**MSH5**, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair

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Using a screen designed to identify yeast mutants specifically defective in recombination between homologous chromosomes during meiosis, we have obtained new alleles of the meiosis-specific genes, HOP1, RED1, and MEK1. In addition, the screen identified a novel gene designated *MSH5* (MutS homolog 5). Although Msh5p exhibits strong homology to the MutS family of proteins, it is not involved in DNA mismatch repair. Diploids lacking the *MSH5* gene display decreased levels of spore viability, increased levels of meiosis I chromosome nondisjunction, and decreased levels of reciprocal exchange between, but not within, homologs. Gene conversion is not reduced. Msh5 mutants are phenotypically similar to mutants in the meiosis-specific gene *MSH4* (Ross-Macdonald and Roeder 1994). Double mutant analysis using *msh4 msh5* diploids demonstrates that the two genes are in the same epistasis group and therefore are likely to function in a similar process—namely, the facilitation of interhomolog crossovers during meiosis.

[Key Words: MSH5; yeast; MutS homolog; recombination]

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A unique problem confronting sexually reproducing organisms is how to maintain the parental chromosome number in offspring produced by fertilization. The solution to this problem is to reduce the number of chromosomes in germ cells by half in a very precise manner such that each gamete receives one, and only one, member of each chromosome pair. Fertilization then restores the diploid chromosome number. The reduction in chromosome number is accomplished by meiosis, during which a single round of chromosome replication is followed by two rounds of chromosome segregation. Failures in meiosis result in gametes that are chromosomally imbalanced or aneuploid.

Proper segregation of homologs at the first meiotic division requires that the chromosomes align correctly on the metaphase plate. For this alignment to occur, the homologs must be physically connected by crossovers or chiasmata (for review, see Hawley 1987). These crossovers must occur in the context of the synaptonemal complex (SC), the proteinaceous structure formed when homologous chromosomes become physically associated, to function in segregation (Engebrecht et al. 1990).

To understand how functional chiasmata are generated in the presence of SCs, it is necessary to first define the proteins involved both in synopsis (i.e., SC formation) and recombination. Antibodies directed against purified SCs have enabled the isolation of genes encoding SC components in mammals (Meuwissen et al. 1992). In yeast a number of different genetic screens have been used to identify genes important for recombination, synopsis, or both (Petes et al. 1991). One of these screens detects mutants specifically defective in recombination between homologs during meiosis (Hollingsworth and Byers 1989). The basis of this approach is to look for mutants that are unaffected for reciprocal recombination within a chromosome, but reduced for recombination between homologs. Because several genes have been shown to be necessary for both intra- and interchromosomal recombination (*MER1, SPO11, and RAD50*), the assumption is that the same recombination machinery mediates both types of events (Wagstaff et al. 1985; Engebrecht and Roeder 1989; Gottlieb et al. 1989). In theory, therefore, this screen should detect mutants defective in synopsis, as well as in processes that facilitate crossovers specifically between homologous chromosomes.

This screen was used successfully to isolate *HOP1*, a structural component of meiotic chromosomes in yeast, which is necessary for homolog synopsis (Hollingsworth...
and Byers 1989; Hollingsworth et al. 1990). Subsequent reconstruction experiments indicated that mutations in two other genes, RED1 and MEK1, also specifically reduce interhomolog recombination and, therefore, should have been detected (Rockmill and Roeder 1990; B. Rockmill, pers. comm.). Like HOP1, RED1 is required for synapsis (Rockmill and Roeder 1990). MEK1 encodes a meiosis-specific kinase required for complete SC formation (Rockmill and Roeder 1990; Leem and Ogawa 1992). Because only a single allele of HOP1 was identified in the original screen, the screen was repeated in an attempt to find additional genes that may be important for synapsis and/or interhomolog recombination.

This paper reports the discovery of a novel MutS homolog designated MSH5 [MutS homolog 5]. MutS homologs function in mismatch repair in a variety of different organisms, including bacteria, yeast, and humans (for review, see Modrich 1991). In addition, msh5 is required to prevent partially diverged sequences from undergoing recombination in bacteria (Rayssiguier et al. 1989). We demonstrate here that, unlike msh5 and the yeast MSH1 and MSH2 family of MutS homologs, the MSH5 gene plays no role in DNA mismatch repair. Instead, MSH5 appears to facilitate reciprocal crossovers between homologs, presumably to prevent nondisjunction at the first meiotic division.

