Analysis of Meiotic Recombination Pathways in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

In the yeast, *Saccharomyces cerevisiae*, several genes appear to act early in meiotic recombination. *HOP1* and *RED1* have been classified as such early genes. The data in this paper demonstrate that neither a *red1* nor a *hop1* mutation can rescue the inviable spores produced by a *rad52 spo13* strain; this phenotype helps to distinguish these two genes from other early meiotic recombination genes such as *SPO11*, *REC104*, or *MEI4*. In contrast, either a *red1* or a *hop1* mutation can rescue a *rad50S spo13* strain; this phenotype is similar to that conferred by mutations in the other early recombination genes (*e.g.*, *REC104*). These two different results can be explained because the data presented here indicate that a *rad50S* mutation does not diminish meiotic intrachromosomal recombination, similar to the mutant phenotypes conferred by *red1* or *hop1*. Of course, *RED1* and *HOP1* do act in the normal meiotic interchromosomal recombination pathway; they reduce interchromosomal recombination to ~10% of normal levels. We demonstrate that a mutation in a gene (*REC104*) required for initiation of exchange is completely epistatic to a mutation in *RED1*. Finally, mutations in either *HOP1* or *RED1* reduce the number of double-strand breaks observed at the *HIS2* meiotic recombination hotspot.

A unique event that affects chromosomes during meiosis is the high frequency recombination and synapsis between homologs that occurs in prophase I. Recombination not only generates variation among genes that reside on the same chromosome, but is, in almost all eucaryotes, necessary for the proper execution of the second unique meiotic event, the reductional division. Much progress has been made in defining the genes required for meiotic recombination and synapsis in the yeast *Saccharomyces cerevisiae* (*e.g.*, PETES *et al.* 1991). Many of the advances in understanding meiotic recombination are due to the existence of a powerful tool in yeast that allows (at least some types of) Rec$^-$ cells to produce viable meiotic products (spores). This tool is a mutation in the *SPO13* gene; a *spo13* mutation can essentially eliminate the reductional division so that there is no longer an absolute demand for recombination for proper chromosome segregation (KLAPHOLZ and ESPOSITO 1980; MALONE and ESPOSITO 1981; KLAPHOLZ *et al.* 1985). A *spo13* mutant diploid produces two diploid spores (a "dyad"). More recently, HUGERAT and SIMCHEN (1993) demonstrated that, in the presence of *spo13*, some chromosomes can undergo a reductional division while others divide equationally. However, in the presence of a Rec$^-$ mutation that eliminates meiotic recombination (such as *rec104*), essentially all chromosomes undergo an equational division in a *spo13* strain and produce almost no (<0.5%) recombinant (or reductional) type dyads for all chromosomes and intervals examined (*e.g.*, GALBRAITH and MALONE 1992). One can conclude from this that any reductional divisions that may occur in a *spo13* meiosis are dependent upon recombination.

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It is possible to place genes affecting meiotic recombination in *S. cerevisiae* into two basic classes, which have been called "Early" and "Late" (MALONE 1983; see also PETES *et al.* 1991). In combination with *spo13*, mutations in early genes (*e.g.*, *spo11*) produce viable, recombinationless spores after meiosis. Mutations in late recombination genes (*e.g.*, *rad52*) produce dead spores even if *spo13* is present. To demonstrate that the late mutations are indeed in the recombination pathway, it is possible to "rescue" them by mutating an early gene like *RAD50* or *SPO11* (*e.g.*, MALONE 1983; ENGEBRECHT and ROEDER 1989). These observations are consistent with the idea that recombination occurs in a linear dependent pathway; blocks in the early genes prevent exchange from starting, whereas blocks in the late recombination genes stop exchange in the middle of the process (MALONE 1983). Bivalents caught in the middle of exchange would presumably have difficulty with either an equational or a reductional division and hence would produce dead spores even in a *spo13* meiosis. Consistent with this view are the observations that cells with mutations in late exchange genes generate some recombinational intermediates (*e.g.*, heteroduplex DNA), while mutations in early exchange genes do not generate any intermediates at all (*e.g.*, BORTS *et al.* 1986; NAG and PETES 1993; NAG *et al.* 1995).

The picture became more complex with the discovery of a second type of early meiotic recombination gene. In a clever screen for mutations that affected interchromosomal, but not intrachromosomal, meiotic ex-

TABLE 1

Genes involved in meiotic recombination

Groups	Genes	Reference
Early exchange (EE)	*RAD50*	GAME *et al.* (1980)
	SPO11	KLAPHOLZ *et al.* (1985)
	MEI4	MENEES and ROEDER (1989)
	MER2	ENGEBRECHT and ROEDER (1990)
	REC102	MALONE *et al.* (1991)
	REC104	MALONE *et al.* (1991)
	REC114	MALONE *et al.* (1991)
	XRS2	IVANOV *et al.* (1992)
	MRE11	AJIMURA *et al.* (1993)
Early synapsis (ES)	*HOP1*	HOLLINGSWORTH and BYERS (1989)
	RED1	ROCKMILL and ROEDER (1988)
	MEK1	ROCKMILL and ROEDER (1991)
Late exchange (LE)	*RAD52*	GAME *et al.* (1980)
	RAD57	GAME *et al.* (1980)
	RAD55	GAME (1983)
	RAD51	GAME (1983)

Genes in the EE group produce viable, recombinationless spores in the presence of *spo13*. No induction of meiotic recombination is observed. The *MER1* gene has many of the phenotypes of an EE gene; mutations are viable with *spo13* and it rescues *rad52 spo13*. However, *mer1* reduces meiotic recombination to only 10% of the normal level (ENGEBRECHT and ROEDER 1989). ENGEBRECHT *et al.* (1991) have shown that *MER1* is needed to splice *MER2* in meiosis. Its modest reduction in recombination could be attributable to some *MER2* activity being present even in the absence of *MER1*. Although *RAD50* null mutations have the phenotype of an EE gene, a special allele of *RAD50, rad50S,* has the phenotype of a LE mutation (ALANI *et al.* (1990) (see text). The activity of *RAD50S* is required after DSB formation (see Table 2). Genes in the ES class also produce viable spores in the presence of *spo13,* but interchromosomal meiotic recombination is only reduced ~10-fold. Intrachromosomal recombination is not generally affected by *hop1* or *red1* mutations. We include *MEK1* in the ES class because it is viable with *spo13,* and reduces recombination to 10%. However, the effect of *mek1* mutations on intrachromosomal recombination has not been directly tested. Genes in the LE class produce inviable spores with *spo13,* but the addition of a mutation in the EE class restores spore viability. Some genes in which a mutation affects meiotic recombination are not shown here. They include *DMC1; dmc1* mutations have been reported to produce inviable spores with *spo13* (a LE phenotype) (BISHOP *et al.* 1992), but *dmc1* mutations have also been reported to generate viable spores with *spo13* (an early phenotype) (ROCKMILL and ROEDER 1994). Likewise *ZIP1,* a gene required for mature tripartite SC formation is not listed because sporulation and spore viability in *zip1* mutants were at wild type levels and meiotic recombination was decreased only about twofold (SYM and ROEDER 1994). Its phenotype is therefore different from the other ES genes.

change, HOLLINGSWORTH and BYERS (1989) found mutations that defined the *HOP1* gene. A *hop1 spo13* diploid produces viable spores with reduced recombination levels, so by definition, the *HOP1* gene is an early meiotic recombination gene. However, mutations in *HOP1* conferred two phenotypes distinct from the other early recombination genes: *hop1* mutations did not affect intrachromosomal exchange and even a null *hop1* mutation reduced interchromosomal meiotic recombination to only ~10% of normal. There is still a 40–100-fold induction of recombination over mitosis in such *hop1* mutants. In contrast, mutations in the other early exchange genes completely eliminated all types of meiotic recombination; there was no induction over the background mitotic level (*e.g.,* WAGSTAFF *et al.* 1985; MENEES and ROEDER 1989; GALBRAITH and MALONE 1992). Another early mutation, *red1,* which conferred a phenotype similar to *hop1,* was discovered by ROCKMILL and ROEDER (1988, 1990). Consistent with the phenotypes conferred by *hop1* and *red1* mutations, antibodies to both the *HOP1* or *RED1* gene products have been found to localize to the synaptonemal complex (*HOP1,* HOLLINGSWORTH *et al.* 1990; *RED1,* cited in NAG *et al.* 1995). We have listed most of the genes involved in meiotic recombination in Table 1. For the purposes of this paper we have sorted many of them into three classes: early exchange ("EE") genes, early synapsis ("ES") genes, and late exchange ("LE") genes (Tables 1 and 2). As indicated above, mutations in the genes in both early groups give viable spores in the presence of *spo13,* whereas LE mutants do not.

