Functional specificity of MutL homologs in yeast: Evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction

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Contributed by Nancy Kleckner, September 8, 1999

The yeast genome encodes four proteins (Pms1 and Mlh1–3) homologous to the bacterial mismatch repair component, MutL. Using two hybrid-interaction and coimmunoprecipitation studies, we show that these proteins can form only three types of complexes *in vivo*. Mlh1 is the common component of all three complexes, interacting with Pms1, Mlh2, and Mlh3, presumptively as heterodimers. The phenotypes of single deletion mutants reveal distinct functions for the three heterodimers during meiosis: in a *pms1* mutant, frequent postmeiotic segregation indicates a defect in the correction of heteroduplex DNA, whereas the frequency of crossing-over is normal. Conversely, crossing-over in the *mlh3* mutant is reduced to ≈70% of wild-type levels but correction of heteroduplex is normal. In a *mlh2* mutant, crossing-over is normal and postmeiotic segregation is not observed but non-Mendelian segregation is elevated and altered with respect to parity. Finally, to a first approximation, the *mlh1* mutant represents the combined single mutant phenotypes. Taken together, these data imply modulation of a basic Mlh1 function via combination with the three other MutL homologs and suggest specifically that Mlh1 combines with Mlh3 to promote meiotic crossing-over.

Meiotic recombination culminates in the formation of crossovers that, in combination with cohesion between sister chromatids, provide a physical connection between homologous chromosomes. This connection permits bipolar orientation on the meiotic spindle and subsequent reductional segregation at the first meiotic division (1, 2).

In eukaryotes, homologs of the *Escherichia coli* MutL and MutS mismatch-repair proteins, are involved in multiple pathways of DNA recombination and repair (3, 4). In *Saccharomyces cerevisiae*, three of the six MutS homologs (Msh2, 3, and 6) and two of the four MutL homlogs (Pms1 and Mlh1) have been shown to play a role in nuclear mismatch repair (3, 4). Also, Mlh3 recently has been implicated in the avoidance of frameshift mutations (5). The function of the fourth MutL homolog, Mlh2, is less clear but a role in processing some forms of DNA damage is implied by the finding that *mlh2* mutants are resistant to cisplatin and related anticancer compounds (6). The eukaryotic MutS and MutL homologs analyzed to date form heterodimers, pairs of which are specialized to process specific forms of DNA mismatches: Msh2-Msh6 plus Mlh1-Pms1 function in repair of single base pair mismatches and small insertion/deletion mispairs whereas Msh2-Msh3 plus Mlh1-Mlh3 are likely involved in repair of specific insertions/deletions (5, 7–14).

In yeast, MutS and MutL homologs also are required for meiotic recombination, irrespective of any involvement in mismatch repair. Mutations in Msh4, Msh5, and Mlh1 reduce the levels of crossing-over (refs. 15–17; see below), even in isogenic situations where the potential for base pair mismatches is minimal. Msh4/5 are meiosis-specific proteins that play no role in mismatch repair; Mlh1 functions in the same pathway as Msh4/5, but with a somewhat different role (refs. 17 and 18; see *Discussion*). The mammalian Msh5 and Mlh1 homologs are implicated similarly in meiotic recombination (refs. 19–24; see *Discussion*). Like other MutS proteins, yeast Msh4/5 appear to function as a heterocomplex (18, 25). The general pattern of protein associations that governs mismatch repair (above) suggests that Mlh1 functions in a complex with another MutL homolog during meiotic recombination.

The work described below uses two-hybrid, coimmunoprecipitation, and genetic assays to address two interrelated issues regarding the roles of MutL homolog proteins in yeast. First, which complexes of MutL homologs can assemble *in vivo*? Second, which MutL protein(s) is involved in meiotic recombination, i.e., as a partner(s) for Mlh1? The results of our studies reveal a pivotal, coordinating role for Mlh1, which acts in combination with each of the other MutL protein homologs to mediate different functions in repair and recombination. Among these, a Mlh1-Mlh3 heterocomplex is implicated specifically in meiotic recombination.

Materials and Methods

Analysis of mutL Homolog Protein Interactions. Two-hybrid screening was performed as described (26, 27). Four yeast Mlh1 protein variants (residues 1–145, 115–554, 501–769, and 721–769) were created by PCR, cloned into pBHA (lexA fusion vector; ref. 26), and used in screens of *S. cerevisiae* genomic libraries. Yeast genomic libraries, in pGAD1 and pGAD3, GAL4 activation domain vectors (28), were provided by Philip James (University of Wisconsin, Madison). Independent clones ($\approx 5 \times 10^6$) were screened for each bait construct.

Interactions between the four yeast mutL protein homologs also were analyzed by two-hybrid assay. Full-length or truncated cDNAs were created by PCR and cloned into pGAD10 (GAL4 activation domain vector; CLONTECH) or pBHA. pBHA and pGAD10 vectors were gifts from Morgan Sheng and Yi-Ping Hsueh (Massachusetts General Hospital, Boston).