Table 1. Saccharomyces cerevisiae strains

| Strain        | Genotype                                                                 |
|---------------|--------------------------------------------------------------------------|
| **A. Disomic haploids** |                                                                 |
| karC2-4       | MATa cdc10-2 LEU2::pNH18 (URA3 CYH2) HIS4 trp1 ura3-52 can1 cyh2 spo13-1 ade2-1 |
|               | MATa CDC10 leu2 his4                                                     |
| B31-89        | karC2-4 but msh5-1                                                       |
| **B. SKI background** |                                                                |
| NH144         | MATa leu2-k HIS4 arg4-nsp ura3 lys2 ho::LYS2                               |
|               | MATa leu2his4-x ARG4 ura3 lys2 ho::LYS2                                   |
| NH145         | NH144 but msh5::URA3                                                     |
|               | msh5::URA3                                                               |
| NH155         | NH144 but msh5::URA3 msh4::LEU2                                          |
|               | msh5::URA3 msh4::LEU2                                                    |
| **C. BR background** |                                                              |
| NH156         | MATa LEU2 his4 ade2 MET13 trp5 cyh3 ade6 ura3 spo13Aura3                  |
| NH157         | NH156 but msh5::URA3                                                     |
|               | msh5::URA3                                                               |
| **D. A364A background** |                                                          |
| PS593         | MATa leu2-3,112 ura3-52 trp1 his3A200                                     |
| PS593/190     | same as PS593 but msh5::URA3                                             |
| **E. Miscellaneous strain background** |                                                              |
| NH111         | MATa LEU2 HIS4 his7 trp1 msh5-1 ura3-52 CAN1 CYH2 spo13-1 ade2-1            |
|               | MATa leu2 his4 HIS7 trp1 msh5-1 ura3-52 can1 cyh2 spo13-1 ade2-1            |
|               | ade2-1::pNH164 (ADE2 SPO13)                                              |

Strains are grouped in isogenic sets (A–E).

Results

A screen for mutants specifically defective in meiotic interchromosomal recombination identifies new alleles of HOP1, RED1, and MEK1, as well as MSH5, a novel MutS homolog

To find mutants defective specifically in meiotic interhomolog recombination, the chromosome III disomic haploid karC2-4 [Table 1] was mutagenized. A total of 9855 colonies were assayed for a change in the segregation pattern of chromosome III, which is indicative of a defect in reciprocal recombination between homologs. At the same time the screen required that efficient reciprocal intrachromosomal recombination still occur [Hollingsworth and Byers 1989]. In this assay a correlation has been observed previously between a decrease in meiotic interhomolog recombination and an increase in the fraction of equationally segregating chromosomes in the single-division spo13 meiosis. Mutants exhibiting increased levels of equationally segregation in the spo13 disomic haploids were tested further for their effects on spore viability in SPO13 diploids. Defects in reciprocal recombination between chromosomes reduce spore viability in SPO13 meiosis because of the requirement for crossovers for proper chromosome disjunction at meiosis I. Mutants exhibiting mitotic phenotypes such as sensi-
tivity to the DNA-damaging agent, methyl methane sulfonate (MMS), or slow growth were excluded.

Eleven new mutants were isolated, which displayed increased levels of equational segregation of chromosome III in spo13 disomic haploids and reduced spore viability in SPO13 diploids (Table 2). All of the mutations are recessive. Complementation tests using spore viability as an assay revealed that of these 11 mutants, 5 carry alleles of MEK1, 2 carry alleles of HOP1, and 3 carry alleles of RED1. Subsequent experiments demonstrated that the remaining mutant, msh5-I, identifies a novel MutS homolog that we designate MSH5 (see below).

msh5 decreases spore viability in SPO13 diploids and causes increased meiosis I nondisjunction

A number of meiotic mutants have been described recently that moderately reduce spore viability in SPO13 strains (zip1, med1, msh4) (Sym et al. 1993; Rockmill and Roeder 1994; Ross-Macdonald and Roeder 1994). Diploids homozygous for msh5-I fall into this class, producing 37% viable spores (Table 2). To determine whether this level of spore viability represents the null phenotype for MSH5, an insertion allele was created using the cloned gene (see Materials and methods). The URA3 gene was inserted into a unique BglII site within the MSH5-coding sequence (Fig. 3). This insertion creates a truncated Msh5 protein, removing two highly conserved functional domains [see below].

Table 2. Assay of equational segregation in spo13 disomic haploids and spore viability in SPO13 diploids for mutants detected by the screen

| Genotype | Disomic haploid strain | Equational segregation of chromosome III (%) | SPO13 diploid (% spore viability) |
|----------|------------------------|---------------------------------------------|----------------------------------|
| +        | karC2-4                | 17                                          | 92 (72)                          |
| mek1-974 | A9 -74                 | 43                                          | 48 (103)                         |
| mek1-3382| A33-82                 | 87                                          | 1 (18)                           |
| mek1-4113| B41-13                 | 90                                          | 10 (20)                          |
| mek1-5069| B50-69                 | 70                                          | 12 (22)                          |
| mek1-5994| B59-94                 | 83                                          | 57 (38)                          |
| hop1-1b  | 2-26                   | 95                                          | 1 (52)                           |
| hop1-742 | B7-42                  | 100                                         | ~1c                              |
| hop1-348 | B3-48                  | 100                                         | ~1c                              |
| red1-2353| B23-53                 | 88                                          | 1 (18)                           |
| red1-867 | A8-67                  | 78                                          | 5 (10)                           |
| red1-6183| B61-83                 | 82                                          | ~1c                              |
| msh5-1   | B31-89                 | 84                                          | 37 (485)                         |

a The value in parentheses represents the number of asci dissected. SPO13 mutspore viability was determined by isolating MATa and MATα haploid derivatives from each mutant disomic haploid, integrating the SPO13 gene into the MATα derivative, crossing the two haploids, and dissecting the ascis obtained from the sporulated diploids.

b hop1-1 data from Hollingsworth and Byers [1989].

c Qualitative results obtained by random spore analysis.

msh5::URA3 allele was then substituted for the wild-type copy in two different strain backgrounds—A364A and SK1. In both backgrounds the level of spore viability observed was similar to the 37% found for msh5-I (50%, 146 tetrads for the A364A background, and 38%, 1370 tetrads, for the SK1 background).

