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 (Engbrecht et al. 1990).

To understand how functional chiasmata are gener-

ated in the presence of SCs, it is necessary to first define the proteins involved both in synapsis (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, synapsis, 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; Engbrecht and Roeder 1989; Gottlieb et al. 1989). In theory, therefore, this screen should detect mutants defective in synapsis, 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 synapsis (Hollingsworth

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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, *mutS* is required to prevent partially diverged sequences from undergoing recombination in bacteria (Rayssiguier et al. 1989). We demonstrate here that, unlike *mutS* 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.

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 equational 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-

Table 1. *Saccharomyces cerevisiae* strains

Strain	Genotype
A. Disomic haploids	
<i>karC2-4</i>	<i>MATα cdc10-2 LEU2::pNH18 (URA3 CYH2) HIS4 trp1 ura3-52 can1 cyh2 spo13-1 ade2-1</i> <i>MATα CDC10 leu2 his4</i>
B31-89	<i>karC2-4</i> but <i>msh5-1</i>
B. SK1 background^a	
NH144	<i>MATα leu2-k HIS4 arg4-nsp ura3 lys2 ho::LYS2</i> <i>MATα leu2Δhisg his4-x ARG4 ura3 lys2 ho::LYS2</i>
NH145	NH144 but <i>msh5::URA3</i> <i>msh5::URA3</i>
NH155	NH144 but <i>msh5::URA3 msh4::LEU2</i> <i>msh5::URA3 msh4::LEU2</i>
C. BR background^a	
NH156	<i>MATα LEU2 his4 ade2 MET13 trp5 cyh3 ade6 ura3 spo13Δura3</i> <i>MATα leu2 HIS4 ade2 met13 TRP5 CYH3 ADE6 ura3 spo13Δura3</i>
NH157	NH156 but <i>msh5::URA3</i> <i>msh5::URA3</i>
D. A364A background	
PS593	<i>MATα leu2-3,112 ura3-52 trp1 his3Δ200</i>
PS593/190	same as PS593 but <i>msh5::URA3</i>
E. Miscellaneous strain background	
NH111	<i>MATα LEU2 HIS4 his7 trp1 msh5-1 ura3-52 CAN1 CYH2 spo13-1</i> <i>MATα leu2 his4 HIS7 trp1 msh5-1 ura3-52 can1 cyh2 spo13-1</i> <i>ade2-1</i> <i>ade2-1::pNH164 (ADE2 SPO13)</i>

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

^a From P. Ross-MacDonald.

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, *msh5-1*, 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 *msh5-1* 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 *Bgl*III site within the *MSH5*-coding sequence (Fig. 3). This insertion creates a truncated Msh5 protein, removing two highly conserved functional domains (see below). The

Figure 1. Comparison of the pattern of viable spores within tetrads in the SK1 isogenic diploids NH144 [*MSH5/MSH5*] and NH145 [*msh5::URA3/msh5::URA3*]. For NH144, 337 tetrads were dissected. For NH145, 1370 tetrads were dissected. (Solid bar) *MSH5*; (hatched bar) *msh5::URA3*.

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 *msh5-1* (50%, 146 tetrads for the A364A background; and 38%, 1370 tetrads, for the SK1 background).

The observation that *spo13* can rescue the *msh5-1* spore lethality suggests that the spore inviability conferred by *msh5-1* 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 *msh5-1 SPO13* diploid produces 37% viable spores (485 tetrads). In the isogenic *msh5-1 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

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

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

^b *hop1-1* data from Hollingsworth and Byers (1989).

^c Qualitative results obtained by random spore analysis.

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 *MATα* 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 *MATα* 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 (χ^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 *α*-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 ^a	Genotype	Intergenic distance (cM) ^b				
		<i>HIS4-LEU2</i>	<i>LEU2-MAT</i>	<i>MET13-TRP5</i>	<i>TRP5-CYH3</i>	<i>CYH3-ADE6</i>
NH156	<i>MSH5 spo13</i> <i>MSH5 spo13</i>	13	38	47	24	19
NH157	<i>msh5::URA3 spo13</i> <i>msh5::URA3 spo13</i>	7 (0.5) ^c	12 (0.3)	15 (0.3)	6 (0.3)	6 (0.3)
NH144	<i>MSH5 MSH4</i> <i>MSH5 MSH4</i>	<i>HIS4-MAT</i> 37				
NH145	<i>msh5::URA3 MSH4</i> <i>msh5::URA3 MSH4</i>	13 (0.3)				
NH155	<i>msh5::URA3 msh4::LEU2</i> <i>msh5::URA3 msh4::LEU2</i>	10 (0.3)				

