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Competing Crossover Pathways Act During Meiosis in *Saccharomyces cerevisiae*

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ABSTRACT

In *Saccharomyces cerevisiae* the MSH4-MSH5, MLH1-MLH3, and MUS81-MMS4 complexes act to promote crossing over during meiosis. MSH4-MSH5, but not MUS81-MMS4, promotes crossovers that display interference. A role for MLH1-MLH3 in crossover control is less clear partly because *mlh1*Δ mutants retain crossover interference yet display a decrease in crossing over that is only slightly less severe than that seen in *msh4*Δ and *msh5*Δ mutants. We analyzed the effects of *msh5*Δ, *mlh1*Δ, and *mms4*Δ single, double, and triple mutants on meiotic crossing over at four consecutive genetic intervals on chromosome XV using newly developed computer software. *mlh1*Δ *mms4*Δ double mutants displayed the largest decrease in crossing over (13- to 15-fold) of all mutant combinations, yet these strains displayed relatively high spore viability (42%). In contrast, *msh5*Δ *mms4*Δ and *msh5*Δ *mms4*Δ *mlh1*Δ mutants displayed smaller decreases in crossing over (4- to 6-fold); however, spore viability (18–19%) was lower in these strains than in *mlh1*Δ *mms4*Δ strains. These data suggest that meiotic crossing over can occur in yeast through three distinct crossover pathways. In one pathway, MUS81-MMS4 promotes interference-independent crossing over; in a second pathway, both MSH4-MSH5 and MLH1-MLH3 promote interference-dependent crossovers. A third pathway, which appears to be repressed by MSH4-MSH5, yields deleterious crossovers.

IN most eukaryotic organisms the correct segregation of chromosomes at the first meiotic division requires reciprocal exchange between homologs. The physical manifestations of these crossover events, chiasmata, provide the contacts between homologous chromosomes that are necessary for segregation (Jones 1987). This cohesion or "chiasma binder" function ensures the generation of a bipolar spindle in which tension is generated at the kinetochores (Maguire 1974). The subsequent "programmed release of sister connections" is thought to be critical for meiosis I segregation (Storlazzi *et al.* 2003). Because of their importance, crossover events are highly regulated both within and among chromosomes. This regulation is clearly seen in *Saccharomyces cerevisiae* where two crossovers rarely occur within the same genetic interval (positive interference) and smaller chromosomes tend to display less positive interference than larger ones (Mortimer and Fogel 1974; Kaback *et al.* 1999). A net result of this regulation is that every chromosome, regardless of size, receives at least one reciprocal exchange (Jones 1987).

How are crossover events generated? Genetic and physical analyses of meiosis in *S. cerevisiae* showed that meiotic recombination is initiated by double-strand breaks that occur at specific chromosomal positions (reviewed in Keeney 2001). The repair of these breaks, preferentially using an unbroken homolog as a template, results in both reciprocal exchanges, termed crossovers (CO), and nonreciprocal exchanges, termed noncrossovers (NCO). The classical double-strand break repair (DSBR) model proposes that these events result from alternative resolutions of a common Holliday junction intermediate (reviewed in Pâques and Haber 1999). Recent studies, however, have suggested that COs and NCOs are processed via separate pathways. In support of this idea, meiotic mutants have been identified that specifically reduce the number of COs or allow NCO formation in the absence of COs (Ross-Macdonald and Roeder 1994; Sym and Roeder 1994; Hollingsworth *et al.* 1995; Storlazzi *et al.* 1995; Hunter and Borts 1997; Chua and Roeder 1998; Nakagawa and Ogawa 1999; Agarwal and Roeder 2000; Allers and Lichten 2001a,b; Hunter and Kleckner 2001; reviewed in Bishop and Zickler 2004; Hollingsworth and Brill 2004). Furthermore, the configuration of heteroduplex DNA seen in NCOs does not fit that predicted by the DSBR model (Porter *et al.* 1993; Gilbertson and Stahl 1996; Merker *et al.* 2003). Finally, the majority of Holliday junctions detected by physical analyses of cells induced for meiosis are processed into COs (Allers and Lichten 2001a,b; Börner *et al.* 2004).

In the budding yeast *S. cerevisiae*, the *MER3*, *EXO1*, *MSH4*, *MSH5*, *MLH1*, *MLH3*, *MMS4*, and *MUS81* genes are each required to achieve wild-type levels of meiotic crossing over (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Hunter and Borts 1997; Nakagawa and Ogawa 1999; Wang *et al.* 1999; Khazanehdari and Borts 2000; Borts *et al.* 2000; Tsubouchi and Ogawa

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2000; de los Santos *et al.* 2001, 2003; Börner *et al.* 2004; Mazina *et al.* 2004). In each of these mutants, crossing over, as measured at specific genetic intervals, is reduced by less than threefold. The proteins encoded by these genes are thought to participate in the biochemical steps that lead to meiotic recombination. EXO1 is a 5′–3′ exonuclease that can act on duplex DNA ends (Tsubouchi and Ogawa 2000), MER3 is a meiosis-specific 3′–5′ helicase that is thought to process double-strand breaks into Holliday junction intermediates that form COs (Nakagawa and Ogawa 1999; Nakagawa and Kolodner 2002a,b; Mazina *et al.* 2004), and MUS81-MMS4 is an endonuclease that appears to preferentially cleave D-loops and half-Holliday junctions (Kaliraman *et al.* 2001; reviewed in Hollingsworth and Brill 2004). How these biochemical activities converge to regulate crossing over and interference remains a major question in the field.

Little is known about the roles of MSH4, MSH5, MLH1, and MLH3 in meiotic crossing over. Biochemical and genetic studies, however, have shown that they act in MLH1-MLH3 and MSH4-MSH5 complexes (Pochart *et al.* 1997; Wang *et al.* 1999; Wang and Kung 2002). While both MSH4 and MSH5 are homologs of the bacterial MutS mismatch repair protein, they do not appear to play a role in eukaryotic mismatch repair (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995). In *S. cerevisiae*, *msh4*Δ and *msh5*Δ mutants display a two- to threefold reduction in crossing over, an increase in meiosis I nondisjunction, the loss of interference, and a subsequent loss in spore viability (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Novak *et al.* 2001). In *Caenorhabditis elegans*, deletion of either the *MSH4* or the *MSH5* homolog results in a complete loss of crossing over that is accompanied by meiotic inviability (Zalevsky *et al.* 1999; Kelly *et al.* 2000). These observations have led to models in which MSH4-MSH5 acts to stabilize and/or resolve Holliday junction intermediates (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Pochart *et al.* 1997). While meiotic crossover defects in *mlh1*Δ and *mlh3*Δ mutants appear less severe than those in *msh4*Δ and *msh5*Δ mutants, these mutants still display relatively high levels of meiosis I nondisjunction (Hunter and Borts 1997; Wang *et al.* 1999; Argueso *et al.* 2003). In contrast to *msh4*Δ strains, interference appears intact in *mlh1*Δ mutants (Argueso *et al.* 2003). *Mlh1*$^{-/-}$ and *Mlh3*$^{-/-}$ mutant mice show severe defects in crossing over, resulting in sterility (Edelmann *et al.* 1996; Woods *et al.* 1999; Lipkin *et al.* 2002). These results, in conjunction with epistasis and cell biological analyses in yeast and mice, suggest that MSH4-MSH5 and MLH1-MLH3 act in a common crossover pathway, with MSH4-MSH5 functioning prior to MLH1-MLH3 (this study; Hunter and Borts 1997; Wang *et al.* 1999; Santucci-Darmanin *et al.* 2000; Moens *et al.* 2002; Wang and Kung 2002).

