RDH54, a *RAD54* Homologue in *Saccharomyces cerevisiae*, Is Required for Mitotic Diploid-Specific Recombination and Repair and for Meiosis

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ABSTRACT

Most mitotic recombination and repair genes of *Saccharomyces cerevisiae* show no specificity of action for the genome ploidy. We describe here a novel repair and recombination gene that is specific for recombination and repair between homologous chromosomes. The *RDH54* gene is homologous to the *RAD54* gene, but *rdh54* mutants do not show sensitivity to methyl methanesulfonate at concentrations that sensitize a *rad54* mutant. However, the *rdh54* null mutation enhances the methyl methanesulfonate sensitivity of a *rad54* mutant and single *rdh54* mutants are sensitive to prolonged exposure at high concentrations of methyl methanesulfonate. The *RDH54* gene is required for recombination, but only in a diploid. We present evidence showing that the *RDH54* gene is required for interhomologue gene conversion but not intrachromosomal gene conversion. The *rdh54* mutation confers diploid-specific lethalities and reduced growth in various mutant backgrounds. These phenotypes are due to attempted recombination. The *RDH54* gene is also required for meiosis as homozygous mutant diploids show very poor sporulation and reduced spore viability. The role of the *RDH54* gene in mitotic repair and in meiosis and the pathway in which it acts are discussed.

THE *RAD54* gene functions in mitotic recombination and repair. As a member of the *RAD52* epistasis group of repair genes, *rad54* mutants are sensitive to ionizing radiation and the alkylating agent methyl methanesulfonate (MMS). These phenotypes suggest a function for the Rad54p in double-strand break (DSB) repair and some of the mitotic recombination defects of *rad54* mutants bear this out. In early studies *rad54* mutant diploid strains were found to have significantly reduced levels of spontaneous and induced mitotic recombination (SAEKI *et al.* 1980). More recently, *rad54* mutant strains have been reported to be defective in healing of an HO-induced DSB when the donor sequences are not accessible or are located on a chromosome, but are proficient in repair of the break when the donor sequences are accessible on a plasmid molecule (SUGAWARA *et al.* 1995). *rad54* mutants are also defective in spontaneous recombination, primarily in gene conversion events (LIEFSHITZ *et al.* 1995; RATTRAY and SYMINGTON 1995). In contrast, MCDONALD and ROTHSTEIN (1994), FAN and KLEIN (1994), and LIEFSHITZ *et al.* (1995) all found that *rad54* mutants exhibited an increased frequency in deletions between direct repeats. This last finding suggests that lesions accumulate in the *rad54* mutant that stimulates direct repeat deletions and that the deletions can occur in the absence of the Rad54 protein.

The *RAD54* gene is a member of the SNF2/SWI2 superfamily (EMERY *et al.* 1991; EISEN *et al.* 1995) and contains the seven motifs found in most DNA helicase proteins. The Rad54 protein is associated with the Rad51 protein both *in vivo* and *in vitro* (JIANG *et al.* 1996), suggesting that it is part of a protein complex, possibly involved in chromatin accessibility during recombination (SUGAWARA *et al.* 1995). A *rad54* null mutant is synthetically lethal with the DNA helicase mutant *srs2* (ABOUSSEKHRA *et al.* 1989; PALLADINO and KLEIN 1992), suggesting either an overlap in function or accumulation of a lethal DNA recombination repair intermediate.

Studies on the types of recombination events that are affected by *rad* mutations have shown that *Saccharomyces cerevisiae* has multiple pathways for mitotic recombination (RATTRAY and SYMINGTON 1995; BAI and SYMINGTON 1996). Mitotic cells can undergo spontaneous mitotic recombination through a strictly gene conversion mechanism or through an associated pathway that links gene conversion and crossing over. *RAD54* functions in the unassociated gene conversion pathway and from the data reported from several labs, this pathway appears to be operative in both haploid and diploid cells. The haploid pathway must involve either sister chromatid recombination in G2 to repair DNA damage or ectopic and intrachromosomal recombination between repeated sequences. The existence of multiple pathways with specific components might explain why several of the *rad* mutants of the *RAD52* epistasis group still retain significant levels of mitotic recombination and in a few cases some meiotic viability.

rad mutants that have a deficiency in mitotic gene

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conversion events usually are not restricted by the cell ploidy for this phenotype. The mutants show a reduction in intrachromosomal gene conversion between repeated sequences in haploid strains and gene conversion between allelic and ectopic gene copies in diploid strains. One exception is the *rad59* mutant, which is reduced in mitotic intrachromosomal recombination but shows a higher than normal interchromosomal recombination rate (BAI and SYMINGTON 1996).

We report here on a novel gene, termed *RDH54* for rad homologue 54, which functions in diploid-specific mitotic recombination and also is required for complete meiotic viability. We propose that the *RDH54* gene acts in repair and recombination in a mitotic pathway that is distinct from *RAD54*.

MATERIALS AND METHODS

Yeast strains: Strains used in this study are listed in Table 1. All strains are isogenic and are in the W303 background (W303 genotype: *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*). The *rdh54::HIS3* disruption was made in the wild-type strain HKY579-10A. A PCR reaction using primers 5′GAGAACAAGCCATTCAAGCCTCCAAGAAGGGTTGGAT CAAATAAGTAGGATCCGCTGCACGGTCCTG3′ and 5′GCG CACTTGTCCATGTCGTAGTGCTACGCTTTCTCAGCTCT ACGGTGGATCCTCGGGGACACCAAA3′ with the plasmid YIp1 amplified the *HIS3* gene to give a product that had 46–47 bp at each end derived from open reading frame (ORF) YBR073w. The product was used to transform HKY579-10A. His^+ transformants were screened by PCR of total DNA using primers 5′GAGAACAAGCCATTCAAGCCTCCAAG3′ and 5′GCGCACTTGTCCATGTCGTAGTGC3′.

Media and growth conditions: Standard media were prepared as described (SHERMAN *et al.* 1986). All strains were grown on solid media or in liquid media at 30°. Percentage sporulation was determined by microscopic examination of diploids after 3 days of culture on sporulation plates at 25°. Strains were transformed according to the lithium acetate procedure of ITO *et al.* (1983) as modified by HILL *et al.* (1991).

