Genetic Control of Recombination Partner Preference in Yeast Meiosis: Isolation and Characterization of Mutants Elevated for Meiotic Unequal Sister-Chromatid Recombination

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ABSTRACT

Meiotic exchange occurs preferentially between homologous chromatids, in contrast to mitotic recombination, which occurs primarily between sister chromatids. To identify functions that direct meiotic recombination events to homologues, we screened for mutants exhibiting an increase in meiotic unequal sister-chromatid recombination (SCR). The *msc* (*m*eiotic *s*ister-*c*hromatid recombination) mutants were quantified in *spo13* meiosis with respect to meiotic unequal SCR frequency, disome segregation pattern, sporulation frequency, and spore viability. Analysis of the *msc* mutants according to these criteria defines three classes. Mutants with a class I phenotype identified new alleles of the meiosis-specific genes *RED1* and *MEK1*, the DNA damage checkpoint genes *RAD24* and *MEC3*, and a previously unknown gene, *MSC6*. The genes *RED1*, *MEK1*, *RAD24*, *RAD17*, and *MEC1* are required for meiotic prophase arrest induced by a *dmc1* mutation, which defines a meiotic recombination checkpoint. Meiotic unequal SCR was also elevated in a *rad17* mutant. Our observation that meiotic unequal SCR is elevated in meiotic recombination checkpoint mutants suggests that, in addition to their proposed monitoring function, these checkpoint genes function to direct meiotic recombination events to homologues. The mutants in class II, including a *dmc1* mutant, confer a dominant meiotic lethal phenotype in diploid *SPO13* meiosis in our strain background, and they identify alleles of *UBR1*, *INP52*, *BUD3*, *PET122*, *ELA1*, and *MSC1–MSC3*. These results suggest that *DMC1* functions to bias the repair of meiosis-specific double-strand breaks to homologues. We hypothesize that the genes identified by the class II mutants function in or are regulators of the *DMC1*-promoted interhomologue recombination pathway. Class III mutants may be elevated for rates of both SCR and homologue exchange.

MEIOSIS reduces the chromosome complement from diploidy to haploidy by a single round of DNA replication followed by two rounds of chromosome segregation. At the first meiotic division (MI), homologous chromosomes, which consist of pairs of sister chromatids, disjoin to opposite poles (reductional division). The second meiotic division (MII) resembles mitosis in that sister chromatids separate and segregate (equational division). For homologues to properly disjoin at MI, they must pair, recombine, and synapse. In MI prophase, homologous chromosomes align and pair with one another along their length. Pairing is followed by formation of the synaptonemal complex (SC; Albini and Jones 1987). SC formation initiates with the assembly of axial elements along the pairs of sister chromatids. A less densely staining central element then forms between the two homologues. In the completed (tripartite) SC, the axial elements are called lateral elements, and structures called transverse filaments extend from the central element to the lateral elements. The chromatin of each pair of sister chromatids is organized into loops attached at the base to the lateral elements (von Wettstein *et al.* 1984; Heyting 1996). Synapsis is defined as the intimate association of homologues in the context of mature SC. At full synapsis, the entire structure (paired homologues plus SC) is called a meiotic bivalent.

In *Saccharomyces cerevisiae*, recombination is induced 100- to 1000-fold in meiosis, and most or all is initiated, concomitantly with SC formation, by meiosis-specific double-strand breaks (DSBs; reviewed in Lichten and Goldman 1995). In this article, "exchange" refers to reciprocal events, and "recombination" refers to the sum of reciprocal and nonreciprocal events. Exchange between homologues in the context of mature SC (Engebrecht *et al.* 1990) is required to form the stable interconnections, cytologically observed as chiasmata (Carpenter 1988), that are necessary to orient the meiotic bivalent with respect to the MI spindle apparatus (reviewed in Bascom-Slack *et al.* 1997). Mutations that disrupt interhomologue exchange result in spore inviability because of missegregation of homologous chromosomes in MI. In addition, sister chromatids are

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closely associated with each other and with proteins of the axial elements when homologues are fully synapsed before the MI division (Moens and Pearlman 1988). It has been proposed that this sister-chromatid cohesion is also necessary for chiasma function (Maguire 1990, 1995). Mutations that disrupt sister-chromatid cohesion result in precocious separation of sister chromatids before the separation of homologues in MI (Miyazaki and Orr-Weaver 1992; Molnar *et al.* 1995).

Although phenotypic analysis of meiotic mutants clearly indicates that chromosome pairing, recombination, and synapsis are interdependent, the exact relationship among these processes remains to be delineated. In yeast, it appears that early steps in the recombination pathway are required for synapsis, which initiates at the sites of recombination events (reviewed in Roeder 1997). For example, mutants that are defective for meiotic recombination do not form SC. However, although early steps in the meiotic recombination pathway promote synapsis, the formation of recombinant products at normal levels depends on proper synapsis (reviewed in Roeder 1997).

Meiotic exchanges occur preferentially between homologous chromatids (reviewed in Petes and Pukkila 1995; Kleckner 1996; Roeder 1997). However, Kadyck and Hartwell (1992) showed that DNA damage induced in G2 of the mitotic cell cycle was repaired preferentially by interaction with the sister chromatid. These observations indicate that as a cell enters meiosis, there is a change in recombination partner preference from intersister to interhomologue. This implies the existence of a meiotic machinery that directs the repair of meiosis-specific DSBs to homologues and/or away from sisters. Mutations inactivating this machinery would increase intersister recombination in meiosis and reduce, but not eliminate, interhomologue exchange.

Several screens have identified genes in yeast required for wild-type levels of meiotic recombination between homologues (reviewed in Petes *et al.* 1991; Roeder 1997). The mutations identified in these screens can be generally classified into two groups: those that eliminate recombination and those that retain a significant level. In the former class are mutations in *SPO11*, which encodes a protein homologous to type II topoisomerases and is the catalytic subunit of the complex responsible for meiosis-specific DSBs (Bergerat *et al.* 1997; Keeney *et al.* 1997). *RAD50* and several others have phenotypes implying involvement at an "early" stage in the meiotic recombination process (Malone and Esposito 1981; Malone *et al.* 1991; Klapholz *et al.* 1985). These mutants do not form meiosis-specific DSBs or SC, but they do proceed through the two divisions of meiosis (Alani *et al.* 1990; Cao *et al.* 1990). In the absence of recombination, the homologous chromosomes missegregate at MI, resulting in aneuploid meiotic products that are largely inviable.

There are several mutations that reduce meiotic interhomologue recombination to 10–25% of the wild-type level. Possible candidates for genes encoding components of the machinery that biases the repair of meiosis-specific DSBs to homologous chromatids may be found in this group. Two of these, *HOP1* and *RED1*, are meiosis-specific genes encoding axial/lateral element components (Hollingsworth and Byers 1989; Hollingsworth *et al.* 1990; Rockmill and Roeder 1990; Smith and Roeder 1997). *MEK1*/*MRE4* encodes a putative meiosis-specific kinase (Rockmill and Roeder 1991; Leem and Ogawa 1992). Genetic evidence indicates that the products of these three genes interact to promote proper SC assembly (Rockmill and Roeder 1990, 1991; Hollingsworth and Johnson 1993; Hollingsworth and Ponte 1997; Friedman *et al.* 1994), and this conclusion was supported by recent cytological studies (Smith and Roeder 1997; Bailis and Roeder 1998). In addition, the *RED1*/*MEK1*/*HOP1* epistasis group is implicated in meiotic sister-chromatid cohesion. *red1* mutants fail to form axial elements (Rockmill and Roeder 1990) and are defective in meiotic sister-chromatid cohesion (Bailis and Roeder 1998). The defect in meiotic sister-chromatid cohesion may explain why the crossovers that do occur in this mutant are not effective in disjunction (Rockmill and Roeder 1990). Phosphorylation of Red1p by Mek1p is required for meiotic sister-chromatid cohesion. *hop1* mutants assemble axial elements, but synapsis is blocked (Hollingsworth and Byers 1989; Loidl *et al.* 1994). Although not absolutely required for axial element formation and sister-chromatid cohesion, Hop1p is required for proper Mek1p localization, and it appears to stabilize the Red1p and Mek1p interaction (Bailis and Roeder 1998). In addition, the interaction of Hop1p with Red1p is enhanced by the presence of *MEK1* (de los Santos and Hollingsworth 1999). Thus, all three genes are likely required to form functional axial elements capable of nucleating synapsis.

RAD51 and *DMC1* encode ubiquitous and meiosis-specific *recA* homologues, respectively. In *rad51* and *dmc1* mutants, meiosis-specific DSBs occur at wild-type levels, but they are unrepaired and hyperresected, indicating that *RAD51* and *DMC1* are required for strand exchange during meiotic recombination (Bishop *et al.* 1992; Shinohara *et al.* 1992). Chromosome pairing is delayed and incomplete in the two mutants (Rockmill *et al.* 1995). In addition, both mutants are delayed in synapsis, are reduced for meiotic recombination to 10% of the wild-type level, and can cause arrest in meiotic prophase subsequent to synapsis (Bishop *et al.* 1992; Rockmill *et al.* 1995).

It has been proposed that one function of the SC-associated proteins encoded by *HOP1*, *RED1*, *MEK1*, and *DMC1* is to bias meiotic recombination events to homologues (Petes and Pukkila 1995; Kleckner 1996; Roeder 1997). A *dmc1* mutant exhibits an increase in intrachromosomal recombination between directly re-

peated sequences (Bishop *et al.* 1992). In addition, there is evidence that *DMC1* functions in a meiotic recombination pathway that is biased toward interhomologue exchange and that this pathway has functions that are independent of those of the ubiquitous *RAD51* pathway (Dresser *et al.* 1997; Schwacha and Kleckner 1997; Shinohara *et al.* 1997; Zenvirth *et al.* 1997). In a *hop1* mutant, meiosis-specific DSBs are reduced to 10% of the wild-type level. Moreover, these DSBs are processed exclusively into intersister recombination intermediates (Schwacha and Kleckner 1994). It has been postulated that meiotic sister-chromatid cohesion reduces the participation of sister chromatids in meiotic recombination events (Smith and Roeder 1997). This suggests that disruption of sister-chromatid cohesion in *red1* and *mek1* mutants would result in an increase in meiotic sister-chromatid recombination (see results and discussion).

RED1 and *MEK1* are also required for the meiotic prophase arrest induced by a *dmc1* mutation (Xu *et al.* 1997), suggesting a link between meiotic sister-chromatid cohesion, recombination, and a surveillance system that monitors the faithful completion of meiotic recombination. The DNA damage checkpoint control genes *RAD24*, *RAD17*, and *MEC1* (Weinert *et al.* 1994) are also required for *dmc1*-induced arrest, which defines the meiotic recombination checkpoint (Lydall *et al.* 1996). Spore viability is reduced in *rad24*, *rad17*, *mec1-1*, and *mec3* mutants in a pattern indicative of a defect in homologue disjunction at MI (Lydall and Weinert 1995; Lydall *et al.* 1996). This suggests that, in addition to the proposed monitoring function, these checkpoint genes have a role in ensuring the fidelity of interhomologue recombination and/or disjunction.

Although many individual functions required for the fidelity of meiotic recombination have been identified, a role in distinguishing sequences on homologues from those on sister chromatids, or other "ectopic" homology, has not yet been confirmed. This distinction is defined as partner choice, which results in an overall preference for homologues in meiotic recombination. We sought to identify components of the machinery that mediates proper meiotic recombination partner choice, using a screen designed specifically to detect mutants exhibiting an increase in meiotic unequal sister-chromatid recombination (SCR). We reasoned that, in recombination-competent mutants, loss of the preference for the homologue in meiotic recombination would be manifest as an increase in the frequency of SCR.

This approach has identified 38 mutants exhibiting the *m*eiotic *s*ister *c*hromatid recombination-elevated phenotype (*msc*). The *msc* mutants were quantified with respect to meiotic unequal SCR frequency, disome segregation pattern, sporulation frequency, and spore viability in the one-division meiosis conferred by the *spo13* allele. In addition, outcrossing the mutants to a *SIR3 SPO13* congenic strain revealed a class that conferred a dominant meiotic lethal phenotype peculiar to our strain background. Analysis of the *msc* mutants according to these criteria defined three classes: Mutants with a class I phenotype identify new alleles of the meiosis-specific genes *RED1* and *MEK1*, DNA damage checkpoint genes *RAD24* and *MEC3* (Weinert *et al.* 1994), and a previously unidentified gene, *MSC6*. The dominant meiotic lethal class II mutants, which include a *dmc1*Δ mutant, identify alleles of *UBR1*, *INP52*, *BUD3*, *PET122*, *ELA1*, and *MSC1-MSC3*. Class III mutants, which identify alleles of *MNR2* and *MSC7*, have characteristics consistent with a meiotic *hyper-rec* phenotype.