The genetic, cytological, and biochemical studies summarized above suggest that crossing over in mice and *C. elegans* occurs primarily through an interference-dependent (MSH4-MSH5, MLH1-MLH3) pathway. Crossing over in *S. cerevisiae*, however, is thought to be controlled by both interference-dependent and interference-independent (MUS81-MMS4) mechanisms (Zalevsky *et al.* 1999; Khazanehdari and Borts 2000; de los Santos *et al.* 2001, 2003). The above observations, which suggest that organisms utilize interference-dependent and -independent crossover pathways to varying degrees, are supported by the following:

1. Mouse and *C. elegans* mutants defective in MSH4-MSH5 and MLH1-MLH3 complexes display severe crossover defects relative to the equivalent *S. cerevisiae* mutants (Edelmann *et al.* 1999; Woods *et al.* 1999; Zalevsky *et al.* 1999).
2. Crossing over, while reduced in *S. cerevisiae mus81*Δ and *mms4*Δ strains, is still subject to interference (de los Santos *et al.* 2001, 2003).
3. Crossing over and spore viability in *S. cerevisiae msh5*Δ *mus81*Δ or *msh5*Δ *mms4*Δ double mutants is significantly lower (approximately fivefold) than that in the single mutants (de los Santos *et al.* 2001, 2003; this study).
4. *Schizosaccharomyces pombe mus81*Δ strains display severe defects in spore viability and crossing over that can be explained by the lack of an interference-dependent pathway in this organism (Egel 1995; reviewed in Hollingsworth and Brill 2004).

To gain a better understanding of the relationships between members of different crossover pathways as well as the contribution of distributive pairing to the meiosis I division, we analyzed the effect of *msh5*Δ, *mlh1*Δ, and *mms4*Δ single, double, and triple mutations on meiotic crossing over at four consecutive genetic intervals on chromosome XV. Data from tetrad dissection and single spores were analyzed using newly developed software. Our data suggest that meiotic crossing over in yeast can occur through three distinct crossover pathways: MUS81-MMS4 promotes interference-independent crossing over in one pathway while both MSH4-MSH5 and MLH1-MLH3 participate in a second interference-dependent pathway (Argueso *et al.* 2003; de los Santos *et al.* 2003). MSH4-MSH5 appears to repress a third pathway that yields deleterious crossovers.

MATERIALS AND METHODS

Media and strains: Yeast strains were grown in either yeast extract-peptone-dextrose (YPD) or minimal selective media (Rose *et al.* 1990). Sporulation plates were prepared as described previously (Detloff *et al.* 1991). All incubations were performed at 30°. When required, geneticin (Invitrogen, San Diego), nourseothricin (Hans-Knoll Institute fur Naturstoff-Forschung), and hygromycin B (Calbiochem, La Jolla, CA) were included in YPD media as described (Wach *et al.* 1994; Goldstein and McCusker 1999).

The strains used in this study were derived from the SK1-congenic strains HTY1212 and HTY1213 (Sym and Roeder 1994; Tsubouchi and Ogawa 2000). Homologous gene replacement was used to insert genetic markers near the centromere and on the right arm of chromosome XV at positions 326272 (*URA3*-cenXVi and *TRP1*-cenXVi), 462712 (*LEU2*-chXVi), and 504881 (*LYS2*-chXVi). The inserted markers are located in intergenic regions predicted to not affect the functions of neighboring genes. The resulting parental haploid strains are EAY1108 (*MAT***a**, *ho::hisG, lys2, ura3, leu2::hisG, trp1::hisG, URA3*-cenXVi, *LEU2*-chXVi, *LYS2*-chXVi) and EAY1112 (*MAT*α, *ho::hisG, lys2, ura3, leu2::hisG, trp1::hisG, ade2::hisG, his3::hisG, TRP1*-cenXVi). These strains were mated to create the reference wild-type diploid strain (Figure 1). For the mutant analyses, at least two independent transformants for each genotype were analyzed.

EAY1108/EAY1112 diploids homozygous for coding-region deletion mutations in *MMS4, MSH5,* and *MLH1* were created by sequential deletion using the *KanMX4, NatMX4,* and *HphMX4* selectable markers, respectively (Wach *et al.* 1994; Goldstein and McCusker 1999). Mutant derivatives of EAY1108 are: EAY1167 (*mms4*Δ*::Kan*), EAY1281 (*msh5*Δ*::Nat*), EAY1271 (*mlh1*Δ*::Hph*), EAY1288 and EAY1289 (*mlh1*Δ*::Hph msh5*Δ*::Nat*), EAY1273 and EAY1274 (*mlh1*Δ*::Hph mms4*Δ*::Kan*), EAY1284 and EAY1285 (*mms4*Δ*::Kan msh5*Δ*::Nat*), EAY1303 and EAY1304 (*mlh1*Δ*::Hph mms4*Δ*::Kan msh5*Δ*::Nat*), and EAY1165 (*pms1*Δ*::Kan*). Mutant derivatives of EAY1112 are: EAY1168 (*mms4*Δ*::Kan*), EAY1279 and EAY1280 (*msh5*Δ*::Nat*), EAY1276 (*mlh1*Δ*::Hph*), EAY1286 and EAY1287 (*mlh1*Δ*::Hph msh5*Δ*::Nat*), EAY1277 and EAY1278 (*mlh1*Δ*::Hph mms4*Δ*::Kan*), EAY1282 and EAY1283 (*mms4*Δ*::Kan msh5*Δ*::Nat*), EAY1290 and EAY1291 (*mlh1*Δ*::Hph mms4*Δ*::Kan msh5*Δ*::Nat*), and EAY1166 (*pms1*Δ*::Kan*).

Genetic analysis: Diploids were sporulated using the zero-growth mating protocol (Argueso *et al.* 2003). Briefly, haploid parental strains were patched together, allowed to mate for 4 hr on complete plates, and then transferred to sporulation plates where they were incubated at 30° for 3 days. Because of our interest in comparing our data to previous studies, all strains were sporulated at 30°. Tetrads were dissected on minimal complete plates and then incubated at 30° for 3–4 days. Spore clones were replica plated onto relevant selective plates and assessed for growth after an overnight incubation.

Recently, Börner *et al.* (2004) examined *zip1*Δ, *zip2*Δ, *zip3*Δ, *mer3*Δ, and *msh5*Δ *S. cerevisiae* mutants for meiotic progression at 23° and 33°. Their studies suggested a coordinated formation of early meiotic recombination intermediates that is important for establishing CO and NCO products. They hypothesized that yeast meiosis can proceed through two recombination modes and that sporulation at 30° represented a mixture of the two. While we would have liked to perform tetrad analyses at 23° and 33°, the meiotic prophase arrest of *msh5*Δ strains at 33° (Börner *et al.* 2004) makes such a study untenable.