MMS sensitivity: Yeast strains were grown in rich medium (YEPD) to saturation phase at 30°. Cells were pelleted and washed once in sterile water. Cells were pelleted again and resuspended in 0.05 M KH_2PO_4 (pH 7.0) at a concentration of 10^7 cells/ml and MMS added to a concentration ranging from 0.1% to 0.5%, depending on the experiment. At 10-min intervals, 0.5 ml MMS-treated cells were added to 0.5 ml 10% $Na_2S_2O_3$ to inactivate the MMS. The cells were then serially diluted, plated on rich medium and incubated at 30°. Survival was determined after 2 days.

Sensitivity to HO endonuclease: Yeast strains transformed with plasmid pGAL-HO, which carries the *HO* gene under control of the *GAL1* promoter, were grown to saturation in selective medium. Cells were diluted 2×10^4-fold into YEPL, containing 3% (wt/vol) lactic acid pH 6.5, and grown overnight at 30° to a density of 1×10^6 to 5×10^6 cells/ml. The cultures were split in half, galactose was added at a final concentration of 2% to one flask from each strain and cells were incubated at 30° for an additional 1.5 hr. Cells were collected by centrifugation, washed with dH_2O, diluted and plated on YEPD medium. Cells were counted after 2 days. The ratio of colony forming units of the galactose-treated cells to glucose only cells was used as an indicator of sensitivity to HO endonuclease.

Genetic analysis and recombination rate determinations: Genetic analyses were performed according to published procedures (SHERMAN *et al.* 1986). Recombination rates were calculated according to the median method of LEA and COULSON (1948) as described (AGUILERA and KLEIN 1988). Strains were streaked on solid YEPD medium and grown at 30° for 2–3 days. Nine colonies from each strain were used for each fluctuation test. Rate determinations in diploid strains were done on fresh zygotes with three zygotes from each cross.

DNA manipulations: Plasmid DNA was isolated from *Escherichia coli* by the alkaline lysis procedure (SAMBROOK *et al.* 1989). Plasmid DNA was treated with restriction endonucleases according to the manufacturer's specifications. Genomic DNA was purified from yeast cells according to ROSE *et al.* (1990). Southern analysis was performed as previously described (FAN *et al.* 1996). For ligation, DNA fragments were gel purified (QIAEX, gel purification kit from QIAGEN) and ligated using T4 DNA ligase (Boehringer Mannheim) at 16° overnight. PCR reactions were performed with Taq DNA polymerase (Boehringer Mannheim) for a total of 25–30 cycles. One hundred picomoles of primers, 1 ng template (genomic or plasmid DNA) in 100 μl buffer (provided by the manufacturer) with 200 μM dNTP were incubated at 92° for 5 min and then cycled at 92° (1 min), 55° (2 min) and 72° (2 min).

RNA manipulation: Northen analysis was performed as previously described (FAN and KLEIN 1994).

RESULTS

Phenotypes of the *rdh54* null mutant: In the report of VAN DER AART *et al.* (1994) on the sequence of a portion of chromosome II of *S. cerevisiae*, an ORF called YBR0715 and now renamed YBR073w was noted to have significant homology to the recombination and repair gene *RAD54*. To ascertain whether this ORF had any functional relationship to *RAD54*, the null allele (*rdh54*Δ) was constructed in strain W303 and examined for any phenotype related to recombination or repair. The *rdh54* null mutant haploid displayed no UV sensitivity (data not shown) or MMS sensitivity to brief exposures in liquid (Figure 1), nor was it sensitive to DSBs induced by the HO endonuclease in either the haploid or diploid state (data not shown). However, we observed that the *rdh54*Δ mutation enhanced the MMS sensitivity of the *rad54* null mutant in diploid strains ~10-fold after exposure for 30 min in liquid and ~100-fold after exposure for 50 min in liquid (Figure 1). This additional sensitivity of the *rad54* mutant by the *rdh54* mutation could be observed only at low MMS concentrations. At higher MMS concentrations the *rad54*Δ mutant was killed too fast to permit a discrimination between the *rad54*Δ *RDH54* and *rad54*Δ *rdh54*Δ strains.

On solid medium the *rdh54*Δ mutant did not display an MMS-sensitive phenotype and the plating efficiency was close to wild type when a MMS concentration of 0.016% was used. At this concentration mutants such as *rad50, rad51, rad52* and *rad54* are sensitive. When higher concentrations of MMS were used, the *rdh54* mutant grew considerably slower than wild type such that at 1–2 days after plating on a YEPD plate containing MMS, no colonies were obtained from the

TABLE 1

Strains

Strain	Genotype
HKY579-10A	*MATa leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY632-3B	*MATa rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY632-2B	*MATα rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY641-3A	*MATa rdh54::HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY641-6C	*MATα rdh54::HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-1B	*MATa leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-2B	*MATα leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-3A	*MATa leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-4D	*MATα leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-2A	*MATa rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-1D	*MATα rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-3C	*MATa rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-8D	*MATα rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-5D	*MATa rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-2D	*MATα rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-9A	*MATa rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-1A	*MATα rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-1C	*MATa rdh54:HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-3B	*MATα rdh54:HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-2C	*MATa rdh54:HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY638-5C	*MATα rdh54:HIS3 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY650-19B	*MATa rdh54::HIS3 srs2::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY650-19C	*MATα rdh54::HIS3 srs2::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY658-2B	*MATa rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY658-8C	*MATα rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY658-2C	*MATa rdh54::HIS3 rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY658-11B	*MATα rdh54::HIS3 rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-2C	*MATa leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-1D	*MATα leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-1A	*MATa rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-2B	*MATα rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-1B	*MATa rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-2D	*MATα rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-2A	*MATa rdh54::HIS3 rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY682-1D	*MATα rdh54::HIS3 rad51::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY681-1B	*MATa rdh54::HIS3 rad51::LEU2 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY681-10D	*MATa rdh54::HIS3 rad51::LEU2 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY681-5C	*MATα rdh54::HIS3 rad51::LEU2 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY681-9B	*MATα rdh54::HIS3 rad51::LEU2 rad54::LEU2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-1B	*MATα mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-11C	*MATa mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-1D	*MATa spo13::URA3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-3A	*MATα spo13::URA3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-8A	*MATa spo13::URA3 mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-2B	*MATα spo13::URA3 mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-3A	*MATa spo13::URA3 rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-1A	*MATα spo13::URA3 rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-5A	*MATa spo13::URA3 rdh54::HIS3 mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY677-2D	*MATα spo13::URA3 rdh54::HIS3 mei4::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-6C	*MATa spo13::URA3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-3B	*MATα spo13::URA3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-1B	*MATa spo13::URA3 rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-1D	*MATα spo13::URA3 rdh54::HIS3 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-2D	*MATa spo13::URA3 red1::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY687-8D	*MATα spo13::URA3 red1::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1*
HKY687-9B	*MATa spo13::URA3 rdh54::HIS3 red1::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 can1-100*
HKY687-6B	*MATα spo13::URA3 rdh54::HIS3 red1::ADE2 leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY661-3C	*MATα leu2-rI::URA3::leu2-bsteII his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY661-1D	*MATa leu2-rI::URA3::leu2-bsteII rdh54::HIS3 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY661-4B	*MATα leu2-rI::URA3::leu2-bsteII rdh54::HIS3 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY669-1B	*MATα leu2-rI his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY670-13C	*MATa leu2-bsteII his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY668-2D	*MATa leu2-rI rdh54::HIS3 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*
HKY667-5A	*MATα leu2-bsteII rdh54::HIS3 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*