MATERIALS AND METHODS

Plasmid construction: Plasmids were constructed using standard procedures (Maniatis *et al.* 1982). The *arg4*::*URA3* fusion gene in pDT113 was constructed as follows: YIP5 (Struhl *et al.* 1979) was digested with *Pst*I and *Ava*I, and the ends of the resulting 901-bp fragment containing the *URA3* coding region were filled in with T4 DNA polymerase. In parallel, pMLC28::*ARG4* (Levinson *et al.* 1984) was cut with *Sac*I, treated with T4 DNA polymerase (New England Biolabs, Beverly, MA) to fill in the ends, and then cut with *Sna*BI to remove the 1558-bp fragment containing the *ARG4* coding region. The resulting 4.4-kb vector fragment was ligated with the aforementioned 901-bp *URA3* fragment to generate pDT113.

pMS12 was constructed by ligating the 3.5-kb *Sna*BI fragment containing a segment of chromosome VIII adjacent to the 3′-end of the *ARG4* gene from pSPO13-1 (Wang *et al.* 1987) into the *Sna*BI site of pMLC28::*ARG4*.

pMS23 was constructed in several steps:

1. An ~1.4-kb, *CUP1*-containing *Bam*HI fragment of pYep36::*CUP1* (Butt *et al.* 1984) was inserted into the *Bam*HI site of pTZ18U (United States Biochemical, Cleveland) to generate pMS4.
2. pAB34 was cut with *Sau*3a, and the ends of the resulting 374-bp fragment containing *ARSH4* were filled in with T4 DNA polymerase. This fragment was then inserted into the *Sma*I site of pMS4 to generate pMS5.
3. pDT113 was cut with *Pst*I, and the ends were filled in with T4 DNA polymerase and then cut with *Eco*RV to liberate an ~1-kb fragment containing a 3′-segment of the *arg4*::*URA3* fusion. In parallel, pMS5 was cut with *Pst*I, and the ends were filled in with T4 DNA polymerase. The resulting pMS5 vector fragment was ligated to the aforementioned ~1-kb fragment containing a 3′-segment of *arg4*::*URA3* to yield pMS6.
4. pDT113 was cut with *Pst*I and *Bsa*I, and the ends of the resulting ~2-kb fragment containing a 5′-segment of the *arg4*::*URA3* fusion gene were made blunt with T4 DNA polymerase. In parallel, pMS6 was cut with *Sac*I, and the 5′-overhang was removed with T4 DNA polymerase. The linear, blunt-ended product was then ligated to the aforementioned ~2-kb fragment containing a 5′-segment of the *arg4*::*URA3* fusion gene to generate pMS7. The 5′ and 3′ *arg4*::*URA3* fragments and the intervening *CUP1* gene comprise the SCR construct.
5. pMS7 was digested with *Eco*RI and *Sph*I, and the ends of the resulting ~5-kb fragment containing the SCR construct were made blunt with T4 DNA polymerase. In parallel, pMLC28::*ARG4* was digested with *Hpa*I to remove an ~2-kb fragment of the *ARG4* gene. The resulting 4-kb fragment

of pMLC28::*ARG4* was ligated to the aforementioned ~5-kb fragment containing the SCR construct to generate pMS13.
6. pMS12 was digested with *Hpa*I to liberate an ~3-kb fragment containing a segment of chromosome VIII adjacent to the 3′-end of *ARG4*, which was then ligated into the *Sna*BI site of pMS13 to generate pMS14.
7. A *Not*I linker was then inserted into the *Stu*I site in the 5′-segment of the *arg4*::*URA3* gene in pMS14 to generate pMS21.
8. A *Pme*I linker was then inserted into the *Xmn*I site in the chromosome VIII *ARG4* 3′-segment in pMS21 to generate pMS22.
9. Finally, pASZ10 (Stotz and Linder 1990) was digested with *Bgl*II to liberate an ~2.5-kb *ADE2*-containing fragment. In parallel, pMS22 was digested with *Bgl*II to remove an ~1.5-kb fragment of the chromosome VIII *ARG4* 3′-segment. The resulting 11.7-kb pMS22 fragment was ligated to the aforementioned ~2.5-kb *ADE2* fragment to generate pMS23.

pMS36 was constructed in several steps.

1. An ~1.4-kb, *CUP1*-containing *Bam*HI fragment of pYep36::*CUP1* was inserted into the *Bam*HI site of pTZ18U to yield pMS4.
2. pAB34 was cut with *Sau*3a, and the ends of the resulting 374-bp fragment containing *ARSH4* were filled in with T4 DNA polymerase. This fragment was then inserted into the *Sma*I site of pMS4 to yield pMS5.
3. pDT113 was digested with *Bsa*I; the ends were filled in with T4 DNA polymerase and subsequently digested with *Eco*RV to liberate a 432-bp fragment containing the middle segment of the *arg4*::*URA3* fusion gene. pMS5 was digested with *Pst*I; the 5′-overhang was removed with T4 DNA polymerase and then ligated to the aforementioned 432-bp fragment containing the middle segment of the *arg4*::*URA3* fusion gene to generate pMS8.
4. pDT113 was digested with *Pst*I; the 5′-overhang was removed with T4 DNA polymerase, and the resulting fragment was then digested with *Eco*RV to liberate an ~1-kb fragment containing a 3′-segment of the *arg4*::*URA3* fusion gene. In parallel, pMS8 was digested with *Sac*I; the 5′-overhang was removed with T4 DNA polymerase and then ligated to the aforementioned ~1-kb fragment containing a 3′-segment of the *arg4*::*URA3* fusion gene to generate pMS9.
5. pMS9 was then digested with *Kpn*I and *Bam*HI to remove the *ARSH4* and *CUP1* sequences. The resulting 4.3-kb fragment of pMS9 was treated with T4 DNA polymerase to make the ends blunt and then ligated to generate pMS10.
6. pMS10 was digested with *Bsa*I; the 5′-overhang was removed with T4 DNA polymerase, and the resulting fragment was then digested with *Msc*I to remove 373 bp of the 3′-segment of the *arg4*::*URA3* gene. The resulting 4-kb fragment of pMS10 was ligated to generate pMS11. The tandem segments of the *arg4*::*URA3* gene comprise the homologue homology (HH) construct.
7. pMS10 was digested with *Eco*RI and *Sph*I, and the ends of the resulting ~1.1-kb fragment containing the HH construct were made blunt with T4 DNA polymerase. In parallel, pMLC28::*ARG4* was digested with *Hpa*I to remove an ~2-kb fragment of the *ARG4* gene. The resulting 4-kb fragment of pMLC28::*ARG4* was ligated to the aforementioned ~1.1-kb fragment containing the HH construct to generate pMS11.
8. pMS12 was digested with *Hpa*I to liberate an ~3-kb fragment containing a segment of chromosome VIII adjacent to the 3′-end of *ARG4*, which was then ligated into the *Sna*BI site of pMS11 to generate pMS17.
9. A *Pme*I linker was then inserted into one of the two *Bgl*II sites in the chromosome VIII *ARG4* 3′-segment in pMS17 to generate pMS35.
10. Finally, pASZ10 was digested with *Bgl*II to liberate an ~2.5-kb fragment containing the *ADE2* gene, which was ligated to *Bgl*II-digested pMS35 to generate pMS36.

pCP3 (Foss and Stahl 1995) was digested with *Eco*RI and *Hin*dIII, and the ends of the resulting ~2.7-kb *LYS2*-containing fragment were filled in with T4 DNA polymerase. In parallel, pLG54 (Gilbertson and Stahl 1996) was digested with *Bst*EII and *Bgl*II to remove a 1.1-kb *URA3*-containing fragment. The ends of the resulting 4-kb pLG54 fragment were filled in with T4 DNA polymerase and then ligated to the aforementioned ~2.7-kb *LYS2*-containing fragment to generate pMS38.

pEF83 (Foss and Stahl 1995) was digested with *Eco*RI; the 3′-overhangs were filled with with T4 DNA polymerase and then ligated to the 2-kb, *ARG4*-containing *Hpa*I fragment of pMLC28::*ARG4* to generate pMS39.

A 4.5-kb, *SIR3*-containing *Sal*I fragment of pJR273 (obtained from George Sprague, Jr.) was ligated into *Xho*I/*Sal*I-digested pRS306 (Sikorski and Hieter 1989) to yield pMS40. pMS41 was constructed by digesting pMS40 with *Nru*I and *Cla*I to remove a 1.6-kb fragment of the *SIR3* gene, making the ends blunt and inserting a *Pme*I linker.

pMS42 was constructed by ligating an ~1.1-kb, *URA3*-containing *Sma*I fragment from pJJ242 (Jones and Prakash 1990) into *Sma*I-digested pB84 (Rockmill and Roeder 1990).

pMS43 was constructed by ligating the ~1.4-kb, KanMX4-containing *Bgl*II/*Eco*RV fragment of KanMX4 (Wach *et al.* 1994) into *Bgl*II/*Msc*I-digested pRSQ303 (constructed by Joe Horeka).

pMS47 was constructed in several steps, beginning with filling in the 3′-overhangs of *Bgl*II-digested pMS43 with T4 DNA polymerase, and then the blunt ends were ligated to destroy the *Bgl*II site. The plasmid was then digested with *Apa*I, the 5′-overhangs were removed, and a *Bgl*II linker was inserted. The plasmid was then digested with *Nde*I, the 3′-overhangs were filled in with T4 DNA polymerase, and then the blunt ends were ligated to destroy the *Nde*I site. The plasmid was then digested with *Sal*I, the 3′-overhangs were filled in with T4 DNA polymerase, and a *Nde*I linker was inserted. Insertion of the *Nde*I linker restores the *Sal*I site.

pMS49 was made in several steps, beginning with ligating the ~1.1-kb, *URA3*-containing *Sma*I fragment from pJJ242 into *Nae*I-digested pMS47. The plasmid was then digested with *Eco*RI, and the ends were filled in with T4 DNA polymerase and then ligated to destroy the *Eco*RI site. The ~3.3-kb *SPO13*-containing *Bam*HI/*Eco*RV fragment of YIP5::*SPO13* (constructed by Larry Gilbertson) was then ligated to the *Bgl*II/*Eco*RV-digested plasmid.

Yeast strains: Yeast strains were constructed and manipulated by standard genetic methods (Sherman *et al.* 1982). Yeast strains were transformed using a standard LiOAC procedure (Ito *et al.* 1983). The genotypes of the yeast strains used in this study are listed in Table 1. DT71was constructed in several steps: (1) DT 60.3a was transformed with *Bam*HI/*Xho*I-digested pEF84 to introduce the *GPA1-3′*::*TRP1* construct by one-step transplacement (Rothstein 1983), generating DT61; (2) DT60.3a was also tranformed with *Bam*HI/*Xho*I-digested pEF154 to introduce the *GPA1-3′*::*LEU2* construct by one-step transplacement, generating DT62; (3) DT61 was transformed with *Eco*RI/*Pme*I-digested pMS23 to introduce the (*ADE2*::SCR) construct by one-step transplacement, generating DT63; (4) DT62 was transformed with *Eco*RI/*Pme*I-digested pMS36 to introduce the (*ADE2*::HH) construct by one-step transplacement, generating DT64; (5) DT65 is a