The segregation data from each replica were converted to a numeric tetrad scoring code and analyzed using the *r*ecombination *ana*lysis software (RANA, available upon request). RANA analyzes tetrad data for spore viability, genetic linkage, genetic interference, and non-Mendelian segregation. The most important feature of the system is that it allows linkage and interference analysis of data from complete tetrads (four viable spores), as well as from single spores present in incomplete tetrads (three, two, and one viable spores). This is especially useful for the analysis of meiotic recombination mutants because direct comparison of recombination frequencies between complete and incomplete tetrads provides a valuable experimental control and may uncover interesting phenotypes. Only tetrads with Mendelian segregation of all markers were used in tetrad analysis, but all spores in the data set were used in single-spore analysis. In the single-spore analysis, the program compares the marker segregation pattern for each individual spore, outside of the context of a tetrad. Spores are classified as parental or recombinant for each marker pair. The total number of recombinant spores is then counted and divided by the total number of viable spores to obtain recombination frequency (Rf) values.

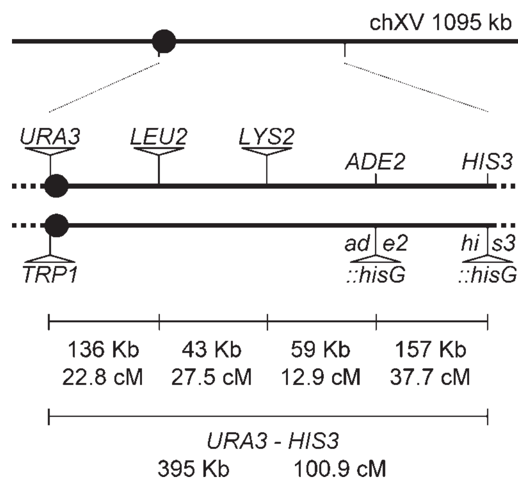

Figure 1.—Distribution of genetic markers on chromosome XV. The solid circle indicates the centromere. The distances between markers are not drawn to scale. The actual physical and genetic distances in the wild-type diploid are given numerically for each interval and for the entire region between *CENXV* and *HIS3*.

Genetic map distances were determined by the formula of Perkins (1949) and the expected number of nonparental ditype tetrads (NPD) was calculated using the equation of Papazian (1952). Interference calculations from three-point intervals were conducted as described (Novak *et al.* 2001; de los Santos *et al.* 2003; Shinohara *et al.* 2003). Statistical analysis was done using the Stahl Laboratory Online Tools (http://groik.com/stahl/), VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html), and the Categorical Statistics Packages (http://engels.genetics.wisc.edu).

RESULTS

Development of genetic and software tools to examine crossing over in *S. cerevisiae*: Tetrad dissection of meiotic recombination mutants can provide information on crossover frequency, crossover interference, spore viability, and chromosome segregation efficiency. Such an analysis, however, can be difficult to perform in mutants that display poor spore viability. To overcome this, we developed a computer program (RANA) that allows us to organize, store, and share information obtained from a large set of tetrad dissections (29,000 in this study). As described in materials and methods, this allows us to analyze complete tetrads as well as single spores and to identify any inconsistencies when the data are compared.

To analyze the above parameters in a single-strain set, two SK1 congenic strains, EAY1108 and EAY1112, were created to measure crossing over at four consecutive genetic intervals on chromosome XV (100.9 cM, 395 kB; Figure 1). Recent studies by Fung *et al.* (2004) indicate

TABLE 1

Genetic map distances (cM) in wild type, *mms4*Δ, *msh5*Δ, and *mlh1*Δ strains

Relevant genotype	No. analyzed	*URA3-LEU2*	*LEU2-LYS2*	*LYS2-ADE2*	*ADE2-HIS3*	*URA3-LYS2*	*LYS2-HIS3*
			Tetrads[a]				
Wild type	1068	21.8–23.8	26.6–28.4	12.1–13.7	36.5–38.9	46.5–49.9	46.0–49.6
*mms4*Δ	153	14.8–18.6	23.8–29.2	7.9–11.1	28.7–32.7	39.2–48.4	32.8–39.0
*mlh1*Δ	616	10.3–12.5	11.8–13.6	6.2–7.6	18.2–21.0	21.8–24.4	24.7–28.3
*msh5*Δ	720	5.0–6.4	11.0–13.0	3.7–4.7	17.2–20.2	15.5–18.1	20.5–23.9
*mlh1*Δ *msh5*Δ	764	8.7–10.9	14.9–17.5	5.2–6.6	19.1–21.9	24.1–27.9	24.5–27.9
*mlh1*Δ *mms4*Δ	201	0.1–0.9	1.7–3.3	0.7–1.7	1.9–3.5	2.2–3.8	2.8–4.6
*mms4*Δ *msh5*Δ	52	0.1–2.0	5.2–10.2	2.0–5.6	2.8–6.8	6.1–11.3	6.1–11.3
*mlh1*Δ *mms4*Δ *msh5*Δ	51	0.6–3.4	7.0–12.6	0.1–2.0	5.3–10.3	7.0–12.6	6.1–11.5
			Single spores[b]				
Wild type	4644	20.6–23.0	25.8–28.4	11.8–13.8	33.3–36.1	38.0–40.8	37.7–40.5
*mms4*Δ	2732	17.1–20.0	22.3–25.5	9.3–11.7	27.9–31.4	33.6–37.3	32.1–35.7
*mlh1*Δ	3792	9.6–11.6	11.7–13.9	6.5–8.1	16.9–19.4	20.2–22.9	21.7–24.5
*msh5*Δ	5674	5.1–6.3	10.3–11.9	4.1–5.3	14.5–16.4	13.8–15.6	17.3–19.3
*mlh1*Δ *msh5*Δ	6509	8.3–9.7	12.1–13.7	5.5–6.6	16.2–18.1	18.4–20.4	20.3–22.3
*mlh1*Δ *mms4*Δ	2260	0.8–1.7	1.8–3.1	0.5–1.3	2.3–3.8	2.5–4.0	3.0–4.6
*mms4*Δ *msh5*Δ	1920	1.7–3.1	6.2–8.6	2.1–3.6	6.2–8.6	7.3–9.9	8.3–11.0
*mlh1*Δ *mms4*Δ *msh5*Δ	1790	2.1–3.7	7.1–9.7	1.8–3.3	8.6–11.4	8.2–11.0	9.9–12.9

All mutants are isogenic derivatives of EAY1108/EAY1112 (MATERIALS AND METHODS).

[a] Intervals correspond to the genetic distance calculated from tetrads ±1 standard error. Standard error was calculated using the Stahl Laboratory Online Tools website (http://groik.com/stahl/).

