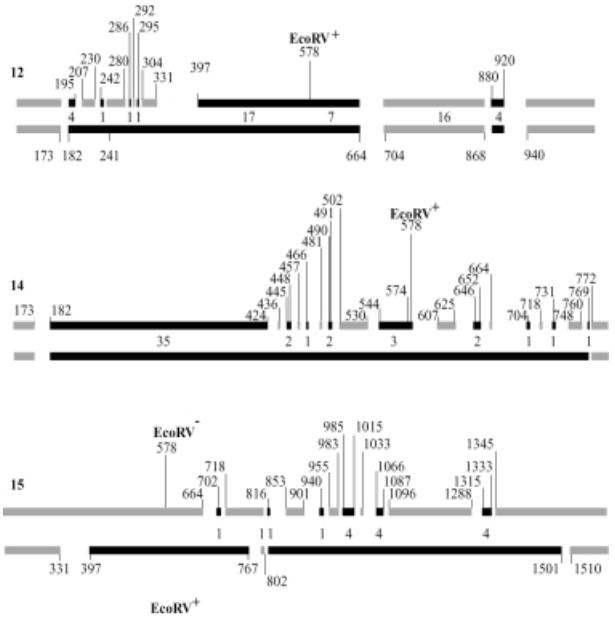

Fig. 4. Detailed structure of recombinant tracts. These three recombinants were recovered in *msh2* cells. They correspond to one mitotic and two meiotic events (case nos 12, 14 and 15 in Figure 3B). The numbers are the coordinates, as defined in the legend of Figure 1, and indicate the position of the first and last polymorphic site that delimits rearranged segments. Numbers between the two strands of each duplex are the numbers of mismatches that were repaired in the conversion and restoration tracts.

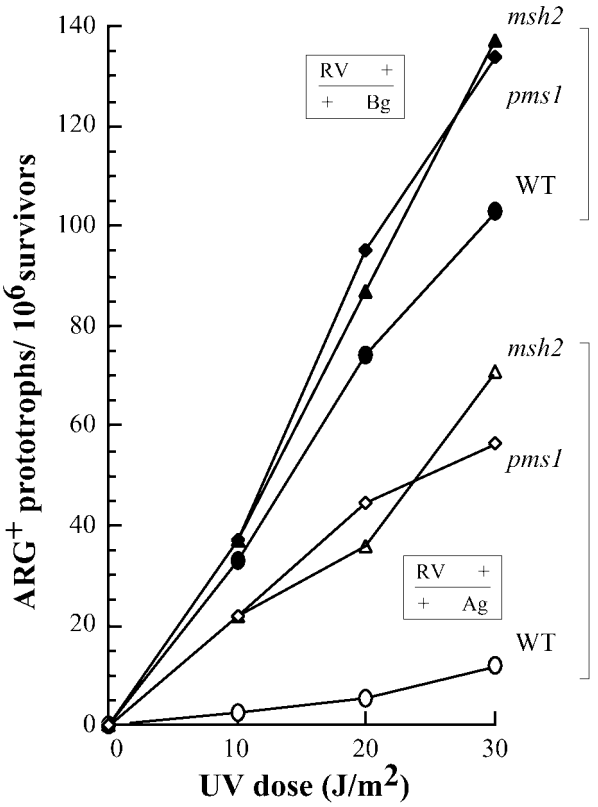

Fig. 5. UV induction of ARG+ recombinants in wild-type and *msh2* cells, heteroallelic for distant or close mutations in *ARG4*. Open symbols: heteroallelic cells with closely spaced mutations (wild type, Ec153; *msh2*, Ec144000; *pms1*, FF181617); closed symbols: heteroallelic cells with distant mutations (wild type, Ec160; *msh2*, FF181379; *pms1*, FF181614).

matches are not found in our sample, which does not mean that these mismatches are not repairable. Since each individual substitution can be generated by repair of two alternative mismatches, we cannot determine which one was involved. Besides G/G and C/C, all other types of mismatch could have been involved in the observed repair events. Cases where adjacent mismatches were co-repaired do not allow the determination of which of them was the initial target since the tract could have resulted from a single or from several independent repair events. The maximal size of an excision tract corresponds to the distance between unrepaired flanking mismatches. For events that involved a single mismatch, this size ranges from 11 to 53 nucleotides. Thus, the excision tracts are very short.