Antibodies. A hexahistidine fusion protein of Mlh1 (amino acids 144–751) was expressed, purified, and used to immunize guinea pig. Specificity of the Mlh1 antisera was confirmed by Western blot analysis of extracts from wild-type and Δ*mlh1* mutant yeast strains (see below) with both pre- and postimmune serum (not shown). Mouse monoclonal anti-myc (9E10) and rat monoclonal anti-hemagglutinin (HA) (3F10) antibodies were purchased from Santa Cruz Biotechnology and Roche Molecular Biochemicals, respectively. Mouse horseradish peroxidase (HRP)-conjugated anti-V5 antibody (Invitrogen) was a gift from Sue-Jane Chen (Harvard University, Cambridge, MA).

Abbreviations: PMS, postmeiotic segregation; NMS, non-Mendelian segregation; HA, hemagglutinin.

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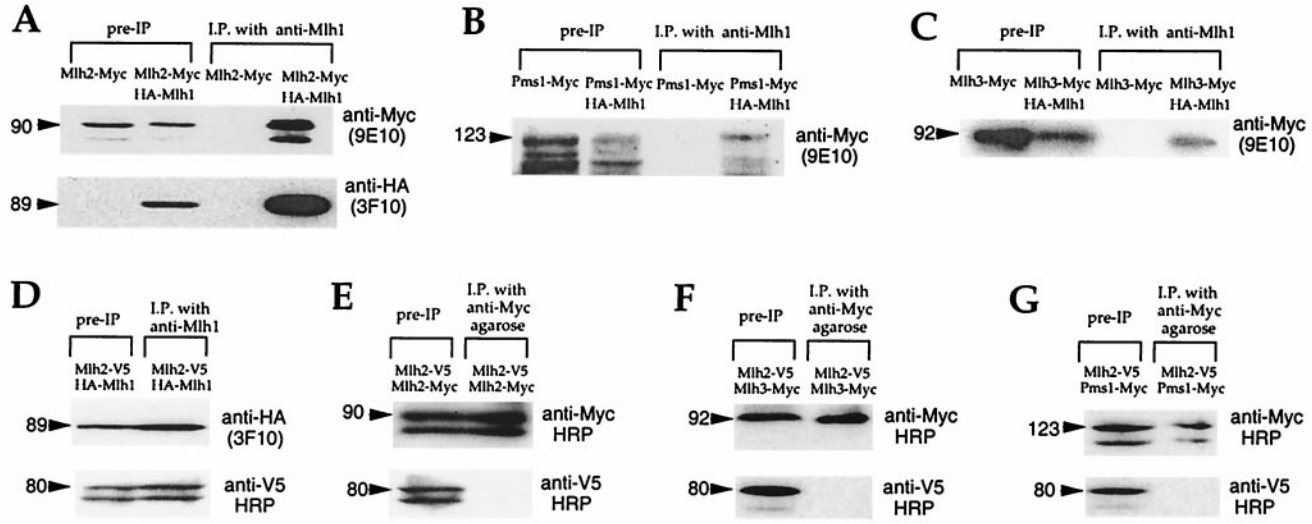

Fig. 1. Coimmunoprecipitation of Mlh1 and the other three yeast MutL proteins. (*A–C*) Anti-Mlh1 antisera can immunoprecipitate Mlh2, Mlh3, and Pms1 in the presence of Mlh1. Mlh2-myc$_9$-tagged (*A*), Pms1-myc$_9$-tagged (*B*), or Mlh3-myc$_9$-tagged (*C*) genes were transfected alone or together with triple HA-tagged Mlh1 into COS7 cells, and extracts were immunoprecipitated with anti-Mlh1 antiserum. The immunoprecipitates were immunoblotted by using myc antibody 9E10 to detect Mlh2-myc$_9$, Mlh3-myc$_9$, Pms1-myc$_9$, and HA antibody 3F10 to detect HA$_3$-Mlh1. Input (preIP) lanes represent 10% of the extract used in the immunoprecipitation reaction. (*D–F*) Mlh2 does not form precipitable complexes with Mlh3, Pms1, or itself. (*D*) Anti-Mlh1 antisera can immunoprecipitate V5-tagged Mlh2 when cotransfected with the Mlh1 expression construct. (*E–G*) Anti-myc antibody does not coprecipitate Mlh2-V5 together with either Mlh2-myc9, Mlh3-myc9, or Pms1-myc9 proteins.

Expression Constructs. Full-length Mlh1 in expression vector pClneo (Promega) was tagged at the C terminus with three HA epitopes; Mlh2 and Mlh3 (in pClneo) and Pms1 (in pcDNA3; Invitrogen) were tagged similarly with nine c-Myc epitopes. The Mlh2-V5 tag was constructed in expression vector pcDNA3.1/V5-HisA (Invitrogen). Details of tagged expression constructs are available on request.