TABLE 1

Yeast strains

Strain[a]	Genotype
DT47.1d	*MAT*α *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 his3*
DT60.3a	*MAT***a** *cup1*::*ura3*::*THR1 trp1 ura3 lys2 leu2 his3 ade2-1*
DT61	*MAT*α *GPA1-3′*::*TRP1 cup1*::*ura3*::*THR1 trp1 ura3 lys2 leu2 his3*
DT62	*MAT*α *GPA1-3′*::*LEU2 cup1*::*ura3*::*THR1 trp1 ura3 lys2 leu2 his3*
DT63	*MAT***a** *GPA1-3′*::*TRP1 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3 lys2 leu2 ade2-1 his3*
DT65	*MAT***a** *GPA1-3′*::*TRP1 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2-1 his3*
DT66	*MAT***a** *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2-1 his3*
DT64	*MAT***a** *GPA1-3′*::*LEU2 ADE2*::*HH cup1*::*ura3*::*THR1 trp1 ura3 lys2 leu2 ade2-1 his3*
DT67	*MAT***a** *GPA1-3′*::LEU2 spo13::LYS2 ADE2::HH *cup1*::*ura3*::*THR1 trp1 ura3 ly2 leu2 ade2-1 his3*
Z140-51c	*MAT*α *arg4-2* + + + *trp5-48 trp1-1 his5-2 ade2-1 leu1-12* + arg4-17 thr1 CUP1
DT68	*MATR*α *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::*SCE cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2-1 his3* + *arg4-17 thr1 CUP1*
DT69	*MAT*α *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3 GPA1-3′*::*LEU2 spo13*::*LYS2 ADE2*::HH *cup1*::*ura3*::*THR1*
DT70	*MAT*α *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3 GPA1-3′*::*ARG4 spo13*::*LYS2 ADE2*::HH *cup1*::*ura3*::*THR1*
DT71	*MAT*α *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3 sir3 GPA1-3′*::*ARG4 spo13*::*LYS2 ADE2*::HH *cup1*::*ura3*::*THR1*
DT83	DT71, except *red1*::*ADE2*
DT84	DT71, except *red1*::*TN+62*
DT84	DT71, except *red1*::*TN+143*
DT86	DT71, except *red1*::*TN+411*
DT87	DT71, except *red1*::*TN+621*
DT88	DT71, except *red1*::*TN+955*
DT89	DT71, except *red1*::*TN+1293*
DT91	DT71, except *red1*::*TN+2075*
DT92	DT71 except, *red1*::*TN+2174*
DT93	DT71, except *mek1*::*TN+944*
DT115	DT71, except *rad24*Δ
DT94	DT71, except *rad24*::*TN+342*
DT95	DT71, except *rad24*::*TN+385*
DT90	DT71, except *rad24*::*TN+509*
DT96	DT71, except *rad24*::*TN+585*
DT97	DT71, except *rad24*::*TN+614*
DT98	DT71, except *rad24*::*TN+625*
DT99	DT71, except *rad24*::*TN+801*
DT100	DT71, except *rad24*::*TN+1346*
DT101	DT71, except *mec3*::*TN+1153*
DT114	DT71, except *rad17*Δ
DT117	DT71, except *mec1-1*
DT113	DT71, except *hop1*::*LEU2*
DT116	DT71, except *dmc1*Δ
DT118	DT71, except *rad24*Δ *red1*::*ADE2*
DT120	DT71, except *dmc1*Δ *red1*::*ADE2*
DT122	DT71, except *spo11*Δ *red1*::*ADE2*
DT121	DT71, except *spo11*Δ *rad24*Δ
DT102	DT71, except *inp52*::*TN+277*
DT103	DT71, except *ubr1*::*TN+330*
DT119	DT71, except *ubr1*Δ
DT104	DT71, except *pet122*::*TN+593*
DT105	DT71, except *yml128c*::*TN+1531*
DT106	DT71, except *ydr205w*::*TN+1255*
DT107	DT71, except *yll067c*::*TN+1891*
DT108	DT71, except *ylr219w*::*TN+751*
DT109	DT71, except *ynl230c*::*TN>216*
DT110	DT71, except *yor354c*::*TN+1608*
DT111	DT71, except *ypl108w*::*TN-25*

(*continued*)

TABLE 1

(Continued)

Strain[a]	Genotype
DT112	DT71, except *mnr2*::*TN+1766*
DT72	*MAT*α *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3 sir3*
DT78	*MAT***a** *GPA1-3′*::*ARG4 ADE2*::HH *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3*
DT124	DT71, except *ydr205w*::*LEU2*
DT125	DT71, except *yml128c*::*LEU2*
DT126	DT71, except *ylr219w*::*LEU2*
DT127	DT71, except *inp52*::*LEU2*
DT154	*MAT***a** *GPA1-3′*::*TRP1 spo13*::*LYS2 ADE2*::SCR *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3* *MAT***a** *GPA1-3′*::*ARG4 spo13*::*LYS2 ADE2*::HH *cup1*::*ura3*::*THR1 trp1 ura3*::*HIS3 lys2 leu2 ade2 his3*
DT155	DT154, except homozygous *red1*::*TN+621*
DT156	DT154, except homozygous *mek1*::*TN+944*
DT157	DT154, except homozygous *rad24*::*TN+801*
DT158	DT154, except homozygous *mec3*::*TN+1153*

[a] All strains were constructed by the authors, except Z140-51c (Maloney and Fogel 1980).

ura3::*HIS3* segregant of a DT63 × DT47.1d cross; (6) DT65 was transformed with *Eco*RI/*Hin*dIII-digested pMS38 to introduce the *spo13*::*LYS2* allele by one-step transplacement, generating DT66; (7) DT64 was transformed with *Eco*RI/*Hin*dIII-digested pMS38 to introduce the *spo13*::*LYS2* allele by one-step transplacement, generating DT67; (8) DT68 (Table 1) is a segregant of a DT66 × Z140-51c cross (Maloney and Fogel 1980); (9) DT69 is a segregant of a DT67 × DT68 cross; (10) DT69 was transformed with *Bam*HI/*Xho*I-digested pMS39 to transplace the *GPA1-3′*::*LEU2* construct with a *GPA1-3′*::*ARG4* derivative, generating DT70; (11) DT70 was transformed with *Xho*I-digested pMS41 to introduce the *sir3*Δ allele by two-step transplacement to yield DT71.

The following mutations were introduced into DT71. The *red1*::*ADE2* allele was introduced by two-step transplacement with *Xho*I-digested pMS42. The *hop1*::*LEU2* allele was introduced by one-step transplacement with *Bgl*II-digested pNH37-2 (Hollingsworth and Byers 1989). The *dmc1*::*LEU2* allele was introduced by one-step transplacement with *Xba*I-digested pNKY422 (Bishop *et al.* 1992). The *rad17*::*LEU2* allele was introduced by one-step transplacement with *Bam*HI/*Xba*I-digested pWL8 (Lydall and Weinert 1995). The *rad24*::*LEU2* allele was introduced by one-step transplacement with *Sma*I-digested pWL62 (Lydall *et al.* 1996). The *spo11*::*hisg* allele was introduced by two-step transplacement with *Bgl*II/*Xba*I-digested pGB518 (C. N. Giroux, unpublished results). The *ubr1*Δ allele was introduced by one-step transplacement with *Hin*dIII-digested pSOB30 (a gift from Alex Varshavsky). The *inp52*::*LEU2* allele was introduced by one-step transplacement with a PCR product generated as described in Stolz *et al.* (1997), using pRS305 (Sikorski and Hieter 1989) as the template. The *msc1*::*LEU2* allele was introduced by one-step transplacement with *Apa*I/*Bsa*I-digested pMS82. The *ydr205w*::*LEU2* allele was introduced by one-step transplacement with *Asc*I-digested pMS84. The *msc3*::*LEU2* allele was introduced by one-step transplacement with *Eco*RI-digested pMS85.

DT72 was obtained by sporulating DT71 and screening the spore colonies from dissected dyads for an aberrant segregant that was monosomic for the SCR-construct-containing derivative of chromosomeVIII (Figure 1). DT78 was constructed in several steps, beginning with the introduction of the *SPO13* allele by two-step transplacement with *Eco*RI-digested pMS49. The *SIR3* allele was then introduced by two-step transplacement with *Xho*I-digested pMS40. Mating type was then switched by transformation with pGAL-*HO* (Herskowitz and Jensen 1991), and transformants were tested for mating type. Southern blot analysis was used to verify the function of the SCR construct and the structure of all strains made by transformation.

The *spo13* homozygous diploid strains were constructed by transforming the haploid disomic strains with the *SIR3*-containing plasmid pJR273 and crossing them to DT78. These diploids were sporulated, and haploid segregants of the appropriate genotype were mated.

Mutant screen protocol: Mutagenesis was with the *Tn3* transposon-mutagenized yeast genomic library constructed by Burns *et al.* (1994). DT71 was transformed with *Not*I-cleaved DNA from 15 pools of the yeast genomic library carrying random *TN3*::*lacz*::*LEU2* insertions. A total of 53,523 individual Leu$^+$ transformants were picked and patched onto YEPEG plates and grown for 3 days. The patches were then replicated to S-raffinose + 5-fluoroorotic acid (5-FOA, 0.1%) plates and grown for 2 days to select against mitotic unequal SCR recombinants. The patches were then replicated to SPO plates and incubated for 3 days. The centromere of the SCR-construct-containing chromosome VIII has been marked with *TRP1* integrated 3′ of the *GPA1* locus located ~2 cM from CEN 8 and the centromere of the chromosome containing the HH construct with *ARG4* at the equivalent location (Figure 1). To eliminate the contribution of mitotic loss events from the analysis, cells were selected that contained both a Trp$^+$ chromosome that had experienced an unequal sister-chromatid recombination event, depicted in Figure 1C, and an Arg$^+$ HH-containing chromosome. For example, mitotic loss of the Arg$^+$ HH-containing chromosome would result in a frequency of meiotic unequal SCR comparable to that observed in the *homologue*Δ strain (Table 2) and, thus, score positive in the screen. Cells of the desired genotype were selected by replica plating to a medium lacking tryptophan, arginine, and uracil and containing the appropriate concentration of copper sulfate (240 μm $CuSO_4 \cdot 5H_2O$). These SD-Ura-Arg-Trp + 240 μm $CuSO_4 \cdot 5H_2O$ plates were incubated for 2 days, after which colonies were clearly visible. All incubations were at 30°. A total of 4 individual colonies from each of the 455 candidates displaying an increase in meiotic unequal SCR in the initial screen were rescreened for this phenotype, revealing 67 candidates in which at least 3 out of the 4 colonies exhibited an increase in meiotic unequal SCR comparable to that of a *red1* mutant. For each putative mutant, dyads were dissected to determine the pattern of segregation of the chromosome VIII pair. Cells were incubated in 12% glusulase for 8 min at 25°, followed by 30 min on ice. The frequencies of reductional,

equational, and aberrant segregations in each strain were determined by replica plating the spore colonies from the dissected dyads to medium lacking tryptophan and medium lacking arginine. Genomic DNA flanking the transposon insertion was recovered from each of the 38 candidates displaying a chromosome VIII segregation pattern differing significantly from that of DT71.

Plasmid rescue and DNA analysis: Genomic DNA flanking the *TN3*::*lacz*::*LEU2* insertion was cloned as described (Burns *et al.* 1994) with the following modifications. Yeast strains were transformed with *Bam*HI/*Not*I-digested pMS43 or pMS47, and transformants were selected on YEPD + G418 (200 μg/ml) plates (Wach *et al.* 1994). Integration into the *TN3*::*lacz*::*LEU2* sequences replaces *LEU2* with an ~1-kb *Eco*RI/*Hpa*I fragment of the *LEU2* gene. G418^r transformants were screened for correct integration of the rescue plasmid on medium lacking leucine. Genomic DNA from Leu$^-$ transformants was isolated according to the Rapid DNA Isolation Protocol (Hoffman 1997) with the addition of one phenol and one chloroform-isoamyl alcohol extraction. Genomic DNA from pMS43 transformants was digested individually with *Eco*RI, *Xho*I, and *Sal*I. Genomic DNA from pMS47 transformants was digested individually with *Eco*RI, *Xho*I, *Sal*I, *Bgl*II, and *Nde*I and then ligated. The KanMX4 module confers resistance to 50 μg/ml kanamycin in *Escherichia coli* cells (Wach *et al.* 1994). The ligated DNA was used to transform *E. coli* strain XLII-Blue (Stratagene, La Jolla, CA), and kanamycin-resistant transformants were screened for plasmids bearing a chromosomal insert. A primer complementary to the *lacZ* fragment (NEB sequencing primer catalog no. 1224) was used to sequence the adjacent chromosomal insert. Sequencing was carried out at the Institute of Molecular Biology sequencing facility at the University of Oregon. The locus of transposon insertion was determined by reference to the Stanford *S. cerevisiae* Genome Database (http://genome-www.stanford.edu/Saccharomyces/).

Recombination assays: Yeast strains were grown to saturation in 2-ml cultures of YEPEG. The entire culture was then used to inoculate 100 ml S-raffinose + 5-FOA (0.1%) and was incubated for ~36 hr to select against mitotic SCR recombinants. Cells were pelleted, washed twice with sterile water, and diluted 1:4 in liquid sporulation medium. Aliquots from the liquid sporulation cultures were washed twice in 250 mm EDTA, pH 8.0, followed by two washes with sterile H_2O, and then plated on SD-Ura-Arg-Trp + 240 μm $CuSO_4 \cdot 5H_2O$ and on YEPD medium to determine the mitotic unequal SCR frequency per viable cell. Cultures were aerated for 3 days to induce sporulation, and the meiotic unequal SCR frequency was determined as described above. All incubations were at 30°. At least three independant colonies were assayed for each strain.

Sporulation frequency and spore viability: Sporulation frequency in liquid sporulation cultures was determined microscopically. Spore viability was determined by dissection of dyads from SPO plates that had been incubated for 3–4 days at 30°. At least 100 individual spores were analyzed for each strain.