In a selection for mutations in EE genes, we found mutations in all of the meiosis-specific EE genes known at that time, as well as in several new genes (MALONE *et al.* 1991). This selection was based on the observation that EE mutations could rescue a LE mutation (in this case *rad52*) in the presence of *spo13* (*e.g.,* MALONE 1983). However, from >170 mutations isolated, no mutations were found in either *HOP1* or *RED1* (MALONE *et al.* 1991). This suggested to us that the ES genes might act in a pathway parallel to the main exchange

TABLE 2

Phenotypes of mutations in the three groups of genes affecting meiotic recombination in *Saccharomyces cerevisiae*

Groups	Meiotic recombination		SC formation	DSB formation	Spore viability	
	Interchromosomal	Intrachromosomal			Double mutants w/*spo13*⁻	Triple mutants w/*LE*⁻ *spo13*⁻
Early exchange (EE)	Eliminated completely	Eliminated completely	No mature SC; may have axial elements	Not present	Viable	Viable
Early synapsis (ES)	Retain 10%	No reduction	No mature SC: usually no axial elements	?	Viable	?
Late exchange (LE)	Retain 1–10% by RTG	Retain 1–10% by RTG	Tripartite SC can be present; often short	Present	Inviable	NR

References for the data presented in this table are given in the text and in Table 1. NR, not relevant; RTG, return to growth experiment. Note that not every gene in each class has been tested for every phenotype.

path. We proposed such a model for the action of the meiotic recombination genes (see Figure 1). The experiments in this paper test that model and find it to be inadequate. We then suggest an alternative pathway and present several experiments which test its predictions.

MATERIALS AND METHODS

Yeast strains and plasmids: The yeast strains and plasmids used in this paper are listed in Table 3. The construction of the yeast strains used is described below.

YM1-32 and YM1-33: Each **a** and α haploid parent of RM69 (MALONE and ESPOSITO 1981) was transformed with the one-step gene disruption plasmid pR1695 (obtained from G. S. ROEDER) or pNH46-1 (obtained from N. HOLLINGSWORTH) (Table 3) to create strains with *red1::URA3* or *hop1::URA3* mutations, respectively. The integrants were confirmed by Southern blotting and complementation tests; the resulting haploids were mated to give YM1-32 and YM1-33 diploids.

YM1-34 and YM1-35: Each **a** and α haploid parent of RM69 was transformed with the one-step gene replacement plasmid pNKY349 (obtained from N. KLECKNER) or pSM22 (MALONE 1983) (Table 3) to create strains with *rad50S-KI81::URA3* (referred to henceforth as *rad50S*) or *rad52::URA3* mutations, respectively. The *rad50S* integrants were tested by their intermediate sensitivity to MMS compared with *rad50Δ* mutants and *RAD50* (ALANI *et al.* 1990). The integrants were then confirmed by Southern analysis. The *rad52::URA3* integrants were verified by high sensitivity to MMS and then by Southern analysis. The correct integrants were then mated to give the YM1-34 and YM1-35 diploids.

YM1-39: Each **a** and α haploid parent of RM69 was transformed with the two-step gene replacement plasmid pAMG404-SSΔ to create *rec104-Δ1* strains. First step transformation was targeted with *ClaI*; correct integrants were determined by Southern and then were plated out on 5-fluoro-orotic acid (5-FOA) (BOEKE *et al.* 1984) to select for Ura⁻ "popouts". The correct

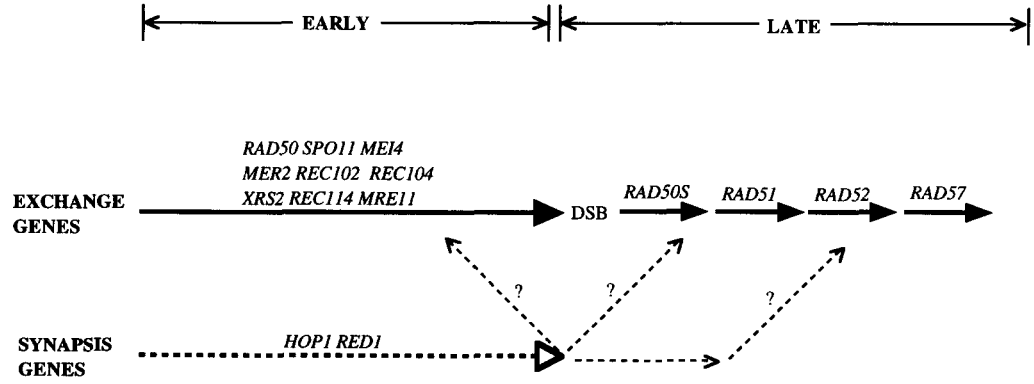

FIGURE 1.—A model for the meiotic recombination pathway (derived from MALONE *et al.* 1991). We propose that there are three groups of meiotic recombination genes. In the exchange pathway, the EE (early exchange) genes are placed before DSBs while the LE (late exchange) genes are placed after DSBs. The function of the *RAD50* gene that is inactivated by the *rad50S* mutation is referred to as *RAD50S* for brevity (see also text). We place the ES (early synapsis) genes in a parallel pathway to the exchange pathway. Not all known genes are shown to simplify the model. See text and Tables 1 and 2 for details. We used "?" to indicate possible points of convergence between the exchange and the synapsis pathway.

TABLE 3

Yeast strains and plasmids

Strain name	Genotype	Source or reference
	A. Yeast strains	
RM69	*MAT***a** *lys2-1 tyr1-1 his7-2 can1^r ura3-13* *MATα lys2-2 tyr1-1 his7-1 CAN1^S ura3-1* *ade5 met13-d CYH2^2 trp5-d leu1-d ade2-1 spo13-1* *ade5 met13-c cyh2^r trp5-c leu1-c ade2-1 spo13-1*	MALONE and ESPOSITO (1981)
YM1-32	RM69 but homozygous *red1::URA3*	This study
YM1-33	RM69 but homozygous *hop1::URA3*	This study
YM1-34	RM69 but homozygous *rad50S::URA3*	This study
YM1-35	RM69 but homozygous *rad52::URA3*	This study
YM1-36	RM69 but homozygous *red1::ura3* and *rad50S::URA3*	This study
YM1-37	RM69 but homozygous *red1::ura3* and *rad52::URA3*	This study
YM1-38	RM69 but homozygous *red1::ura3* and *rec104-Δ1*	This study
YM1-39	RM69 but homozygous *rec104-Δ1*	This study
YM1-40	RM69 but homozygous *red1::ura3, rec104-Δ1,* and *rad52::URA3*	This study
YM1-41	RM69 but homozygous *rec104-Δ1* and *rad52::URA3*	This study
RM26	*MAT***a** *lys2-1 can1^r ura3-1 HOM3 his1-19, 34* *MATα lys2-1 can1^r ura3-13 hom3 HIS1* *ade5 trp5-d leu1-d ADE6 ade2-1* *ade5 trp5-d leu1-d ade6 ade2-1*	MALONE *et al.* (1991)
RM186	*MAT***a** *lys2-2 tyr1-1 his7-2 can1^r ura3-13* *MATα lys2-1 tyr1-1 his7-1 CAN1^S ura3-1* *ade5 met13-d CYH2^S trp5-d leu1-d ade2-1 rad52-1 spo13-1* *ade5 met13-c cyh2^r trp5-c leu1-c ade2-1 rad52-1 spo13-1*	MALONE and ESPOSITO (1981)
C3-16	*MAT***a** *CAN1^S ura3-1 HOM3* *MATα can1^r ura3-52 hom3* *ADE5 met13-c cyh2^r trp5-c leu1-c ade6 lys2-2 tyr1-1* *ade5 met13-d CYH2^S trp5-d leu1-d ADE6 lys2-1 tyr1-1* *ade2-1 his4::LEU2 leu2 spo13-1* *ade2-1 HIS4 LEU2 spo13-1*	COOL and MALONE (1992)
C3-13	*MAT***a** *lys2-2 tyr1-1 his7-2 his4::LEU2 leu2* *MATα lys2-1 tyr1-1 his7-1 his4::LEU2 leu2* *CAN1^S ura3-1 HOM3 ade2-1* *can1^r ura3-52 hom3 ade2-1* *met13-c cyh2^r trp5-c leu1-c ade6 rec102-1 spo13-1* *MET13 CYH2^S trp5-d leu1-d ade6 rec102-1 spo13-1*	COOL and MALONE (1992)
C3-25	C3-13 but homozygous *rad52:URA3*	This study
C3-26	C3-13 but homozygous *rad50S::URA3*	This study
A7-7	*MAT***a** *lys2-1 tyr1-1 can1^r ura3-13 ade5 met13-d trp5-c ade6* *MATα lys2-1 tyr1-1 can1^r ura3-13 ADE5 met13-d trp5-d ade6* *ade2-1 rad50S::URA3 spo13-1* *ade2-1 rad50S::URA3 spo13-1*	GALBRAITH and MALONE (1992)
A7-2	*MAT***a** *lys2-2 tyr1-1 CAN1^S ura3-1* *MATα lys2-1 tyr1-1 can1^r ura3-13* *ADE5 met13-c cyh2^r trp5-c leu1-c ADE6 ade2-1* *ade5 met13-d CYH2^S trp5-d leu1-d ade6 ade2-1* *rad50S::URA3 rec104-1 spo13-1* *rad50S::URA3 rec104-1 spo13-1*	GALBRAITH and MALONE (1992)
YM1-7	*MAT***a** *LEU2 LYS2 tyr1-2 his7-2* *MATα leu2-3,112 lys2-1 tyr1-1 HIS7* *ADE5 met13-c cyh2^r trp5-c leu1-c red1::ADE2 rad52-1* *ade5 met13-d CYH2^S trp5-d leu1-d red1::ADE2 rad52-1* *spo13-1* *spo13-1*	This study