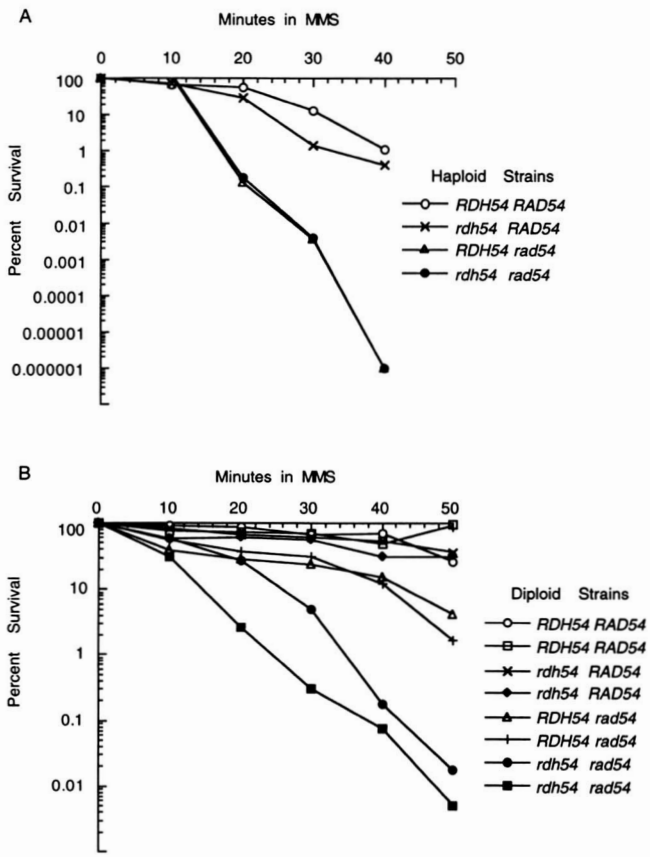

FIGURE 1.—MMS sensitivity of *rdh54* and *rdh54 rad54* strains. Strains were grown in rich medium overnight, pelleted, washed and resuspended in 0.05 M KH_2PO_4 (pH 7.0) at 10^7 cells/ml. MMS was added and cells were incubated at 30° with constant aeration in a roller drum. Aliquots (0.5 ml) were removed at the indicated times and exposure to MMS was stopped by the addition of 0.5 ml 10% $Na_2S_2O_3$. Cells were diluted and plated on YEPD. After 2 days incubation at 30°, survival was determined. (A) Survival of haploid strains following treatment with 0.5% MMS. Strains used were as follows: HKY638-1B (*RDH54 RAD54*), HKY638-2A (*rdh54 RAD54*), HKY638-5D (*RDH54 rad54*) and HKY638-1C (*rdh54 rad54*). (B) Survival of diploid strains following treatment with 0.1% MMS. Strains used were as follows: HKY638-1B × HKY638-2B and HKY638-3A × HKY638-4D (*RDH54 RAD54*), HKY638-2A × HKY638-1D and HKY638-3C × HKY638-8D (*rdh54 RAD54*), HKY638-9A × HKY628-2D and HKY638-5D × HKY628-2D (*RDH54 rad54*), and HKY638-1C × HKY638-3B and HKY638-2C × HKY638-5C (*rdh54 rad54*).