The absence of any significant difference concerning the short-patch repair events among the cases recovered from mitotic and meiotic cells suggests that the processes involved in their formation are similar during mitosis and meiosis.

Differential effect of msh2 and pms1 mutations on recombination between homologous alleles carrying close or distant mutations

Recombination in heteroallelic cells can result in the formation of a wild-type gene either by a reciprocal exchange between the mutations or by non-reciprocal transfer covering one mutation. In both mitotic and meiotic yeast cells, the majority of the events are due to conversion resulting from heteroduplex DNA formation followed by MMR (Roman, 1956; Fogel *et al*., 1981; Petes *et al*., 1991). If two sites are close together, the probability of them being included in the same heteroduplex and being repaired by a single long-patch excision tract is high. Such events will not form wild-type genes. However, if a short-patch repair process is able to act independently on these sites, a wild-type gene can be generated. Therefore, mutations like *msh2* or *pms1*, which eliminate the long-patch repair, may uncover a short-patch repair mechanism by increasing prototroph frequency. Such a hyper-recombination effect is not expected if the mutated sites are located far apart, since they would rarely be included together in a heteroduplex DNA.

We tested the effect of *msh2* and *pms1* mutations on recombination in homologous heteroallelic cells containing mutations that are either closely spaced (*RV* and *Ag*, 69 bp apart) or distant (*RV* and *Bg*, 1016 bp apart). The *RV* mutation is a 2 bp deletion and the *Bg* and *Ag* mutations are each a 4 bp insertion. The alleles are located in an ectopic position, the *Ag* or the *Bg* allele being integrated on chromosome V. Mitotic recombination was measured in haploids, and meiotic recombination in diploids obtained by crossing the same haploids with cells deleted for the *ARG4* locus. UV-induced and spontaneous rates of mitotic recombination were measured. As seen in Figure 5, both the *msh2* and the *pms1* mutations increased the UV induction of intragenic ARG+ recombinants as compared with the wild-type response. This increase is modest when the *arg4* mutations are far apart (1.1–1.3 according to the

Table I. Mitotic and meiotic rates of recombination between close or distant mutations

RV-Bg			*RV-Age*		
(A)					
WT	*msh2*	*pms1*	WT	*msh2*	*pms1*
(FF181246)	(FF181379)	(FF181614)	(Ec153)	(Ec144000)	(FF181617)
3 (1)	4.9 (1.6)	3.2 (1.1)	0.3 (1)	2.2 (7.3)	1.9 (6.3)
msh2	*msh2-rad1*	*msh2-rad14*	*msh2*	*msh2-rad1*	*msh2-rad14*
(FF181379)	(Ec168)	(Ec167)	(Ec144000)	(Ec144001)	(Ec144003)
4.9 (1)	3.5 (0.7)	5.8 (1.2)	2.2 (1)	2.2 (1)	2.8 (1.3)
pms1	*pms1-rad1*	*pms1- rad14*	*pms1*	*pms1-rad1*	*pms1- rad14*
(181614)	(FF181615)	(FF181616)	(FF181617)	(FF181619)	(FF181620)
3.2 (1)	4.6 (1.4)	4.4 (1.6)	1.9 (1)	1.6 (0.8)	1.7 (0.9)
WT	*rad1*	*rad14*	WT	*rad1*	*rad14*
(FF181246)	(Ec160)	(Ec166)	(Ec153)	(Ec153000)	(Ec153002)
3 (1)	2.9 (1)	3.2 (1)	0.3 (1)	0.36 (1.2)	0.27 (0.9)
(B)					
WT	*msh2*		WT	*msh2*	
(Ec183)	(Ec184)		(Ec185)	(Ec186)	
1.2 (1)	1 (0.8)		0.2 (1)	0.75 (3.8)	

(A) Mitotic rates for 10^7 divisions, between *arg4-RV* and *arg4-Bg* (columns 1–3) or between *arg4-RV* and *arg4-Ag* (columns 4–6). In parentheses is the increase over the wild-type, *msh2* or *pms1* rates. (B) Meiotic rates for 10^3 asci.