TABLE 3

Continued

Strain name	Genotype	Source or reference
	A. Yeast strains	
YM2-13	*MATa HIS4 LEU2 LYS tyr1-2 his7-2* *MATα his4-289,519 leu2-3,112 lys2-1 tyr1-1 HIS7* *ADE5 met13-c cyh2^r trp5-c leu1-c ura3-13 ade2-1* *ade5 met13-d CYH2^S trp5-d leu1-d ura3-1 ade2-1* *trp1-289 red1::ADE2 spo13-1 thr1-1* *TRP1 red1::ADE2 spo13-1 thr1-1*	This study
YM5-14	YM2-13 but homozygous *rad50S::URA3*	This study
YM1-16	*MATa lys2-1 try1-1 CAN1^S ura3-1 HIS1* *MATα lys2-1 tyr1-1 can1^r ura3-1 his1* *ADE5 met13-c cyh2^r trp5-c leu1-c ade2-1 hop1::URA3* *ade5 met13-d CYH2^S trp5-d leu1-d ade2-1 hop1::URA3* *rad52-1 spo13-1* *rad52-1 spo13-1*	This study
YM2-1	*MATa lys2-1 tyr1-1 his7-2 can1^r ura3-1 his1* *MATα lys2-1 tyr1-1 his7-1 CAN1^S ura3-1 HIS1* *ade5 met13-d CYH2^S trp5-d leu1-d ade2-1 hop1::URA3* *ade5 met13-c cyh2^r trp5-c leu1-c ade2-1 hop1::URA3* *spo13-1* *spo13-1*	This study
YM5-11	*MATa can1^r ura3-1 his1 ade5 MET13 trp5-d ade2-1* *MATα can1^r ura3-1 HIS1 ade5 met13-d trp5-d ade2-1* *lys2-1 TYR1 hop1::URA3 rad50S::URA3 spo13-1* *lys2-1 tyr1-1 hop1::URA3 rad50S::URA3 spo13-1*	This study
SK5-11	*MATa CAN1^S ura3-13 ADE5 ADE2 trp1-1 leu2-1 met2-1* *MATα can1^r ura3-1 ade5 ade2-1 trp1-1 LEU2 MET2* *HIS2::ΔAha rad50S::URA3* *his2-xho::ΔAha rad50S::URA3*	BULLARD *et al.* (submitted)
YM5-36	SK5-11 but homozygous *red1::URA3*, and homozygous *rad50S::ura3*	This study
YM5-37	SK5-11 but homozygous *hop1::URA3*, and homozygous *rad50S::ura3*	This study
YM5-23	*MATa his3-Δ200 leu2 trp1 CAN 1^S ura3-52 ade2-1* *MATα his3-Δ200 leu2 trp1 can1::BYA112 ura3-52 ade2-1*	This study
YM5-24	YM5-23 but homozygous *rad50S::URA3*	This study
YM1-42	YM5-23 but homozygous *red1::URA3*	This study
MEI57-14A	*MATα spo13-1 sir4::HIS3 his4-3′Δ::LEU2::his4-260 can1^r lys2 ura3-52 ade2 ade5*	MALONE *et al.* (1991)
MEI57-14A-10	MEI57-14A but *rad50S::URA3*	This study
MEI57-14A-11	MEI57-14A but *red1::URA3*	This study

B. Plasmids

Plasmid name	Description	Restriction sites for transformation	Reference
pR1695	pHSS6 with *red1::URA3* gene disruption for one-step gene replacement of *RED1*	*Eco*RI and *Xba*I	SYM and ROEDER (1995)
pNH46-1	YCP50 with *hop1::URA* gene disruption for one-step gene replacement of *HOP1*	*Eco*RI and *Sph*I	HOLLINGSWORTH and BYERS (1989)
pNKY349	pSP65 with *rad50S::URA3* gene disruption for one-step gene replacement of *RAD50*	*Eco*RI and *Bam*HI	ALANI *et al.* (1990)
pSM22	pBR322 with *rad52::URA3* gene disruption for one-step gene replacement of *RAD52*	*Bam*HI	MALONE 1983
pAMG404-SSΔ	YIp*lac*211 with *rec104-Δ1* gene disruption for two-step gene replacement of *REC104*	*Cla*I	This study
pRM9	YEp24 with *HIS2* gene for isolating *Bgl*II-*Eco*RI probe to detect DSBs	—	MALONE *et al.* (1994)

Ura⁻ *rec104-Δ1* popouts were verified by *rec104* complementation tests and by Southern blot analysis. Those were then mated to obtain desired diploids. The *rec104-Δ1* plasmid (pAMG404-SSΔ) was constructed as follows. The *Hin*dIII-*Bam*HI 2.8-kb fragment containing *REC104* (GALBRAITH and MALONE 1992) was cloned into YIp*lac*211 (GIETZ and SUGINO 1988). The 0.8-kb *Ssp*I fragment containing *REC104* was deleted by a partial *Ssp*I digestion followed by religation to obtain the resulting plasmid pAMG404-SSΔ.

YM1-36, YM1-37 and YM1-38: Each *red1::URA3* haploid parent of YM1-32 (see above) was plated out on 5-FOA to select for Ura⁻ ectopic recombinants between the *ura3* mutation on chromosome *V* and the *red1::URA3* construct (BOEKE *et al.* 1984). The correct **a** and α *red1::ura3* recombinants were verified again by *red1* complementation tests and by Southern analysis. These were transformed with pNKY349, pSM22, and pAMG404-SSΔ to create *red1::ura3 rad50S, red1::ura3 rad52::URA3,* and *red1::ura3 rec104-Δ1* strains, respectively (described above). Correct integrants were confirmed by Southern, MMS, and complementation tests and were then mated to obtain the desired diploids.

YM1-40 and YM1-41: To construct *rad52 rec104 red1* and *rad52 rec104* diploids, the haploid parents of YM1-38 and YM1-39 (see above) were transformed with pSM22 (described above). These were then checked by Southern and MMS tests; correct haploids were mated to obtain YM1-40 and YM1-41, respectively.

YM1-7 and YM2-13: These two "outcross" diploids were created as follows. The **a** *red1::ADE2* haploid (BR1373-6Da) (provided by G. S. ROEDER) was mated with RM127-6D, an α *rad52-1 spo13-1* haploid congenic to RM69. Diploids were selected, sporulated, and dissected, and **a** and α *red1::ADE2 rad52-1 spo13-1* segregants as well as **a** and α *red1::ADE2 spo13-1* segregants obtained. These were all verified by *red1* complementation and MMS tests for *rad52*. The **a** and α *red1::ADE2 rad52-1 spo13-1* segregants were mated to obtain YM1-7. The **a** and α *red1::ADE2 spo13-1* segregants were crossed again to the haploid parents of RM69. These diploids were than dissected to obtain **a** and α *red1::ADE2 spo13-1* segregants with good diagnostic markers. These segregants were then mated to obtain YM2-13.

YM5-14: Each haploid **a** and α parent of YM2-13 was transformed with pNK349; *rad50S* transformants were confirmed by their intermediate MMS sensitivity phenotype and by Southern analysis (see above). The integrants were then mated to create YM5-14.

YM1-16 and YM2-1: RM26-26C, an α strain congenic to RM69, was transformed with pNH46-1 (described above) to obtain a *hop1::URA3* disruption transformant, which was confirmed by *hop1* complementation tests and by Southern analysis. It then was mated with the **a** haploid parent of YM1-35 (isogenic to RM69 but containing *rad52-1 spo13-1*; see above). Diploids were selected, sporulated, tetrads dissected, and **a** and α *hop1::URA3* segregants were isolated. At least four backcrosses were done for each construct. Then **a** and α *hop1::URA3 rad52-1 spo13-1* segregants were mated to make YM1-16. Likewise, **a** and α *hop1::URA3 spo13-1* segregants were isolated and mated to give YM2-1.

YM5-11: RM26-26C-*hop1::URA3* was mated with A7-5-1B, also derived from the parents of RM69. Diploids were selected, sporulated, tetrads dissected, and **a** and α *hop1::URA3 rad50S spo13-1* segregants were obtained and mated to obtain YM5-11.

C3-25 and C3-26: Each haploid **a** and α *rec102-1* parent of C3-13 (congenic with the haploid parents of RM69) was transformed with pSM22 (*rad52::URA3*) or pNKY349 (*rad50S*); transformants were confirmed by MMS and Southern analysis (see above), then were mated to create C3-25 and C3-26, respectively.

YM5-36 and YM5-37: Each haploid **a** and α parent of SK5-11 was plated out on 5-FOA to select for Ura⁻ ectopic recombinants (described above). The correct **a** and α *rad50S::ura3* recombinants were verified by MMS and Southern analysis and were transformed with pR1695 or pNH46-1 (described above). The correct integrants were also checked by Southern and complementation tests and then mated to obtain the desired diploids YM5-36 and YM5-37. Thus, all strains used to measure DSBs were isogenic. Note that SK5-11 is from the same genetic background as RM69, although not strictly congenic.