Linkage analysis of the *msc* mutants: Each of the *msc* mutants was transformed with the *SIR3*-containing plasmid pJR273 and subsequently crossed to DT78. For each cross, the spore colonies from at least 20 four-spore-viable tetrads were analyzed for growth on SD-Arg, SD-Trp, SD-Leu, and SD-Lys media. In all crosses producing live spores, the *LEU2* marker segregated in a 2:2 pattern, indicating that these *msc* mutants were carrying a single-transposon insertion. Linkage of the *msc* phenotype to the transposon insertion was determined by assaying meiotic unequal SCR in at least four Leu$^+$ and four Leu$^-$ segregants of the appropriate genotype from each cross. In the class II mutants, which did not produce viable spores when crossed to DT78, linkage was tentatively assessed by deleting the ORF identified by the transposon insertion in DT71 and assaying meiotic unequal SCR in the resulting mutant.

RESULTS

Isolation of mutants defective in directing meiotic recombination events to homologous chromatids: Yeast mutants defective in directing the repair of meiosis-specific DSBs to homologous chromatids were isolated using a screen based on the strategy developed by Hollingsworth and Byers (1989). They isolated mutants unaffected for intersister and/or intrachromatid recombination, but reduced for recombination between homologues.

We reasoned that, in meiotic recombination-competent mutants, loss of the preference for the homologue would be manifest as an increase in the frequency of meiotic SCR. To specifically detect an increase in meiotic SCR, we designed an SCR construct on the basis of those described in Fasullo and Davis (1987). The mutations we were seeking were expected to result in an elevation of meiotic SCR at the expense of interhomologue exchange. Since mutations that reduce interhomologue exchange alter chromosome disjunction (reviewed in Hawley 1988), our putative mutants exhibiting an increase in meiotic unequal SCR were also screened for an alteration in chromosome disjunction (Figures 1 and 2).

The strain used in the screen, DT71, is a *spo13*Δ *sir3*Δ haploid, disomic for chromosome VIII. The haploidy facilitates isolation of recessive mutations. The *sir3*Δ mutation results in the derepression of the normally silent mating-type loci *HML* and *HMR*, which leads to coexpression of **a** and α (Shore *et al.* 1984), resulting in a haploid strain competent to undergo meiosis. The spore inviability of mutants affecting interhomologue exchange can be rescued by a mutation in the *SPO13* gene. Meiotic recombination occurs at wild-type levels in *spo13* mutants, which then skip one meiotic division and produce dyads containing two viable spores (Klapholz *et al.* 1985). The elimination of one meiotic division serves to bypass the requirement for recombination and/or synapsis to produce viable spores in *spo13* meiosis (Klapholz *et al.* 1985; Rockmill and Roeder 1988; Hollingsworth and Byers 1989; Malone *et al.* 1991). Thus, *spo13* mutations have been exploited in the characterization of mutations that affect these processes. In addition, the single-division meiosis in the *spo13*Δ mutant permits a haploid to sporulate and produce two viable spores (Wagstaff *et al.* 1982).

In *spo13* disomic haploids, a homologous chromosome pair exhibits three types of segregation in the single-division meiosis: reductional (as in MI), equational (as in MII), and aberrant (one spore monosomic and one spore trisomic; Wagstaff *et al.* 1982; Figure 1B). In *spo13* meiosis, the distribution of the three types

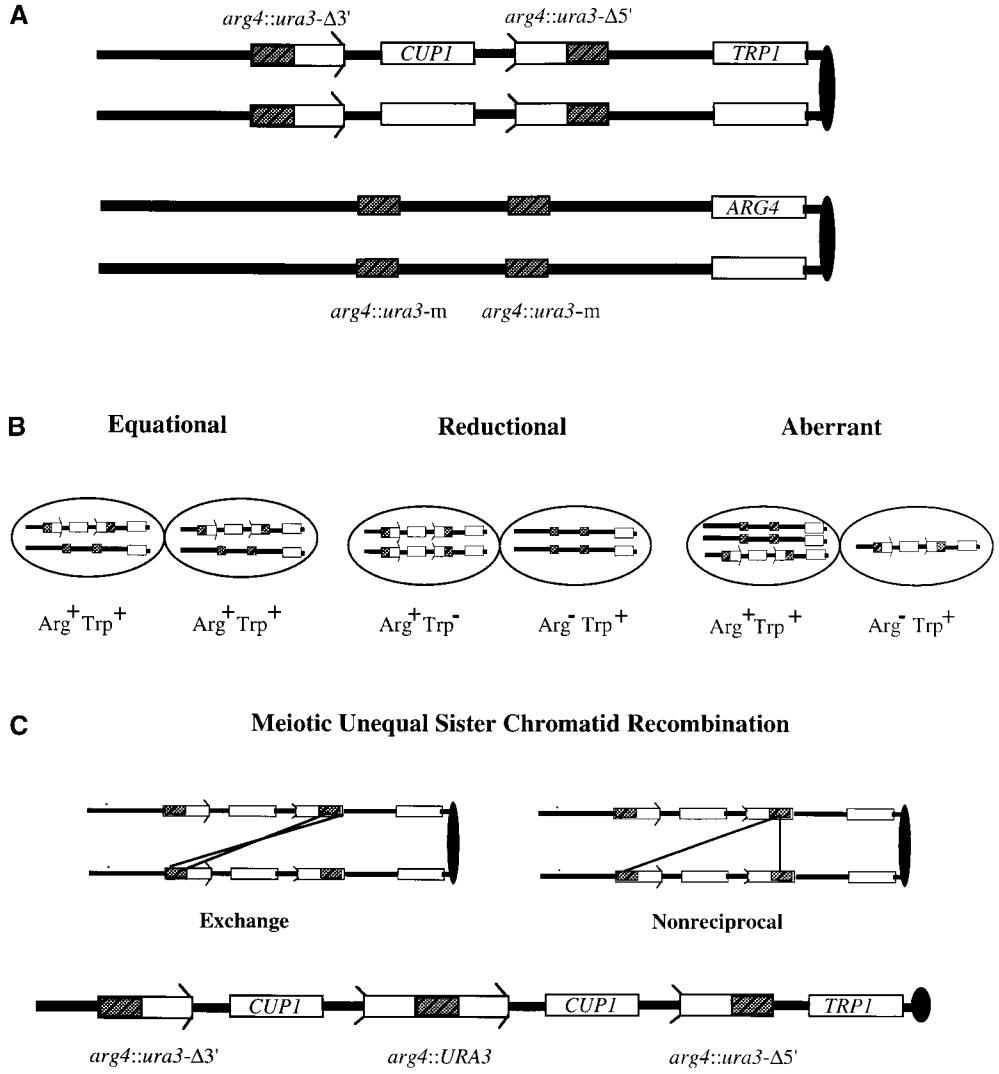

Figure 1.—Experimental design. (A) Marker configuration on each homologue of the chromosome VIII disome in the *sir3 spo13::LYS2* haploid strain, DT71, used to screen for mutants that do not prefer the homologue over the sister chromatid in meiotic recombination. The centromere-linked markers *TRP1* and *ARG4* allow the determination of the chromosome VIII meiotic segregation pattern. The Trp^+ chromosome carries the SCR construct transplaced at the normal *ARG4* locus. The SCR construct consists of a tandem pair of *arg4*::*ura3* gene fragments with 432 bp of overlapping homology (shaded regions) separated by the *CUP1* gene. The *arg4*::*ura3*-Δ*3′*, marked with an arrowhead, is missing sequences 3′ of the shaded homologous region, while the *arg4*::*ura3*-Δ*5′*, marked with feathers, lacks the 5′-segment. The Arg^+ chromosome carries the HH construct, which consists of a tandem pair of 432-bp segments, labeled *arg4*::*ura3-m*, that are homologous to the shaded regions in the SCR construct. (B) The three types of segregation occurring in a *spo13* disomic haploid. (C) Two types of unequal SCR events (exchange and nonreciprocal gap repair) in the homologous segments of the *arg4*::*ura3* segments (shaded areas) will generate an intact *arg4*::*URA3* gene conferring a Ura^+ phenotype. Both unequal SCR events also duplicate the *CUP1* gene. The copper-resistance phenotype conferred by *CUP1* is sensitive to copy number. The products of unequal SCR are selected on medium lacking uracil, tryptophan, and arginine + 240 μm $CuSO_4 \cdot 5H_2O$. Interactions with the homologue cannot produce an intact *arg4*::*URA3* gene.

of segregation appears to depend on the frequency of interhomologue exchange, chromosome pairing, and/or synapsis. Mutations that disrupt any or all of these processes result in a shift in favor of equational segregation (Wagstaff *et al.* 1982; Hollingsworth and Byers 1989; Rockmill and Roeder 1990; Hollingsworth *et al.* 1995). In addition, mutations that reduce interhomologue exchange and/or pairing of homologous chromosomes increase spore viability in haploid disomic strains undergoing *spo13* meiosis (Wagstaff *et al.* 1985; Hollingsworth and Byers 1989; Rockmill and Roeder 1990).

To monitor the segregation of the chromosome VIII pair in DT71, one homologue is marked with a *TRP1* gene integrated just 3′ of the *GPA1* locus. The other homologue is marked with an *ARG4* gene at the equivalent location. The *GPA1* locus is ~2 cM from CEN 8 (Miyajima *et al.* 1987; Fujimura 1989). The frequency of reductional, equational, and aberrant segregations was determined in each strain by dyad dissection. The resulting spore colonies were tested for the centromere-linked *ARG4* and *TRP1* markers. The segregation pattern of the chromosome VIII pair in DT71 is 60.2% equational, 14.4% reductional, and 25.4% aberrant (Table 2).

Meiotic unequal SCR assay: One of the chromosome VIII homologues carries a tandem pair of *arg4*::*ura3* segments separated by the *CUP1* gene (SCR recombination construct, Figure 1). The *arg4::URA3* gene, from which the segments were derived, was created by removing the *ARG4* coding region and replacing it with that of *URA3*. The DNA sequences corresponding to the well-characterized *ARG4* hotspot were retained, but the activity in the construct used has not been tested. Un-

equal exchange or nonreciprocal gap repair (which may or may not be accompanied by exchange) between the *arg4*::*ura3* segments on sister chromatids can generate a functional *arg4*::*URA3* gene and duplicate the intervening *CUP1* gene (Figure 1). The level of copper resistance is sensitive to the copy number of *CUP1* (Hamer *et al.* 1985). Intrachromatid events that generate *arg4*::*URA3* would not duplicate the *CUP1* gene. The unequal SCR recombinant is dominant, eliminating any significant contribution of chromosome segregation pattern in this initial analysis. Southern analysis confirmed that our SCR recombination construct functioned as expected (data not shown). There is homology to the SCR construct on the homologue (HH construct), but interhomologue recombination events cannot generate an intact *arg4*::*URA3* gene (Figure 1). Mutants elevated for meiotic unequal SCR were identified using the pick-and-patch plate assay described in detail in materials and methods (Figure 2).

The frequency of meiotic unequal SCR in each strain was quantified by plating aliquots from liquid sporulation medium onto medium lacking uracil, arginine, and tryptophan and containing 240 μm $CuSO_4 \cdot 5H_2O$. Viable titer was determined by plating on rich (YEPD) medium. Addition of 5-FOA to the pregrowth regimen (see materials and methods) eliminated any significant contribution of mitotically generated Ura^+ cells (data not shown). Sporulation frequency was determined by microscopic examination of liquid sporulation cultures.

In the wild-type haploid disomic strain (DT71), we assume that the majority of the meiotic recombination events occur between homologues, resulting in a characteristic frequency of meiotic unequal SCR, chromosome segregation pattern, and spore viability in *spo13* meiosis (Table 2). In contrast, we expected that a mutant defective in meiotic recombination partner choice would increase meiotic unequal SCR at the expense of interhomologue recombination, resulting in a change in chromosome disjunction in favor of equational segregation and increased spore viability. The *red1*::*ADE2* mutant illustrates the spectrum of phenotypes exhibited by a mutation affecting interhomologue exchange, pairing, synapsis, and meiotic recombination partner choice in *spo13* meiosis (Rockmill and Roeder 1990; Hollingsworth *et al.* 1995; Table 2; see below).

A *red1* mutant is increased for meiotic unequal SCR: The hypothesis that meiotic sister-chromatid cohesion suppresses meiotic sister-chromatid exchanges suggests that disruption of sister-chromatid cohesion will result in an increase in meiotic SCR. The product of the *RED1* gene is required for meiotic sister-chromatid cohesion (Smith and Roeder 1997; Bailis and Roeder 1998). We compared the frequency of meiotic unequal SCR in a *red1*::*ADE2* mutant (Rockmill and Roeder 1990) with that in *RED1* strains. The *red1*::*ADE2* mutant is increased 3.6-fold for meiotic unequal SCR, and the frequency of equational segregation is increased to 95% at the expense of the reductional and aberrant classes. In addition, sporulation frequency ($P < 0.001$) and spore viability ($P < 0.001$) are increased (Table 2). We used the *red1*::*ADE2* mutant as a positive control in the screen for mutants with a comparable elevation in meiotic unequal SCR.