The observation that spo13 can rescue the msh5-I spore lethality suggests that the spore inviability conferred by msh5-I is attributable to the mis-segregation of homologs at the first meiotic division. The single-division spo13 meiosis has been shown to rescue a large number of recombination-deficient mutants [Petes et al. 1991]. The msh5-I SPO13 diploid produces 37% viable spores (485 tetrads). In the isogenic msh5-I spo13 diploid, spore viability is improved to 66% (327 dyads). This value is also an improvement over the 22% (660 dyads) viability observed in the isogenic MSH5 spo13 diploid, consistent with previous observations that defects in recombination improve spore viability in spo13 meiosis.

Further evidence for a Meiosis I defect for msh5 was obtained by analyzing the pattern of spore viability in tetrads produced by the msh5::URA3 SPO13 diploid NH145. The pattern of spore death in NH145 is nonrandom, with an excess of two- and zero- viable spore tetrads at the expense of tetrads with four viable spores (Fig. 1). This pattern is indicative of meiosis I nondisjunction (Fig. 2). The tetrads with two viable spores result when one or more pairs of homologs segregate to the same pole. The spores that survive are disomic for the mis-segregated homologs. Tetrads with zero viable spores result when two or more pairs of homologs disjoin to opposite poles.

It is possible in S. cerevisiae to show definitively that
MutS homolog facilitates meiotic crossing-over

Figure 2. Diagram of tetrads undergoing, no nondisjunction (A); nondisjunction of chromosome III (B); nondisjunction of chromosome III and chromosome VIII (C). No recombination is assumed. Sister centromeres are indicated by white or shaded circles. Shaded spores are inviable.

meiosis I nondisjunction is occurring by taking advantage of the codominant MATa and MATo alleles present on chromosome III. As depicted in Figure 2, meiosis I nondisjunction of chromosome III results in tetrads with two viable spores, where both spores are nonmaters because of the presence of both MATa and MATo information. Of 308 two-viable spore tetrads analyzed for NH145, 15.3% contained two nonmating spores, demonstrating that in the msh5::URA3 diploid, chromosome III is mis-segregating frequently.

Analysis of the segregation of the chromosome VIII centromere-linked marker arg4 indicates that other chromosomes besides chromosome III are mis-segregating as well. If spore death is attributable to nondisjunction of a pair of homologous chromosomes to the same pole at meiosis I, equational segregation of the properly disjoined homologs at meiosis II will result in the two surviving spores containing sister chromatids (such spores are designated sister spores). Assuming that no recombination exists between arg4 and the centromere, sister spores should have the phenotype Arg+ :Arg+ or Arg- :Arg- (Fig. 2). We observed that the majority (83%) of 135 two-viable spore tetrads scored for NH145 are comprised of chromosome VIII sister spores. This difference is significantly different from the 50% predicted if spore death were random \( \chi^2, P<0.001 \). A similar statistically significant bias for chromosome III sister spores in two-viable spore tetrads where chromosome III had properly segregated was also observed (data not shown). The effect of msh5::URA3 on chromosome nondisjunction therefore appears to be general.

Another type of chromosome mis-segregation event that may occur is precocious separation of sister chromatids (PSSC), where sister chromatids of one homolog fall apart prior to meiosis I and then segregate randomly to either pole. In some cases, mutants that cause an increase in meiosis I nondisjunction also result in an increase in PSSC (Miyazaki and Orr-Weaver 1992; Rockmill and Roeder 1994). Precocious sister chromatid separation of chromosome III is manifested by three-viable spore tetrads in which one spore is a nonmater, one is an a-mater, and one is an s-mater. Of 87 three-viable spore tetrads analyzed for NH145, 3 exhibited this pattern. In the same strain background, Sym and Roeder (1994) observed no chromosome III disomic spores of 57 three-viable spore tetrads. We conclude, therefore, that whereas the major defect caused by the absence of MSH5 is an increase in nondisjunction of homologous chromosomes at the first meiotic division, other types of mis-segregation events may be elevated as well.
msh5 decreases meiotic reciprocal exchange between homologs but does not reduce gene conversion

To test whether crossing-over is affected by the absence of MSH5, intervals on chromosome III and chromosome VII were assayed by ascus dissection for recombination. A pair of isogenic spo13 diploids, NH156 and NH157, were constructed that are homozygous for MSH5 and msh5::URA3, respectively. As discussed earlier, the single-division meiosis conferred by spo13 eliminates the need for crossovers to ensure proper reductional segregation of homologs at the first meiotic division. One can therefore analyze recombination in spo13 strains without having to account for potential bias introduced by poor spore viability. As is the case with the other genes identified by this screen, HOP1, RED1, and MEK1 (Hollingsworth and Byers 1989, Rockmill and Roeder 1990, 1991), the msh5::URA3 and msh5-1 mutations reduce, but do not eliminate, reciprocal exchange (Table 3; N.M. Hollingsworth, unpubl.). The msh5::URA3 mutation resulted in a statistically significant reduction in recombination of either two- or threefold in five intervals on two different chromosomes (Table 3). A similar reduction in recombination was observed for the HIS4-MAT interval when four-viable spore tetrads from the SK1 SPO13 diploids NH144 and NH145 were compared (Table 3).