MEI57-14A-10 and MEI57-14A-11: MEI57-14A was transformed with pNKY349 to create a haploid *rad50S spo13-1* strain (verified by MMS and Southern analysis), or with pR1695 to create a haploid *red1::URA3 spo13-1* strain (verified by *red1* complementation and Southern analysis). Thus, all three strains used to measure haploid intrachromosomal recombination were isogenic. Note that MEI57-14A is from the same strain background as RM69, although it is not strictly congenic.

YM5-23, YM5-24 and YM1-42: Both haploid **a** and α parents (SSL231 and SSL204A) of YM5-23 were kindly provided by KAYTOR and LIVINGSTON (1994); they were transformed with pNKY349 (verified by MMS tests and Southern analysis), then mated to obtain YM5-24. They were also transformed with pR1695 (verified by *red1* complementation tests and Southern analysis), and then mated to obtain YM1-42. Thus, all three strains used to measure diploid intrachromosomal recombination were isogenic. Note that all three diploids (YM5-23, YM5-24 and YM1-42) come from DENNIS LIVINGSTON's background.

Genetic procedures: Yeast strains were grown on either YPD (rich medium), HIS dropout medium, or URA dropout medium (synthetic medium with all required nutrients except histidine or uracil, respectively) (SHERMAN *et al.* 1986). YPA and sporulation media are described in MALONE *et al.* (1991). 5-FOA medium was prepared as described by BOEKE *et al.* (1984). Mating, diploid isolation, and tetrad analysis were carried out by standard procedures (SHERMAN *et al.* 1986). Spheroplast and lithium acetate transformation methods were used to transform yeast (HINNEN *et al.* 1978; ITO *et al.* 1983).

Measurements of interchromosomal recombination: Mitotic crossing-over was measured as the frequency of drug-resistant colonies in diploids that were heterozygous for a mutation (*e.g., can'*) conferring a recessive drug resistance phenotype. Drug-resistant colonies may potentially be the result of crossing-over, gene conversion, chromosome loss, or missegregation. However, crossing-over occurs at a greater frequency than the other events (ROMAN 1956). Mitotic gene conversion was monitored in diploids by measuring the frequency of prototroph formation from heteroalleles at several different loci. At least three independent cultures were measured for each strain. Meiotic gene conversion frequencies were determined by allowing diploids to go through meiosis and measuring the frequency of prototroph formation at several heteroallelic loci. This prototrophy results mainly from gene conversion (ROTH and FOGEL 1971; MALONE *et al.* 1988). Crossing-over in *spo13* diploids was measured in meiotic cultures by the frequency of drug-resistant colonies. Standard yeast genetic techniques were used for dissection of dyad asci as another measure of crossing-over (SHERMAN *et al.* 1986).

Measurements of intrachromosomal recombination: The duplication used for intrachromosomal recombination assays are shown in Figure 2. The strain containing the *HIS4* duplication was made and provided by MERL HOEKSTRA (ICOS

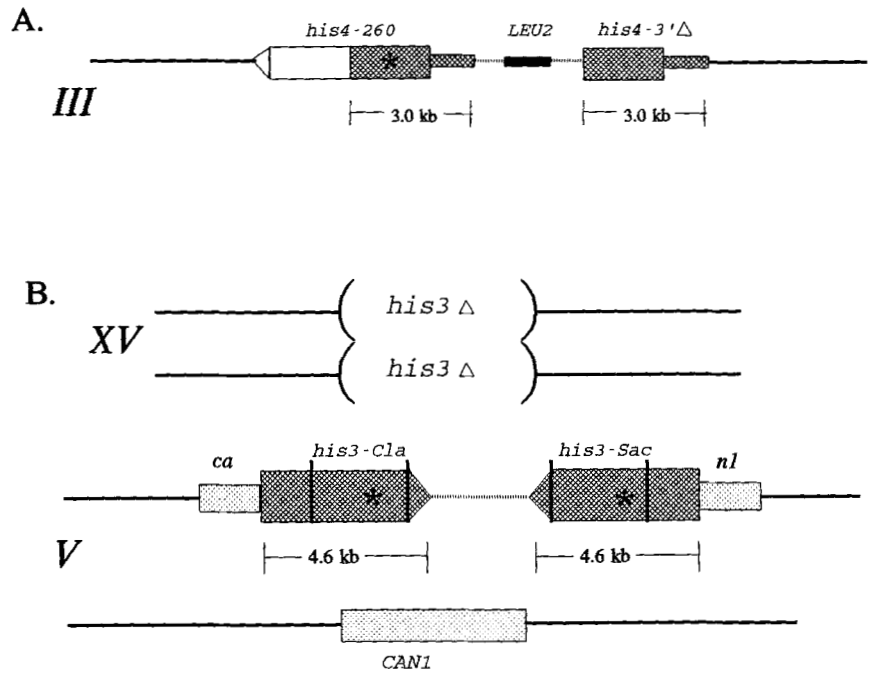

FIGURE 2.—Duplications used to study intrachromosomal recombination. (A) Structure of the *his4* direct repeats in haploid MEI57-14A. The extent of the homology between the *his4* direct repeats is 3.0 kb and indicated by the crosshatched area. Thin line, chromosomal *III* around *HIS4* region; dotted line, pBR322 vector sequences; filled block, *LEU2* gene; *, the relative position of the *his4-260* mutation (DONAHUE *et al.* 1982); *his4-3'Δ*, a mutation produced by deletion of the 3′ coding sequences from *HIS4*. (B) Structure of the *his3* inverted repeats on chromosome *V* in diploid YM5-23 (KAYTOR and LIVINGSTON 1994). The *HIS3* gene on chromosome *XV* was completely deleted in both haploid parents. The extent of homology between the *his3* repeats is 4.6 kb. Thin line, chromosome *XV* and *V* sequences; dotted line, pBR322 vector sequences; rectangle with dots, *CAN1* gene; crosshatched rectangle, region containing *HIS3* that is repeated. The dark vertical lines indicate the region of the repeat that is the *HIS3* coding region. *, the relative positions of the restriction site linkers used for creating the *his3* heteroalleles.

Corp.). In the haploid *his4* direct repeats system, intrachromosomal recombination between the *his4-3'Δ* and *his4-260* alleles in the duplication was monitored by measuring the frequency of His^+ prototrophs. In the diploid system, intrachromosomal recombination between the alleles in the *his3* inverted repeats (which were located at the *CAN1* locus) was monitored by measuring the frequency of His^+ prototrophs (KAYTOR and LIVINGSTON 1994). Single colonies were inoculated into YPA cultures at a concentration of ~1000 cells/ml and grown to approximately 2×10^7 cells/ml, washed twice with water and once with sporulation medium, and then transferred to sporulation medium at 4×10^7 cells/ml. At 24 hr in meiosis, 2.5-ml aliquots were taken, washed with 0.2 M PO_4 buffer and resuspended into an equal volume of PO_4 buffer. From this suspension 0.5 ml was added to 0.5 ml of 8% formaldehyde to fix a portion of cells for examining sporulation. The remaining cells were diluted and plated onto synthetic complete medium to measure the amounts of viable cells and on histidine dropout medium to measure His^+ prototrophic recombinants. The plates were incubated at 30° for 2.5–3 days. Recombination frequency was determined as the concentration of His^+ recombinants at a given time divided by the total viable cell concentration at that time. In the diploids YM5-23, YM5-24 (*rad50S*) and YM1-42 (*red1*), return to growth experiments (SHERMAN and ROMAN 1963) were performed to determine the amount of intrachromosomal recombination.

Physical examination of double-strand breaks: Diploid cells were grown to 2×10^7 cells/ml in YPA presporulation medium, spun down, and washed twice with water and once with sporulation medium before resuspending in sporulation medium at 4×10^7 cells/ml. At various times throughout meiosis, 0.5-ml aliquots were taken into 0.5 ml of 8% formaldehyde to be counted and DAPI stained to verify the progression of the cells in meiosis (data not shown). In addition, 20-ml samples were taken into 20 ml of 100% ethanol and stored at −20°. To make genomic DNA, these cell samples were spun down, treated with 100 T zymolyase (ICN Biochemicals) and proteinase K (Sigma), and extracted twice with phenol:chloroform:isoamyl-alcohol (25:24:1) and once with chloroform. The DNA was digested with *Bgl*II, subjected to electrophoresis in a 0.9% agarose gel for 18 hr at 60 V, transferred to Hy-Bond N (Amersham), and probed with the 1.1-kb *Bgl*II/*Eco*RI *HIS2* fragment isolated from pRM9 (MALONE *et al.* 1994) (see Figure 4). The fragment was labeled with [α-^{32}P]dCTP using the random primer kit (BRL). Filters were then analyzed on the Molecular Dynamics PhosphorImager Model 445SI as per instructions from the manufacturer.