COS7 Cell Transfection, Immunoprecipitation, and Immunoblotting. COS7 cells were transfected with tagged, expression constructs by using the Lipofectamine method (Life Technologies, Rockville, MD). Cell lysates were incubated with guinea pig anti-Mlh1 antisera (1:500 dilution) at 4°C for 2 hr. Protein A-Sepharose (Amersham Pharmacia) was added, and incubation was continued for a further 2 hr. Immunoprecipitates were washed with lysis buffer, eluted in SDS-loading buffer, separated by SDS/PAGE, transferred to nitrocellulose, and immunoblotted with anti-Myc(9E10) or anti-HA(3F10). One-tenth volume of COS7 cell lysates were analyzed as input controls. Proteins were visualized with HRP-conjugated, anti-mouse, or anti-rat secondary antibodies (Roche Molecular Biochemicals) and the Renaissance chemiluminescence reagent (DuPont). Myc-tagged proteins were immunoprecipitated with anti-Myc antibody-conjugated agarose; HRP-conjugated mouse antibodies (anti-Myc or anti-V5) were used for Western analysis.

Media and Genetic Techniques. Yeast extract/peptone/dextrose (YPD), drop-out, synthetic complete (SC), and sporulation media were as described (29). Geneticin (G418) was added to YPD medium at 400 mg/liter to select transformants. Yeast strains were transformed by using the lithium acetate method as modified by Gietz *et al.* (30). After a brief mating (17, 31), tetrad ascospores were dissected, and all spore clones were analyzed for His, Leu, and Ura phenotypes and mating type. Meiosis I nondisjunction of chromosome III was assigned to tetrads producing two viable spores that were nonmating, Ura+, His+, and Leu+. Disomy of chromosome VIII was determined by virtue of the *CUP1* gene that confers dosage-dependent resistance to copper (32). Tetrads with two viable spores resistant to 100 μM copper sulfate (in SC medium) were assigned as chromosome VIII meiosis I nondisjunctions. Statistical analysis was performed by using z- and G-tests (33), taking a P value of <0.05 as significant.

***S. cerevisiae* Strains.** All strains are derivatives of SK1 (34). NKY3231 (*mlh1*), 3232 (*mlh2*), 3233 (*mlh3*), and 3234 (*pms1*) are isogenic to the wild-type diploid, NKY3230:

HIS4::*LEU2*-(*NBam*) *leu2*::*hisG* *MAT***a** *ho*::*hisG* *ura3*(Δ*Pst-Sma*)
his4-X::*LEU2*-(*NBam*)-*URA3* *leu2*::*hisG* *MATα* *ho*::*hisG* *ura3*(Δ*Pst-Sma*).

The *HIS4*::*LEU2*-(*NBam*) locus is a modification of that described by Schwacha and Kleckner (35). Deletion mutations of *mutL* homolog genes were marked with the *Kan*MX4 cassette (36). Details of strain construction are available on request.

Results

Mlh1 Interacts with the Three Other MutL Protein Homologs. To identify proteins that interact with Mlh1, four different LexA-Mlh1 fusion proteins were used as "baits" in two-hybrid screens. With the LexA-Mlh1 bait (residues 551–769), we isolated two independent genomic clones of *MLH2* (ORF YLR035c) and one each of *MLH3* (ORF YPL164c) and *PMS1* (ORF YNL082w).

Physical interaction between Mlh1 and the other three yeast MutL proteins was confirmed by coimmunoprecipitation experiments. Mlh1 was tagged at the amino terminus with a triple HA epitope. Mlh2, Mlh3, and Pms1 each were tagged with nine c-Myc epitopes at their carboxyl termini. The c-Myc-tagged constructs were individually transfected into COS7 cells with or without the HA-Mlh1 expression construct. Cell lysates then were subjected to immunoprecipitation with guinea pig anti-Mlh1 antisera. As shown in Fig. 1, anti-Mlh1 antisera was able to precipitate HA-Mlh1 together with Myc-tagged Mlh2, Pms1, or Mlh3 only when the HA-Mlh1 construct was cotransfected (Fig. 1 *A*, *B*, and *C*, respectively). No signals for either HA- or Myc-tagged proteins were detected in immunoprecipitates when the HA-Mlh1-tagged construct was absent. Coimmunoprecipitation of Mlh1 with Mlh2, Mlh3, and Pms1 confirms the inference from two-hybrid analysis that Mlh1 is able to form heterocomplexes with each of the three other yeast MutL proteins. Interactions of Mlh1 with Pms1 and Mlh3 were reported previously (5, 10, 37).