[b] Data shown as 95% confidence intervals around the recombination frequency determined from single spores, calculated using the VassarStats website (http://faculty.vassar.edu/lowry/VassarStats.html). To facilitate comparisons to the tetrad data, recombination frequencies obtained from single-spore data were multiplied by 100 to yield genetic map distances (in centimorgans).

that chromosome XV was an appropriate choice because interference appears constant throughout its length. The diploid strain created by mating EAY1108 × EAY1112 displays high spore viability and chromosome XV genetic map distances (Table 1) that correspond well with previously published data (*Saccharomyces* Genome Database at http://www.yeastgenome.org/). Strains isogenic to the EAY1108/EAY1112 diploid and homozygous for the *mlh1*Δ, *msh5*Δ, and *mms4*Δ deletions were generated as described in MATERIALS AND METHODS. These mutations were chosen because previous studies had shown that mutants bearing these single mutations displayed phenotypes indistinguishable from those defective in both partners (*msh5*Δ *vs. msh5*Δ *msh4*Δ, *mlh1*Δ *vs. mlh1*Δ *mlh3*Δ, and *mms4*Δ *vs. mms4*Δ *mus81*Δ; HOLLINGSWORTH *et al.* 1995; ARGUESO *et al.* 2003; DE LOS SANTOS *et al.* 2003).

Effect of *mlh1*Δ, *msh5*Δ, and *mms4*Δ mutations on spore viability and chromosome segregation: As shown in Figure 2, *mlh1*Δ, *msh5*Δ, and *mlh1*Δ *msh5*Δ strains displayed spore viability patterns (4, 2, 0 viable spores >3 and 1) consistent with high levels of meiosis I nondisjunction (ROSS-MACDONALD and ROEDER 1994; HOLLINGSWORTH *et al.* 1995; HUNTER and BORTS 1997; WANG *et al.* 1999; ARGUESO *et al.* 2003). The defect in spore viability appears more severe in *msh5*Δ than in *mlh1*Δ strains. In addition, the *msh5*Δ *mlh1*Δ strain displayed a spore viability phenotype similar to that observed in *msh5*Δ strains, suggesting that MSH4-MSH5 and MLH1-MLH3 act in the same pathway in meiosis (HUNTER and BORTS 1997; KNEITZ *et al.* 2000; LIPKIN *et al.* 2002; MOENS *et al.* 2002). *mlh1*Δ strains displayed defects in both mismatch repair (MMR) and meiotic crossing over and showed 68% spore viability. Because PMS1, MLH1's major partner in MMR, does not appear to play a role in meiotic crossing over, we examined strains homozygous for the *pms1*Δ mutation with the goal of determining the contribution of defects in mismatch repair to meiotic spore viability. As shown in Figure 2, the MMR defect in *pms1*Δ strains contributed <10% decrease in spore viability compared to wild type, suggesting that a reduction of ~20% in spore viability in *mlh1*Δ strains was due to meiotic defects.

*mms4*Δ strains did not display a spore viability pattern consistent with meiosis I misegregation despite displaying defects in meiotic crossing over (DE LOS SANTOS *et al.* 2001, 2003). Presumably such a pattern was not observed because *mms4*Δ strains display defects associated with DNA metabolism that result in random spore death (MULLEN *et al.* 2001). Double- and triple-mutant combinations involving *mms4*Δ, *mlh1*Δ, and *msh5*Δ yielded a spore viability pattern that appeared as a mixture of the *mms4*Δ and *mlh1*Δ/*msh5*Δ spore viability profiles. Consistent with this, the double- and triple-mutant analysis did not reveal an epistatic relationship between *mms4*Δ and *mlh1*Δ or *msh5*Δ mutations with respect to spore viability. Strikingly, spore viability was significantly lower in *msh5*Δ *mms4*Δ strains (19%) than in *mlh1*Δ *mms4*Δ strains (42%).

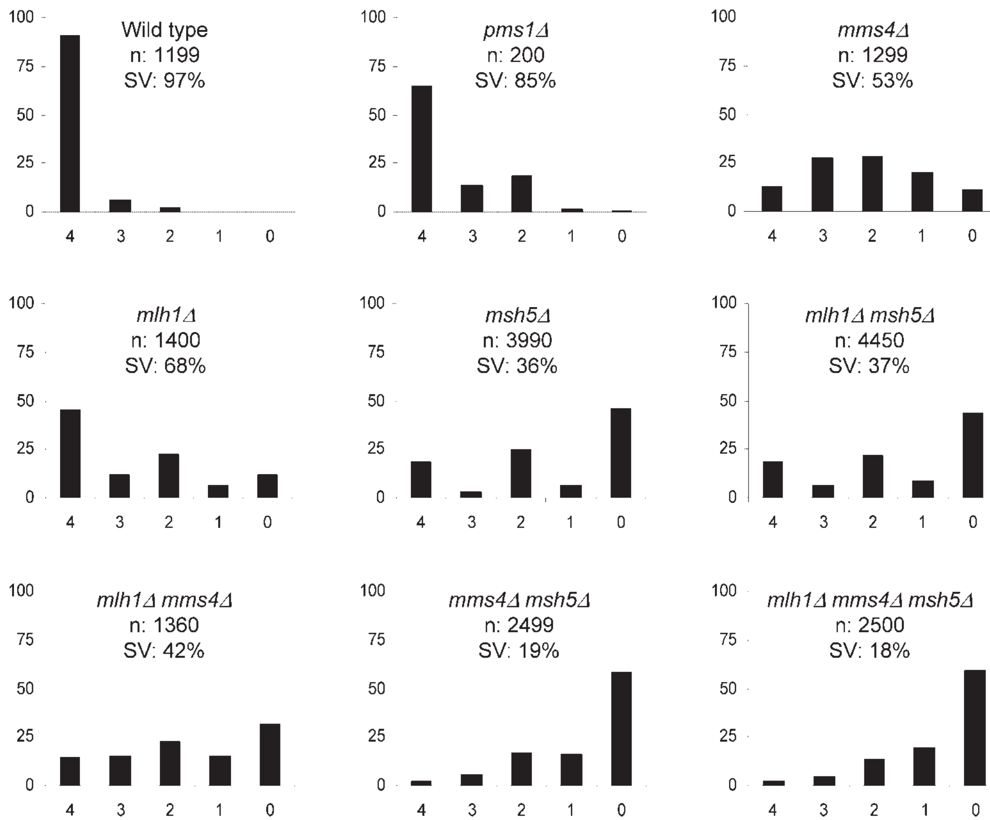

Figure 2.—Plots showing the distribution of viable spores in tetrads of each genotype. In all plots, the horizontal axis corresponds to the classes of tetrads with 4, 3, 2, 1, and 0 viable spores, and the vertical axis corresponds to the frequency of each class given in a percentage. The total number of tetrads dissected (*n*) and the overall spore viability (SV) are shown for each genotype.

The presence of centromere-linked markers at chromosome XV in the EAY1108/EAY1112 diploid allows us to analyze two viable spore tetrads for a chromosome disjunction phenotype. The detection of a large percentage of sisters (Trp^+/Ura^-, Trp^-/Ura^+, or Trp^+/Ura^+) in this two-viable-spore class is suggestive of a meiosis I defect (*e.g.*, Khazanehdari and Borts 2000). In contrast, a large percentage of nonsister spores (one Trp^+/Ura^- and the other Trp^-/Ura^+) suggests spore death unrelated to the meiosis I division. Only a small number of two-spore-viable tetrads, 32, were observed for wild type, with 38% displaying the sister pattern. Between 323 and 974 two-spore tetrads were observed in each mutant study. Consistent with the spore viability data (Figure 2), *mlh1*Δ (72%), *msh5*Δ (95%), and *mlh1*Δ *msh5*Δ (85%) strains displayed high percentages of two-spore-viable sister tetrads. In contrast, *mms4*Δ strains, which displayed a spore viability distribution consistent with random spore death, displayed a frequency of two-spore-viable sister tetrads (37%) that was similar to wild type. *mlh1*Δ *mms4*Δ (68%), *mms4*Δ *msh5*Δ (73%), and *mlh1*Δ *msh5*Δ *mms4*Δ (62%) strains displayed intermediate frequencies, relative to the single mutants, of two-spore-viable tetrads that were sisters.