Our hypothesis is that the high levels of nondisjunction observed in msh5 diploids are attributable to the reduction in crossing-over. If this is the case, then the chromosome III disomic spores found in the two viable spore tetrads of NH145 should both be heterozygous at HIS4 and therefore prototrophic for histidine (Fig. 2). Of the 47 tetrads observed to have undergone meiosis I nondisjunction of chromosome III, 2 contained one histidine prototrophic and one histidine auxotrophic spore. These spores could arise either by recombination between HIS4 and the centromere or by gene conversion of the HIS4 allele to his4. Given the lack of a centromere-linked marker and the high frequency of conversion at HIS4 in this strain (see below), we could not distinguish between these two possibilities. Further work is therefore necessary to confirm whether there is a mechanistic link in msh5 mutants between crossing-over and nondisjunction.

HOP1, RED1, and MEK1 mutants not only reduce crossing-over between chromosomes, they also reduce

| Table 3. Effect of msh5::URA3 on reciprocal recombination between homologs |
|---------------------------------|-------------------|----------------|-----------------|-----------------|-----------------|
| Strain                          | Genotype          | HIS4-LEU2 | LEU2-MAT | MET13-TRP5 | TRP5-CYH3 | CYH3-ADE6 |
| NH156  | MSH5             | 13       | 38       | 47       | 24       | 19       |
|       | spo13            |          |          |          |          |          |
|       | MSH5             |          |          |          |          |          |
|       | spo13            | 7 (0.5)  | 12 (0.3) | 15 (0.3) | 6 (0.3) | 6 (0.3)  |
| NH157  | msh5::URA3       | 13 (0.3) |          |          |          |          |
|       | spo13            |          |          |          |          |          |
|       | msh5::URA3       |          |          |          |          |          |
|       | spo13            | 13 (0.3) |          |          |          |          |
| NH144  | MSH5             |          |          |          |          |          |
|       | MSH4             |          |          |          |          |          |
|       | MSH5             |          |          |          |          |          |
|       | MSH4             |          |          |          |          |          |
| NH145  | msh5::URA3       | 13 (0.3) |          |          |          |          |
|       | MSH4             |          |          |          |          |          |
|       | msh5::URA3       |          |          |          |          |          |
|       | MSH4             |          |          |          |          |          |
| NH155  | msh5::URA3       | 10 (0.3) |          |          |          |          |
|       | msh4::LEU2       |          |          |          |          |          |
|       | msh5::URA3       |          |          |          |          |          |
|       | msh4::LEU2       |          |          |          |          |          |

*a NH156 and NH157 are isogenic. NH144, NH145, and NH155 are isogenic with the SK1 background.

*b Map distances for the spo13 strains were calculated by the formula

$\frac{|2 \times SCO_e| + SCO_r + 6 \times \text{four strand DCO}|}{\text{Total dyads}} \times \frac{100}{2} = \text{cM}$

where SCO_e and SCO_r are single crossovers that have segregated equationally and reductionally, respectively.

For the SPO13 diploids, the following formula was used (Perkins 1949):

$\frac{|T + 6 \times \text{NPD}|}{\text{Total tetrads}} \times \frac{100}{2} = \text{cM}$

For NH156, 99 dyads were analyzed for chromosome III markers and 104 dyads were analyzed for chromosome VII markers. For NH157, 104 dyads were analyzed for chromosome III and 115 for chromosome VII. For NH144, NH145, and NH155, 279, 229, and 65 tetrads were examined, respectively.

*c Numbers in parenthesis represent the ratio of the msh5 map distance over the wild-type distance. All of the mutant values differ significantly from wild type by $\chi^2$ analysis ($P < 0.001$).
gene conversion [Hollingsworth and Byers 1989, Rockmill and Roeder 1990, 1991; Leem and Ogawa 1992]. Using isogenic diploids heteroallelic for ade2, ura3, or leu2, we assayed mitotic and meiotic gene conversion in MSH5, msh5-1, and msh5::URA3 diploid strains by selection for the formation of prototrophs. No significant differences between the msh5 diploids and wild type were observed [N.M. Hollingsworth, L. Ponte, and C. Halsey, unpubl.]. In addition, gene conversion was assayed in NH144 and NH145 at three loci [HIS4, ARG4, and LEU2] by dissection (Table 4). A statistically significant increase in gene conversion was observed at ARG4 in the msh5::URA3 diploid. In contrast, no effect was observed for msh5::URA3 on the conversion frequency at HIS4 or LEU2. MSH5 is therefore phenotypically distinct from HOP1, RED1, and MEK1 in that it does not generally reduce interhomolog recombination but does reduce reciprocal exchange.