RESULTS

Verification of the phenotypes of *red1* and *hop1* mutations: It has been demonstrated that some mutations that affect meiotic recombination have different phenotypes in different yeast strain backgrounds [*e.g.*, *dmc1* (BISHOP *et al.* 1992; ROCKMILL and ROEDER 1994); see also Table 1]. We therefore wished to verify that the reported phenotypes conferred by *hop1* and *red1* mutations were similar in our strain background. Table 4 illustrates that a *red1* null mutation reduces heteroallelic recombination (gene conversion) by an average of

TABLE 4

The effect of *hop1* and *red1* mutations on meiotic recombination in *spo13* diploids

Diploid name	Relevant genotype	Interchromosomal recombination								
		Prototroph frequency ($\times 10^5$)						Drug-resistant colony frequency ($\times 10^4$)		
		lys2-1/*lys2-2*	*his7-1*/*his7-2*	*trp5-c*/*trp5-d*	*leu1-c*/*leu1-d*	*met13-c*/*met13-d*	Average reduction[a]	*cyh2*^{r}/*CYH2*^{s}	*can1*^{r}/*CAN1*^{s}	Average reduction[b]
RM69	*spo13-1*	14 (1.0)	117 (1.0)	3400 (1.0)	1600 (1.0)	4300 (1.0)	— (1.0)	2400 (1.0)	3400 (1.0)	— (1.0)
YM1-32	*red1::URA3 spo13-1*	5.0 (0.36)	7.2 (0.06)	67 (0.02)	57 (0.04)	250 (0.06)	— (0.11)	350 (0.15)	360 (0.11)	— (0.13)
YM1-33	*hop1::URA3 spo13-1*	1.2 (0.09)	3.4 (0.03)	19 (0.006)	15 (0.01)	91 (0.02)	— (0.03)	140 (0.06)	280 (0.08)	— (0.07)
YM2-1	*hop1::URA3 spo13-1*	NT	5.5 (0.05)	33 (0.01)	16 (0.01)	154 (0.04)	— (0.03)	230 (0.1)	250 (0.07)	— (0.09)

RM69, YM1-32, and YM1-33 are isogenic. YM2-1 is a congenic strain. NT, not tested. Each diploid was sporulated and plated on various selective media and the frequency of recombination was determined. The frequencies given are the geometric means of at least three independent meiotic cultures. The numbers in parentheses represent the amount of recombination relative to wild type. Note that none of the differences between YM1-33 and YM2-1 was significant (Student *t*-test).

[a] Average reduction is the arithmetic mean of the relative frequency of all the heteroallelic loci shown.

[b] Average reduction is the arithmetic mean of the relative frequency of the two heterozygous drug-resistant loci.

about ninefold; the frequency of drug resistant colonies (which reflects crossing-over) is reduced by an average of eightfold. These reductions are similar to those observed by ROCKMILL and ROEDER (1990), although we analyzed different loci and alleles. We note, as they did, that different loci are affected differently by a *red1* mutation (*e.g.*, recombination at the *lys2* locus is reduced only threefold—see Table 4). The *hop1* null mutation reduced gene conversion by an average of ~30-fold; the drug-resistant colony frequency was reduced 14-fold (Table 4). These results are similar to those observed by HOLLINGSWORTH and BYERS (1989). For both the *hop1* and the *red1* mutations, our work not only confirms the reported phenotypes in our strains, but extends the observations to several more loci on more chromosomes. Finally, we note that the recombination frequencies observed in the isogenic and the congenic *hop1* diploids are not significantly different. The similarities between the phenotypes observed in our strains (with both *hop1* and *red1*) and the published work suggests that the phenotypes conferred by these mutations may be general. [In a screen for meiotic recombination mutants in an SK1 background, AJIMURA *et al.* (1993) found several alleles of *HOP1* that were reported to eliminate meiotic recombination. However, only one heteroallelic locus was examined. Given the variable effect of *hop1* on different loci, the phenotype of the mutation in an SK1 background is still unclear.] Clearly, in our strains, interchromosomal meiotic recombination is reduced by mutations in ES genes ~10–20-fold. To further examine the effect these two mutations have on crossing-over, we also measured the frequency of recombinant ("R") type dyad asci after dissection. [R type asci are almost completely dependent upon recombination (*e.g.*, MALONE 1983).] In examination of eight intervals on three chromosomes, we found an average reduction of 6.1-fold in the *hop1* mutant and an average reduction of 11-fold in the *red1* mutant (data not shown). Thus, we conclude that both *hop1* and *red1* confer the standard recombination phenotypes in our strains. Since on average the ES mutations reduce meiotic recombination only 10-fold, a substantial amount of meiotic recombination still remains (Table 4) (HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990).

Does either the *hop1* or the *red1* mutation "rescue" the spore lethality found in a *rad52 spo13* strain? If the ES genes were required in a pathway parallel to the main exchange pathway, as predicted in Figure 1, combining them with *rad52* should still result in dead spores. The data in Table 5 indicate that diploids of genotype *hop1 spo13* or *red1 spo13* produce viable spores after meiosis (as expected for the ES mutations), but when combined with *rad52*, only dead spores are produced. The failure to rescue *rad52* is observed both in congenic diploids of *hop1* (see MATERIALS AND METHODS) and in isogenic and outcrossed diploids of *red1* (see MATERIALS AND METH-

TABLE 5

Mutations in ES genes do not rescue *rad52 spo13* diploids

Diploid name	Relevant genotype	Sporulation (%)	Spore viability (%)	No. of spores examined
Controls				
RM26[a]	*SPO13*	66	97	300
RM69[b]	*spo13-1*	65	62	112
C3-16[a]	*spo13-1*	58	71	204
YM1-35[b]	*rad52::URA3 spo13-1*	4.8	0	40
RM186[a]	*rad52-1 spo13-1*	5.5	0	160
Early exchange genes				
C3-13[a]	*rec102-1 spo13-1*	52	93	396
YM1-39[b]	*rec104-Δ1 spo13-1*	49	90	50
C3-25[a]	*rec102-1 rad52::URA3 spo13-1*	27	78	46
YM1-41[b]	*rec104-Δ1 rad52::URA3 spo13-1*	44	76	68
Early synapsis genes				
YM1-33[b]	*hop1::URA3 spo13-1*	75	95	64
YM2-1[a]	*hop1::URA3 spo13-1*	38	89	66
YM1-32[b]	*red1::URA3 spo13-1*	85	60	70
YM2-13[c]	*red1::ADE2 spo13-1*	25	64	206
YM1-16[a]	*hop1:URA3 rad52-1 spo13-1*	15	0	40
YM1-37[b]	*red1::ura3 rad52:URA3 spo13-1*	7.8	0	42
YM1-7[c]	*red1::ADE2 rad52-1 spo13-1*	5	0	120

Diploids were sporulated and spores were dissected. Spore viability was calculated as the number of viable spores divided by the total number of spores dissected (×100).

[a] These diploids are congenic to RM69 (see MATERIALS AND METHODS).

[b] These diploids are isogenic to RM69 (see MATERIALS AND METHODS).

[c] These diploids are outcrosses (see MATERIALS AND METHODS).

ODS) and with two different (though null) alleles of *red1*. For comparison, Table 5 shows the effect of mutations in the EE genes *REC104* and *REC102*; they do rescue *rad52*. All of these observations are consistent with the model proposed in Figure 1.

Does either the *hop1* or the *red1* mutation rescue the spore lethality found in a *rad50S spo13* strain? The *rad50S* mutation eliminates an activity of the *RAD50* gene that is required to process double-strand breaks (DSBs) (CAO *et al.* 1990). (For brevity, we will use the term *RAD50S* activity in this paper to refer to the function of the *RAD50* gene that is lost in *rad50S* mutants.)

TABLE 6

Mutations in ES genes do rescue *rad50S spo13* diploids

Diploid name	Relevant genotype	Sporulation (%)	Spore viability (%)	No. of spores examined
Controls				
RM26[a]	*SPO13*	66	97	300
RM69[b]	*spo13-1*	65	62	112
C3-16[a]	*spo13-1*	58	71	204
YM1-34[b]	*rad50S spo13-1*	2.1	9	32
A7-7[a]	*rad50S spo13-1*	1	8	48
Early exchange genes:				
A7-2[a]	*rec104-1 rad50S spo13-1*	63	94	36
C3-26[a]	*rec102-1 rad50S spo13-1*	54	93	80
Early synapsis genes:				
YM5-11[a]	*hop1::URA3 rad50S spo13-1*	63	64	104
YM1-36[b]	*red1::ura3 rad50S spo13-1*	68	61	100
YM5-14[c]	*red1::ADE2 rad50S spo13-1*	69	60	104

Diploids were sporulated and spores were dissected. Spore viability was calculated as the number of viable spores divided by the total number of spores dissected (×100). All data for single EE and ES mutations in combination with *spo13-1* are shown in Table 5.

[a] These diploids are congenic to RM69 (see MATERIALS AND METHODS).

[b] These diploids are isogenic to RM69 (see MATERIALS AND METHODS).

[c] This diploid is an outcross (see MATERIALS AND METHODS).