Crossing over is reduced 13- to 15-fold in *mlh1*Δ *mms4*Δ strains: A major advantage of using the EAY1108/EAY1112 strain set is that the genetic intervals can be expanded to measure crossing over in mutants strongly defective in crossing over (Tables 1 and 2). Because each of the four genetic intervals in EAY1108/EAY1112 appeared to be similarly affected by the *mms4*Δ, *mlh1*Δ, and *msh5*Δ mutations, the data can be examined as composite graphs (Figure 3). It is important to note that due to high levels of spore inviability, only a small number of complete tetrads could be recovered for the double- and triple-mutant combinations containing the *mms4*Δ mutation. This limitation was partly overcome by the use of RANA software, which helped us recover and analyze genetic recombination data from a very large number of viable single spores (1790–2260, Table 1) from these same strains. As shown previously, the *mms4*Δ, *msh5*Δ, and *mlh1*Δ mutations caused small increases in the frequency of aberrant segregation events (Table 3; Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Hunter and Borts 1997; Argueso *et al.* 2003; de los Santos *et al.* 2003). However, the finding that gene conversions represented only a small proportion of events in the entire strain set allowed us to include information from single spores. As shown in Table 1 and Figure 3, the crossover frequencies obtained in the two analyses matched extremely well, suggesting that the crossover events in complete tetrads did not represent a subset of events that permitted all spores from a single tetrad to be viable.

As shown in Figure 3 and Table 1, *mms4*Δ strains displayed an ~20% reduction in crossing over. This value is similar to that observed by de los Santos *et al.* (2003) in their analysis of large chromosomes similar in size to

TABLE 2

Distribution of parental and recombinant progeny for strains presented in Table 1

	Single spores[a]			Tetrads			
Relevant genotype	Parental	Recombinant	Rf	cM	PD	TT	NPD
		URA3-LEU2					
Wild type	3635	1009	0.217	22.8	607	456	5
*pms1*Δ	539	140	0.206	20.9	74	53	0
*mms4*Δ	2227	505	0.185	16.7	102	51	0
*mlh1*Δ	3393	399	0.105	11.4	486	128	2
*msh5*Δ	5352	322	0.057	5.7	643	76	1
*mlh1*Δ *msh5*Δ	5928.5	580.5	0.089	9.8	639	120	5
*mlh1*Δ *mms4*Δ	2234	26	0.012	0.5	199	2	0
*mms4*Δ *msh5*Δ	1876	44	0.023	1.0	51	1	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1739.5	50.5	0.028	2.0	49	2	0
		LEU2-LYS2					
Wild type	3388	1256	0.270	27.5	496	569	3
*pms1*Δ	504	175	0.258	26.8	64	62	1
*mms4*Δ	2081	651	0.238	26.5	77	75	1
*mlh1*Δ	3309	438	0.127	12.7	459	157	0
*msh5*Δ	5047	627	0.111	12.0	562	155	3
*mlh1*Δ *msh5*Δ	5672	837	0.129	16.2	557	199	8
*mlh1*Δ *mms4*Δ	2206	54	0.024	2.5	191	10	0
*mms4*Δ *msh5*Δ	1779	141	0.073	7.7	44	8	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1641.5	148.5	0.083	9.8	41	10	0
		LYS2-ADE2					
Wild type	4052	592	0.127	12.9	803	263	2
*pms1*Δ	591	88	0.130	12.2	96	31	0
*mms4*Δ	2447	285	0.104	9.5	124	29	0
*mlh1*Δ	3517	275	0.073	6.9	531	85	0
*msh5*Δ	5409	265	0.047	4.2	659	61	0
*mlh1*Δ *msh5*Δ	6118	391	0.060	5.9	679	84	1
*mlh1*Δ *mms4*Δ	2242	18	0.008	1.2	196	5	0
*mms4*Δ *msh5*Δ	1867	53	0.028	3.8	48	4	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1745.5	44.5	0.025	1.0	50	1	0
		ADE2-HIS3					
Wild type	3033	1611	0.347	37.7	343	709	16
*pms1*Δ	477	202	0.297	31.5	57	68	2
*mms4*Δ	1923	809	0.296	30.7	59	94	0
*mlh1*Δ	3104	688	0.181	19.6	400	211	5
*msh5*Δ	4797	877	0.155	18.7	496	215	9
*mlh1*Δ *msh5*Δ	5394	1115	0.171	20.5	495	260	9
*mlh1*Δ *mms4*Δ	2193	67	0.030	2.7	190	11	0
*mms4*Δ *msh5*Δ	1779	141	0.073	4.8	47	5	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1611.5	178.5	0.100	7.8	43	8	0
		URA3-LYS2					
Wild type	2815	1829	0.394	48.2	264	759	45
*pms1*Δ	390	289	0.426	52.8	28	92	7
*mms4*Δ	1764	968	0.354	43.8	49	98	6
*mlh1*Δ	2976	816	0.215	23.1	351	261	4
*msh5*Δ	4843	831	0.146	16.8	513	300	7
*mlh1*Δ *msh5*Δ	5248.5	1260.5	0.194	26.0	481	260	23
*mlh1*Δ *mms4*Δ	2188	72	0.032	3.0	189	12	0
*mms4*Δ *msh5*Δ	1757	163	0.085	8.7	43	9	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1619.5	170.5	0.095	9.8	41	10	0
		LYS2-HIS3					
Wild type	2829	1815	0.391	47.8	278	744	46
*pms1*Δ	451	228	0.336	39.8	46	77	4
*mms4*Δ	1806	926	0.339	35.9	53	98	2
*mlh1*Δ	2917	875	0.231	26.5	344	261	11
*msh5*Δ	4638	1036	0.183	22.2	465	242	13
*mlh1*Δ *msh5*Δ	5127	1382	0.212	26.2	438	311	15
*mlh1*Δ *mms4*Δ	2177	83	0.037	3.7	186	15	0
*mms4*Δ *msh5*Δ	1736	184	0.096	8.7	43	9	0
*mlh1*Δ *mms4*Δ *msh5*Δ	1587.5	202.5	0.113	8.8	42	9	0

Rf refers to the recombination frequency in single spores determined by parental/(parental + recombinant) and cM indicates the genetic distance in tetrads calculated using the formula of Perkins (1949): $50 \times \{TT + (6 \times NPD)\}/(PD + TT + NPD)$.

[a] In rare cases sectored spores were observed. They were assigned as half parental (0.5) and half recombinant (0.5).