Cloning and mapping of MSH5 identifies it as a new gene

To determine whether msh5-1 identifies a new gene, the MSH5 gene was cloned and mapped [see Materials and methods]. Hybridization to an overlapping set of yeast genomic fragments mapped the cloned piece of DNA to chromosome IV. Meiotic mapping confirmed that the MSH5 gene is located 1.9 cM from CDC36 on chromosome IV. This map position does not coincide with that of any previously identified genes.

Various subclones of the original cloned insert were tested for their ability to complement the spore inviability of the msh5-1 diploid NH111. The smallest complementing piece is a 4.7-kb HindIII-SphI fragment contained in pNH181. pNH181 restores spore viability in NH111 from 50% with the vector YCp50 (48 tetrads) to 92.5% (20 tetrads).

The DNA sequence of the MSH5 gene was obtained by sequencing the majority of the 4.5-kb BglII-HindIII complementing fragment as described in Materials and methods (Fig. 3). When the DNA sequence was used to search the GenBank data base, an exact match was found between one end of the fragment containing the last 161 bp of the submitted sequence that contains the CLB3 [cyclin B] gene (Fitch et al. 1992). The sequence of MSH5 is not present in the GenBank data base [see below]. This fact, coupled with the mapping data, demonstrates that MSH5 represents a new gene.

To confirm that the cloned DNA represents MSH5 and not a suppressor, a complementation test was performed between msh5::URA3 and msh5-1. Dissection of tetrads from a msh5::URA3/msh5-1 heteroallelic diploid gave 42.3% spore viability (55 tetrads), demonstrating that the cloned gene is MSH5.

MSH5 encodes a MutS homolog

The MSH5 open reading frame is composed of 2703 nucleotides and encodes a protein of 901 amino acids [predicted molecular mass = 102 kD]. An 8/9 match with the URS1 consensus sequence is located at position −99 relative to the MSH5-initiating methionine. This cis-acting element has been found upstream or within a number of meiotically regulated genes (for review, see Mitchell 1994); however, meiosis-specific expression of MSH5 has not yet been demonstrated.

When the Msh5p amino acid sequence was compared with other proteins in the GenBank data base [Altschul et al. 1990], strong homology to the MutS family of proteins was found [Fig. 4]. Msh5p exhibits 23.5% identity and 49.0% similarity with the entire Escherichia coli MutS protein. Of the four previously identified yeast MutS homologs, Msh5p is most homologous to Msh2p [25.6% identity, 50.3% similarity] and least homologous to Msh4p [21.0% identity, 45.6% similarity]. As with the other members of the MutS family, the strongest regions of homology for Msh5p are located in the carboxy-terminal part of the protein. Two functional domains—a nucleotide-binding site and a helix-turn-helix DNA-binding domain—are both present in Msh5p. A consensus sequence for the nucleotide-binding site region is depicted in Figure 4. All four of the domains involved in NTP binding found in the superfamily of recombination/repair proteins defined by Gorbalenya and Koonin (1990) are present in Msh5p. Also depicted is a comparison of the Msh5p helix-turn-helix region with the consensus sequence derived by Ross-MacDonald and Roeder (1994). The placement of the conserved domains within Msh5p is similar to that seen for the other members of the family.

| Table 4. Effect of msh5::URA3 on gene conversion |
|-----------------------------------------------|
| Strain | Genotype | HIS4/his4-x | ARG4/arg4-hsp1 | leu2-K/leu2ΔhisG |
|--------|----------|-------------|----------------|----------------|
| NH144  | MSH5     | 5.1 [297]   | 1.7 [293]      | 2.6 [193]      |
|        | msh5::URA3 | 7.6 [264]   | 10.4 [259]     | 3.5 [114]      |
| NH145  | msh5::URA3 | 7.6 [264]   | 10.4 [259]     | 3.5 [114]      |

*a Only four-viable spore tetrads were analyzed. For HIS4 and ARG4, gene conversions were scored as tetrads exhibiting 3+:1− or 3−:1+ segregation for the marker. For LEU2, the presence of a Leu + spore indicated gene conversion. A single PMS event (5+:3−) was observed for the arg4-hsp1 marker in NH144. The underlined value differs significantly from wild type (χ², P < 0.001). Numbers in parentheses indicate the number of tetrads assayed.
MSH5 is not involved in mismatch repair in mitosis or meiosis

The homology of Msh5p with MutS suggested that Msh5p may play some role in mismatch repair in yeast. A failure to undergo mismatch repair results in a mutant phenotype in vegetatively growing cells. To test whether msh5::URA3 has a mutant phenotype, the rate of mutation from Can^+ to Can^- was determined in the isogenic strains PS593 and PS593/190 (msh5::URA3) (see Materials and methods). Mutation in the yeast MutS homolog MSH2, a gene that has been demonstrated to be required for mitotic and meiotic mismatch repair, results in an 85-fold increase in the rate of mutation to Can^- [Reenan and Kolodner 1992b]. In contrast, no difference was found for the mutation rate in the isogenic strains served to exhibit moderate amounts of PMS in wild-type and his4--x and arg4--nsp strains. The lack of PMS events for msh2 and msh5::URA3 could be made. The resulting diploid [NH145] is heterozygous for the his4--x and arg4--nsp alleles. Although gene conversion frequencies at these loci are either the same or increased relative to the MSH5 diploid NH144 [Table 4], no PMS events were observed at either ARG4 or HIS4 [see Materials and methods]. In contrast, Reenan and Kolodner [1992b] reported that 46% of the gene conversion events involving the his4--x allele exhibited PMS in msh2 strains. The lack of PMS events for arg4--nsp was somewhat surprising given that this allele has been observed to exhibit moderate amounts of PMS in wild-type strains [Lichten et al. 1990, Ross-MacDonald and Roeder 1994]. In our hands, the gene conversion frequency in NH144 (MSH5) is also significantly reduced from that observed by other groups [e.g., 1.7% vs. 9.2% found by Ross-MacDonald and Roeder (1994)]. It is clear from these data, however, that the lack of PMS events in the msh5::URA3 diploid indicates that MSH5 is not required for mismatch repair during meiosis.
A. Nucleotide binding site region:

| Bacteria | MutS family | Consensus: 
|-----------|-------------|--------------------------------------------------------|
| Ecoli 609 | MSH2 | +ITITPHWS GES.Y-RQ A +L+AOQVS VPAH+ ... +D+.+XNG |
| Ecoli 609 | MutS | +ITITPHWS GES.Y-RQ A +L+AOQVS VPAH+ ... +D+.+XNG |
| MutS | MSH2 | +ITITPHWS GES.Y-RQ A +L+AOQVS VPAH+ ... +D+.+XNG |
| MutS | MutS | +ITITPHWS GES.Y-RQ A +L+AOQVS VPAH+ ... +D+.+XNG |

B. Helix-turn-helix consensus:

| Bacteria | MutS family | Consensus: 
|-----------|-------------|--------------------------------------------------------|
| Ecoli | MutS | +.IITGPNMG GKS.YRQ.A +I.++AQIGS +VPA,.+.+. ++D.+.+RVG |
| MutS | Ecoli | +.IITGPNMG GKS.YRQ.A +I.++AQIGS +VPA,.+.+. ++D.+.+RVG |
| MutS | MutS | +.IITGPNMG GKS.YRQ.A +I.++AQIGS +VPA,.+.+. ++D.+.+RVG |
| MutS | MutS | +.IITGPNMG GKS.YRQ.A +I.++AQIGS +VPA,.+.+. ++D.+.+RVG |

Figure 4. Alignment of Msh5 protein with MutS homologs known to be involved in mismatch repair and/or recombination. [A] Alignment of the region containing the 4 nucleotide-binding site motifs of Msh5 protein with seven other members of the MutS family. The consensus sequence was generated using the following parameters: A bold uppercase letter means that the amino acid is conserved in all family members except Msh4 and Msh5 proteins (also designated Msh4p and Msh5p). Numbers in parentheses indicate the positions of the amino acids in the proteins. [B] Comparison of the Msh5 protein helix-turn-helix domain to the consensus defined by Ross-MacDonald and Roeder (1994).

Discussion

Using a screen specific for mutants defective in reciprocal crossing-over between homologs during meiosis in yeast, we have isolated new alleles of HOP1, RED1, and MEK1. The screen also identified MSH5, a gene encoding a novel member of the MutS family of proteins. MutS has been studied extensively in E. coli and has been shown to be a protein important for mismatch repair and for preventing recombination between partially diverged DNA sequences [homologous recombination]. MutS protein binds to DNA mismatches and then recruits other proteins including MutL and MutH to facilitate meiotic recombination between homologs.

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MutS homolog facilitates meiotic crossing-over

MSH4 and MSH5 belong to the same genetic epistasis group

MSH4 is a gene from S. cerevisiae that encodes a MutS homolog that also specifically affects meiotic reciprocal recombination and does not appear to have a role in mismatch repair (Ross-MacDonald and Roeder 1994). Mutants in either MSH4 or MSH5 decrease spore viability to 40%–60%. The residual spore viability observed in msh4 and msh5 strains may result because [1] the two genes are functionally redundant and can partially substitute for each other, [2] the two genes act on separate pathways, either of which is capable of generating approximately 50% viable spores, or [3] the two genes function in the same process which, when abolished, decreases spore viability by half. If either of the first two cases is true, disruption of both genes in the same diploid should lead to a reduction in spore viability below the viability observed for either single mutant alone. Isogenic diploids were constructed that differ only at MSH4 and/or MSH5. These diploids were sporulated, and the resulting tetrads dissected to assess their spore viability. For the MSH4 MSH5 diploid NH144, spore viability was 97% [377 tetrads]. The msh4::LEU2 msh5::URA3 diploid NH155 produced 41% viable spores [297 tetrads], similar to the 38% observed for msh5::URA3 alone [1370 tetrads].