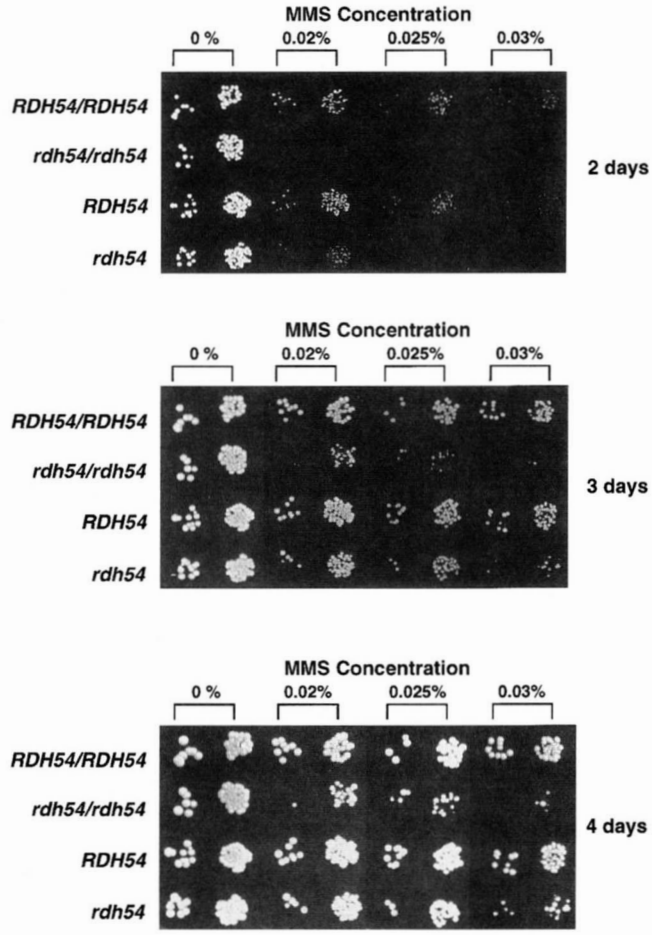

FIGURE 2.—MMS sensitivity of *rdh54* haploid and diploid strains with constant exposure. Cells were grown overnight in liquid YEPD to stationary phase. Cells were diluted and 10-μl aliquots were spotted onto freshly made YEPD plates with MMS as indicated. Plates were incubated at 30° for the indicated times. For each MMS concentration the left column is plated from a dilution of 10^5 and the right column is plated from a dilution of 10^4. Strains used were as follows: HKY638-1B × HKY638-2B (*RDH54/RDH54*), HKY638-2A × HKY638-1D (*rdh54/rdh54*), HKY638-1B (*RDH54*) and HKY638-2A (*rdh54*).

*rdh54*Δ strains, whereas the wild-type strains did grow. By 3 days some growth was seen for the *rdh54*Δ strains and by 4 days the *rdh54*Δ strains were grown, although with reduced viability and plating efficiency compared to wild type. This MMS sensitivity was stronger in the *rdh54*Δ diploid strain (Figure 2).

Double mutant analyses with the *rdh54* null allele: Since the *rad54* null allele is lethal in combination with the *srs2* null allele (ABOUSSEKHRA *et al.* 1989; RONG *et al.* 1991), we examined the phenotype of the *srs2*Δ *rdh54*Δ double mutant. The haploid double mutant showed normal growth and viability. However, the homozygous diploid *srs2*Δ/*srs2*Δ *rdh54*Δ/*rdh54*Δ was inviable. Fifty-six out of 56 zygotes from crosses of the double mutant haploid parents failed to grow beyond two divisions. Since the *srs2*Δ mutant had been proposed to undergo lethal recombination events in diploids (ABOUSSEKHRA *et al.* 1989) and the lethality with *rdh54*Δ was diploid specific, we asked if this was related to recombination. If this were so, then prevention of recombination through a *rad51* mutation should restore viability. The *srs2*Δ/*srs2*Δ *rdh54*Δ/*rdh54*Δ *rad51*Δ/*rad51*Δ triple mutant showed normal viability and growth in 10 out of 10 zygotes tested. The inviability appeared to be related to the diploid chromosome state rather than heterozygosity at the mating type locus. We introduced a *MAT*α plasmid into a *MAT***a** *srs2*Δ *rdh54*Δ haploid strain carrying the wild-type *SRS2* allele on a plasmid. This strain could readily lose the wild-type *SRS2*-containing plasmid, indicating that the haploid

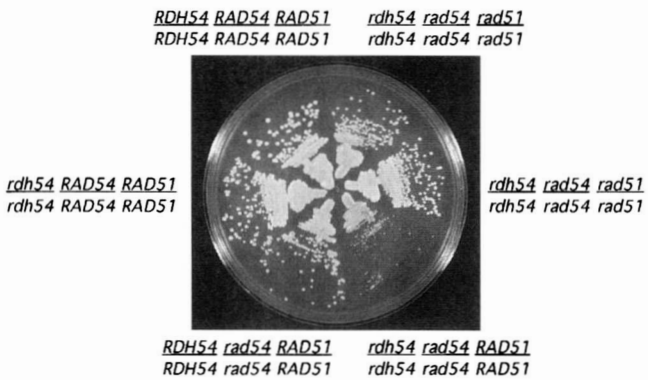

FIGURE 3.—The *rdh54 rad54* slow growth in diploids is suppressed by a *rad51* mutation. Strains of the indicated genotypes were streaked from zygotes onto YEPD medium and incubated at 30° for 2 days. Strains used were HKY638-1B × HKY638-2B (*RDH54 RAD54 RAD51* diploid), HKY638-2A × HKY638-1D (*rdh54 RAD54 RAD51* diploid), HKY638-5D × HKY638-2D (*RDH54 rad54 RAD51* diploid), HKY638-1C × HKY638-3B (*rdh54 rad54 RAD51* diploid), and HKY681-1B × HKY681-5C and HKY681-9B × HKY681-10D (*rdh54 rad54 rad51* diploids).

*srs2*Δ *rdh54*Δ strain that was heterozygous at the *MAT* locus was viable. We obtained a similar result introducing a *MAT***a** plasmid into a *MATα srs2*Δ *rdh54*Δ strain carrying the *SRS2* allele on a plasmid. To investigate this further, we crossed a *MATα srs2*Δ *rdh54*Δ strain by a *MATD::hisG-URA3-hisG srs2*Δ *rdh54*Δ strain. Eight out of 14 zygotes formed colonies, while the remaining six failed to grow beyond eight cells. We verified that the eight colonies were in fact diploids. These results suggest that ploidy is important in the *srs2*Δ *rdh54*Δ diploid lethality, but that mating type heterozygosity also is an important factor.

The *rad54*Δ *rdh54*Δ double mutant haploid strain showed no growth defect, but the diploid double mutant grew slower than wild type (Figure 3). This slow growth was related to recombination as the triple mutant strain *rad54*Δ*/rad54*Δ *rdh54*Δ*/rdh54*Δ *rad51*Δ*/rad51*Δ grew as well as the *rad54*Δ*/rad54*Δ or *rdh54*Δ*/rdh54*Δ single mutant diploid strains (Figure 3). This suppression was not observed with the *dmc1*Δ allele. *DMC1* is a meiotic homologue of *RAD51* and has been shown to interact with *RDH54* in a two-hybrid test (DRESSER *et al.* 1997).

The *rdh54*Δ *rad51*Δ double mutant showed no mitotic growth defect in either the haploid or diploid state (data not shown). We tested other mutants in the *RAD52* epistasis group of genes for growth defects when combined with the *rdh54*Δ mutation. *rdh54*Δ *rad52*Δ, *rdh54*Δ *rad55*Δ and *rdh54*Δ *rad57*Δ double mutants were viable in both the haploid and diploid states and showed no reduction in growth. In contrast, while the *rdh54*Δ *rad50*Δ haploid showed normal growth, the *rdh54*Δ *rad50*Δ double mutant diploid grew extremely poorly.