The frequency of meiotic unequal SCR in a monosomic (*homologue*Δ) strain carrying the SCR construct represents the maximum detectable frequency in this system: The frequency of recombination between duplicated *HIS4* sequences is ~10-fold higher in haploid meiosis than it is in the same construct in diploid meiosis (Jackson and Fink 1985; Wagstaff *et al.* 1985). This observation was corroborated in a study that compared intersister and ectopic exchanges in isogenic diploid and haploid strains (Loidl and Nairz 1997). In addition, meiosis-specific DSBs occur at wild-type levels and are processed efficiently in *spo13* haploids (de Massy *et al.* 1994; Gilbertson and Stahl 1994). These results imply that recombination between sister chromatids is suppressed in a diploid. In haploid meiosis, however, chromatids with DSBs are able to use the homology available on the sister chromatid for recombinational repair. Similarly, the frequency of meiotic unequal SCR in the monosomic (*homologue*Δ) haploid strain is increased 5.8-fold compared to that of the disomic haploid strain (DT71, Table 2). We assume that this is the maximum frequency of meiotic unequal SCR we can expect in this system. The difference in the frequency of meiotic unequal SCR in our monosomic strain and the frequency of intersister recombination reported by others in haploid meiosis is likely attributable to differences in the construct used to monitor meiotic SCR.

***msc* mutants define three classes:** DT71 was mutagenized by integrative transformation with a transposon-mutagenized yeast genomic library carrying random *TN3*::*LEU2* insertions (Burns *et al.* 1994). We screened 53,523 colonies for an increase in meiotic unequal SCR (for details see materials and methods). The putative mutants were then screened by dyad dissection for an alteration in the segregation pattern of the chromosome VIII disome. For candidates that satisfied both criteria, DNA flanking the transposon insertion was recovered and sequenced. The locus of transposon insertion was determined by reference to the Yeast Genome Database (see materials and methods).

To ensure that they exhibited phenotypes relevant to meiosis, the *msc* (meiotic sister chromatid recombination) mutants were quantified in *spo13* meiosis with respect to meiotic unequal SCR frequency, disome segregation pattern, sporulation frequency, and spore viability (see above and materials and methods). In addition, outcrossing the mutants to a *SIR3 SPO13* strain revealed a class that conferred a dominant meiotic lethal phenotype peculiar to our strain background (see class II be-

TABLE 2

Phenotypic and molecular characterization of *msc* mutants

Genotype[a]	Meiotic unequal SCR frequency ($\times 10^3$)[b]	Fold increase	Chromosome VIII segregation (%)[c] Equ.	Red.	Aber.	Dyad no.	Sporulation (%)[d]	Spore viability (%)[e]
Wild type	0.3 ± 0.02	1	61	15	24	251	42 ± 0.7	44
red1::*ADE2*	1.1 ± 0.15	3.6	94	1	5	176	55 ± 0.7	75
*homologue*Δ	1.7 ± 0.48	5.7	—	—	—	—	ND	ND
Class I								
red1::*TN+62*	1.2 ± 0.15	4.0	95	2	3	58	41 ± 5	62
red1::*TN+143*	0.72 ± 0.08	2.4	89	0	11	46	42 ± 5	44
red1::*TN+411*	1.1 ± 0.01	3.6	96	1	3	73	49 ± 3	61
red1::*TN+621*	0.9 ± 0.1	3.0	97	1	2	64	53 ± 5	59
red1::*TN+953*	1.2 ± 0.13	4.0	94	1	5	140	54 ± 1	54
red1::*TN+1293*	1.6 ± 0.3	5.3	94	1	5	158	55 ± 3	63
red1::*TN+2075*	1.3 ± 0.20	4.3	90	2	8	224	53 ± 3	50
red1::*TN+2174*	1.3 ± 0.09	4.3	91	2	7	104	42 ± 2	74
mek1::*TN+944*	1.1 ± 0.1	3.6	93	2	5	60	53 ± 5	47
hop1::*LEU2*	0.27 ± 0.02	0.9	90	0	10	52	56 ± 5	67
*rad24*Δ	0.68 ± 0.06	2.3	90	0	10	20	42 ± 3	43
rad24::*TN+342*	1.5 ± 0.15	5.0	89	2	9	97	44 ± 7	41
rad24::*TN+385*	1.0 ± 0.09	3.3	90	1	9	81	42 ± 3	47
rad24::*TN+509*	1.4 ± 0.23	4.7	83	5	12	66	55 ± 2	53
rad24::*TN+585*	1.0 ± 0.11	3.3	87	1	12	77	42 ± 1	41
rad24::*TN+614*	1.0 ± 0.01	3.3	91	1	8	97	41 ± 3	36
rad24::*TN+625*	1.8 ± 0.14	6.0	77	9	14	58	41 ± 1	42
rad24::*TN+801*	1.4 ± 0.16	4.7	89	0	11	63	37 ± 7	39
rad24::*TN+1346*	1.1 ± 0.06	3.6	93	0	7	62	47 ± 1	53
mec3::*TN+1152*	0.84 ± 0.05	2.8	91	1	8	76	31 ± 1	44
yor354c::*TN+1608* (*MSC6*)	1.4 ± 0.23	4.7	78	3	20	31	30 ± 1	35
609[f]	0.86 ± 0.05	2.9	95	2	3	38	44 ± 0.5	47
133[f]	1.4 ± 0.19	4.7	87	3	10	105	45 ± 1	54
85[f]	1.3 ± 0.07	4.3	82	8	10	39	32 ± 5	37
*rad17*Δ	0.66 ± 0.03	2.2	91	3	6	32	27 ± 1	40
mec1-1	0.20 ± 0.04	0.7	—	—	—	—	25 ± 9	<0.1
*rad24*Δ *red1*::*ADE2*	0.59 ± 0.02	2.0	98	0	2	61	34 ± 1	57
*spo11*Δ	0.001 ± 0.0009	—				ND	55 ± 7	ND
*spo11*Δ *red1*::*ADE2*	0.002 ± 0.0004	—				ND	52 ± 2	ND
*spo11*Δ *rad24*Δ	0.001 ± 0.0006	—				ND	49 ± 3	ND
Class II								
*dmc1*Δ	0.86 ± 0.06	2.9	94	6	0	17	44 ± 3	27
*dmc1*Δ *red1*::*ADE2*	1.72 ± 0.01	5.7	91	0	9	32	50 ± 3	61
inp52::*TN+277*	0.87 ± 0.05	2.9	100	0	0	46	45 ± 3	48
yml128cTN+1531 (*MSC1*)	0.86 ± 0.09	2.9	99	0	1	81	61 ± 1	50
ela1::*TN>216* (*MSC4*)	1.0 ± 0.1	3.3	89	1	10	153	41 ± 1	46
ylr219w::*TN+751* (*MSC3*)	0.83 ± 0.07	2.8	100	0	0	37	34 ± 2	35
bud3::*TN+2396*[g]	1.2 ± 0.13	4.0	95	0	5	94	69 ± 5	55
pet122::*TN+593*[g]	1.8 ± 0.15	6.0	76	2	22	64	12 ± 1	48

(*continued*)

TABLE 2

(Continued)

Genotype[a]	Meiotic unequal SCR frequency ($\times 10^3$)[b]	Fold increase	Chromosome VIII segregation (%)[c] Equ.	Red.	Aber.	Dyad no.	Sporulation (%)[d]	Spore viability (%)[e]
ubr1::*TN+330*[h]	0.82 ± 0.06	2.7	97	1	2	75	51 ± 2	43
*ubr1*Δ	0.18 ± 0.01	0.6				ND	59 ± 2	ND
ydr205w::*TN+1255* (*MSC2*)[h]	1.2 ± 0.1	4.0	100	0	0	65	52 ± 1	40
360.41[h]	1.3 ± 0.09	4.3	99	0	1	78	43 ± 5	49
471[h]	1.5 ± 0.09	5.0	96	0	4	108	34 ± 3	34 ± 2
227[g]	1.4 ± 0.12	4.7	95	0	5	65	36 ± 3	54
Class III								
yhr039c::*TN+1583* (*MSC7*)[g]	0.65 ± 0.10	2.2	75	16	9	43	36 ± 2	42
mnr2::*TN+1766*[g]	1.0 ± 0.12	3.3	65	15	20	55	40 ± 3	32
625[f]	1.5 ± 0.19	5.0	57	23	20	109	40 ± 2	50
455[f]	0.91 ± 0.13	3.0	52	27	21	52	42 ± 3	32
116-42[f]	0.53 ± 0.03	1.8	46	21	33	43	35 ± 5	33
1589[g]	1.1 ± 0.08	3.6	62	23	15	86	42 ± 2	38

—, The experiment is not applicable to a particular genotype. ND, not determined.

[a] The symbol TN+ designates the transposon insertion position relative to the translational start site ATG +1 for each mutant allele, and TN> denotes an insertion position upstream of the ATG +1 site of the designated gene. The locus designation listed in the *S. cerevisiae* genome database is denoted for all previously uncharacterized (*MSC*) genes. The ± symbol denotes standard error.

[b] To determine the frequency of meiotic unequal SCR, yeast strains were grown to saturation in 2-ml cultures of YEPEG. The entire culture was then used to inoculate 100 ml S-raffinose + 5-FOA (0.1%) and incubated for ~36 hr to select against mitotic SCR recombinants. Cells were pelleted, washed twice with sterile water, and diluted 1:4 in liquid sporulation medium. Aliquots were washed twice in 250 mm EDTA, pH 8.0, followed by two washes in sterile H_2O, and then plated on SD-Ura-Arg-Trp + 240 μm $CuSO_4 \cdot 5H_2O$ and YEPD medium to determine mitotic unequal SCR frequency per viable cell. Cultures were aerated for 3 days to induce sporulation, and meiotic unequal SCR frequency was determined as described above. All incubations were at 30°. At least three independent colonies were assayed for each strain. As a result of 5-FOA counterselection, the frequency of mitotic unequal SCR was negligible in all experiments (data not shown).

[c] Segregation of the *ARG4* and *TRP1* markers, integrated on each homologue, ~2 cM from *CEN* 8, was used to determine the frequency of reductional, equational, or aberrant segregations in each strain. Reductional segregation resulted in a dyad containing one Arg^+ Trp^- and one Arg^- Trp^+ spore. In an equational segregation, both spores are Arg^+ Trp^+. Aberrant segregations give rise to dyads containing either one Arg^+ Trp^- and one Arg^+ Trp^+ or one Arg^- Trp^+ and one Arg^+ Trp^+ spore (Figure 1). Only dyads with two viable spores were included in these data. The frequency of equational segregation was significantly different from DT71 (WT, $P < 0.01$ to < 0.001) in all class I and II mutant strains, with the exception of the *dmc1*Δ ($P < 0.05$), *rad24*Δ, and *yor354c*::*TN+1608* (*MSC6*) strains. At their current size, the data sets for *rad24*Δ and *yor354c*::*TN+1608* are not significantly different from DT71 ($P > 0.08$). In addition, the frequency of reductional segregation in the current data sets for the class III mutant strains is not significantly different from that in DT71 ($P > 0.08$). The paucity of two-spore viable dyads produced impedes the analysis (see results).

[d] Sporulation percentage was calculated from a minimum of 200 individual cells plus asci.

[e] Spore viability was calculated from a minimum of 120 individual spores.

[f] The mutant phenotype not linked to transposon insertion.

[g] Linkage of the mutant phenotype to the transposon insertion not yet confirmed.

[h] Deletion of the ORF did not result in *msc* phenotype.

low). Analysis of the *msc* mutants according to these criteria defines three classes (Tables 2 and 3).

Class I: Mutants in class I are increased in meiotic unequal SCR, and they are increased in equational segregation at the expense of the reductional and aberrant classes (Tables 2 and 3). A total of 23 mutants fall into this class. These mutations identify alleles of *RED1*, *MEK1*, *RAD24*, *MEC3*, and *MSC6*. In the remainder, the phenotype was unlinked to the locus of transposon insertion; these mutants are denoted in Table 2 by strain number (see footnote *f*) and were not pursued further. Our observation that mutations in several of the meiotic recombination checkpoint genes result in an elevation of meiotic unequal SCR suggests that these genes encode functions required for proper meiotic recombination partner choice.

The nine *red1*::*TN* alleles represent eight different insertion positions spanning the open reading frame.