Disruption of MSH4 in this background gives rise to 56% viable spores [Ross-Macdonald and Roeder 1994]. Similar results were obtained when these genes were disrupted in the A364A strain background [N.M. Hollingsworth, unpubl.]. NH155 was also analyzed for recombination between HIS4 and MAT. A threefold reduction in recombination similar to that found for msh5::URA3 was observed for the double mutant [Table 2]. [MSH4 reduced recombination twofold in this interval [Ross-MacDonald and Roeder 1994]]. MSH4 and MSH5 therefore belong to the same epistasis group both in terms of spore viability and recombination. Furthermore, it appears that MSH5 is epistatic to MSH4. One interpretation of these results is that MSH4 and MSH5 work in the same pathway to facilitate meiotic recombination between homologs.
drial genome. MSH2 is involved in mismatch repair both in mitosis and meiosis and in the prevention of homeologous recombination (Reenan and Kolodner 1992b; Selva et al. 1995). MSH3 may have a small role in mismatch repair and in certain situations acts to inhibit homeologous recombination (Selva et al. 1995). MSH4 differs from the previously identified MutS homologs in that it plays no role in mismatch repair but is instead exclusively required for wild-type levels of reciprocal recombination during meiosis in yeast (Ross-Macdonald and Roeder 1994).

To test whether MSH5 is involved in mismatch repair we assayed two phenotypes characteristic of mismatch repair defective mutations—an increase in mutation rate in vegetatively growing cells and increased levels of post-meiotic segregation. In both cases, the msh5 mutant was indistinguishable from the isogenic MSH5 control. Spore viability in the msh5 spo13 disomic haploid is slightly improved relative to wild type (N.M. Hollingsworth, unpubl.), indicating that lethal lesions are not being introduced during premeiotic DNA synthesis (Ross-Macdonald and Roeder 1994). Therefore, despite its strong homology to MutS, the role of MSH5 is not in mismatch repair.

The MSH5 gene appears to be restricted to meiosis. No mitotic phenotypes have been observed so far in msh5 strains (MMS sensitivity, spontaneous reciprocal recombination, gene conversion, and growth rate have been examined; data not shown). Strains lacking MSH5 show an average threefold reduction in meiotic crossing-over between homologs measured on two different chromosomes. This defect in interhomolog reciprocal exchange is probably responsible for increased levels of Meiosis I nondisjunction and a reduction in spore viability. The role of MSH5 therefore appears to be in facilitating crossovers between homologs during meiosis.

The phenotypes observed for msh5 are similar to those found in strains lacking the meiosis-specific MutS homolog, MSH4. Interestingly, for spore viability and recombination, the MSH5 defect consistently seems to be slightly more severe. The msh5::URA3 allele was introduced into the same spo13 strains used to analyze recombination for msh4 and so direct comparisons may be made. In every interval the msh5 mutant exhibited at least 10% less recombination than msh4 (Table 3; Ross-Macdonald and Roeder 1994). This result is consistent with the observation that the msh5::URA3 mutation results in fewer viable spores (38% vs. 56%) than the msh4 disruption in the SK1 background.

Ross-Macdonald and Roeder [1994] have proposed that the twofold reduction in reciprocal recombination caused by msh4 may be attributable to the existence of two independent pathways of recombination, only one of which requires MSH4. We tested the idea that MSH5 might be involved in the MSH4-independent pathway by assaying spore viability in a msh4 msh5 diploid. In two different strain backgrounds the number of viable spores in the double mutant was no lower than the more severe single mutant msh5. Recombination between HIS4 and MAT in the double mutant was also not reduced beyond the level of msh5 alone. This result indicates that rather than working in separate pathways, MSH4 and MSH5 function in the same process. One model to account for this result would be if Msh4p and Msh5p interact to form heterodimers. If this is the case, the fact that MSH5 is epistatic to MSH4 would require that MSH5 homodimers also be important. Ross-MacDonald and Roeder (1994) have noted that the DNA-binding domain of Msh4p differs in several conserved residues from the MutS family. Interestingly, these sites are much more conserved in Msh5p (Fig. 4). In the heterodimer model it may be the Msh5p subunit that is responsible for binding DNA while the Msh4 protein is important for interacting with other proteins. In support of the heterodimer model, Drummond et al. [1995] have shown recently that the human mismatch repair activity hMutSo1 is a heterodimer of MutS homologs.

The role of MutS in mismatch repair is to bind DNA duplexes containing mispaired bases (Su and Modrich 1986). MutS protein therefore recognizes a structure as opposed to a specific sequence. The fact that a human MutS homolog, hMSH2, and yeast Msh2p are capable of binding mismatches comprised of loops of DNA as large as 14 nucleotides but E. coli MutS cannot [Fishel et al. 1994; Alani et al. 1995], suggests that during evolution different MutS family members have diverged to recognize different structures. Because MSH4 and MSH5 are not required for mismatch repair and because both exhibit a mutant phenotype in homothallic diploids where no mismatches exist during meiosis [Ross-Macdonald and Roeder 1994; N.M. Hollingsworth and C. Halsey, unpubl.], it seems likely that these proteins bind a DNA structure other than a mismatch.

MSH5 was isolated using a screen that demanded efficient intrachromosomal reciprocal recombination. Furthermore, we have demonstrated that gene conversion at LEU2 is not decreased in the same strains in which reciprocal crossing-over between HIS4 and MAT, two markers that flank LEU2, is reduced threefold. These results suggest that normal levels of heteroduplex are forming to allow for gene conversion but that the resolution of such intermediates is somehow biased against crossovers. One possibility is that Msh5p binds a configuration of DNA that is formed specifically between homologs. Perhaps binding of such substrates by Msh5p stabilizes recombination intermediates formed between non-sister chromatids (Collins and Newlon 1994, Schwacha and Kleckner 1994), thereby channeling the recombination mechanism to favor events between homologs.