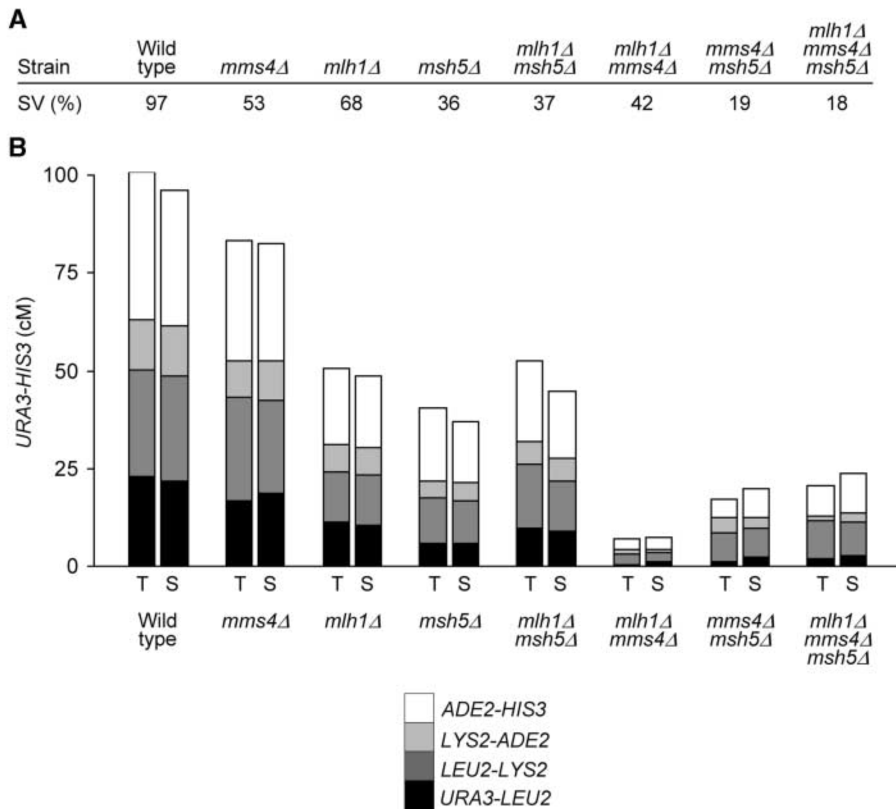

Figure 3.—Summary of the relationship between spore viability and meiotic crossing over. (A) Percentage of spore viability. (B) Cumulative genetic distances between *URA3* and *HIS3* measured from tetrads (T) and single spores (S). Each bar is divided into four sectors corresponding to the four genetic intervals in the region of chromosome XV analyzed. The size of the sectors is proportional to the contribution of each interval to the total *URA3-HIS3* genetic distance.

XV. In addition, *mlh1Δ* (50% reduction) and *msh5Δ* (60% reduction) strains displayed decreases in crossing over similar to that reported previously (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Hunter and Borts 1997; Wang *et al.* 1999; Argueso *et al.* 2003). The *mlh1Δ msh5Δ* double mutant showed a decrease in crossing over that was similar to that observed in each single mutant. *mms4Δ msh5Δ* strains displayed a four- to sixfold decrease in crossing over that was consistent with a physical analysis of this mutant (de los Santos *et al.* 2003).

Strikingly, *mms4Δ mlh1Δ* strains displayed a 13- (single spore) to 15- (complete tetrads) fold decrease in crossing over. This and the finding that a wild-type cell experiences ~87–95 crossovers in meiosis (Mortimer *et al.* 1992; Cherry *et al.* 1997; Winzeler *et al.* 1998) predicts that a *mms4Δ mlh1Δ* cell would experience 6–7 crossovers in meiosis. If we extrapolate the observed map for chromosome XV (100.9 cM in a 395-kb interval) over the entire yeast genome (12,300 kb), only 4.4 crossovers are predicted to occur in a *mms4Δ mlh1Δ* cell. It is important to note that the calculation for the total num-

TABLE 3

Percentage of aberrant segregation events observed in tetrads from wild-type, *mms4Δ*, *mlh1Δ*, and *msh5Δ* strains

Relevant genotype	Tetrads	All markers	*TRP1*	*URA3*	*LEU2*	*LYS2*	*ADE2*	*HIS3*
Wild type	1087	1.7	0	0	0.2	0.6	0.1	0.8
pms1Δ	130	2.3	0	0	0	0.8	0	1.5
mms4Δ	167	9.0	0	0	0.6	1.8	3.0	3.6
mlh1Δ	635	3.0	0	0.2	0.8	0.6	0.5	0.9
msh5Δ	757	5.0	0.1	0.1	1.6	1.2	0.8	1.2
mlh1Δ msh5Δ	815	7.0	0.4	0.5	2.1	1.8	0.9	1.3
mlh1Δ mms4Δ	203	1.0	0	0	0.5	0	0	0.5
mms4Δ msh5Δ	59	11.9	0	0	1.7	3.4	1.7	5.1
mlh1Δ mms4Δ msh5Δ	55	10.8	1.8	1.8	3.6	3.6	0	0

Aberrant events were identified from the wild type, *mms4Δ*, *mlh1Δ*, and *msh5Δ* tetrad data presented in Tables 1 and 2. For the entire data set, 97% of the aberrant events were 3:1 or 1:3 tetrads; the rest were 4:0 or 0:4 tetrads. No postmeiotic segregation events were detected.

TABLE 4

Analysis of crossover interference in wild-type (EAY1108/EAY1112) and *mms4*Δ, *mlh1*Δ, and *msh5*Δ derivatives

	NPD ratios (NPD observed/NPD expected)			Coefficients of coincidence (COC observed/COC expected)			
				URA3-LEU2-LYS2		*LEU2-LYS2-ADE2*	
Relevant genotype	*ADE2-HIS3*	*URA3-LYS2*	*LYS2-HIS3*	Tetrads	Spores	Tetrads	Spores
Wild type	0.097**	<0.253**	<0.258**	0.717**	0.799**	0.458**	0.550**
	(16/165.6)	(45/>178)	(46/>178)	(177/246.9)	(218/272.9)	(65/141.9)	(88/160.1)
*mms4*Δ	0**	0.321**	0.107**	0.789	0.781**	0.555	0.633**
	(0/15.5)	(6/18.7)	(2/18.7)	(20/25.3)	(94/120.3)	(8/14.4)	(43/67.9)
*mlh1*Δ	0.415*	0.197**	0.540*	0.573**	0.649**	0.646	0.628*
	(5/12.1)	(4/20.4)	(11/20.4)	(19/33.1)	(33/50.8)	(14/21.7)	(22/35.0)
*msh5*Δ	0.880	0.808	0.965	1.184	1.658[a]	0.747	0.990
	(9/10.2)	(7/8.7)	(13/13.5)	(20/16.9)	(59/35.6)	(10/13.4)	(29/29.3)
*mlh1*Δ *msh5*Δ	0.611	1.562[a]	0.658	1.092	1.052	0.651	1.352[a]
	(9/14.7)	(23/14.7)	(15/22.8)	(37/33.9)	(78.5/74.6)	(15/23.0)	(68/50.3)

Interference was calculated from data presented in Tables 1 and 2. Asterisks indicate that the observed number of NPDs or COCs deviated significantly from the expected number based on a two-tail binomial test (Categorical Statistics Package, http://engels.genetics.wisc.edu), suggesting that interference is present in the interval (*$P < 0.05$; **$P < 0.01$).