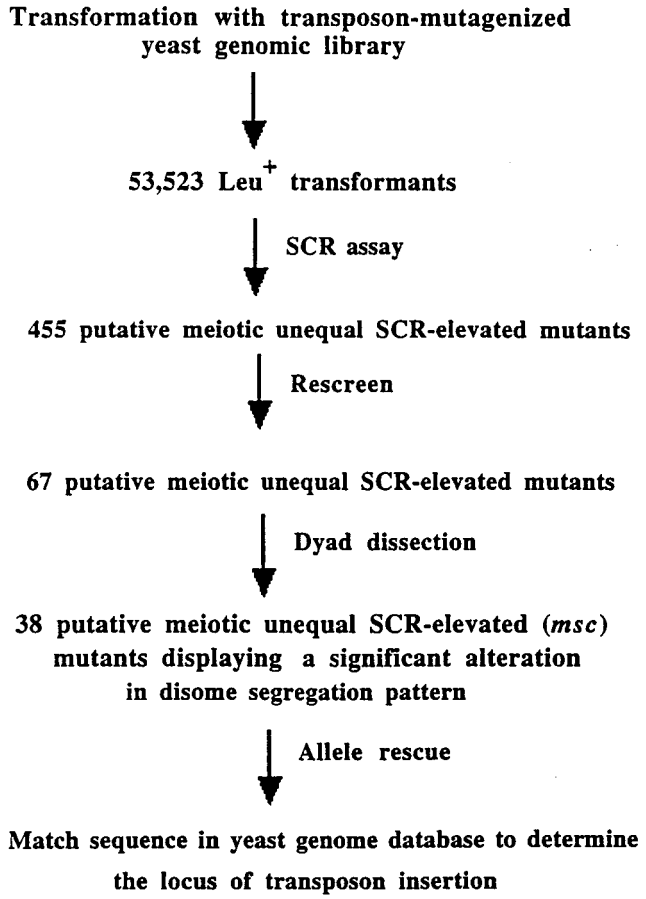

Figure 2.—Strategy and summary of the *msc* mutant screen.

Most of the *red1*::*TN* mutants exhibit increases in sporulation frequency ($P < 0.001$) and spore viability ($P < 0.001$) in *spo13* meiosis (Table 2). The nine *rad24*::*TN* alleles represent individual insertions spanning the open reading frame. All the *rad24*::*TN* insertion alleles have a frequency of meiotic unequal SCR that is higher than the twofold increase observed in the *rad24*Δ mutant. Most notably, the insertion at position +625 exhibits a sixfold increase (Table 2).

Single insertions in *MEK1* and *MEC3* were identified, and linkage of the *msc* phenotype to the transposon insertion was confirmed. In the *mek1*::*TN+944* mutant, the sporulation frequency is not significantly different from that observed in the majority of *red1*::*TN* alleles, but the spore viability approximated that of DT71 (Table 2).

The *HOP1* gene is in the same epistasis group as *RED1* and *MEK1* (Rockmill and Roeder 1990, 1991). In a reconstruction experiment, a *hop1* mutant was not elevated for meiotic unequal SCR, but it did favor equational chromosome segregation and significantly increased sporulation and spore viability (Table 2).

No alleles of *RAD17* or *MEC1*, both of which participate in the mitotic DNA damage and meiotic recombination checkpoints, were identified. In a reconstruction experiment, the phenotype of a *rad17*Δ mutant was not significantly different from that of the *rad24*Δ mutant (Table 2). Failure to identify any new alleles of *RAD17* may imply that the screen was not performed to saturation. *MEC1/ESR1* is an essential gene, and the *mec1-1* allele is the only viable mutant isolated to date (Kato and Ogawa 1994; Weinert *et al.* 1994). It was subsequently shown that the viability of *mec1-1* mutants required an additional mutation in the *SML1* gene which results in an increase in dNTP pools (Zhao *et al.* 1998). We were able to introduce the *mec1-1* mutation into our strain, assesed by sensitivity to the DNA-damaging agent methyl methanesulfonate (MMS), suggesting that our strain also carries a mutation in *SML1*. A *mec1-1* mutant is not elevated in the meiotic unequal SCR in our system. However, the spore inviability conferred by this mutation was not rescued by the *spo13* mutation (Table 2). Thus, in *mec1-1* mutants, any increase in meiotic unequal SCR may have been obscured by spore inviability.

The observed sporulation frequencies and spore viabilities in *rad17*Δ, *mec3*::*TN+1152*, and most alleles of *rad24*::*TN* were similar to those of the DT71 strain. The *rad24* ::*TN+509* allele is exceptional and resembled a *red1* mutant in these respects (Table 2).

The strain carrying the *yor354c*::TN+*1608* (*mcs6*) mutation was not MMS sensitive (data not shown), suggesting that this gene does not function in the DNA damage checkpoint. Expression of the *MSC6* gene was not induced in the large-scale study of the transcriptional program of sporulation described in Chu *et al.* (1998).

The product of the *SPO11* gene catalyzes meiosis-specific DSBs (Bergerat *et al.* 1997; Keeney *et al.* 1997). The lack of meiotic induction of SCR events in both *spo11 red1*::*ADE2* and *spo11 rad24*Δ double mutants (Table 2) indicates that the meiotic unequal SCR events in the single *red1* and *rad24* mutants are initiated by meiosis-specific DSBs.

***red1 rad24* epistasis:** A *red1*::*ADE2 rad24*Δ double mutant has a meiotic unequal SCR indistinguishable from that of the *rad24*Δ mutant, a reduction in sporulation frequency compared to the *rad24*Δ mutant ($P < 0.01$), and a spore viability that is intermediate to the viabilities of the component single mutants (Table 2). The observation that meiotic unequal SCR in the *red1*::*ADE2 rad24*Δ double mutant is not significantly different from that in the *rad24*Δ single mutant indicates that *RAD24* is required for the elevated levels of meiotic unequal SCR in *red1* mutants.

Class II: Mutants in class II are increased in meiotic unequal SCR, increased in equational segregation at the expense of the reductional and aberrant classes (Tables 2 and 3), and confer a dominant meiotic lethal phenotype when crossed to a congenic *SIR3 SPO13* strain monosomic for chromosome VIII. The spore viability, assessed by tetrad dissection, in each of the corresponding diploids was ≤1% (data not shown). A total of 11 mutants fall into this class. In two of the mutants,

the phenotype was unlinked to the locus of transposon insertion; these mutants are denoted in Table 2 by strain number (see footnote *f*) and were not pursued further. Single-transposon insertions were identified in *INP52*, *UBR1*, *BUD3*, *PET122*, and *MSC1-MSC3*. In one mutant, the transposon insertion position was 246 bp upstream of *ELA1*, which encodes a yeast elongin A homologue (C. Koth, personal communication).

Linkage of the class II phenotype to the transposon insertions in *INP52*, *MSC1*, and *MSC3* was confirmed by transplacement of deletion derivatives of these three genes, respectively, into DT71 and assaying meiotic unequal SCR (see materials and methods). *INP52* encodes an inositol polyphosphate 5-phosphatase that is similar to synaptojannin proteins, which regulate Ca^{2+} levels during neurotransmission (Stolz *et al.* 1997). *MSC1-MSC3* were sequenced as part of the Yeast Genome Project (http://genome-www.stanford.edu/Saccharomyces/) and code for YML128c, YDR205w, and YDR219w, respectively. The gene products presumed to be encoded by *MSC1* and *MSC3* are not homologous with any proteins in the database. The gene product of *MSC2* is a predicted transmembrane protein with homology to *S. cerevisiae* Cotp, which functions in cobalt ion transport (Conklin *et al.* 1992), and to a cation efflux protein in *Alcaligenes eutrophus* (Nies *et al.* 1989). In a reconstruction experiment, a strain carrying a *msc2*Δ mutation was not elevated for meiotic unequal SCR (data not shown), suggesting either that the transposon insertion is not linked to the *msc* phenotype in this mutant or that this phenotype is specific to the *ydr205w*::*TN+1255* allele.

An allele of *UBR1* was isolated in the screen. *UBR1* encodes the E3 ubiquitin protein ligase, which associates with the ubiquitin-conjugating enzyme encoded by *RAD6* to carry out N-end rule ubiquitin degradation (Bartel *et al.* 1990). *UBR1* is also required for peptide transport into cells (Alagramam *et al.* 1995). The observation that a *ubr1*Δ mutant is not elevated for meiotic unequal SCR suggested either that the transposon insertion is not linked to the *msc* phenotype in this mutant or that this phenotype is specific to the *ubr1*::*TN+330* allele. It was observed that overexpressing peptides with the N-end rule sequence, recognized by Ubr1p (Bartel *et al.* 1990), results in a meiotic delay beginning with the appearance of meiotic recombinants in *UBR1* strains. However, no delay is observed in *ubr1*Δ strains, which proceed through meiosis faster than *UBR1* strains (L. Bulté, K. Madura and A. Varshavsky, personal communication). This suggests that partial function of Ubr1p results in the delay in recombinant formation. It may be that, analogously to the case presented above, partial function of Ubr1p is responsible for the *msc* phenotype of the mutant carrying the *ubr1*::*TN+330* allele.

The *msc* phenotype of the transposon insertion upstream of *ELA1* was complemented by tranformation with a plasmid bearing a wild-type allele of *ELA1*, indicating that the insertion disrupts expression of *ELA1*. Experiments to determine linkage of the *msc* phenotype to the transposon insertions in *PET122* and *BUD3* are in progress. *PET122* encodes a translational activator of cytochrome c oxidase subunit III (Kloeckener-Gruissem *et al.* 1988). The *pet122*::*TN+1593* mutant is able to grow on medium that selects against *petite* mutants, although it does so more slowly than does DT71. *BUD3* encodes a protein required for the axial budding pattern in haploid strains (Chant *et al.* 1995).

A *dmc1*Δ mutant has a class II phenotype: No alleles of *DMC1* were identified in the screen. A *dmc1*Δ mutant was shown previously to be elevated for intrachromosomal exchange (Bishop *et al.* 1992). In a reconstruction experiment, a *dmc1*Δ mutant was shown to have a class II *msc* phenotype (Table 2). This suggests that *DMC1* plays a role in directing events to homologous chromatids.

The dominant meiotic lethality of these mutants, when heterozygous, was unexpected, since the *dmc1*Δ allele used was shown previously to be recessive for completion of meiosis (Bishop *et al.* 1992). In addition, a dominant meiotic lethal phenotype has not been reported for mutations in any of the other previously identifed genes in this class (Kloeckener-Gruissem *et al.* 1988; Bartel *et al.* 1990; Chant *et al.* 1995; Stolz *et al.* 1997; C. Koth, personal communication). Thus, this phenotype apears to be peculiar to our strain background (see discussion). Since the meiotic phenotypes of the mutants in class II resemble those of *dmc1*Δ, we suggest that the genes identified by these mutations function in or are regulators of the *DMC1*-promoted interhomologue exchange pathway.

***RED1* and *DMC1* act independently in partner choice:** A *red1*::*ADE2 dmc1*Δ double mutant exhibits an additive increase in meiotic unequal SCR, the sporulation frequency approximates that in the single *red1*::*ADE2* mutant, and the spore viability is intermediate to those of the single mutants (Table 2). The frequency of meiotic unequal SCR in the *red1*::*ADE2 dmc1*Δ double mutant is identical to that in the *homologue*Δ strain, suggesting that meiotic recombination events in this background occur predominantly between sister chromatids. This result corroborates the observation of Schwacha and Kleckner (1997) that *red1 dmc1* mutants produce only intersister recombination intermediates in meiosis. The additive increase in meiotic unequal SCR in the double mutant suggests that *RED1* and *DMC1* act independently to bias the repair of meiosis-specific DSBs to homologues.

Class III: Mutants in class III are increased in meiotic unequal SCR. In contrast to the mutants in classes I and II, they have increased reductional segregation and generally have a spore viability lower than that of DT71

($P < 0.05$ to < 0.01, Tables 2 and 3). In addition, the mutants in this class exhibit mitotic marker loss, which is likely to be caused by mitotic chromosome loss. There are six mutants in this class; the location of the transposon insertion has been determined in five of them. In three of these, the phenotype was unlinked to the locus of transposon insertion; these mutants are denoted in Table 2 by strain number (see footnote *f*) and were not pursued further. Experiments to determine linkage of the *msc* phenotype to the transposon insertions in *MNR2* and a previously uncharacterized gene, *MSC7*, are in progress. The protein encoded by *MNR2* has 52% identity to the *S. cerevisiae* aluminum-resistant protein Alr2p over 98 amino acids (Dujon *et al.* 1994). Overexpression of *MNR2* overcomes manganese toxicity. *MSC7* was sequenced as part of the Yeast Genome Project and codes for YHR039c, which has some similarity to aldehyde dehydrogenases.