Materials and methods

Yeast strains and media

The genotypes of strains used in this work are listed in Table 1. Standard yeast genetic methods were employed (Mortimer and Hawthorne 1969). Liquid and solid media have been described [Hollingsworth and Johnson 1993]. All gene disruptions created by transformation were confirmed by Southern blot analysis.
Plasmid construction

Plasmids for this study were constructed using standard procedures [Maniatis et al. 1982]. pNH181 was constructed by deleting first a HindIII fragment and then a SpHl fragment from the original clone pMSHS-2. pNH180-13 was made by cloning a 4.4-kb HindIII fragment from pMSHS-2 into the HindIII site of Ylp5. pNH180-13 was targeted to the MSH5 region of chromosome IV by digestion with XhoI [Orr-Weaver et al. 1981]. pNH189-2 was generated by ligating a 4.3-kb HindIII–XhoI fragment that contains the entire MSH5 gene from pMSHS-2 into pVZ1 cut with HindIII and SalI. The msh5::URA3 insertion allele was constructed by cloning a 1.1-kb BglII–BamHI fragment containing URA3 into the BglII site of pNH189-2. Digestion of the resulting plasmid, pNH190, with EcoRI and Clal releases a 3.2-kb fragment that can be used to replace the wild-type copy of the MSH5 by one-step gene transplacement [Rothstein 1983]. pNH187-2 contains a 1.9-kb BglII fragment from pNH181 cloned into the BamHI site of pVZ1. The msh4::LEU2 disruption was generated using p6H from P. Ross-MacDonald (Yale University, New Haven, CT).

The meiotic interhomolog recombination mutant screen

The interhomolog recombination screen has been described previously in detail in Hollingsworth and Byers [1989]. The screen was performed with some minor modifications. The disomic haploid karC2-4 was mutagenized with ethylmethane sulfonate cell viability were used. Of 9855 patched colonies, 243 were initially scored as having elevated levels of His+ Leu+ Cys+ cells. Failure to restet was observed in 114 candidates, whereas 59 were either increased for intrachromosomal exchange in vegetative cells or had a mutant cyh2 allele now resident in the duplication and were therefore discarded.

The remaining 70 candidates were analyzed further in two ways. First the frequency of intrachromosomal recombination in both mitotic and meiotic cells was quantitated as described in Ivanov et al. [1992]. To ensure that we could detect PMS events, prototrophic and auxotrophic spores were micromanipulated adjacent to each other and the resulting colonies were tested as described. In such cases, sectoring was readily observed.

Cloning the MSH5 gene

The MSH5 gene was cloned by complementation of the msh5-1 spore viability phenotype using the random spore method described in Hollingsworth and Byers [1989]. The msh5-1 NH111 diploid was transformed with a genomic yeast DNA library constructed in YCp50 [Rose et al. 1987]. Two different plasmids with overlapping inserts of 17 and 11.6 kb (designated pMSHS-1 and pMSHS-2) were found to complement msh5-1. The smallest MSH5 complementing activity was found to reside in a 4.7-kb HindIII–SpHl fragment. The MSH5 gene was placed on the physical map of the S. cerevisiae genome by hybridizing fragments from either pMSHS-1 or pMSHS-2 (0.9- and 1.9-kb SpHl fragments, respectively) to a set of overlapping genomic fragments contained in a series of λ clones (available from the American Type Culture Collection). Both fragments identified the same two λ clones (ATCC no. 71144 and 70858), indicating that the two inserts carry overlapping sequences. These λ clones are adjacent to one carrying CDC36, leading to the prediction that tight genetic linkage should be observed between MSH5 and CDC36. The MSH5 region was marked with URA3 using pNH180-13 and crossed to a ura3 cdc36 strain. Tetrad analysis of the resulting diploid demonstrated that MSH5 and CDC36 are ∼1.9 cM apart [50PD: 0NPD: 2T, map distance calculated using the formula of Perkins [1949]].

Mapping MSH5

The DNA sequence of the MSH5 gene was obtained by first constructing exonuclease III deletion derivatives of plasmids pNH187-2 and pNH189-2 [Henikoff 1984]. These plasmids were used as templates for the dideoxy method of DNA sequencing using T7 or T3 primers [Sanger et al. 1977]. To fill in gaps in the sequence not covered by the deletion series, specific oligonucleotides were synthesized and used as primers. The DNA sequence of both strands was determined. The GenBank accession number is L42517.

Mutation rate analysis

Ten independent single colonies of PS593 or PSS93/190 were inoculated into YEPD and grown at 30°C to saturation. The cells were plated onto YPAD to obtain the viable cell titer and onto Arg+ Can plates to determine the number of spontaneously arising Can+ mutants. The mutation rates were calculated as described in Ivanov et al. [1992].

Gene conversion and PMS analysis

Tetrads were dissected onto YPAD plates, and the spore colonies transferred by replica plating to appropriate dropout media. To ensure that we could detect PMS events, prototrophic and auxotrophic spores were micromanipulated adjacent to each other and the resulting colonies were tested as described. In such cases, sectoring was readily observed.

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