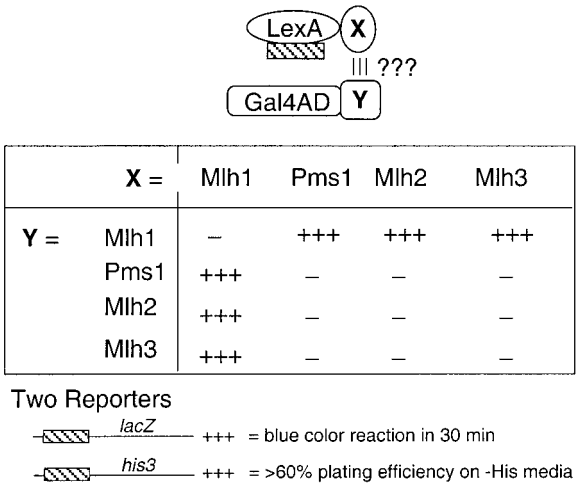

Fig. 2. Two-hybrid analysis of MutL protein interactions. Interaction was determined by the ability to induce the two-hybrid assay reporter genes *HIS3* and β-galactosidase. *HIS3* prototroph assay, as plating efficiency on histidine-lacking medium: +++ (>60%), ++ (30–60%), + (10–30%), − (no significant growth). β-galactosidase activity, as time taken for yeast colonies to turn blue on an 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) filter assay at room temperature: +++ (<30 min), ++ (30–90 min), + (90–240 min), − (no visible β-galactosidase activity).

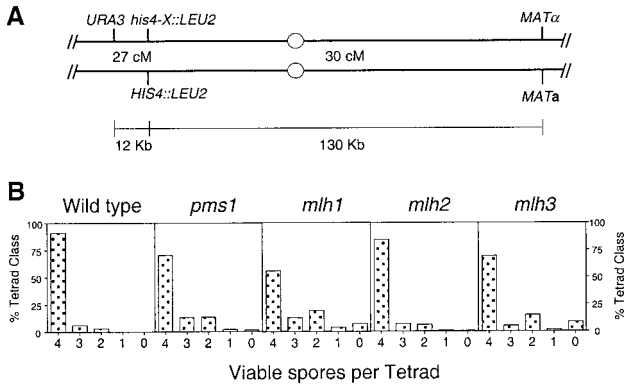

Fig. 3. Genetic intervals and spore viability. (*A*) Intervals on chromosome III examined in this study. Physical distances and genetic distances in the wild-type strain are shown. An open circle represents the centromere. (*B*) Distributions of tetrads with four, three, two, one, and zero viable spores for wild-type and mutant strains. Data for between 498 and 1,373 tetrads are represented by each graph. Spore viability is shown as percent viable spores: wild type, 96.9; *pms1*, 87.3; *mlh1*, 76.3; *mlh2*, 93.6; *mlh3*, 81.5.

Since the LexA-Mlh1 bait used in two-hybrid analysis contains only the carboxyl portion of Mlh1, residues 551–769, that region is likely to be responsible for association with each of the other three MutL homologs. From DNA sequence analysis, one of the two *MLH2* clones isolated in the two-hybrid screen (above) was found to comprise only 98 aa, residues 598–695, of the Mlh2 carboxyl terminus. Furthermore, the same 98-aa fusion exhibited a similar intensity of interaction with Mlh1 as the second, larger Mlh2 two-hybrid clone (residues 463–695) or even the full-length protein (residues 1–695; data not shown), strongly suggesting that this region contains the major Mlh1-interaction domain.

All Identified MutL Homolog Complexes Contain Mlh1 Plus One Other MutL Family Member. Since Mlh1 can interact with each of the other three MutL homologs, we investigated whether these were the only interactions that could occur among the four MutL proteins or whether other hetero- and/or homocomplexes could form *in vivo*. The GAL4 activation domain was fused to each of the four, full-length MutL proteins that were used as baits in two-hybrid analysis against each of four -lexA "prey" fusions, which contained, respectively, full-length Mlh1, Mlh2, and Pms1 and C-terminal truncated Mlh3 (residues 481–715). A truncated Mlh3 fusion was used because full-length Mlh3 gave a very high, nonspecific signal in the two-hybrid assay. In Fig. 2 we show that Mlh1 interacts with each of the other three MutL homologs, in accord with the results of the two-hybrid library screen described above. In contrast, none of the four proteins exhibits self–self interactions or interactions with members of the family other than Mlh1.

We also extended the coimmunoprecipitation analysis by examining the potential pairwise interactions between Mlh2 and the other MutL homologs by coimmunoprecipitation. An additional epitope-tagged Mlh2 protein was constructed by adding the V5 epitope at the carboxyl terminus. Coimmunoprecipitation revealed that only Mlh1 (Fig. 1*D*) but not Mlh2, Mlh3, or Pms1 (Fig. 1 *E*, *F*, and *G*, respectively) can interact physically with the Mlh2-V5-tagged protein. Thus, whereas Mlh1 forms precipitable complexes with each of the other four MutL homologs, Mlh2-Mlh2, Mlh2-Mlh3, and Mlh2-Pms1 complexes do not form. Self–self interactions for Pms1, Mlh1, and Mlh3 were not examined because analysis by other workers suggested that these complexes do not form (5, 37).