[a] Although double crossovers deviated significantly from the expected number in this interval, the COC (NPD ratio) is >1, indicating negative interference.

ber of crossovers in *mms4*Δ *mlh1*Δ strains is based on an extrapolation of map distances obtained in a single chromosome arm. This calculation may be inaccurate if chromosomes of different size act differently with respect to crossover distribution (Kaback *et al.* 1999). In contrast, the *mms4*Δ *mlh1*Δ *msh5*Δ triple mutant displayed a decrease in crossing over (5-fold) that was similar to *mms4*Δ *msh5*Δ strains, providing further evidence that *MSH5* functions upstream of *MLH1*.

Interference observed in *mlh1*Δ strains is no longer observed in *msh5*Δ *mlh1*Δ strains: Two distinct analyses of crossover interference are shown in Table 4: (1) observed NPD/expected NPD, which represents the ratio of observed nonparental ditypes (NPDs) to NPDs predicted by the number of single crossovers detected, and (2) a coefficient of coincidence (COC), the ratio of double crossovers observed in adjacent genetic intervals to the number predicted. Because so few crossovers were observed in double-mutant combinations involving the *mms4*Δ mutation, statistically significant measures of interference could be obtained only in single mutants and in *mlh1*Δ *msh5*Δ strains (Table 4). The measure of interference using the COC value appeared less robust than NPD ratios because of the large genetic intervals that were examined. Such large intervals were needed to allow us to measure recombination in mutants that display a large decrease in crossing over.

In wild type, interference was significant at all intervals analyzed in chromosome XV, with NPD ratios (<0.258) and COC values (0.458–0.799) significantly <1.0. These values did not significantly change in *mlh1*Δ and *mms4*Δ strains, which were shown previously to maintain interference. *msh5*Δ strains, however, displayed NPD ratios and COC values that were not significantly <1.0, indicating that interference could not be detected. A similar situation was observed in *mlh1*Δ *msh5*Δ strains. It is important to note that for the *URA3-LYS2-HIS3* interval the wild type, *mms4*Δ, *mlh1*Δ, *msh5*Δ, and *mlh1*Δ *msh5*Δ strains all displayed 1:2:1 ratios for single crossovers involving two, three, and four chromatids, respectively (Table 5). This indicates an absence of chromatid interference. Together, the NPD ratios and COC values for all the intervals analyzed provide further evidence that *MSH5* functions upstream of *MLH1*.

DISCUSSION

This study was initiated to understand how MLH1 acts in meiotic crossover control. In particular, we were interested in understanding why the *mlh1* meiotic crossover defect is less severe in *S. cerevisiae* compared to mice (Hunter and Borts 1997; Woods *et al.* 1999). To determine this, we examined the effect of *mlh1*Δ, *msh5*Δ, and *mms4*Δ single, double, and triple mutations on meiotic crossing over at four consecutive genetic intervals on chromosome XV. As shown in Table 1 and Figure 3, *mlh1*Δ *mms4*Δ double mutants displayed a decrease (13- to 15-fold) in crossing over that was similar to that observed in mouse $Mlh1^{-/-}$ female meiosis (Woods *et al.* 1999). In contrast, *msh5*Δ *mms4*Δ and *msh5*Δ *mms4*Δ *mlh1*Δ mutants displayed smaller decreases in crossing over, 4- to 6-fold, yet were less viable than *mlh1*Δ *mms4*Δ strains (18–19% *vs.* 42%). We hypothesize that competing and overlapping crossover pathways exist in yeast, some of which are deleterious to meiosis.

Recently de los Santos *et al.* (2003) showed in physical and genetic analyses that the MUS81-MMS4 complex acts in an interference-independent crossover pathway

TABLE 5

Analysis of chromatid interference at the *URA3-LYS2-HIS3* interval

Relevant genotype	No. of double-crossover tetrads involving			*P*-value
	Two strands	Three strands	Four strands	
Wild type	138	287	114	0.11
*mms4*Δ	16	25	19	0.37
*mlh1*Δ	30	55	26	0.86
*msh5*Δ	17	24	16	0.48
*mlh1*Δ *msh5*Δ	24	48	27	0.87

Tetrads displaying the tetratype class at both the *URA3-LYS2* and the *LYS2-HIS3* intervals were examined for chromatid interference. *P*-values derived from χ^2 analysis indicate the probability that the number of tetrads with exchanges involving two, three, and four chromatids follows a 1:2:1 neutral distribution of double crossovers. *P*-values <0.05 indicate a deviation from neutrality.

during *S. cerevisiae* meiosis. Their physical analysis of crossover products in *mms4*Δ *msh5*Δ double mutants showed that crossing over was reduced ~5-fold compared to wild type. Genetic analysis of *mms4*Δ *msh5*Δ strains, which revealed a 5-fold decrease in crossing over compared to wild type, is consistent with their physical studies (Table 1, Figure 3). In addition, our study of *msh5*Δ *mlh1*Δ mutants suggested that *MSH5* and *MLH1* act in the same crossover pathway, with *MSH5* acting in an upstream step that enforces the crossover interference decision and *MLH1* acting in a step after which crossover interference has been established (Börner *et al.* 2004; Fung *et al.* 2004). Surprisingly, we found that *mlh1*Δ *mms4*Δ strains displayed a much more severe defect in crossing over (13- to 15-fold decrease) than *msh5*Δ *mms4*Δ strains did, but showed significantly higher spore viability. The introduction of the *msh5*Δ mutation to *mlh1*Δ *mms4*Δ strains resulted in an increase in crossing over and a decrease in spore viability that was indistinguishable from that seen in *msh5*Δ *mms4*Δ strains. These data provide additional support for the idea that MSH4-MSH5 acts upstream of MLH1-MLH3 (Hunter and Borts 1997; Santucci-Darmanin *et al.* 2000; Moens *et al.* 2002); more significantly, they support the idea that compensating and competing crossover pathways function during yeast meiosis (Zalevsky *et al.* 1999; de los Santos 2003; reviewed in Hollingsworth and Brill 2004).

In Figure 4 we present a model consistent with the presented data. In this model, crossing over in wild-type yeast occurs primarily by MUS81-MMS4- and MSH4-MSH5-dependent pathways with MLH1-MLH3 acting in a downstream step in the MSH4-MSH5 pathway. In the absence of MUS81-MMS4, only the interference-independent pathway is compromised. The net result is a mild defect in crossing over and a spore inviability phenotype that is difficult to distinguish from inviability due to defects in DNA metabolism previously seen in *mms4* and *mus81* mutants (Mullen *et al.* 2001; de los Santos *et al.* 2001, 2003). In the absence of MSH4-MSH5, a significant defect in a crossover pathway subject to interference is observed, but this defect is partly compensated for by the MUS81-MMS4 pathway. In *mms4*Δ *msh5*Δ mutants, however, the two critical pathways for crossing over are absent, resulting in a modest 4- to 6-fold decrease in crossing over. The fact that a significantly higher (13- to 15-fold) decrease in crossing over was observed in *mms4*Δ *mlh1*Δ mutants suggests that recombination intermediates destined to become crossovers are shunted in *mms4*Δ *msh5*Δ mutants to a deleterious crossover pathway that results in increased spore death. According to this idea, deleterious crossovers do not arise in *mms4*Δ *mlh1*Δ but do so in *mms4*Δ *msh5*Δ *mlh1*Δ mutants because commitment to a MSH4-MSH5-dependent crossover pathway prevents the activation of the deleterious pathway. Under this model, crossing over, but not spore viability, was dramatically decreased in