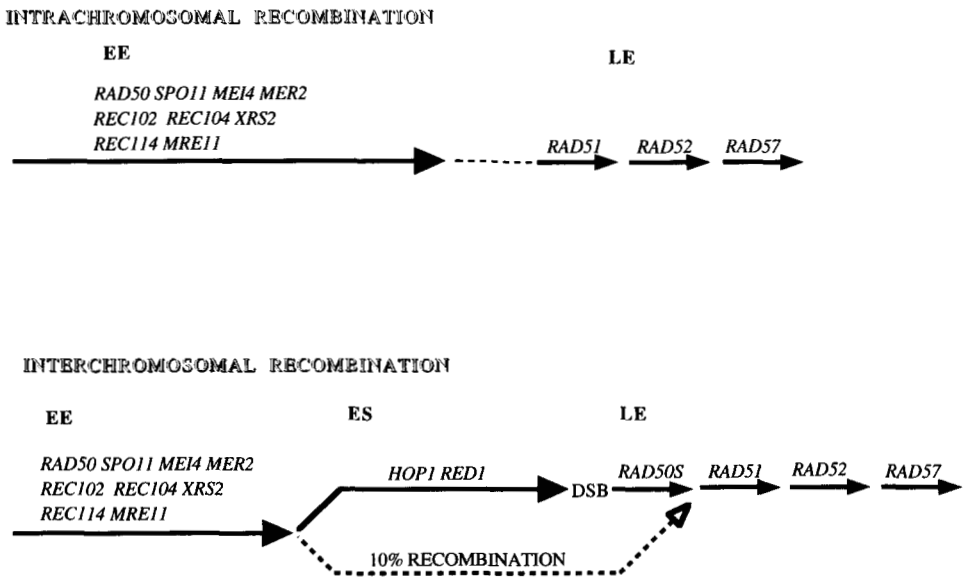

FIGURE 3.—A revised model for the meiotic recombination pathway. In this pathway, the ES genes (*e.g., RED1*) are located on the main interchromosomal exchange pathway. The pathway for the residual 10% interchromosomal recombination that occurs in the absence of the ES genes is hypothesized to return to the main exchange pathway after the point at which *RAD50S* activity is required (see DISCUSSION). The dotted line in the intrachromosomal pathway indicates potential (and unknown) steps occurring between the EE genes and the LE genes. These steps are proposed not to require ES genes because of the observations by HOLLINGWORTH and BYERS (1989) and ROCKMILL and ROEDER (1990) that mutations in the ES genes do not affect intrachromosomal recombination. Furthermore, we propose that the *RAD50S* activity is not needed in this pathway, because *red1 rad50S spo13* and *hop1 rad50S spo13* spores are viable. Since we suggest that *RAD50S* activity is not needed in intrachromosomal exchange, it is formally possible that DSBs are not part of the intrachromosomal recombination pathway. Tests of some of the predictions of this model are presented in the text (see RESULTS and DISCUSSION).

We find, as CAO *et al.* (1990) did, that a *rad50S spo13* diploid strain has poor spore viability (8–9%) after meiosis, compared with 60–70% viable spores produced in a *spo13* diploid (Table 6). For this reason, we classify *RAD50S* activity as a LE function. If the ES genes were really part of a parallel pathway (Figure 1), they should not be able to rescue *rad50S*. The data in Table 6 indicate that both *hop1* and *red1* can rescue the *rad50S* allele. The spore viability of the *hop1 rad50S spo13* and the *red1 rad50S spo13* diploids is as good as a Rec$^+$ *spo13* diploid. The EE mutations *rec102* and *rec104*, as expected, also rescue *rad50S*, although we do note that the spore viability is even higher than in strains with mutations in the ES genes.

Clearly, the model as proposed in Figure 1 cannot be correct. How can it be that mutations in ES genes prevent the spore inviability in *rad50S spo13* stains, but not in *rad52 spo13* strains? HOLLINGSWORTH and BYERS (1989) found that *hop1* mutations did not affect intrachromosomal meiotic recombination. ROCKMILL and ROEDER (1990) found that *red1* did not affect intrachromosomal recombination between 11.5-kb repeats of the *LEU2-HIS4* region of chromosome *III*, nor between 13.2-kb repeats of the *HIS4* gene region. ROCKMILL and ROEDER (1990) did find that *red1* modestly reduced intrachromosomal recombination when measured by a 2.8-kb duplication. They suggested that *red1* may be required in intrachromosomal exchange only for relatively short segments of homology (see DISCUSSION).

To explain the observations above, we would like to suggest that, for almost all intrachromosomal recombination, neither *RED1* nor *HOP1* is required. Furthermore, we suggest that *RAD50S* activity also is not required for intrachromosomal recombination events. This would lead to the results observed in the experiments above; a proposed model is shown in Figure 3. In this scheme, the failure of either *hop1* or *red1* mutations to prevent the dead spores produced in *rad52 spo13* strains is due to the fact that *RAD52* is required in the meiotic intrachromosomal exchange pathway (GAME 1983; KLEIN 1988; DORNFELD and LIVINGSTON 1992).

Is *RAD50S* activity required for intrachromosomal recombination in meiosis? If the pathway shown in Figure 3 is correct, *RAD50S* activity should not be needed for intrachromosomal recombination. We tested intrachromosomal recombination in two ways (Table 7). First, we examined a haploid strain containing a repeated sequence of *HIS4*. (The amount of homology between the two repeats was 3.0 kb; see Figure 2A.) There is no interchromosomal recombination possible in a haploid strain; only intrachromosomal exchange can occur. The data in Table 7A clearly indicate that the *rad50S* mutation does not affect intrachromosomal recombination in this *HIS4* repeat. As a control, we examined the effect of the *red1* mutation; it confers no reduction in intrachromosomal exchange. These results are consistent with the pathway in Figure 3.

TABLE 7

The *rad50S* mutation has no effect on intrachromosomal meiotic recombination

Strain name	Experiment no.	Intrachromosomal recombination His$^+$ rate: Mitotic ($\times 10^3$)	Intrachromosomal recombination His$^+$ rate: Meiotic ($\times 10^3$)	Intrachromosomal recombination His$^+$ rate: Induction	Percentage sporulation[a]	Spore viability (%)[b]
A. Haploid (*spo13*$^-$)						
ME157-14A (*RAD50 RED1*)	1	0.052	35	673	37	57.5
	2	0.102	57	558	17	37.5
ME157-14A-10 (*rad50S*)	1	0.052	57	1096	25	37.5
	2	0.045	60	1333	23	32.5
ME157-14A-11 (*red1::URA3*)	1	0.056	33	589	25	62.5
	2	0.079	50	633	25	40.0
B. Diploid (*SPO13*)						
YM5-23 (*RAD50 RED1*)	1	0.072	1.3	18.1	17	92.5
	2	0.060	1.1	18.3	24	95.0
YM5-24 (*rad50S*)	1	0.114	2.2	19.3	16	5.0
	2	0.088	1.6	18.2	13	7.5
YM1-42 (*red1::URA3*)	1	0.084	1.5	17.8	10	2.5
	2	0.071	1.2	16.9	11	2.5

All the haploid strains are isogenic to the MEI57-14A control; relevant genotypes are given in parentheses. All the diploids are isogenic to the YM5-23 control. Cells were sporulated and plated on complete medium and medium lacking histidine at 0 hr in sporulation medium to determine the mitotic His$^+$ frequency and after 24 hr in sporulation medium to determine the meiotic His$^+$ frequency. The mitotic His$^+$ frequencies were converted to recombination rates for each culture from the equation $r = 0.4343f/(\log N - \log N_0)$ where r = rate, f = frequency; N = final total cell number, and N_0 = initial cell number (DRAKE 1970). The meiotic His$^+$ rate is the same as the meiotic His$^+$ frequency. Induction of meiotic recombination was calculated as the ratio of meiotic His$^+$ rate to mitotic His$^+$ rate.

[a] At least 200 cells were counted from each culture at the 24-hr time point to determine percent sporulation.

[b] Spore viability was determined by dissection from plates. At least 80 spores were examined for each strain.

Second, we tested a repeat of the *HIS3* gene in diploid strains, originally constructed by KAYTOR and LIVINGSTON (1994) (Table 7B). The amount of homology between the repeats is 4.6 kb (Figure 2B). The data demonstrate that neither *rad50S* nor *red1* reduce intrachromosomal recombination. The average induction in Rec$^+$ cells is 18.2-fold, in the *rad50S* strain 18.8-fold, and in the *red1* strain 17.4-fold. These results are also consistent with the pathway shown in Figure 3.

Is an early exchange mutation epistatic to an early synapsis mutation? The model presented in Figure 3 indicates that a mutation in an EE gene should stop recombination before a block in an ES gene such as *red1*. We therefore asked the question in two ways. First, we determined the frequency of meiotic recombination in a *rec104 red1 spo13* strain. The *rec104* mutation completely abolishes meiotic recombination and the *red1* mutation reduces it to ~10% of normal. The data in Table 8 indicate that the EE mutation *rec104* is epistatic to the ES mutation *red1*, consistent with the pathway drawn in Figure 3. Second, since the EE and the ES mutations have different effects on *rad52 spo13* strains (see above), we also tested epistasis in the presence of *rad52*. It is clear that *rec104* is epistatic to *red1* for spore viability (Table 8).