Mitotic recombination is reduced in the *rdh54*Δ*/rdh54*Δ diploid but not in the *rdh54*Δ haploid: The diploid-specific lethalities of the *rdh54*Δ mutant are related to recombination as these are suppressed by ablation of the major recombinational repair pathway. This suggested that the *rdh54*Δ mutant might have an altered mitotic recombination level. We first tested recombination in the *rdh54*Δ haploid using the *leu2-rI::URA3::leu2-bsteII* direct repeat. The *leu2* alleles are ablations of the indicated restrictions sites. We measured gene conversion events by determining the rate of Leu$^+$ prototrophs and deletion events by determining the rate of Ura$^-$ events. The wild-type haploid *RDH54* strains and the mutant haploid *rdh54*Δ strains had similar rates of recombination (Table 2). Since the other *rdh54*Δ phenotypes we had observed were diploid-specific, we thought that recombination might be affected in a diploid strain. We constructed diploids that were heteroallelic at the *LEU2* locus, using the same *leu2-rI* and *leu2-bsteII* alleles that were used in the duplication system. We observed that spontaneous mitotic recombination, measured as gene conversion Leu$^+$ events, was reduced 10-fold in the *rdh54*Δ*/rdh54*Δ diploid (Table 2).

To further demonstrate that the diploid specific reduction in gene conversion was restricted to interhomologue gene conversion, we examined intrachromosomal gene conversion in the *rdh54*Δ*/rdh54*Δ diploid. One chromosome III homologue carried the *leu2-rI::URA3::leu2-bsteII* duplication while the other chromosome III homologue carried a single copy of the *LEU2* gene that had the double mutation genotype of *leu2-bsteII, rI*. Thus all Leu$^+$ prototrophs are derived from intrachromosomal events. The *rdh54*Δ mutation did not reduce the rate of Leu$^+$ formation in the diploid for intrachromosomal recombination (Table 2), demonstrating that the *RDH54* gene is specifically required for interhomologue recombination.

To determine the effect of the *rdh54*Δ mutation on intrachromosomal gene conversions not associated with crossing over *vs.* intrachromosomal gene conversions associated with deletions, we determined the percentage of Leu$^+$ events that were associated with deletions (Leu$^+$ Ura$^-$) in haploids and diploids. From 100 independent Leu$^+$ events each, 45 were also Ura$^-$ in the wild-type haploid and 36 were also Ura$^-$ in the *rdh54*Δ haploid. These numbers are not significantly different ($\chi^2 = 0.66$, $P > 0.3$). Of 100 independent intrachromosomal Leu$^+$ events in each diploid strain, 24 were also Ura$^-$ in the wild-type strain while 34 were also Ura$^-$ in the *rdh54*Δ strain. Again these numbers are not significantly different ($\chi^2 = 1.96$, $P > 0.1$). These results show that the *rdh54*Δ mutation has no detectable effect on intrachromosomal recombination.

Since many of the *rdh54*Δ phenotypes are observed only in the diploid strains, we were concerned that the *RDH54* gene might only be expressed in diploid cells. To test this we performed Northern analysis on log

TABLE 2

Recombination rates in the *rdh54* mutant

Genotype	Recombination rate ($\times 10^{-5}$)[a] Leu$^+$	Ura$^-$
Intrachromosomal		
RDH54	1.1	1.9
rdh54	2.2, 2.3	1.9, 1.2
RDH54/RDH54	1.4 ± 0.13	1.5 ± 0.75
rdh54/rhd54	2.8 ± 0.85	3.4 ± 0.71
Interchromosomal		
RDH54/RDH54	1.0 ± 0.34	
rdh54/rdh54	0.093 ± 0.008	

[a] Rates for the haploids strains were determined using the direct repeat *leu2-rI::URA3::leu2-bsteII*. Leu$^+$ rates reflect intrachromosomal gene conversion events and deletion events associated with gene conversions or deletions occurring between the mutant alleles. Ura$^-$ rates measure deletion events without regard to gene conversions or other events at the *leu2* duplications. Intrachromosomal rates in diploids were determined using strains carrying duplication *leu2-rI::URA3::leu2-bsteII* on one homologue and the *leu2-bsteII, rI* double mutant allele on the other homologue. Interchromosomal rates were determined with the heteroalleles *leu2-rI* and *leu2-bsteII* and reflect only gene conversion events. Rates in the haploid strains were determined using strains HKY661-3C (*RDH54*), and HKY661-1D and HKY661-4B (*rdh54*). The two rates for the haploid *rdh54* entry are from the two strains. For the diploid strains rates were determined for three fresh zygotes from each cross. Strains used were HKY660-2B × HKY716-1D (*RDH54/RDH54*) and HKY661-1D × HKY716-6B (*rdh54/rdh54*) for intrachromosomal recombination, and HKY669-1B × HKY670-13C (*RDH54/RDH54*) and HKY668-2D × HKY667-5A (*rdh54/rdh54*) for interchromosomal recombination.

phase wild-type diploid and haploid cells and a haploid *rdh54Δ* null mutant strain as a control. Using the actin gene expression as a control we found no difference in expression of the *RDH54* gene between haploid and diploid cells (data not shown). The Northern analysis also revealed that expression of the *RDH54* gene was quite low.