Meiotic Phenotypes of Yeast Mutants Lacking MutL Homologs. To investigate the meiotic functions of the four MutL homologs we constructed isogenic wild-type and single-mutant derivatives of a strain with genetic markers on chromosome III (Fig. 3). Tetrads from the five strains were dissected and analyzed for spore viability, crossing-over, chromosome nondisjunction, and non-Mendelian segregation (NMS) [including postmeiotic segregation (PMS), indicative of unrepaired heteroduplex DNA].

Sporulation and spore viability. All mutants sporulated essentially as efficiently as wild type, producing more than 90% mature spores; also, the fraction of asci containing 4 or 3, 2, and 1 spores was not different from wild type (not shown). In all mutant strains, however, spore viability was reduced compared with wild type by 3.4–21.2%, depending on the mutant (Fig. 3). Since mismatch-repair-defective strains are mutators, their reduced spore viability has been attributed to the accumulation of haplo-lethal mutations before and during meiosis (31, 38). We do not, however, observe a simple correlation between the relative severity of mutator phenotype and the amount of spore death in the mutant strains. Notably, the *mlh3* mutant, shown to be a very weak mutator (5), produces fewer viable spores than a *pms1* mutant, which is known to be a very strong mutator (ref. 39; $P \ll 0.001$).

Crossing-over. Crossing-over was examined in the intervals flanking the *HIS4*::*LEU2* recombination hot spot (refs. 35 and 40; see *Materials and Methods*) on chromosome III (Fig. 3 and Table 1). In both *mlh1* and *mlh3* mutants, map distances were reduced by 21–33% ($P < 0.001$); moreover, the *mlh1* and *mlh3* data sets are not different from each other ($P > 0.5$). In *pms1* and *mlh2* mutants, in contrast, map distances were not different from wild type.

Nondisjunction. In the *mlh3* mutant, spore death is restricted to the tetrad classes having two or zero viable spores (Fig. 3). This pattern usually implies high levels of homolog nondisjunction (without conspicuous premature sister separation); further analysis confirms this possibility. In the *mlh3* mutant, among 1,270 tetrads, 20 contained two spores that were disomic for chromosome III plus two dead spores, and 19 contained two spores disomic for chromosome VIII plus two dead spores. Similarly, in 1,632 tetrads from the *mlh1* mutant, 20 contained two chromosome III disomes and two dead spores. These frequencies correspond to nondisjunction rates of $\approx 1.5 \times 10^{-2}$ per meiosis, a strong elevation over rates observed for these chromosomes in wild-type strains (e.g., 7×10^{-4}

Table 1. Tetrad analysis

	Wild type				*pms1*				*mlh1*				*mlh2*				*mlh3*			
Interval	P	T	N	cM	P	T	N	cM	P	T	N	cM	P	T	N	cM	P	T	N	cM
URA3-HIS4LEU2(NB)	483	483	7	27.0	323	277	8	26.7	564	345	8	21.4	354	316	9	27.2	697	370	6	18.9
HIS4LEU2(NB)-MAT	506	451	24	30.3	309	280	24	29.7	603	301	12	20.4	366	298	15	28.6	689	388	17	21.9
URA3-MAT	258	646	72	55.2	163	405	38	52.2	391	502	37	38.9	187	444	43	52.1	450	585	47	40.1

P, parental ditype: T, tetratype; N, nonparental ditype; cM centimorgans. Only tetrads with four viable spores and without non-Mendelian segregation were used to calculate map distance as described by Perkins (41).

per meiosis for chromosome III in the SK1 strain background; K. Haack and N.K., unpublished data; $\approx 3 \times 10^{-6}$ for chromosome VIII in the BR strain background; ref. 32). In contrast, no homolog nondisjunction events were detected in wild-type, *pms1*, or *mlh2* strains, corresponding to rates of chromosome III homolog nondisjunction of $<1.5 \times 10^{-3}$. Also, no nondisjunction events were observed in tetrads containing three viable spores in any of the mutant strains or in wild type.

Non-Mendelian segregation. Frequencies of NMS and PMS were examined for the *his4-X/HIS4* and *MATa/α* alleles (Table 2). In the wild-type strain, NMS at *his4-X/HIS4* and *MATa/α* occurred in 1.1% and 0.4% of tetrads, respectively. No PMS events were observed, indicating that the alleles form efficiently repaired mispairs in heteroduplex DNA. The *mlh3* mutant did not differ from wild type for either the frequency of NMS or the occurrence of PMS.

The other three mutants all exhibited differences from wild type, each with a distinct pattern (Table 2). In the *pms1* mutant, NMS was elevated at both loci: 2.3-fold at *his4-X/HIS4* and 5.7-fold at *MATa/α* ($P < 0.05$ and <0.01, respectively), and high frequencies of PMS were observed. The *mlh1* strain also had elevated NMS (3.9- and 5.5-fold) and PMS at both loci. In the *mlh2* strain, NMS was elevated more than 3-fold at *MATa/α* ($P < 0.05$), but for *his4-X/HIS4*, the frequency of NMS was not different from wild type. No PMS was detected at either locus in the *mlh2* mutant.