Do mutations in ES genes reduce DSBs? The model shown in Figure 3 predicts that a *red1* or *hop1* mutation should reduce the number of DSBs that occur in meiosis. We have been studying recombination at the *HIS2* recombination hotspot (MALONE *et al.* 1994). Recently, we have found that two DSBs occur during meiosis and that they are dependent upon such EE recombination functions as *REC104*, (A. M. GALBRAITH and R. E. MALONE, unpublished data) (see Figure 4). We have examined the effect of *red1* and *hop1* on meiotic DSBs at *HIS2* in the presence of the *rad50S* mutation (Figure 5). In the wild-type strain, 10.7% of the total DNA is found in the DSB bands by 12 hr into meiosis. We cannot detect any DSB bands in the *hop1* or the *red1* mutants; the amount of background present at the positions of the break bands is ~0.5% of the total DNA present. This puts an upper limit upon the amount of breaks that could be present in the *red1* and *hop1* strains. In a reconstruction experiment, we mixed known amounts of purified DNA the size of the DSB bands with a restriction digest of total genomic DNA from mitotic cells that have no breaks. We could detect breaks when present at a 0.5% level but not at a 0.1% level (data not shown). Thus, the reconstruction experiment is consistent with the answer obtained in Figure 5 and indicates that DSBs are reduced ≥20-fold by mutations in ES genes.

DISCUSSION

It is possible to distinguish between two types of mutations affecting meiotic recombination that produce via-

TABLE 8

A *rec104* mutation is epistatic to a *red1* mutation

A. Interchromosomal meiotic recombination[a]

Diploid name	Relevant genotype	Prototroph frequency ($\times 10^5$)					Drug-resistant colony frequency ($\times 10^4$)		Average reduction
		lys2-1/*lys2-2*	*his7-1*/*his7-2*	*trp5-c*/*trp5-d*	*leu1-c*/*leu1-d*	*met13-c*/*met13-d*	*cyh2*^{r}/*CYH2*^{S}	*can1*^{r}/*CAN1*^{r}	
RM69	*spo13-1*	14 (1.0)	117 (1.0)	3400 (1.0)	1600 (1.0)	4300 (1.0)	2400 (1.0)	3400 (1.0)	– (1.0)
YM1-32	*red1::URA3 spo13-1*	5.0 (0.36)	7.2 (0.06)	67 (0.02)	57 (0.04)	250 (0.06)	350 (0.15)	360 (0.11)	– (0.11)
YM1-39	*rec104-Δ1 spo13-1*	0.23 (0.02)	0.49 (0.004)	3.6 (0.001)	3.3 (0.002)	1.7 (0.0004)	2.2 (0.001)	4.6 (0.001)	– (0.004)
YM1-38	*red1::ura3 rec104-Δ1 spo13-1*	0.27 (0.02)	0.48 (0.004)	3.5 (0.001)	2.0 (0.001)	1.7 (0.0004)	4.0 (0.002)	7.6 (0.002)	– (0.004)

B. Sporulation and viability[b]

Diploid name	Relevant genotype	Sporulation (%)	Spore viability (%)	No. of spores examined
YM1-35	*rad52::URA3 spo13-1*	4.8	0	40
YM1-37	*red1::ura3 rad52::URA3 spo13-1*	7.8	0	42
YM1-41	*rec104-Δ1 rad52::URA3 spo13-1*	44	76	68
YM1-40	*red1::ura3 rec104-Δ1 rad52::URA3 spo13-1*	24	80	40

All the diploids in this table are isogenic.

[a] Each diploid was sporulated and plated on various selective media and the frequency of recombination was determined. The frequencies given are the geometric means of at least three independent meiotic cultures. The numbers in parentheses represent the amount of recombination relative to wild type. Average reduction is the arithmetic mean of the relative frequency of all seven loci shown.

[b] Diploids were sporulated and spores were dissected. Spore viability was calculated as the number of viable spores divided by the total number of spores dissected × 100. At least 200 cells were counted to determine percent sporulation.

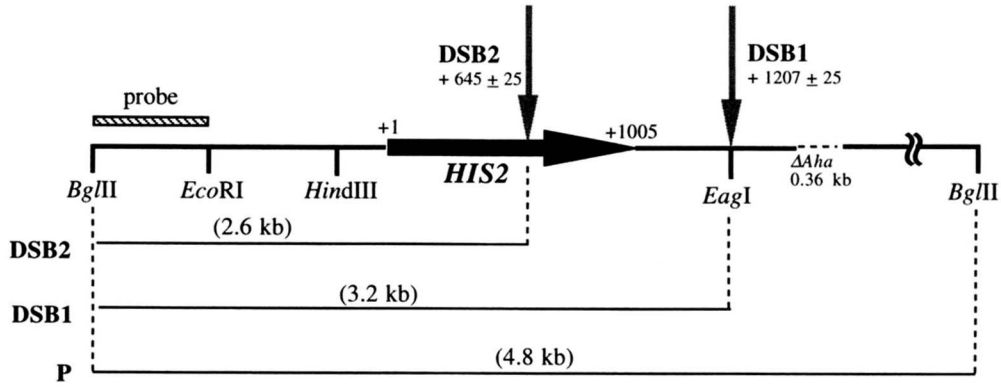

FIGURE 4.—Physical map of the *HIS2* region. The two *Bgl*II sites produce a parental fragment of 4.8 kb in a *HIS2::ΔAha* strain. The 0.36-kb *Aha-Aha* deleted region is shown as the dotted line. The two DSB fragments are 3.2 and 2.6 kb. The 1.1-kb *Bgl*II-*Eco*RI fragment used as a probe is also indicated on the map.

ble spores in the presence of a mutation in the *spo13* gene. Mutations in early exchange (EE) genes have been shown to completely abolish both inter- and intrachromosomal meiotic recombination (*e.g.*, WAGSTAFF *et al.* 1985; MENEES and ROEDER 1989; MALONE *et al.* 1991; SCHWACHA and KLECKNER 1994), consistent with EE genes playing a role in the initiation of all types of meiotic recombination. In addition, in all cases where it has been tested, mutations in EE genes are epistatic to mutations in genes required later in recombination (*e.g., rad52*) (*e.g.*, MALONE 1983; ENGEBRECHT and ROEDER 1989; COOL and MALONE 1992). The other type of early genes consists of *HOP1, RED1,* and *MEK1.* We have called them early (because they are viable with *spo13*) synapsis (because at least *HOP1* and *RED1* gene products have been reported to be components of the SC) genes (HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990; cited in NAG *et al.* 1995). Mutations in these ES genes reduce interchromosomal recombination only to ~10% of the normal level and have no (or in one case, only a modest) effect on intrachromosomal events. The work in this paper indicates that mutations in ES genes differ in yet another way from mutations in EE genes, because *hop1* and *red1* mutations are not epistatic to a *rad52* mutation. Thus, there are at least three mutant phenotypes that distinguish the EE and the ES genes.

The simple pathway suggested at the beginning of this work (Figure 1) was based on the known differences in the phenotypes of mutations in EE and ES genes. It was also based on the failure to obtain mutations in either *HOP1, RED1,* or *MEK1* during a selection

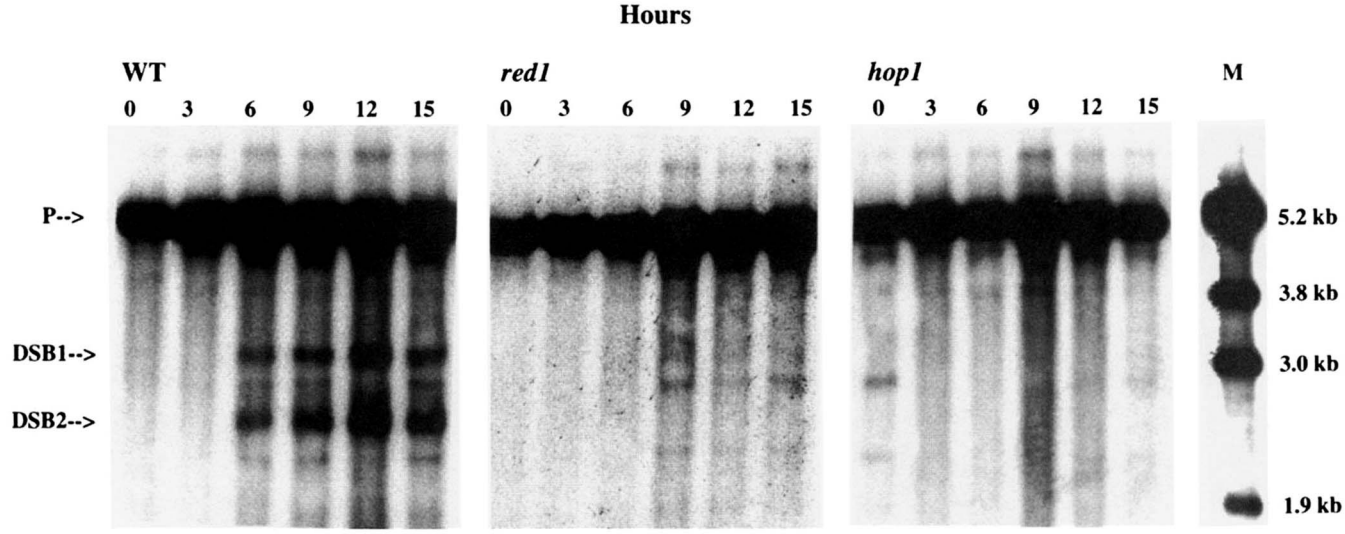

FIGURE 5.—Formation of meiotic DSBs at the *HIS2* locus in *hop1*, *red1*, and wild type diploids. Time course of DSB formation in isogenic *HIS2::ΔAha* diploids homozygous for *red1 rad50S* (YM5-36), *hop1 rad50S* (YM5-37), or *rad50S* (SK5-11); the SK5-11 diploid is referred to as "WT" in the figure. Numbers above the lane refer to the time in hours that the sample was taken from the sporulating culture. P, parental 4.8-kb *Bgl*II-*Bgl*II *HIS2::ΔAha* fragment; DSB1, DSB fragment No. 1 (3.2 kb); DSB2, DSB fragment No. 2 (2.6 kb). Note that the 12-hr time point in the SK5-11 series has approximately twice as much DNA as the other lanes; this was done to make the DSB bands really pronounced. M indicates markers representing different size DNA fragments.