The mutants in this class have the phenotype expected for a meiotic *hyper-rec* mutation in *spo13* meiosis. An elevation in meiotic unequal SCR is expected in a mutant with a meiotic *hyper-rec* phenotype, since both intersister and interhomologue events exhibit meiotic induction. In addition, a meiotic *hyper-rec* mutant, in which more interhomologue connections would occur, is expected to display an increase in reductional segregation and a decrease in spore viability. This expectation is supported by the phenotype of recombinationless *spo11* mutants in *spo13* meiosis, which is an increase in equational segregation and a concomitant increase in spore viability (Wagstaff *et al.* 1982). The observed increase in the frequency of reductional segregation in the *hyper-rec* strains compared to that in DT71 in not statistically signficant ($P > 0.08$), but is likely to be an underestimate because of the spore inviability correlated with interhomologue exchange. In support of this possibility is the observation that spore death in *spo13* disomic haploid meiosis is nonrandom (Wagstaff *et al.* 1982). Dyads in which neither or both spores survive occur more frequently than predicted by random spore death, indicating that the majority of spore inviability results from events that are lethal to both spore products in a given meiosis. Class III mutants display an excess of dyads with two inviable spores compared to DT71 (data not shown), supporting the proposal that these mutants are increased for interhomologue exchange.

Meiotic unequal SCR in diploid *spo13* strains: To confirm that the increase in meiotic unequal SCR in the *msc* mutants is not specific to disomic haploids, the frequency of meiotic unequal SCR was determined in wild-type, *red1*, *mek1*, *rad24*, and *mec3* derivatives of a *spo13* diploid strain isogenic to DT71. Meiotic unequal SCR was elevated in all mutants compared to the wild type, indicating that the increase in meiotic unequal SCR in the mutant backgrounds is not specific to haploid meiosis (Table 4).

DISCUSSION

Using a strain designed specifically to detect mutants exhibiting an increase in meiotic unequal SCR, we conducted a screen for components of the machinery that directs meiotic exchange events to homologous chromatids. This approach has identified 38 *msc* mutants comprising three phenotypic classes. Class I mutants identified genes known and likely to be required for the meiotic recombination checkpoint, class II mutants have a phenotype similar to a *dmc1*Δ mutant, and class III mutants are putative meiotic *hyper-rec* mutants.

Class I: Genes involved in the meiotic recombination checkpoint also play a role in meiotic recombination partner choice. The meiosis-specific genes *RED1* and *MEK1* and the DNA damage checkpoint genes *RAD24*, *RAD17*, and *MEC1* are required for a checkpoint control that monitors meiotic recombination (Lydall *et al.* 1996; Xu *et al.* 1997). Mutations in these genes and *MEC3*, which is also required for the DNA damage checkpoint, exhibit a similar spectrum of phenotypes in the genetic system used in this work (Table 2, class I). A key finding of this work is that meiotic unequal SCR is elevated by mutations in genes known and likely to be required for *dmc1*-induced meiotic prophase arrest. Possible roles for these genes in meiotic recombination partner choice and in the meiotic recombination checkpoint are discussed below.

Meiosis-specific genes: The products of the *RED1*, *MEK1*, and *HOP1* genes interact to promote SC assembly, which is essential for wild-type levels of meiotic recombination. *RED1* and *MEK1* are required for meiotic sister-chromatid cohesion (Bailis and Roeder 1998), and *RED1* and *HOP1* are required for wild-type levels of homologue pairing (Nag *et al.*1995). Our observation that meiotic unequal SCR is elevated in *red1* and *mek1* mutants is compatible with the hypothesis that these genes encode functions required for proper partner choice. In accordance with these observations, alleles of *RED1* and *MEK1* were isolated in a screen for meiotic mutants that were competent for ectopic recombination (Engebrecht *et al.* 1998). In addition, a *hop1* mutant reduces the frequency of DSBs to 10% of the wild-type level, and the breaks that do occur are processed exclusively into intersister recombination intermediates (Schwacha and Kleckner 1994), indicating a defect in partner choice. This is supported by our observation that the frequency of meiotic unequal SCR in a *hop1* mutant was only slightly less than that in wild type, which indicates, assuming that DSBs are similarly reduced in our strain, that the majority of meiotic recombination events that occur are between sisters.

Since the *RED1*/*MEK1*/*HOP1* epistasis group participates in meiotic sister-chromatid cohesion, homologue pairing, and synapsis, it is possible that one or all of these functions mediate proper meiotic recombination partner choice. *red1* and *mek1* exhibit similar phenotypes

TABLE 3

General phenotypic characteristics of the *msc* mutant classes

	Meiotic Unequal SCR	Disome segregation			General description
		Equational	Reductional	Aberrant	
Class I					
RED1	2- to 6-fold increase Avg. 3.8-fold	Increased	Reduced	Reduced	Meiotic recombination checkpoint genes
MEK1					
RAD24					
RAD17					
MEC3					
MSC6					
Class II					
DMC1	2.7- to 6-fold increase Avg. 3.8-fold	Increased	Reduced	Reduced	Dominant meiotic lethal
ELA1					
INP52					
MSC1					
MSC3					
Linkage not yet confirmed[a]					
BUD3					
PET122					
Null mutant not elevated[b]					
UBR1					
MSC2					
Class III	1.8- to 5-fold increase Avg. 3.1-fold	Slightly reduced	Increased	Slightly reduced	Meiotic *hyper-rec*
MNR2					
MSC7					

[a] Linkage of the *msc* phenotype to the transposon insertion at these loci has not yet been confirmed.
[b] Reconstruction experiments revealed that the null mutant did not exhibit the *msc* phenotype.

with respect to meiotic recombination, sister-chromatid cohesion, and checkpoint function (Rockmill and Roeder 1990, 1991; Xu *et al.* 1997; Bailis and Roeder 1998). However, *red1* mutants have a greater defect in pairing of homologous chromosomes (30% of the wild-type level) than do *mek1* mutants (90% of wild type, Nag *et al.* 1995). SC formation does not occur in *red1* mutants (Rockmill and Roeder 1990), whereas *mek1* mutants form SC, but the stretches appear shorter than normal (Rockmill and Roeder 1991). Our observation that the increase in meiotic unequal SCR in the *mek1* mutant is comparable to that in the *red1* mutant suggests that chromosome pairing and synapsis is not sufficient to promote proper recombination partner choice, indicating that meiotic sister-chromatid cohesion is likely to make the most significant contribution to the restriction of sister-chromatid and ectopic exchanges in meiosis. However, it cannot be ruled out that the SC formed in *mek1* mutants is structurally abnormal, leading to a defect in partner choice that is unrelated to the sister-chromatid cohesion defect.

A *hop1* mutant is defective in partner choice, but is only slightly defective for meiotic sister-chromatid cohesion. This suggests that meiotic sister-chromatid cohesion is necessary but not sufficient to promote partner choice. This is supported by the observation that Red1p but not Hop1p localizes to the nucleolus (Smith and Roeder 1997), where meiotic interhomologue recombination is normally suppressed. However, interhomologue recombination occurs in the rDNA locus in a *pch2* mutant in which Hop1p is mislocalized to the nucleolus (San-Segundo and Roeder 1999). A *hop1* mutant forms axial elements that do not synapse, suggesting that, in addition to meiotic sister-chromatid cohesion, full synapsis is required for partner choice. However, in a *zip1* mutant, which similarly lacks only the central element, the total frequency of interhomologue events is not reduced (Sym and Roeder 1994, 1995). The observation that the *zip1* mutant is not defective in partner choice suggests that some function of Hop1p, other than promotion of synapsis, mediates partner choice.

How does meiotic sister-chromatid cohesion act to constrain meiotic intersister/ectopic recombination? The results of several genetic studies raised the possibility that meiotic sister-chromatid cohesion suppresses only intersister and ectopic exchange events and has no effect on nonreciprocal events. For example, these studies have shown that the frequency of intersister plus intrachromatid gene conversion (nonreciprocal events) does not differ significantly from the observed fre-

TABLE 4

Meiotic unequal SCR in *spo13* diploids

Genotype[a]	Meiotic unequal SCR frequency ($\times 10^3$)	Fold increase	Sporulation (%)[a]
Wild type	0.15 ± 0.02	1	40 ± 6
red1::TN+621	1.59 ± 0.07	10.6	38 ± 9
mek1::TN+944	0.66 ± 0.05	4.4	48 ± 7
rad24::TN+801	1.0 ± 0.02	6.6	52 ± 9
mec3::TN+1152	0.94 ± 0.02	6.2	34 ± 7

[a] Experiments carried out as described in Table 2.

quency of interhomologue gene conversion. However, in contrast to interhomologue conversions, which are frequently associated with exchanges, these intersister and intrachromatid conversions were only rarely associated with exchange (reviewed in Petes and Pukkila 1995). This led to the proposal that exchange between sister chromatids occurs only rarely because of a constraint on resolution of the recombination intermediate imposed by the axial elements and/or mature SC (Petes and Pukkila 1995). However, in the absence of exchange, it is not possible to determine what proportion of the intrachromosomal (intersister plus intrachromatid events) nonreciprocal events represent intersister and intrachromatid events, respectively. Intrachromatid events may not be subject to meiotic constraints (see below). Thus, it remains to be determined if only intersister exchanges are suppressed or if both intersister exchange and nonreciprocal events are suppressed in wild-type diploid strains.

In light of these previous observations, we propose explanations for how a defect in meiotic sister chromatid cohesion increases meiotic unequal SCR in our system: (1) Meiosis-specific DSBs are repaired with a bias toward the sister chromatid such that both exchanges and nonreciprocal events are increased; (2) the actual number of intersister recombination events does not change, but the number of intersister recombination intermediates resolved as exchanges is increased; and (3) a combination of both scenarios mentioned above contributes to the observed increase in SCR. Since we do not know what proportion of our SCR events are exchanges, we cannot at present distinguish among these possibilities.

Thus, we suggest that meiotic sister-chromatid cohesion mediated by axial elements acts in two ways to reduce meiotic sister-chromatid and ectopic exchange events: (1) The axial/lateral elements render the sister chromatids less accessible than homologous chromatids for repair of meiosis-specifc DSBs and (2) the structure of the SC constrains the geometry of the intersister and ectopic recombination intermediates to a configuration that favors nonexchange resolution.

A different proposal put forth by Schwacha and Kleckner (1997) is that *RED1* is required to promote the differentiation of a meiosis-specific DSB down an interhomologue-specific recombination pathway. In a *red1* mutant in which meiosis-specific DSBs are reduced to 25% of the wild-type level, they observed a specific reduction in the level of interhomologue joint molecules, while the level of intersister joint molecules remained at the wild-type level. This predicts that the level of intersister recombinant products in a *red1* mutant would be equivalent to that observed in the wild-type strain, in contradiction to the results presented in this work.

An explanation for the apparent differences in SCR in our work and that of Schwacha and Kleckner (1997) might be found among the following possibilities:

1. The total frequency of intersister events is unchanged, but the frequency of intersister events resolved as exchanges is increased in *red1* mutants. This relies on the assumption that the majority of the meiotic unequal SCR events we detect arise by exchange.
2. The meiotic unequal SCR events observed in our genetic system do not arise via a joint molecule intermediate. In support of this possibility, those authors report that no joint molecules of either type were observed in a *dmc1* mutant, even though recombinant products occurred at 10% of the wild-type level. This level is consistent with previous estimates of meiotic recombination in a *dmc1* mutant, which were assessed by genetic methods (Bishop *et al.* 1992; Rockmill *et al.* 1995; Shinohara *et al.* 1997).
3. In a *red1* mutant, some proportion of the DSBs are processed into intermediates that are rapidly resolved into intersister products, escaping detection in the physical assay of Schwacha and Kleckner (1997).
4. The presence or absence of *SPO13* function may affect the frequency of intersister events. In *SPO13* haploid strains competent to undergo meiosis, meiosis-specific DSBs occur at near wild-type levels, but the appearance and processing of DSBs is significantly delayed. On the basis of this observation, de Massy

et al. (1994) suggested that completion of intersister recombinants in haploid meiosis is inefficient. However, intrachromosomal recombination assessed genetically is induced to meiotic levels (Wagstaff *et al.* 1985; Loidl and Nairz 1997), and DSB processing is not delayed in *spo13* haploids (de Massy *et al.* 1994; Gilbertson and Stahl 1994). The level of intersister recombinant products in *SPO13* haploid meiosis is yet to be determined. If intersister products in *SPO13* haploid meiosis are significantly reduced compared to those in a *spo13* haploid, this would indicate a role for *SPO13* in the suppression of intersister recombination. This could account for the observed increase in meiotic unequal SCR in our *red1 spo13* strain as compared to no increase in intersister recombination intermediates in the *red1 SPO13* strain of Schwacha and Kleckner (1997). However, the frequency of ectopic recombination was found to be increased in a *red1 SPO13* diploid (M. Shinohara and D. K. Bishop, personal communication), indicating that *SPO13* cannot be solely responsible for proper partner choice.