Sporulation and spore viability are reduced in the *rdh54Δ/rdh54Δ* mutant: Essentially all mutants of *S. cerevisiae* that reduce spontaneous mitotic recombination also reduce meiotic recombination, which may be manifest as reduced or no spore viability. Since the *rdh54Δ/rdh54Δ* diploid was reduced in mitotic recombination, we examined the effect of the null mutation on meiotic spore viability. We found that the *rdh54Δ* diploid had reduced sporulation and spore viability (Table 3). Sporulation levels ranged from 10 to 20%. The spore viability was ~64% (122 viable spores out of 192 spores dissected) and only 14 tetrads out of 48 tetrads dissected gave four viable spores. The spore viability appeared random (13 four-spore viable tetrads, 10 three-spore viable tetrads, 12 two-spore viable tetrads, 11 one-spore viable tetrads, two zero-spore viable tetrads). There were no excesses of four-, two- and zero-

TABLE 3

Effect of the *rdh54Δ* mutation on spore viability in *spo13Δ* and *spo13Δ red1Δ* backgrounds

Genotype[a]	% sporulation	% spore viability	No. of spores examined
Wild type	90	94	192
mei4	28	0	48
spo13	66	51	98
rdh54[b]	10–20	64	192
spo13 mei4	45	80	48
spo13 rdh54	20	35	104
spo13 mei4 rdh54	50	76	204
spo13	51	44	200
spo13 rdh54	8	38	200
spo13 red1	30	74	200
spo13 rdh54 red1	36	81	200

[a] All strains are homozygous diploids in the W303 background. The *mei4* and *rdh54* diploid strains gave tetrads upon sporulation whereas the remaining strains, in the *spo13Δ* background, gave dyads upon sporulation. Strains used in the first group were as follows: HKY677-1B × HKY677-11C (*mei4*), HKY677-1D × HKY677-3A (*spo13*), HKY632-2B × HKY632-3B and HKY641-3A × HKY641-6C (*rdh54*), HKY667-2B × HKY677-8A (*spo13 mei4*), HKY677-1A × HKY677-3A (*spo13 rdh54*) and HKY677-2D × HKY677-5A (*spo13 mei4 rdh54*). Strains used in the second group were as follows: HKY687-3B × HKY687-6C (*spo13*), HKY687-1B × HKY687-1D (*spo13 rdh54*), HKY687-2D × HKY687-8D (*spo13 red1*) and HKY687-6B × HKY687-9B (*spo13 rdh54 red1*).

[b] Results from four separate determinations of percentage sporulation and spore viability were combined.

viable spore tetrads. This pattern of spore inviability is reminiscent of the sporulation properties of the *srs2Δ* diploid (PALLADINO and KLEIN 1992).

We used return-to-growth experiments and random spore analysis to assess the ability of *rdh54Δ* diploids to undergo meiotic gene conversion at the *LEU2* locus. In two separate return-to-growth experiments the *rdh54Δ* diploids showed a 38- and 40-fold increase in Leu$^+$ prototrophs after 24 hr in sporulation medium, while the wild-type diploids were increased 82 and 264-fold in Leu$^+$ prototrophs. These experiments show that *rdh54Δ* diploids are proficient in initiating meiotic recombination. The rate of Leu$^+$ prototrophs among random spores was 4.6×10^{-2} for the wild-type diploid and 6.6×10^{-3} for the *rdh54Δ* diploid, reflecting an increase of 270-fold in the wild-type strain and 130-fold increase in the *rdh54Δ* strain during meiosis. These results show that the viable spores in the *rdh54Δ* diploid are recombination proficient.

Since the *RDH54* gene is homologous to the *RAD54*, we asked whether high copy expression of the *RAD54* gene could rescue some of the phenotypes of the *rdh54Δ* mutant. We constructed a *rdh54Δ* diploid with a wild-type copy of *RAD54* on a YEp70 plasmid and compared sporulation and spore viability of this strain to the parental *rdh54Δ* diploid strain. Sporulation of the *rdh54Δ* diploid was slightly improved from 10% (20

tetrads out of 200 cells) to 17% (34 tetrads out of 200 cells) and 32% (64 tetrads out of 200 cells) from two separate transformants. Spore viability in the *rdh54Δ* diploid was improved slightly from 68% (196 viable spores out of 288 dissected) to 79% (152 viable spores out of 192 dissected). The more significant change was in the percentage of tetrads with four viable spores, which rose from 36% (26/72) to 58% (28/48) ($\chi^2 = 4.88$, $P < 0.05$). However, the high copy *RAD54* plasmid could not rescue the inviability of *srs2Δ/srs2Δ rdh54Δ/rdh54Δ* diploids.

We next examined the sporulation properties of *rdh54Δ* double mutants. The *rad54Δ/rad54Δ* mutant by itself gave good sporulation (over 70%) but had reduced spore viability (129 viable spores out of 192 spores dissected) with 13/48 tetrads having four viable spores. As described above, the *rdh54Δ/rdh54Δ rad54Δ/rad54Δ* diploid grew very slowly. This diploid was unable to sporulate and the cells appeared grossly enlarged after 3 days on sporulation medium. Introduction of the *rad51Δ* mutation into this strain rescued the slow growth phenotype but prevented any sporulation. Indeed, the *rdh54Δ/rdh54Δ rad51Δ/rad51Δ* diploid failed to sporulate, whereas the single mutant *rad51Δ/rad51Δ* sporulates to a level of 30–35% and the *rdh54Δ/rdh54Δ* diploid sporulates to a level of 10–20%.

To determine when the *RDH54* gene might act in meiosis, we asked if the *spo13Δ* mutation could rescue the *rdh54Δ* mutant. Mutants defective in genes that act early in recombination can be rescued by the *spo13Δ* mutation by circumventing the requirement of recombination for chromosome segregation. However, if cells are defective in functions required for the later events in recombination, they cannot be rescued by the *spo13Δ* mutation (MALONE and ESPOSITO 1981; MALONE *et al.* 1991). Presumably the cells have initiated recombination and contain unresolved or lethal recombination intermediates. As can be seen in Table 3, the *spo13Δ* mutation can rescue the lethality of the early recombination mutant *mei4Δ* but fails to elevate the percentage sporulation or spore viability of the *rdh54Δ* mutant to *spo13Δ* levels. However, when the *rdh54Δ mei4Δ* mutant is examined, sporulation levels and spore viability are at *spo13Δ mei4Δ* levels, indicating that *MEI4* acts before *RDH54*.