Additional differences in the segregation patterns of wild type, *pms1, mlh1*, and *mlh2* strains can be discerned (Table 2). First, at *MATa/α,* in the wild-type strain, only 2:6 segregations were observed, a significant bias ($P < 0.05$), termed "disparity." In contrast, in the *mlh2* mutant (which exhibits no PMS; see above), a situation close to parity is observed. Moreover, the frequency of 2:6 segregations is similar to that of wild type whereas the frequency of 6:2 segregations appears to be increased specifically. Second, at *his4-X/HIS4*, the wild-type strain exhibits parity of NMS: the numbers of 6:2 and 2:6 segregations are similar (there is no PMS). For the *pms1* mutant, in contrast, disparity is observed for total NMS events: 5:3 and 6:2 segregations are 3-fold more frequent than 6:2 and 3:5 segregations ($P < 0.01$). For the *mlh1* mutant, however, parity is again observed, but with an accompanying increase in the frequency of NMS relative to the *pms1* mutant ($P < 0.05$). Thus, the *mlh1* phenotype could be explained as the *pms1* pattern of NMS plus an additional increase of 2:6 and/or 3:5 segregations, with resultant loss of the disparity; it appears that 3:5 segregations are specifically elevated in the *mlh1* mutant compared with *pms1* ($P < 0.01$). The other classes of NMS also may be elevated in the *mlh1* mutant relative to the *pms1* strain, but this is not significant with our data set.

Discussion

Mlh1 Coordinates Interactions Among MutL Homologs *in Vivo*. Our examination of protein/protein interactions among yeast MutL homologs revealed only three types of complexes: those between Mlh1 and each of the other three family members. Neither homotypic nor other heterotypic associations were observed. The complexes identified likely reflect the existence of three types of Mlh1-containing heterodimers, i.e., Mlh1+Pms1, Mlh1+Mlh2, and Mlh1+Mlh3. Our functional analysis (discussed below) is consistent with the existence of three heterocomplexes. The direct biochemical identification of a Mlh1-Pms1 heterodimer (10, 42) and the fact that *E. coli* MutL protein functions as a (homo)dimer (43) supports the proposal that the oligomeric forms of Mlh complexes *in vivo* are (hetero)dimers.

C-Terminal Interaction Domains in Mlh1 and Mlh2. In the current study, two-hybrid analysis indicates that the C-terminal one-third of Mlh1

Table 2. Non-Mendelian segregation

	Allele											
	his4-X/HIS4						*MATa/MATα*					
Genotype	5:3	6:2	2:6	3:5	% NMS	% PMS/total	5:3	6:2	2:6	3:5	% NMS	% PMS/total
Wild type	0	6	5	0	1.1 (11/1,018)	—	0	0	4	0	0.4 (4/998)	—
pms1	5	7	3	1	2.5 (16/638)	37.5 (6/16)	2	7	4	2	2.3 (15/637)	26.7 (4/15)
mlh1	10	12	7	12	4.3 (42/978)*	52.3 (22/42)	5	7	9	1	2.2 (22/981)	27.3 (6/22)
mlh2	0	5	1	0	0.9 (6/696)	—	0	6	4	0	1.4 (10/696)	—
mlh3	0	7	7	0	1.3 (14/1,093)	—	0	1	4	0	0.5 (5/1,094)	—

Non-Mendelian segregation was analyzed for tetrads with four viable spores. Patterns of segregation were assigned according to the nomenclature used for eight spored fungi, 5:3 and 3:5 segregations have a single-sectored colony, indicating unrepaired heteroduplex. For *MAT* segregations, 6:2 and 2:6 segregations were arbitrarily assigned to the **aaa**α and $\alpha\alpha\alpha$**a** patterns, respectively. The *his4-X* mutation will create a 4-bp heterology in hybrid DNA; *MATa/MATα* theoretically creates a 642/747-bp double heterology.
*One 0:8 segregation was observed.

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can interact with the other three MutL homologs; moreover, a small C-terminal fragment of Mlh2, just 14% of the full-length protein, gives a strong interaction signal with the Mlh1 C-terminal region (above). Similarly, Pang *et al.* (37) showed that a 260-aa C-terminal fragment of Pms1 was sufficient for interaction with Mlh1. Indeed, all the MutL family proteins examined appear to interact via their C-terminal regions (37, 44, 45). Pang *et al.* also defined three subdomains that are conserved between the C-terminal regions of Pms1 and Mlh3; these domains were suggested to play a role in dimer formation with Mlh1 (37). In our analysis, however, we found that subdomains I and III were not present in the 98-aa Mlh2 fragment that interacts with Mlh1; furthermore, subdomains II and III are not conserved in Mlh2. Thus, subdomains I, II, and III seem not to be relevant for the Mlh1-Mlh2 interaction. By extension, the interaction domains of Pms1 and Mlh3 may lie elsewhere in the C-terminal regions.