5. Finally, it is possible that only unequal sister-chromatid events are elevated in *red1* mutants in our system, whereas equal sister-chromatid events, representing the majority of intersister events, are not. The lack of meiotic sister-chromatid cohesion may facilitate the unequal pairing of duplicated sequences, but it has no effect on the equal pairing of unique sequences along the sister-chromatid pairs.

Intrachromatid *vs.* intersister events in meiosis: Our observation that meiotic unequal SCR is elevated in a *red1* mutant is in contrast to the failure of Rockmill and Roeder (1990) to see a significant difference in the frequency of meiotic intrachromosomal recombination in *red1*::*ADE2* and wild-type derivatives of the Kar2-4C strain. In Kar2-4C, the intrachromosomal recombination assay selects for loss of the intervening sequence that separates direct repeats (Hollingsworth and Byers 1989). These events can arise by exchanges occurring between sisters or events occuring within a single chromatid, which can occur by exchange or by a *RAD52*-independent, single-strand annealing mechanism (reviewed in Petes and Pukkila 1995). The SCR construct in our system is specific for events between sisters that are dependent on *RAD52* function (D. Thompson, unpublished results). We suggest that the majority of intrachromosomal events observed by Rockmill and Roeder (1990) were intrachromatid events and that *RED1* functions to constrain intersister recombination, but not intrachromatid recombination.

Checkpoint function of *RED1* and *MEK1*: Mutations in *RED1* and *MEK1* may alleviate *dmc1*-induced arrest by either eliminating the event that is monitored or by inactivating a component of the monitoring apparatus. For example, a *spo11* mutation eliminates meiosis-specific DSBs, which alleviates *dmc1*-induced arrest. Mutations in *RED1* and *MEK1* may also bypass the meiotic recombination checkpoint by eliminating meiotic constraints imposed by the SC, thereby rendering recombination unmonitorable by the checkpoint system (Xu *et al.* 1997; Grushcow *et al.* 1999). For example, an unrepaired DSB might be constrained by the proteins of the axial/lateral elements to a specific configuration recognized by the checkpoint control (Xu *et al.* 1997), or in the absence of axial/lateral elements, the checkpoint may be bypassed by the observed *RAD51*-dependent repair of meiosis-specific DSBs by intersister recombination (Shwacha and Kleckner 1997). However, whether the meiotic recombination checkpoint genes act as signal transducers independently of their role in promoting normal meiotic recombination has yet to be resolved.

DNA damage checkpoint genes: We observed that meiotic unequal SCR is elevated in *rad24*, *rad17*, and *mec3* mutants. In addition, ectopic recombination events in meiosis are increased in *rad24*Δ, *rad17*Δ, and *mec1-1* mutants, and interhomologue recombination was reduced approximately twofold at the same locus in these mutants (Grushcow *et al.* 1999). Taken together, these results are consistent with the hypothesis that the DNA damage checkpoint genes function to direct meiotic recombination events to allelic sites on homologous chromatids. The spore viability in *mec1-1*, *rad17*, *mec3*, and *rad24* homozygous diploids is reduced compared to that of the wild type in a pattern that is consistent with a defect in homologue disjunction (Lydall and Weinert 1995; Lydall *et al.* 1996; D. Thompson, unpublished results). We speculate that the spore inviability in these *checkpoint* mutants is caused by a defect in interhomologue exchange, which results from diversion of meiotic DSBs to other "nonallelic" targets.

A *mec1-1* mutant was not elevated for meiotic unequal SCR in our system, and the spore inviability of this mutant was not rescued by *spo13* mutation. The observation that the spore inviability of a *mec1-1* mutant is not rescued by a *spo13* mutation suggests that *MEC1* acts at a different point in the meiotic recombination process than do *RAD24*, *RAD17*, and *MEC3*. The meiotic lethality conferred by the *mec1-1* allele may have obscured any increase in meiotic unequal SCR, or the failure to detect an increase might be attributed to the fact that the *mec1-1* allele is not a null.

How do the DNA damage checkpoint genes function to ensure homologue preference in meiotic recombination? An increase in Zip1p polycomplex formation in *rad24*, *rad17*, and *mec1-1* mutants compared to wild type suggests that synapsis is defective in *checkpoint* mutants (Grushcow *et al.* 1999). This raises the possiblity that the elevation in intersister and ectopic events, accompanied by a reduction in interhomologue events, results from disruption of meiotic constraints imposed by the SC, analogously to the case made for *red1* and *mek1*

mutants. However, several lines of evidence suggest that the role of the checkpoint proteins in meiosis is distinct from that of the SC components. In contrast to Red1p, which is a major constituent of meiotic chromosomal architecture, the human homologue of Rad17p has been shown to bind to meiotic chromatin in a punctate pattern (Freire *et al.* 1998). In addition, the phenotypes of the structural mutants differ from those of the checkpoint mutants in several important respects. Both *red1 dmc1* and *checkpoint dmc1* double mutants exhibit additive increases in the levels of intersister and ectopic recombination, respectively, compared to that in the single mutants (this work; Grushcow *et al.* 1999). However, meiosis-specific DSBs are repaired with normal kinetics in *red1 dmc1* double mutants (Schwacha and Kleckner 1997; Xu *et al.* 1997), whereas DSB repair is defective in *checkpoint dmc1* double mutants (Lydall *et al.* 1996). This suggests that both the mechanisms of bypass of *dmc1*-induced arrest and recombinational repair of DSBs differs in these mutant backgrounds.

If the increase in intersister recombination in the *checkpoint* mutants is caused solely by disruption of the SC, then the level of meiotic unequal SCR observed in a *red1 rad24* double mutant should approximate that in the asynaptic *red1* mutant. The frequency of unequal meiotic SCR in the *red1 rad24* double mutant is similar to that in the single *rad24* mutant, suggesting that the role of the checkpoint proteins in homologue preference is distinct from SC assembly. The synapsis defect in the *checkpoint* mutants may be either a secondary consequence of the defect in partner choice or due to the absence of another function of the checkpoint proteins.

An alternative model contends that the checkpoint proteins act analogously to their role in vegetative cells to ensure that recombinational repair of meiosis-specific DSBs is complete before MI (Grushcow *et al.* 1999). In this model, the meiotic cell cycle proceeds past MI with a fraction of DSBs left unrepaired in the *checkpoint* mutants, and post-MI repair of these DSBs, after SC dissolution, results in the increase in ectopic and concomitant decrease in interhomologue events. To address this possibility, Grushcow *et al.* (1999) prevented meiotic progression and SC dissolution in a *rad17* mutant with a mutation in *NDT80*, a meiosis-specific gene required for exit from MI prophase (Xu *et al.* 1995). Ectopic recombination in the *rad17 ndt80* double mutant is comparable to that in the *rad17* single mutant. These results indicate that prevention of both meiotic progression past MI and SC dissolution does not restore proper meiotic recombination partner choice.

A third possibility states that in addition to their classic monitoring function, the checkpoint proteins act directly to bias the repair of meiosis-specific DSBs to homologous chromatids. Either the checkpoint proteins have two separate functions, or the same checkpoint function acts both as monitor and director of partner choice. There are separation-of-function mutations in the *S. pombe* homologue of *RAD24* that confer radiation sensitivity but retain a normal checkpoint delay (Griffiths *et al.* 1995), indicating the existence of different functional domains in a checkpoint protein.

Several studies have suggested a role for the checkpoint genes in meiotic recombination. A *mec1-1* mutation results in reductions in both heteroallelic and reciprocal interhomologue recombination when the meiotic program is interrupted and the cells are returned to vegetative growth (Kato and Ogawa 1994). In addition, homologues of *MEC1* in Drosophila are required for the normal number and distribution of interhomologue exchanges (Carpenter 1979; Hari *et al.* 1995).

Meiosis-specific DSBs and processing occur at normal levels in *rad24*, *rad17*, and *mec1-1* mutants (Lydall *et al.* 1996), suggesting that the checkpoint proteins exert their influence subsequently to 5′–3′ resection of DSB ends. In addition, the meiotic recombination checkpoint is activated in *dmc1*, *rad51*, and *rad52* mutants in which DSBs are resected but not repaired, but is not activated in a *rad50s* mutant in which breaks occur but resection is blocked (Lydall *et al.* 1996). This suggests that 5′–3′ resection of DSBs is required for checkpoint activity. We offer the following model for checkpoint protein function in meiotic prophase. The resection of DSB ends generates a checkpoint-dependent signal that facilitates the recruitment of Dmc1p to Rad51p foci and/or stabilizes Rad51p-Dmc1p-containing complexes. Rad51p is required for Dmc1p colocalization to chromosomes (Bishop 1994). Successful formation of a Rad51p-Dmc1p strand-exchange complex "modifies" a checkpoint protein(s) generating an independent signal, which promotes meiotic cell cycle progression. In a *dmc1* mutant, in the absence of Rad51p-Dmc1p complexes, "unmodifed" checkpoint proteins mediate prophase arrest.

In this model, the elevation in intersister and ectopic recombination in *checkpoint* mutants results from a subset of Rad51p foci that fail to recruit and/or stabilize an association with Dmc1p. Recombination at these sites is carried out solely by the RAD51-promoted pathway, which is not biased toward allelic sites on homologues. In this scenario, a checkpoint signal acts to "enforce" partner choice (Kleckner 1996) by ensuring that recombinational repair of the majority of meiosis-specific DSBs is carried out by Dmc1p-Rad51p strand-exchange complexes, which are biased toward allelic sites on homologues.

The mutants in class II have meiotic phenotypes like those of a *dmc1* mutant: The observation that meiotic unequal SCR is elevated in a *dmc1* mutant indicates that the meiosis-specific RecA homologue Dmc1p has a role in partner choice. This result is consistent with previous observations indicating that *DMC1* functions in a pathway biased toward interhomologue reciprocal exchange (Dresser *et al.* 1997; Schwacha and Kleckner 1997;

Shinohara *et al.* 1997; Zenvirth *et al.* 1997). For example, in *dmc1* mutants, interhomologue reciprocal exchange is reduced sevenfold compared to a threefold reduction in a *rad51* mutant (Shinohara *et al.* 1997).

The additive increase in meiotic unequal SCR in the *red1 dmc1* double mutant indicates that *DMC1* has a function in partner choice that is independent of meiotic sister-chromatid cohesion and/or SC assembly.

Since the mutants in class II have phenotypes similar to those of a *dmc1* mutant, it is tempting to propose that these mutations identify functions that participate in or are regulators of the *DMC1* recombination pathway. Experiments to determine the epistatic relationships between *DMC1* and the other genes in this class are in progress.

A plausible explanation for the dominant meiotic lethality of the class II mutants is suggested by the following observations: Deletion of *TID1*, a gene implicated in *DMC1*-promoted recombination (Dresser *et al.* 1997), results in a metaphase I block that is suppressed by *DMC1*/*dmc1* heterozygosity (sporulation at about one-half that of wild type, reasonable spore viability). In addition, overexpression of *DMC1* causes an elevation in nondisjunction or, at the highest levels, a complete block to meiotic cell cycle progression (M. Dresser, unpublished results). These observations, taken together, imply a fine tuning of relative levels of gene products in the *DMC1*-promoted meiotic recombination pathway (M. Dresser, personal communication). Thus, the class II mutants may alter the relative levels of gene products in the *DMC1*-promoted recombination pathway when heterozygous in this strain background. This perturbation would disrupt interhomologue recombination, resulting in the observed dominant meiotic lethality caused by homologue nondisjunction.

Class III mutants have *hyper-rec* characteristics: The mutants in this class exhibit an increase in meiotic unequal SCR, an increase in the frequency of reductional segregation, and a reduction in spore viability, which is the phenotype expected for a meiotic *hyper-rec* mutant in *spo13* meiosis. Experiments are in progress to determine if these mutants confer the predicted increase in genetic map distance in *SPO13* diploid meiosis.

Class III mutations may have altered the regulation of *SPO11*, which catalyzes meiosis-specific DSBs, leading to higher DSB rates. Another possibility follows from the observation that, in yeast, small chromosomes have a recombination rate (centimorgans per kilobase) twofold higher than that of larger chromosomes. Bisection of a large chromosome results in an increase in the recombination rate, indicating that the rate of recombination is not solely intrinsic to a particular DNA sequence (Kaback *et al.* 1992). This suggests a genomewide mechanism that functions to ensure that small chromosomes experience an exchange required for proper disjunction at MI. We speculate that the mutants in class III have perturbed the function of this control mechanism, resulting in a higher rate of meiotic recombination along all chromosomes. Finally, the observation that the class III mutants exhibit mitotic marker instability, most likely caused by mitotic chromosome loss, is consistent with a chromosome structural defect. It has been proposed previously that mutations known to affect mitotic sister-chomatid cohesion will alter the rate of meiotic recombination (Michaelis *et al.* 1997).

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