Next we examined the effect of the *red1Δ* mutation on the *rdh54Δ* diploid. The *RED1* gene has been termed an early synapsis gene (MAO-DRAAYER *et al.* 1996) and is required for most interchromosomal meiotic recombination events. The Red1 protein localizes to the synaptonemal complex (SMITH and ROEDER 1997) and has been proposed to repress sister chromatid exchanges during meiosis. The *red1Δ* mutant fails to repress these exchanges. A *red1Δ* mutant can partially rescue the inviability associated with the late exchange mutant *dmc1Δ* (XU *et al.* 1997; D. BISHOP, personal communication), presumably by allowing sister chromatid exchanges to occur and repair the meiotic DSBs that accumulate in the *dmc1* mutant. The Dmc1 protein has been found to interact with the Rdh54 protein by two-hybrid analysis (also called Tid1, for two hybrid interaction with Dmc1) (DRESSER *et al.* 1997). The data of Table 3 show that the *red1Δ* mutation acts epistatically to the *rdh54Δ* mutation. This suggests that *RDH54* acts later in meiosis. However, the *rdh54Δ* mutant is not completely analogous to other late acting mutants such as *rad52* (MALONE and ESPOSITO 1981; MALONE *et al.* 1991). It is similar to *rad52* in that the meiotic phenotype is not rescued by a *spo13Δ* mutation (see Table 3), but is rescued by the early acting mutation *mei4Δ* (see Table 3). Nonetheless, meiotic spore viability is significant in the *rdh54Δ* mutant whereas *rad52* spores are inviable. This suggests that *RDH54* could act in a pathway separate from *RAD52* or could act at a late stage in recombination after the *RAD52* step.

DISCUSSION

We have described the null phenotype of the *rdh54Δ* mutant. The mutant shows defects in mitotic recombination and in meiosis. Unlike most mutants that are defective in mitotic recombination, the *rdh54Δ* mutant shows no sensitivity to DNA damage from MMS in liquid cultures and is not sensitive to MMS concentrations of 0.012–0.016% in solid medium, conditions that do sensitize recombination-defective *rad* mutants such as *rad50, rad51, rad52, rad54, rad55* and *rad57*. However, the *rdh54Δ* mutant is sensitive to continual higher levels of MMS in solid medium. Many of the mitotic phenotypes appear to be limited to homozygous diploids.

Diploid-specific mitotic phenotypes result from recombination: *rdh54Δ* haploids and diploids show no sensitivity to UV irradiation. When *rdh54Δ* diploids are exposed continuously to MMS on solid media, they show slower growth than the isogenic wild-type strains and a reduced plating efficiency that is more severe in the homozygous diploid than in the mutant haploid. The *rdh54Δ* haploid shows no alteration in mitotic intrachromosomal recombination rates, for both gene conversion and deletions. However, the homozygous diploid is reduced 10-fold in gene conversion. This reduction is restricted to interhomologue recombination. We suggest that the reduced growth of the diploid in the presence of MMS is related to the reduced ability of *rdh54Δ* mutants to undergo interhomologue recombination.

There are only a few reported cases of mutants that reduce mitotic recombination rates yet do not have a strong DNA damage sensitivity phenotype (PETES *et al.* 1991). ELIAS-ARNANZ *et al.* (1996) described a novel allele of *SRS2* and two mutants with defects in unknown genes that reduce gene conversion ~10-fold, yet are not sensitive to UV or X-ray irradiation. The recombination defects were seen in haploid strains.

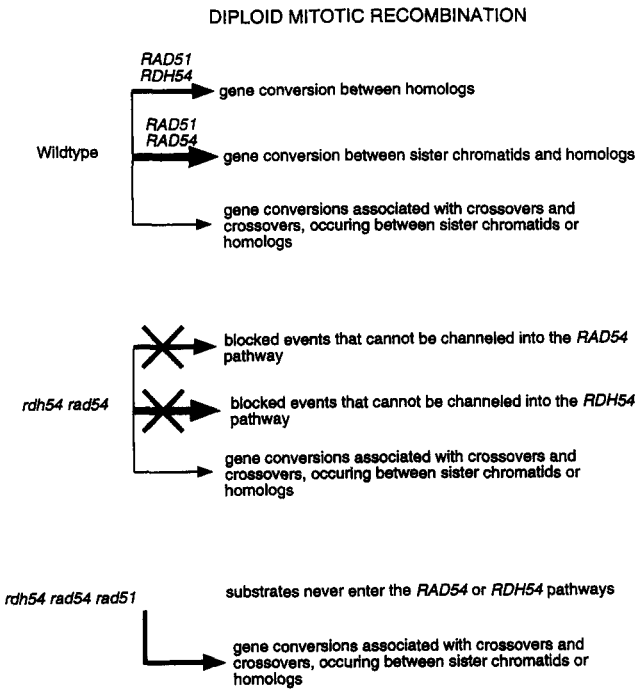

FIGURE 4.—Proposed pathways for diploid mitotic recombination. Three branches are shown, but more may exist. A lesion is channeled into a minor *RDH54* homologue gene conversion pathway, the major *RAD54* pathway using sister chromatids and homologues, or the gene conversion pathway that is associated with crossing over. In *rdh54 rad54* double mutants recombination intermediates are prevented from completion or being channeled into an alternate pathway. In the *rdh54 rad54 rad51* triple mutant the substrates never enter the *RDH54* or *RAD54* pathways and thus can be channeled into the *RAD51*-independent recombination pathway (BAI and SYMINGTON 1996), restoring viability.

While *rdh54*Δ mutants showed no MMS sensitivity in liquid, the null mutation did enhance the MMS sensitivity of *rad54*Δ mutants in liquid. The *rdh54*Δ *rad54*Δ mutant showed a 100-fold increase in MMS sensitivity relative to the *rad54*Δ single mutant after 50 min exposure. This enhanced sensitivity was most apparent in double mutant diploid strains, as opposed to double mutant haploid strains, and was observed only at low MMS concentrations where the killing of the *rad54* mutant was not so rapid as to mask any enhanced sensitivity.