^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

$$\frac{(2 \times \text{SCO}_e) + \text{SCO}_r + 6(\text{four strand DCO})}{\text{Total dyads}} \times \frac{100}{2} = \text{cM}$$

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):

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

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 χ^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 *MSH5* and 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 Gorbelenya 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	Gene conversion (%) ^a		
		<i>HIS4/his4-x</i>	<i>ARG4/arg4-hsp1</i>	<i>leu2-K/leu2ΔhisG</i>
NH144	<i>MSH5</i>			
	<i>MSH5</i>	5.1 (297)	1.7 (293)	2.6 (193)
	<i>msh5::URA3</i>			
NH145	<i>msh5::URA3</i>	7.6 (264)	<u>10.4</u> (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-nspl* marker in NH144. The underlined value differs significantly from wild type (χ^2 , $P < 0.001$). Numbers in parentheses indicate the number of tetrads assayed.

A. Nucleotide binding site region:

Ecoli (609)	MLIITGPNMG	GKSTYMRQTA	LIALMAYIGS	YVPAQKVEIG	PIDRIFTRVG
Styp (609)	MLIITGPNMG	GKSTYMRQTA	LIALLAYIGS	YVPAQNVEIG	PIDRIFTRVG
Hum (664)	FHIITGPNMG	GKSTYIRQTA	VIVLMAQIGC	FVPCSAEVS	IUDCILARVG
Msh1 (766)	LWVITGPNMG	GKSTFLRQNA	IIVLMAQIGC	FVPCSAEVS	IUDKLFVRVG
Msh2 (683)	FLIITGPNMG	GKSTYIRQVQ	VISLMAQIGC	FVPCSAEIA	IUDAILCRVG
Msh3 (815)	INIITGPNMG	GKSSYIRQVA	LITMAQIGS	FVPAEETRLS	IFENVLITRIG
Msh4 (629)	LQIITGPNMG	GKSVYLQKVA	LICIMAQMS	GIPALYGSFP	VFKRLHARV
Msh5 (638)	IIVVTGANAS	GKSVYLQKNG	LIVVLAQIGC	FVPAERARIG	IADKILTRIR
Consensus:	+..IITGPNMG	GKS.Y+RQ.A	+I..+AQIGS	+VPA..+..	++D..+RVG

Ecoli	AADDLASGRS	TFMVEMTETA	NILHNATEYS	LVLMDIEGRG	TSTYDGLSLA
Styp	AADDLASGRS	TFMVEMTETA	NILHNATENS	LVLMDIEGRG	TSTYDGLSLA
Hum	AGDSQLKGVY	TFMAEMLETA	SILRSATKDS	LIILDEIGRG	TSTYDGLSLA
Msh1	SADDLYNEMS	TFMVEMTETS	FILQGATERS	LAILDEIGRG	TSGKGISIA
Msh2	AGDSQLKGVY	TFMVEILETA	SILKNASKNS	LIIVDEIGRG	TSTYDGLSLA
Msh3	AHDDIINGDS	TFKVEMLDIL	HILKNCKNRS	LLLLDEVGGR	TGTHDGLAIS
Msh4	CNDSMELTSS	NFGFEMKEMA	YFLDDINTET	LLILDEIGRG	SSIADGFCVS
Msh5	TQETVYKTOS	SFLILDSQMA	KSLSLATEKS	LILDEYKGG	TDILDGPSLF
Consensus:	..D.....S	TF..EM.E.A	..IL..A..S	L+++DE+GRG	TS..DG+..

Ecoli	WACAENLANK	IKA.LTLFAT	HY
Styp	WACAENLANK	IKA.LTLFAT	HY
Hum	WASEYIATK	IGA.FCMFAT	HF
Msh1	YATLKYLLEN	NQCR.TLFAF	HF
Msh2	WAIABHIASK	IGC.FALFAF	HF
Msh3	YALIKYFSEL	SDCPILFTT	HF
Msh4	LAVTEHLRLT	EAT..VFLST	HF
Msh5	GSIMLNMSKS	EKCPRIATCT	HF
Consensus:	+A...+....	+F.T	HF

B. Helix-turn-helix consensus:

MutS consensus: g + . V A . + + g + p . - + + . r A . .