dose) and was much more pronounced when the sites are close together (6–9.5). Comparable results were obtained for spontaneous mitotic rates (Table I): increases over the wild-type levels in long-patch MMR defective mutants were found to be 1.1 (*pms1*) and 1.6 (*msh2*) for cells with distant mutated sites, while for cells with close sites the increase was 6.3 (*pms1*) and 7.3 (*msh2*). In meiotic cells (Table I), the *msh2* mutation exhibits a hyper-recombination effect (four times) only when the alleles are located close together

These results would be expected if a short-patch repair activity, uncovered in the *msh2* or *pms1* mutants, acts independently on mismatches separated by 69 bp.

The short-patch repair is not controlled by genes required for NER

In *S.pombe*, a short-patch MMR activity independent of *MSH2* and *PMS1* was shown to be controlled by NER genes (Fleck *et al*., 1999). *msh2* and *pms1* mutations display a stimulation effect on recombination between closely spaced alleles, largely abolished by mutations in *SWI10*, *RHP14* and *RAD16* genes involved in an NER pathway. It led us to ask whether the hyper-recombination effect of *msh2* and *pms1* that we observed is also dependent on the NER process. We therefore studied the effects of mutations in *RAD14* or *RAD1*, two genes essential for the removal of UV-induced dimers (Unrau *et al*., 1971; Reynolds and Friedberg, 1981). *RAD14* and *RAD1* are the respective *S.cerevisiae* orthologs of the *S.pombe RHP14* and *RAD16* genes (Carr *et al*., 1994; Fleck *et al*., 1999).

As seen in Table I, neither *rad1* nor *rad14* mutation decreases significantly the elevated spontaneous recombination rates between the close *RV* and *Ag* sites observed in *msh2* or *pms1* mutants. Therefore, these NER genes do not control the short-patch MMR responsible for the differential hyper-recombination effect of *pms1* or *msh2* described above.

Discussion

In this study, we provide evidence for a short-patch MMR activity in *S.cerevisiae*, independent of the long-patch MMR system and of the NER pathway. It was first uncovered by sequence analysis of intragenic recombinant tracts that involved parental homeologous *arg4* alleles, and further supported by a recombination assay involving heteroalleles with either distant or close mutations.

Molecular evidence for an alternative short-patch mismatch repair process

In wild-type cells, 12 of 15 tracts analyzed were found to be continuous, while three others contained a short intervening recipient sequence (Figure 3A). A majority of continuous tracts have also been found in previous studies on meiotic and mitotic conversion. This was observed not only when the parental DNAs differ by a few mutations (Petes *et al*., 1991), but also when they are homeologous (Harris *et al*., 1993; Chen and Jinks-Robertson, 1998, 1999). In both meiotic and mitotic cells, the tract continuity is believed to result from a long-patch excision, governed by *MSH2*, that can cover most if not all of the heteroduplex DNA (Bishop and Kolodner, 1986; Detloff and Petes, 1992). If the 'Msh2' repair process is the only one that repairs mismatches, continuous recombinant tracts will also be found in an *msh2* mutant, with only one of the DNA strands being recombined. We found that this is not always the case.

In *msh2* cells, ~50% of the tracts analyzed contain one or more interruptions. The four cases where both strands were recovered indicate that the alternate stretches of parental DNAs correspond essentially to alternate regions of conversion and unrepaired heteroduplex DNA tracts (nos 11, 12, 14 and 15 in Figure 3B). Events nos 12, 14 and 15 (Figure 4) uncover regions with very short alternate tracts close together. We do not see any alternative for their formation other than by short-patch MMR. This

repair favors the donor DNA, as does repair by the long-patch system. For example, event no. 14 has the recipient strand patched while the donor strand is continuous. In the four cases where both strands were recovered, there are 23 apparent stretches of conversion and four of restoration.

The preferential repair by excision of the recipient strand may well account for the relatively high frequency of continuous tracts (and of tracts with few patches) found in *msh2* mutants. Indeed, by selecting ARG^+ cells, the donor strand, most often unaffected by repair, should be preferentially recovered. This may not be the only explanation for the recovery of continuous tracts. It could also be that not all cells underwent short-patch repair, that replication segregated the strands before repair has occurred, or, finally, that some events involved double-strand gaps and no intermediary heteroduplex DNA (Orr-Weaver *et al*., 1981; Szostak *et al*., 1983).