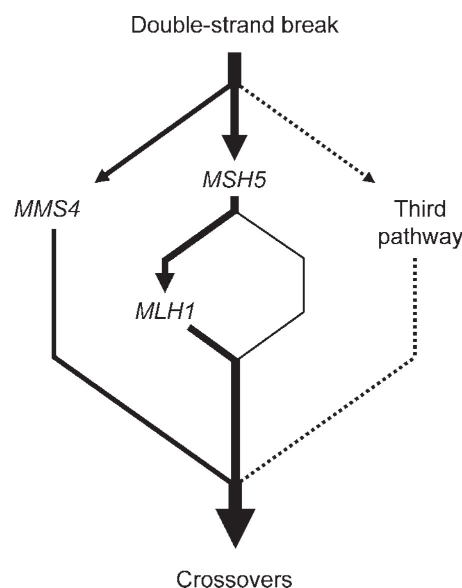

Figure 4.—Proposed organization of the meiotic crossover control pathway. The thickness of each line corresponds roughly to the relative contribution of each branch to the overall generation of crossovers. See text for details.

*mms4*Δ *mlh1*Δ strains because a deleterious crossover pathway was not activated. At present we do not have a sense of what genes or mechanisms could function in such a deleterious pathway. We cannot exclude the possibility that the high level of spore inviability in *msh5*Δ *mms4*Δ strains was due to a general defect in DNA metabolism unrelated to meiotic crossing over. However, the facts that *MSH4* and *MSH5* are specifically expressed in meiosis and *msh4*Δ and *msh5*Δ strains do not display a vegetative growth defect suggest that this was not the case (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995).

It is important to note that the model in Figure 4 proposes the presence of a MSH4-MSH5-dependent, MLH1-independent recombination pathway (represented by the thin line). This is based on the low level of crossing over observed in *mms4*Δ *mlh1*Δ strains and the observation that crossing over in this mutant is roughly equivalent to the difference in crossing over between *mlh1*Δ and *msh5*Δ strains. We hypothesize that this MSH5-dependent, MLH1-independent branch is the only path that is available in the *mlh1*Δ *mms4*Δ mutant; the crossovers that occur in this branch are capable of promoting meiosis I disjunction.

We were initially surprised by the high spore viability observed in *mms4*Δ *mlh1*Δ strains, which are predicted to experience only a small number of total crossovers (four to seven) in a single meiosis. Genetic studies performed in Drosophila females and *S. cerevisiae*, however, have shown that unrecombined chromosomes can properly segregate with varying levels of efficiency. In female Drosophila, a distributive segregation system allows chromosome IV to segregate with high fidelity even though this chromosome never undergoes reciprocal exchange (reviewed in Hawley and Theurkauf 1993; Harris *et al.* 2003). This distributive segregation system is disrupted in *nod* and *mtrm* mutants (Carpenter 1973; Rasooly *et al.* 1991; Harris *et al.* 2003). In *S. cerevisiae*, studies performed with both artificial and homeologous chromosome pairs suggest the presence of a distributive pairing system that allows for a relatively high level of disjunction at meiosis I, estimated at 89–93%, for nonexchanged chromosomes (Dawson *et al.* 1986; Mann and Davis 1986; Guacci and Kaback 1991; Sears *et al.* 1992; Ross *et al.* 1996; Maxfield Boumil *et al.* 2003).

Can the high spore viability observed in *mms4*Δ *mlh1*Δ strains be reconciled by an efficient distributive segregation system? If we assume that *S. cerevisiae* strains display a distributive segregation system in which each of the 16 chromosomes has an 89–93% probability of undergoing meiosis I disjunction in the absence of exchange, then 15–28% (0.89^{16}–0.93^{16}) of yeast cells undergoing a crossover-deficient meiosis would yield four-spore-viable tetrads in which all 16 chromosome pairs would disjoin correctly. While this calculation is simplistic, it is interesting to note that the calculated spore viability is not significantly different from that observed in *mms4*Δ *mlh1*Δ strains (14.9%). However, this correlation is complicated by the fact that residual crossing over, defects in MMR, and increased chromosome instability influence spore viability in *mms4*Δ *mlh1*Δ strains.

In mutants such as *spo11*Δ, which are completely defective in initiating both meiotic gene conversion and crossing over, spore viability is significantly lower than in *mlh1*Δ *mms4*Δ strains (*e.g.*, Keeney *et al.* 1997). What accounts for this difference in viability? Unlike *spo11*Δ, *mlh1*Δ and *msh4*Δ mutants display gene conversion frequencies that are not dramatically different from wild type, and *msh5*Δ and *mms4*Δ mutants display wild-type levels of meiotically induced double-strand breaks (DSBs; Ross-Macdonald and Roeder 1994; Hunter and Borts 1997; de los Santos *et al.* 2001; Argueso *et al.* 2003; Börner *et al.* 2004). These observations suggest that, despite showing defects in promoting crossing over, *msh4*Δ, *msh5*Δ, and *mms4*Δ strains are functional in the formation of interstitial connections that appear between homologs in early meiotic prophase (Giroux *et al.* 1989; Ross-Macdonald and Roeder 1994; Weiner and Kleckner 1994; Hollingsworth *et al.* 1995; de los Santos *et al.* 2001; Börner *et al.* 2004). In *spo11*Δ strains, however, recombination initiation is disrupted and the interstitial connections are absent (Giroux *et al.* 1989; Weiner and Kleckner 1994). An attractive possibility is that these connections are important for a DNA homology search in early meiotic prophase that is essential for distributive meiosis I segregation (Weiner and Kleckner 1994; Keeney *et al.* 1997).

In *mms4*Δ *msh5*Δ mutants, crossing over is approximately three times higher, but spore viability is twofold lower, than that in *mms4*Δ *mlh1*Δ strains. Studies in a variety of organisms have indicated that crossing over alone does not guarantee the proper disjunction of paired homologs in meiosis I (see Ross *et al.* 1996 and references therein). This work also suggests that the location of a crossover in a chromosome pair can affect the efficiency of disjunction. For a crossover to mediate meiosis I segregation, it should be present within the context of sister chromatids that are held together along their lengths or at least at the site of exchange. On the basis of this information, we hypothesize that crossing over in *mms4*Δ *msh5*Δ strains ("the third pathway") interferes with the distributive pairing system. This could occur if crossovers in this strain are not resolved, are resolved after the programmed release of sister connections, or if resolution does not occur through the generation of a chiasma binder at the site of exchange. In such a model the MSH4-MSH5 pathway ensures both the formation and the dissolution of a "chiasma binder." Alternatively, excessive crossing over takes place in *mms4*Δ *msh5*Δ mutants (negative interference) that results in inviability due to a difficulty in separating homologs at anaphase I (Carpenter 1987). A physical analysis of *mms4*Δ *mlh1*Δ and *mms4*Δ *msh5*Δ strains in meiosis that allows for the measure of DSB formation, single- and double-ended

invasion intermediates, as well as physical crossovers, would allow one to test these ideas.

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