Mlh1-Mlh3 Promotes Meiotic Crossing-Over. Functional analysis demonstrates that Mlh1 and Mlh3 both are involved in meiotic recombination. For each of the intervals examined, the corresponding single mutations confer indistinguishable reductions in crossing-over. In addition, *mlh1* and *mlh3* mutants exhibit comparable defects in homolog disjunction, a defect that frequently accompanies reductions in crossing-over (1, 2). Although an *mlh1 mlh3* double mutant has not been analyzed, these results, when taken together with the interaction studies described above, strongly suggest that a Mlh1-Mlh3 heterodimer promotes meiotic crossing-over.

Since the *mlh3* mutant fails to exhibit alterations in NMS/PMS, the mismatch-repair and crossover functions of Mlh1 appear to be, for the most part, genetically separable. Hence, any model of Mlh1 function in crossing-over no longer needs to account for effects on NMS, which appear, instead, to be controlled primarily by the other two Mlh1 heterodimers (17). Furthermore, in contrast to *mlh1* and *mlh3* mutations, neither *mlh2* nor *pms1* confers defects in either crossing-over or chromosome segregation. Thus, during meiosis, the Mlh1-Mlh3 heterocomplex may be dedicated to recombination. In mitotic cells, however, Mlh1-Mlh3 suppresses frame-shift mutations (5); hence, we cannot exclude the possibility of additional, mismatch repair roles for Mlh1-Mlh3 in meiotic cells. On the other hand, the mitotic role for this complex could involve a recombinational pathway, in which case the situations would be similar in both cell types.

The Msh4 and Msh5 proteins function together during meiotic recombination in yeast (15, 16, 18). The reduction in crossing-over conferred by *msh4* and *msh5* mutations is greater than that of *mlh1* and *mlh3* mutations, implying some difference in the roles of the MutL and MutS homologs during meiotic recombination. Nonetheless, Mlh1/3 and Msh4/5 function in a single pathway, because a *msh4 mlh1* double mutant has the crossover defect of a *msh4* single mutant (17). In *msh4* (and, by extension, *msh5*) mutants, the crossovers that occur fail to exhibit interference (2). Whether the same is true of *mlh1* and *mlh3* mutants has not been reported.

In $mlh1^{-/-}$ mice, meiotic crossing-over is reduced ≈10-fold (21, 24). Also, immunolocalization studies implicate Mlh1 as a component of late recombination nodules and, in one case, show specific localization of Mlh1 foci to chiasmata at diplotene (21–23). Correspondingly, two recent analyses of mouse and human spermatocytes indicate that Mlh1 foci on pachytene chromosomes closely correspond to chiasmata/crossovers in both frequency and distribution (22, 23).

In a study of oocytes of $mlh1^{-/-}$ mice, Mlh1 foci were detected along early-pachytene chromosomes in twice the number observed at later stages, with a sharp reduction by midpachytene (ref. 21, but see ref. 22). Since this behavior parallels the behavior of recombination nodules in this organism, i.e., twice the number of early nodules as late nodules with a rapid transition at early/midpachytene (46), these findings may point to a role for Mlh1 in early stages of recombination, i.e., in processing intermediates that mature as both noncrossovers and crossovers. Such a role is not excluded for other cases, where, perhaps, the level of Mlh1 in early recombination nodules is below the level of immunofluorescence detection. Alternatively (or in addition), early Mlh1 foci may represent recombination-related mismatch-repair functions. In light of the functional specialization revealed by the current study, the pattern of cytological localization of yeast Mlh3, in comparison with that of the Pms1 and Mlh2 proteins, may help to resolve these complexities.

Roles for Mlh1-Pms1 and Mlh1-Mlh2 Heterodimers in Mismatch Correction During Meiosis. For the mutants and loci examined in this study, PMS was observed only for the *mlh1* and *pms1* mutants. These data are consistent with a principal role for the Mlh1-Pms1 heterodimer in the repair of heteroduplex DNA (17, 31, 38, 39). Furthermore, the detection of PMS in a *pms1* mutant implies that for a high proportion of the heteroduplexes, this activity cannot be substituted efficiently, by either another MutL complex or an alternative repair activity, and mispairs thus evade correction.

Mismatches in heteroduplex DNA formed during meiotic recombination can undergo one of four fates: first, repair producing a non-Mendelian ratio of alleles that will be detected as a NMS (without PMS), or "gene conversion" event; second, repair that results in a Mendelian (2:2) ratio of alleles that is genetically silent, termed "restoration"; third, removal of the mismatch by unwinding the mispaired region of hybrid DNA, which also will be undetected, termed "heteroduplex rejection"; fourth, no repair resulting in PMS (47–52).