Our data further show that this activity is not or not always processive along the DNA. If some repaired tracts involve several mismatches in a row, others involve a single mismatch. Rather long regions of unrepaired heteroduplex DNA are also observed, e.g. in case no. 15 (Figure 4) where three regions of 156, 192 and 267 bp, which contain 10, 13 and 24 mismatches, respectively, are left unrepaired. The nature of the mismatch does not explain why some of them escape repair, since identical mismatches are sometimes but not always repaired. It seems that mismatches are repaired individually with a limited probability. The size of the excision tract can be very short, with an upper limit of 11 nucleotides (conversions at positions 241, 286 and 295 in event no. 12, Figure 4).

The spectrum of action of the system is large. The different base substitutions found in tracts that involve a single mismatch indicate that all mismatches except C/C or G/G are potential substrates for short-patch repair. We cannot conclude that C/C and C/G are not repaired. First, the C→G or G→C mutations that differentiate the parental sequences and that could form these mismatches are rare, and, secondly, G→C or C→G transversions are observed in tracts uncovering repair of several mismatches. Specifically designed experiments will be needed to obtain the answer. The pertinence of this question lies in the fact that C/C mismatches are the only ones that are not a good substrate for the long-patch MMR (Kramer *et al*., 1989; Detloff *et al*., 1991).

Recombinant tracts are not longer in msh2 than in wild-type cells

Alani *et al*. (1994) proposed that MMR proteins control heteroduplex DNA length by binding to mismatches, which would explain the abolition by *msh2* of meiotic conversion gradients seen in *S.cerevisiae*. If so, one can predict that recombinant tracts in homeologous systems will be longer in *msh2* than in wild-type cells. We compared the recombinant tract length observed in wild-type and *msh2* cells (Figure 3A). In wild-type haploid cells, the minimal length of mitotic tracts ranges from 247 to 1217 bp, with a mean of 605 bp, while in *msh2* cells, it ranges from 196 to 955 bp, with a mean of 525 bp. These mean values are not significantly different, indicating that the mitotic tract length is not regulated by *MSH2*. For meiotic events, the small number of cases analyzed makes the conclusion less definitive. In wild-type cells, the tract lengths range from 7 to 1419 bp, with a mean size of 763 bp, while in *msh2* cells the three tracts are 580, 856 and 1105 bp long, respectively (Figure 3B). These values fall into the range observed for tracts analyzed in spores from wild-type diploids, which argues that meiotic tract length is not under the control of *MSH2*.

The possible control by *MSH2* of heteroduplex length was also recently questioned by Chen and Jinks-Robertson (1998, 1999) who used an artificial system of inverted 350 bp homeologous repeats. These authors reached different conclusions than ours. They found that meiotic conversion tracts were ~50% longer in *msh2* than in wild-type cells. For mitotic events, they obtained a similar result in haploid cells, but not for diploid cells in which *msh2* had no effect. It might be that the differences in the results obtained in the two laboratories relate to the different genetic systems used.

Genetic evidence for short-patch repair activity

Providing that an independent short-patch MMR mechanism exists, the elimination of the long-patch MMR by mutations should confer a hyper-recombination phenotype in homologous heteroallelic cells containing closely spaced mutations. Our results (Figure 5; Table I) show that the absence of Msh2 increases recombination by a factor of 4–9 when sites are close together, as compared with a factor of at most 1.6 for distant sites. The effect of *pms1* was not tested in meiosis, but is similar to that of *msh2* in mitotic cells. These results confirm the existence of a short-patch repair mechanism independent of *MSH2* and *PMS1*. They also indicate that this process efficiently repairs mismatches due to short insertions or deletions.