Msh5p (821-840) G I Y C A K V C G L S R D I V E R A E E

Msh4p (811-830) G I R V V K K I F N P D I I A E A Y N I

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; an uppercase letter means that the amino acid is found in at least six of eight members; (+) a hydrophobic position; (−) a hydrophilic position; (.) no similarity. Positions bearing two letters mean that either amino acid may be present. Asterisks (*) mark amino acids that are 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. (Ecoli) *E. coli* MutS, Schlensog and Bock (1991); (Styp) *S. typhimurium* MutS, Haber et al. (1988); (Hum) hMSH2, Leach et al. (1993); (Msh1 and Msh2) Reenan and Kolodner (1992a); (Msh3) New et al. (1993); (Msh4) Ross-Macdonald and Roeder (1994). (B) Comparison of the Msh5 protein helix-turn-helix domain to the consensus defined by Ross-MacDonald and Roeder (1994). Amino acids found in all of the MutS members are uppercase, whereas those present in the majority of proteins are lowercase. (+) A hydrophobic residue; (−) a hydrophilic residue.

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.

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 (homeologous recombination). MutS protein binds to DNA mismatches and then recruits a complex of other proteins including MutL and MutH to effect the repair of the mismatches (for review, see Modrich 1991). Recently a MutS homolog in humans has been implicated in the development of hereditary non-polyposis colon cancer (Fishel et al. 1993; Leach et al. 1993).

Four other MutS homologs are known in *S. cerevisiae*. *MSH1*, *MSH2*, and *MSH3* were isolated by the polymerase chain reaction using primers directed against conserved regions of MutS (Reenan and Kolodner 1992a; New et al. 1993). *MSH4* was identified by a transposon insertion that resulted in meiosis-specific *lacZ* expression (Burns et al. 1994). *MSH1* is targeted to mitochondria and is important for the stability of the mitochondria

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 cross-overs 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 hMutS α 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 *Hind*III fragment and then an *Sph*I fragment from the original clone pMSH5-2. pNH180-13 was made by cloning a 4.4-kb *Hind*III fragment from pMSH5-2 into the *Hind*III site of Ylp5. pNH180-13 was targeted to the *MSH5* region of chromosome IV by digestion with *Xba*I (Orr-Weaver et al. 1981). pNH189-2 was generated by ligating a 4.3-kb *Hind*III-*Xho*I fragment that contains the entire *MSH5* gene from pMSH5-2 into pVZ1 cut with *Hind*III and *Sal*I. The *msh5::URA3* insertion allele was constructed by cloning a 1.1-kb *Bgl*II-*Bam*HI fragment containing *URA3* into the *Bgl*II site of pNH189-2. Digestion of the resulting plasmid, pNH190, with *Eco*RI 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 *Bgl*II fragment from pNH181 cloned into the *Bam*HI 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 in two independent rounds at 23°C. Time points exhibiting 15% cell viability were used.

Of 9855 patched colonies, 243 were initially scored as having elevated levels of His⁺ Leu⁺ Cyh^r cells. Failure to retest 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 Hollingsworth and Byers (1989) to ensure that the effect was meiosis-specific. Second, dyads from the sporulated disomic haploid strains were dissected to determine the percent spore viability. The segregation of markers on chromosome III was scored in the dyads to assess the effects of the mutations on equational versus reductional segregation and on recombination between homologs.

To determine the spore viability of the mutants in a *SPO13* meiosis, *SPO13* diploids homozygous for each mutation were constructed. Haploid derivatives of both mating types were obtained as described in Hollingsworth and Byers (1989). The *MATa* derivatives were converted to Spo13⁺ by the integration of the *SPO13* gene (Hollingsworth and Byers 1989). After crossing the two haploids, the resulting diploids were sporulated and dissected to ascertain the number of viable spores.

Cloning the *MSH5* gene

The *MSH5* gene was cloned by complementation of the *msh5-1* spore inviability 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 pMSH5-1 and pMSH5-2) were found to complement *msh5-1*. The smallest *MSH5* complementing activity was found to reside in a 4.7-kb *Hind*III-*Sph*I fragment.

Mapping *MSH5*

The *MSH5* gene was placed on the physical map of the *S. cerevisiae* genome by hybridizing fragments from either pMSH5-1 or pMSH5-2 (0.9- and 1.9-kb *Sph*I 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 70585), 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: ONPD: 2T, map distance calculated using the formula of Perkins (1949)].

Sequencing *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 PS593/190 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^r 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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MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair.

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