The increases in total NMS events in *pms1, mlh1*, and *mlh2* strains likely derive from reductions in the proportion of heteroduplexes that are corrected as restorations or undergo heteroduplex rejection. This explanation assumes that mismatch-repair proteins do not affect the frequency of initiation of recombination. In support of this assumption, Vedel and Nicolas (53) have shown that double-strand break frequency at the *CYC3* locus is not affected by mutations in *MSH2* and *PMS1*.

The effect of *mlh2* mutation on NMS at the mating-type locus is consistent with a role for the Mlh1-Mlh2 complex in the correction of some heteroduplexes that is distinct from that of Mlh1-Pms1. Specifically, **aaa**α segregations seem to be prevented by Mlh1-Mlh2. Parity between 5:3/6:2 and 3:5/2:6 segregations is thought to reflect an equal frequency of initiation on both parental homologs; the disparity in wild type therefore could be caused by asymmetric initiation of recombination (but see above), prevention of one type of heteroduplex, or a bias in the direction of correction of $MATa/\alpha$ heteroduplexes. The absence of PMS in *mlh2* mutants, however, suggests that Mlh1-Mlh2 activity is not essential for repair *per se* (although a low level of PMS may not have been detected). This observation appears to support the possibility that heteroduplex formation is prevented by a mechanism that involves Mlh1-Mlh2. Alternatively, Mlh1-Mlh2 may be antagonistic and partially redundant with the Mlh1-Pms1 heterodimer (or some other repair activity) for the processing of some mispairs.

At *his4-X/HIS4,* the specific elevation of 3:5 PMS events in the *mlh1* mutant compared with *pms1* suggests that an alternative Mlh1 complex (presumably Mlh1-Mlh2 or Mlh1-Mlh3) is involved in restorational repair of some mismatches in the *pms1* mutant background. Previously observed differences between *mlh1* and *pms1* mutants are consistent with this proposal (17, 54). Our data did not, however, allow us to discern which complex was responsible for the restoration events observed in the *pms1* mutant. That the Mlh1-Pms1 and Mlh1-Mlh2 complexes have different repair functions is supported further by the observation that *mlh2* but not *pms1* mutants are resistant to cisplatin (6).

In summary, the genetic data described here are consistent with the inference from biochemical analysis, namely, that there are likely only three functionally relevant Mlh heterocomplexes in yeast, each with a distinct function during meiosis.

Modulation of Basic Mlh1-Encoded Activities via Heterocomplex Formation. Since Mlh1 is the mutual component of all MutL heterodimers, it might possess the basic activities common to all MutL-mediated reactions. For example, Mlh1 may interact with the processive polymerase clamp, proliferating cell nuclear antigen, to recruit a polymerase to the site of DNA damage, to modulate polymerase processivity, or to signal a checkpoint response (55–57). The role of Mlh1 for MutL family members is analogous to the role of Msh2 in the two yeast MutS heterodimers, Msh2-Msh6 and Msh2-Msh3 (3, 4, 7, 8). Msh2 has been shown to interact with the Mlh1-Pms1 heterodimer although the exact nature of the protein–protein contacts is unknown (10); perhaps the common components of the two families, Msh2 and Mlh1, interact directly. The basic Mlh1 activities then would be modulated by association with each of the other MutL homologs to permit interaction with additional components specific to one of the three pathways identified above.

Regulation of Mlh Heterodimers. Given our observation that Mlh1 is the common component of all Mlh complexes, the expression patterns of Mlh genes suggest that relative abundance of the three Mlh complexes is regulated via changes in gene expression. Although the transcript level of *MLH1* appears to be constant throughout mitotic and meiotic cell cycles, both *PMS1* and *MLH3* transcripts are periodically induced (58, 59). For example, during vegetative growth, *PMS1* transcription is induced around the time of DNA replication, coordinately with other replication components and together with the *mutS* homologs, *MSH2* and *MSH6* (but not *MSH3*; refs. 59–61), suggesting strong dependence on Msh2-Msh6 plus Mlh1-Pms1 for the correction of replicative errors. In meiosis, transcription of *PMS1* is induced similarly around the time of replication. Transcription of *MLH3* also is up-regulated during meiosis, interestingly somewhat later than *PMS1,* consistent with a primary role in recombination that would be required after bulk DNA replication (58). Unlike *PMS1* and *MLH3,* transcription of *MLH2* is not induced during meiosis, suggesting that the Mlh1-Mlh2 complex may be relatively abundant during G_1 and perhaps functionally most significant during this period of the cell cycle. Programmed expression of Pms1 and Mlh3 could promote preferential formation of specific Mlh1-containing heterocomplexes and subsequent channeling of repair/recombination substrates into the appropriate pathway.

We thank Sue-Jane Chen, Philip James, Michael Liskay, Kim Nasmyth, Morgan Sheng, and Yi-Ping Hsueh for materials used in this study. We are grateful to Job Dekker, Jean-Luc Ferat, and members of N.K.'s laboratory for comments on the manuscript. N.H. was supported by a Prize Travelling Research Fellowship from The Wellcome Trust. This work was supported by National Institutes of Health Grant RO1-GM44794 to N.K.

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