The efficiency of this system is quite high. For instance, after UV treatment, the induction of recombinants in wild-type cells is ~10 times less when the mutations are close to each other than if they are distant. In *msh2* or *pms1* cells, this induction is only 50% smaller than in cells with the distant alleles. In other words, the distance effect on recombination is largely suppressed. Furthermore, the short-patch MMR events are probably more frequent than detected genetically since not all the possible repair events lead to the formation of a wild-type *ARG4* gene. In this respect, it may be relevant to recall the effects of MMR mutations on meiotic segregation of heterozygous markers. The *pms1* and *msh2* mutations increase the class of post-meiotic segregation (unrepaired heteroduplex DNA) at the expense of the conversion class. However, the level of residual conversions remains high and the proportion of conversions among the non-Mendelian segregants can reach 50% (Williamson *et al*., 1985; Alani *et al*., 1994). This was notably reported by Alani *et al*. (1994) for the same *Eco*RV mutation that is present in our strains. A likely explanation is that these convertants are formed by an alternative efficient MMR activity that could well be the same as the short-patch system that we report here.

In wild-type cells, mismatches are predominantly repaired by the long-patch system, as indicated earlier in this discussion. This predominance of long- versus short-patch repair may be due to a close link between the long-patch MMR system and recombination as well as replication. Besides binding to mismatches (Alani *et al*.,

Table II. Strains list

Strain	Genotype
FF181248	*MATα arg4C-RV ura3-52-arg4D-Bg-URA3*
FF181378	*MATα arg4C-RV ura3-52-arg4D-Bg-URA3 msh2Δ*
FF181246	*MATα arg4D-RV ura3-52-arg4D-Bg-URA3*
Ec160	*MATα arg4C-RV ura3-52-arg4C-Bg-URA3 rad1::LEU2*
Ec166	*MATα arg4D-RV ura3-52-arg4D-Bg-URA3 rad14::LEU2*
FF181379	*MATα arg4D-RV ura3-52-arg4D-Bg-URA3 msh2Δ*
Ec168	*MATα arg4D-RV ura3-52-arg4D-Bg-URA3 msh2Δ rad1::LEU2*
Ec167	*MATα arg4D-RV ura3-52-arg4D-Bg-URA3 msh2Δ rad14::LEU2*
FF181614	*MATα arg4C-RV ura3-52-arg4C-Bg-URA3 pms1::TRP1*
FF181615	*MATα arg4C-RV ura3-52-arg4C-Bg-URA3 pms1::TRP1 rad1::LEU2*
FF181616	*MATα arg4C-RV ura3-52-arg4C-Bg-URA3 pms1::TRP1 rad14::LEU2*
Ec153	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3*
Ec153000	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 rad1::LEU2*
Ec153002	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3rad14::LEU2*
Ec144000	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 msh2::Tn10LUK7-7*
Ec144001	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 msh2::Tn10LUK7-7 rad1::LEU2*
Ec144003	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 msh2::Tn10LUK7-7 rad14::LEU2*
FF181617	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 pms1::TRP1*
FF181619	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 pms1::TRP1 rad1::LEU2*
FF181620	*MATα arg4C-RV ura3-52-arg4C-Ag-URA3 pms1::TRP1 rad14::LEU2*
Ec182	MAT**a**/MATα, *arg4D-RV/arg4C-Bg, msh2::URA3/msh2::Tn10LUK7-7*
FF181384	MAT**a**/MATα, *arg4C-RV/arg4Δ, ura3-52-arg4D-Bg-URA3/ura3-52*
FF181396	MAT**a**/MATα, *arg4C-RV/arg4Δ, ura3-52-arg4D-Bg-URA3/ura3-52 msh2Δ/msh2::URA3*
Ec183	MAT**a**/MATα, *arg4D-RV/arg4Δ, ura3-52-arg4D-Bg-URA3/ura3-52*
Ec184	MAT**a**/MATα, *arg4D-RV/arg4Δ, ura3-52-arg4D-Bg-URA3/ura3-52, msh2Δ/msh2::URA3, PMS1/pms1::TRP1*
Ec185	MAT**a**/MATα, *arg4C-RV/arg4Δ, ura3-52-arg4C-Ag-URA3/ura3-52*
Ec186	MAT**a**/MATα, *arg4C-RV/arg4Δ, ura3-52-arg4C-Ag-URA3/ura3-52, msh2::URA3/msh2::Tn10LUK7-7, PMS1/pms1::TRP1*