for mutations that could rescue *rad52*; >170 mutations in eight meiosis specific EE genes were isolated, but no mutations were found in any of the three ES genes (MALONE *et al.* 1991). The idea that the ES genes act in a parallel pathway (Figure 1) is consistent with the failure of mutations in either *RED1* or *HOP1* to rescue a *rad52 spo13* strain. However, the idea of a parallel synapsis pathway is not consistent with the ability of *red1 rad50S spo13* and *hop1 rad50S spo13* strains to produce live spores (Table 6). We therefore proposed a pathway (Figure 3), which supposes that recombination occurring within a chromosome occurs in a different fashion than that occurring between homologous chromosomes. This is not a novel idea; indeed, HOLLINGSWORTH and BYERS (1989) proposed it when they isolated *HOP1*. Likewise, ROCKMILL and ROEDER (1990) have suggested it in discussions of *RED1*. In addition, the new model does place the ES genes in the main interchromosomal exchange pathway, in a position between the EE genes and the LE genes.

The pathway in Figure 3 was motivated, in part, by the difference in the interactions between mutations in the ES genes and *rad52 vs. rad50S*. The data we have presented (Table 7) suggest that *RAD50S* activity, like the activities encoded by *HOP1* and *RED1*, is not required for intrachromosomal exchange. Since it has been elegantly demonstrated that *rad50S* mutants cannot process the DSBs that occur during recombination in meiosis (CAO *et al.* 1990), this would appear to be consistent with the proposal that DSB's do not normally play a role in intrachromosomal meiotic recombination. However, we note that GILBERTSON and STAHL (1994) and DEMASSEY *et al.* (1994) have demonstrated that meiotic DSB's can be observed in a haploid meiosis. DEMASSEY *et al.* (1994) further demonstrated that the haploid DSBs accumulate in a *rad50S* strain, yet are processed in a *RAD50* strain. How can one resolve the apparent paradox that DSBs occur and accumulate in *rad50S* haploids but that intrachromosomal recombination is not affected? We can think of at least four possible reasons. A trivial explanation is that the haploid DSB experiments done by both labs above were in *SPO13* strains, whereas our haploid experiments utilized *spo13* mutants. We do not see why this should make a difference, but it remains a formal possibility. A second possibility is that the breaks that occur in haploids are acted upon by another function that substitutes for *RAD50S* activity. This putative function must act later than *RAD50S* activity, however, since GILBERTSON and STAHL (1994) saw no decrease in the DSBs that accumulate in a *rad50S* haploid meiosis ≤10 hr in meiosis. DEMASSEY *et al.* (1994) reported one haploid experiment in which haploid *rad50S* DSBs begin to decrease at ~12 hr and a second in which they were constant until 24 hr. A third possibility is that the DSBs that have been observed in haploids are not used in the intrachromosomal recombination we measure. In this view, even if *RAD50S* activity were needed to process the breaks, a *rad50S* mutation would not decrease recombination frequency. The fact that both *red1* and *hop1* mutations reduce meiotic DSBs by a factor of 20-fold yet do not reduce intrachromosomal recombination is consistent with this third idea. A fourth explanation is that the DSBs observed in haploid meiosis are actually healed in mitosis after germination of the haploid spores.

ROCKMILL and ROEDER (1990) observed that *RED1* was not needed for intrachromosomal recombination between two different large (>10 kb) repeat constructs, consistent with its other ES phenotypes. They also reported that a *red1* mutation did reduce recombination between two 2.8-kb repeats by a factor of 12-fold. They suggested that perhaps *RED1* was only needed for small repeats. We find that *RED1* is unnecessary for recombination in either of the repeat constructs we used; one has 3.0 kb and the other has 4.6 kb of homology. Taking all the data together, we would argue that the "majority" phenotype (4/5 cases) of a *red1* mutation is that it does not affect intrachromosomal exchange.

In the absence of *RED1* or *HOP1*, 10% of the normal level of meiotic recombination still occurs. Our data cannot clearly distinguish whether this 10% residual interchromosomal meiotic recombination utilizes DSBs or not. The upper limit for DSBs in the *hop1* and *red1* mutant strains shown in Figure 5 is ~0.5% of the total DNA. The normal amount of DSBs observed at *HIS2* in the strains used is 10.7%. Since we have shown that the amounts of DSBs at *HIS2* correlate with the amount of recombination (S. A. BULLARD and R. E. MALONE, unpublished data), 10% of the meiotic level of recombination should therefore correspond to ~1.1% DSBs (*i.e.*, 10 × 10.7% DSBs). The difference between ≤0.5% and 1.1% of the total DNA is too slight to make an argument that the residual pathway occurs without DSBs. Why then do we draw the alternative pathway (in Figure 3) joining the late exchange genes after DSBs and *RAD50S* activity? We propose this primarily because of the epistasis of the *red1* and *hop1* mutations with *rad50S*. If *RAD50S* activity were required for recombination in the residual 10% *HOP1* and *RED1* independent pathway, then it is not obvious why *red1* or *hop1* mutations should produce viable spores with *rad50S spo13*.

It is clear from the data presented here that a *rec104* mutation is epistatic to a *red1* mutation, consistent with the pathway drawn in Figure 3. It is formally possible that this observation would not apply to other EE genes and other ES genes, but considering the similar phenotypes conferred by mutations in the different genes within each group, we predict that this observation will be general. That is, blocking recombination by a mutation in any EE gene will prevent the phenotypes conferred by a mutation in any ES gene. To the best of our knowledge, the data presented here is the first test of the epistasis between

mutations in an EE and an ES gene. In their study of *mek1* mutations, ROCKMILL and ROEDER (1991) showed that *mer1 mek1* double mutants showed no induction of meiotic recombination, whereas both *mer1* and *mek1* alone still had ~10% residual meiotic recombination. That is, neither *mek1* nor *mer1* is epistatic to the other; the effect in the double mutant is synergistic. The difficulty in applying this observation to predictions about the EE and ES genes is that *mer1* mutants are atypical EE mutants; *mer1* reduces recombination to 10% of normal, not to zero (see Table 1). The *MER1* gene is needed to splice *MER2* mRNA (and perhaps some other meiotic genes as well) (ENGEBRECHT *et al.* 1991), rather than acting directly in the recombination pathway.

Since, wherever it has been tested, mutations in EE genes prevent the formation of meiotic DSBs (*e.g.*, *rad50* and *spo11*—CAO *et al.* 1990; *xrs2*—IVANOV *et al.* 1992; *mer2*—ROCKMILL *et al.* 1995; *rec104*—A. M. GALBRAITH and R. E. MALONE, unpublished data), LICHTEN and colleagues have pointed out that one possible activity of the EE genes is to make DSBs (LIU *et al.* 1995). The observations that meiotic DSBs at the *HIS2* locus are greatly reduced in *hop1* and *red1* mutants and that *red1 rad50S spo13* and *hop1 rad50S spo13* strains produce viable spores suggest that these ES genes also may be required for most DSB formation. In a study of the kinetics of DSB formation at the *HIS4::LEU2* hotspot and SC formation, PADMORE *et al.* (1991) found that DSBs could be detected before mature tripartite SC. This observation is not inconsistent with our data, which indicates that mutations in *RED1* and *HOP1* reduce DSBs. Although *RED1* and *HOP1* are required for mature SC formation, they may act at a relatively early step in its formation. It is not unreasonable to suppose that some SC components are assembled on the DNA before DSBs are created, DSBs then occur, and that only then is the mature SC assembled.

The pathway shown in Figure 3 is unlikely to be correct in every detail. However, it is consistent with the tests presented in this paper. The fact that the ES genes can be placed on the main interchromosomal exchange path is encouraging, and predicts that there may be (direct or indirect) interactions between EE and ES genes. One such potential interaction has been found by HOLLINGSWORTH and JOHNSON (1993) and FRIEDMAN *et al.* (1994). The *REC104* gene on a high copy plasmid can suppress several different alleles of the *HOP1* gene. We are currently testing possible ways that this suppression might occur.

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