1995), Msh2 was also reported to bind Holliday junctions (Alani *et al*., 1997; Marsischky *et al*., 1999) and proliferating cell nuclear antigen (Umar *et al*., 1996). Msh2 may bind mismatches and initiate their repair before any other repair protein can act. In support of this view is the very rapid *PMS1*-dependent repair observed after the formation of a mismatch during mating-type switching (Haber *et al*., 1993). However, in the literature, there are data that could indicate the activity of short-patch repair in wild-type cells. In most analyses of conversion tracts, a minority of them were reported to be 'complex' and in some cases could have involved short-patch repair. Weng and Nickoloff (1998) postulated a short-patch activity to explain some of the tracts that they observed. In our study, the three tracts from wild-type cells that had a short stretch of recipient DNA could reflect restoration events by a short excision tract (Figure 3A).

Fleck *et al*. (1999) also found a hyper-recombination effect of *msh2* and *pms1* in heteroallelic cells bearing mutations at sites located close together. They further demonstrated that this effect depends on a short-patch MMR system governed by NER genes. In our test, we found no effect of *rad1* or *rad14* mutations coupled to either *msh2* or *pms1* mutations. We only studied mitotic cells, while in *S.pombe* the test involved meiotic recombination. However, from our data, we see no reason to believe that the process might differ during meiotic and mitotic recombination and we therefore conclude that the short-patch MMR activities in the two yeasts are under different genetic control. We noticed in the work of Fleck *et al*. that heteroallelic cells bearing closely spaced mutations (which would generate non-C/C mismatches) produce prototrophs at higher rates when defective for both the NER and the long-patch systems than when wild type. This suggests the activity of another short-patch MMR system. It might be that this activity involves the *UVE1* gene. Uve1p is a nuclease involved in an alternative NER pathway (Bowman *et al*., 1994; Freyer *et al*., 1995). The mutant exhibits a strong mutator phenotype and the protein possesses an *in vitro* nicking activity on the 5′ side of mismatches (Kaur *et al*., 1999). Similar activities were reported in *S.cerevisiae* cell extracts (Chang and Lu, 1991) and are possibly involved in the short-patch MMR described here. When genes controlling the *S.cerevisiae* system are identified, it will be possible to ask whether they also play a role in mutation avoidance.

Besides this potential role, a short-patch MMR activity is also expected to have important biological consequences related to recombination, at least in mutants affected in the long-patch repair pathway. First, as shown in *S.pombe* (Fleck *et al*., 1999) and in this study, it can separate efficiently two closely spaced mutations. Secondly, it can generate highly patched products during a single round of recombination between diverged DNAs. Not only the rate at which sequence diversification occurs, but also the structure of the outcome products will be different from those observed in cells where the *MSH2*-controlled long-patch MMR process predominates. This is likely to play a role in adaptation and evolution.

Materials and methods

Yeast strains, plasmids and media

Table II gives the list of strains used and their relevant genotype. All strains are MGD derivatives. The parental strains, kindly provided by A.Nicolas, used to derive our strains are ORT118-2 (*MAT***a**, *arg4-Eco*RV, *leu2*, *trp1*, *ura3*, *cyh2*) and ORT126 (*MAT*α, *arg4-Bgl*II, *trp1*, *ura3*, *ade2*, *his3*). The corresponding strains containing an *arg4-Hpa*I deletion (2.1 kb) are MGD131-2C and MGD131-102-a. These strains and their

pms1::TRP1 or *msh2::Tn10LUK7-7* derivatives have been described previously (Rocco *et al*., 1992; Alani *et al*., 1994). The replacement of the *arg4C* allele by the corresponding *arg4D* allele was performed by Adjiri (Adjiri, 1993).

To construct ectopic strains, the 2.1 kb *Hpa*I–*Hpa*I fragments containing the desired allele of *ARG4* were first cloned into the *Nru*I site of YIp5. This integrative vector contains the yeast *URA3* reporter gene (Johnston and Davis, 1984). The plasmid was digested by *Nco*I, a unique site in *URA3*, in order to direct its integration into the *ura3-52* chromosomal allele by transformation. The plasmid bearing the *arg4-AgeI* mutation ('Ag') has a 3.5 kb *Hin*dIII–*Hin*dIII fragment of *S.cerevisiae* integrated into YIp5. The mutation was created by filling in the ends of the *Age*I-cut plasmid and re-ligation, resulting in a 4 bp insertion. On these different plasmids, the orientation of *arg4* with respect to *URA3* is such that, after integration, the two *arg4* alleles are in reverse orientation with respect to the centromeres. Ectopic diploids were obtained by crossing the ectopic haploids with cells bearing a genomic deletion of the *Hpa*I region. These diploids are heterozygous for *leu2*, *ade2*, *his3*, *cyh2*. This is not indicated in Table II for the sake of clarity. Diploids containing the *arg4* alleles in allelic position (Ec182) are also heterozygous for the same markers.

The different plasmids used are pFA4 (*arg4D-Bg*), pNM22 (*arg4C-Bg*) and pNM23 (*arg4C-Ag*). They have the *Hpa*I–*arg4*–*Hpa*I fragment (2.1 kb) integrated into YIp5. pNM3, pNM4 and pNM14 have a larger *Hin*dIII fragment (3.5 kb from the *ARG4C* region) integrated into YIp5, with the internal *Hpa*I fragment containing *ARG4C*, *argD-RV* or *argD-Bg*, respectively. To disrupt *RAD14* and *RAD1* we used the plasmids pBM190 (*rad14::LEU2*) (Bankmann *et al*., 1992) and pWJ153 (*rad1::LEU2*), given by R.Rothstein. pEN63 (*msh2::URA3*), from E.Alani, was used in some cases to delete *MSH2* and to derive cells that had lost *URA3*, indicated in Table II as *msh2*Δ.

Standard yeast genetic and molecular techniques were used (Guthrie and Fink, 1991). YPD medium and SD medium, supplemented with the desired nutrients, were used for mitotic growth. Pre-sporulation and sporulation procedures were according to Resnick *et al*. (1983).

Determination of meiotic and mitotic recombination rates

The frequency of meiotic ARG$^+$ recombinants was determined by plating sporulated cultures on synthetic medium lacking arginine and diluted aliquots on YPD supplemented with 10 mg/l cycloheximide. Because the recessive *cyh* mutation is heterozygous in our diploid cells and segregates during sporulation, the cycloheximide in the medium selects colonies derived from asci.

Spontaneous rates of recombination were determined by the method of the median (Lea and Coulson, 1949). The median was from seven independent cultures.

For UV induction of recombinants, UV light (260 nm) was applied to cell suspensions (2×10^7/ml) in saline (0.9% NaCl). The UV fluence was 1 J/m^2, as determined with a Latarjet dosimeter. Aliquots were plated on medium lacking arginine for recombinant selection and on rich medium for survival determination. The survival of the different strains was similar and was ~50% at 30 J/m^2.

All experiments were repeated at least three times.

Selection and sequencing of the ARG$^+$ alleles

To select spontaneous mitotic recombinants from ectopic haploids, cells were plated on rich medium and the colonies were replica-plated on medium lacking arginine. ARG$^+$ papillae derived from individual colonies were picked, ensuring that they originated from independent recombination events. Meiotic recombinants were selected as described above. They derived from ectopic diploids, except for one case (no. 15) where allelic diploids were used to select ARG$^+$/arg$^-$ sectored monosporic clones. Individual cells from each recombinant colony were plated on rich medium and their genotype analyzed by replica-plating. Since five independent markers segregated, finding the same genotype for all cells of a colony greatly minimized the possibility that it derived from two spores.

The genomic DNA from ARG$^+$ clones was extracted and the allele present on chromosome VIII was specifically PCR amplified using primers that anneal on sequences located outside of the *Hpa*I region. The upper primer (arg4EU) was GTTGGCGCAGGCAATTAATT and the lower one (arg4EL) was AGAATGGCCGGTTCAGACAT. The amplified product was submitted to restriction digestion by *Eco*RV and *Bgl*II, allowing it to be determined whether this allele codes for ARG4$^+$. The whole amplified *Hpa*I–*ARG4*–*Hpa*I fragments were sequenced by the dideoxy method using a Perkin Elmer ABI310 sequencer. Some of the sequences were determined by the E.S.G.S. Company, group Cybergen.

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