Most recombinational repair of DNA damage in G2 occurs between sister chromatids (KADYK and HARTWELL 1992). The observation that the *rdh54* mutant shows no DNA damage sensitivity at standard MMS concentrations in solid medium or in liquid culture suggests that the *RDH54* gene is not normally involved in recombinational repair by sister chromatids. This is further strengthened by the finding that only interhomologue recombination is reduced in the *rdh54* mutant. We suggest that *RDH54* is specifically required for recombination between homologues (Figure 4). This could explain the enhanced MMS sensitivity of *rad54* diploids by the *rdh54* mutation. A small fraction of damage could be repaired by recombination between homologues in diploids. *RAD54* would function normally in sister chromatid recombinational repair and the absence of the Rad54 protein results in damage sensitivity. At low amounts of damage, *i.e.*, low amounts of MMS, most repair is mediated by *RAD54* through the sister chromatid repair pathway, but a small fraction may be channeled to the interhomologue recombination repair pathway. Since *RDH54* would function specifically in this type of repair, a *rdh54* mutation would enhance the MMS sensitivity of the *rad54* mutant, but only in diploid strains. At continuous high MMS concentrations the *RAD54* gene might not be sufficient for repair of all of the damage. In this case *RDH54* might be able to function in the sister chromatid repair pathway. This would explain the sensitivity of *rdh54*Δ haploids and diploids to high MMS concentrations in solid medium and reconcile the observation that the *RDH54* gene is expressed in haploid cells.

The *rad54* haploid, although sensitive to DNA damage, shows no inviability in the absence of induced damage. One possible explanation for this is that *RDH54* can partially substitute for *RAD54* in the haploid. However, we do not favor this explanation for the repair of spontaneous damage as it predicts that the haploid double mutant *rad54*Δ *rdh54*Δ should have reduced growth, which we do not observe. Moreover, we have not recovered *RDH54* in a screen for high copy suppressors of the *srs2*Δ *rad54*Δ lethality. Other repair pathways, such as those described by BAI and SYMINGTON (1996), must be operative in the *rad54*Δ haploid.

Most mitotic recombination repair genes are not limited in their action to interhomologue events. *RDH54* is unusual in this regard. Some conditional alleles of *RAD52* have been reported to have no effect on mitotic intrachromosomal recombination but are reduced for mitotic interchromosomal recombination (KAYTOR and LIVINGSTON 1994). The fold reduction in recombination of the *rad52* alleles is not as great as that of the *rdh54*Δ mutant. *RAD52* does not in general show diploid specific effects when mutant.

Unlike other members of the *RAD52* epistasis group, the *RAD50* gene is not required for mitotic recombination between homologues, and in fact, *rad50* mutants are reported to be increased in spontaneous intragenic recombination (MALONE and ESPOSITO 1981). This observation together with the G2 gamma-ray sensitivity of *rad50*Δ haploids but partial resistance in G1 and G2 *rad50*Δ diploids has led IVANOV *et al.* (1992) to suggest that *rad50*Δ mutants are specifically defective in sister chromatid recombination in mitosis. Our observation that the *rdh54*Δ *rad50*Δ double mutant shows a diploid specific slow growth is consistent with independent defects in sister chromatid recombination repair and interhomologue recombination repair in this strain. However, this would not explain the viability of other diploid double mutant combinations, such as *rdh54*Δ *rad51*Δ

where both repair pathways are ablated or the slow growth of the haploid *srs2Δ rad50Δ* strain. In these mutants other as yet undefined pathways for repair must be available or some of these mutants arrest the recombination repair irreversibly.

Recombination between homologues is lethal in the *rdh54* mutant diploid: The postreplication repair DNA helicase gene *SRS2* is involved in regulating repair of damaged substrates (ABOUSSEKHRA *et al.* 1989; RONG *et al.* 1991). In its absence repair substrates are channeled into recombination pathways. The *srs2* null mutation is synthetically lethal with a *rad54Δ* mutation and this is the result of attempted recombination. We therefore examined the effect of mutation of the *RAD54* homologue *RDH54* on the *srs2Δ* mutant. In contrast to the *srs2Δ rad54Δ* mutant, the haploid *srs2Δ rdh54Δ* double mutant was fully viable. This is because the *RDH54* gene does not function in haploid recombination. However, the homozygous diploid *srs2Δ rdh54Δ* was completely inviable. We suggest that in the *srs2Δ* mutant many substrates that are normally destined for a repair pathway are channeled into recombination pathways. Some enter the pathway in which *RAD54* operates while others enter into the *RDH54* pathway. So many lesions may enter into these recombination pathways that the pathways become overloaded. When one pathway is rendered inoperative by mutational ablation, the unrepaired lesion or the repair intermediate becomes lethal. When recombination is completely prevented by a *rad51* mutation, the lesions never enter these recombination pathways and hence viability is restored.

The lethality of the *rdh54Δ rad54Δ* and *rdh54Δ srs2Δ* diploid double mutants could result from attempted homologue recombination, but be lethal for different reasons. The *rdh54Δ rad54Δ* diploid lethality may result from blockage of both gene conversion repair pathways with unresolvable structures whereas the *rdh54Δ srs2Δ* diploid lethality would result from an overload of the *RDH54* pathway such that the accumulation of blocked intermediates is too great to be accomodated by other pathways. *RDH54* may not be able to substitute for *RAD54,* but *RAD54* may be able to partially substitute for *RDH54.* The suppression of the lethal mutant combinations by a *rad51Δ* mutation suggests that Rad51 protein acts early in mitotic recombination. The observation that overexpression of *RAD54* does not rescue the inviability of a *rdh54Δ srs2Δ* diploid (H. KLEIN, unpublished observations) argues against completely overlapping functions for *RAD54* and *RDH54.*

RAD54 and *RDH54* encode proteins that are members of the SNF2/SWI2 superfamily (EMERY *et al.* 1991; EISEN *et al.* 1995). They contain the seven consensus motifs of DNA helicases, but no *in vitro* helicase activity has been demonstrated for any of the SNF2/SWI2 superfamily members. The Rad54p and Rdh54p may act as motor proteins to facilitate repair by reorganizing chromatin or may be involved in moving a repair complex on the DNA substrate. The specificity for sister chromatid *vs.* interhomologue recombination may be provided by interaction with other factors.

The mitotic roles of *RDH54* and *DMC1* are not identical: The *RDH54* gene has also been identified from a two-hybrid screen for proteins that interact with the meiotic specific gene *DMC1* that encodes a protein similar to Rad51p and *E. coli* RecA (DRESSER *et al.* 1997). We examined the mitotic growth phenotype of *srs2Δ dmc1Δ* and *rad54Δ dmc1Δ* haploids and diploids and did not observe any reduction in growth rate or viability. This indicates that the mitotic functions of the two genes are not identical. Indeed, there is no evidence that *DMC1* is expressed in vegetative cells. The Rad54 protein has been shown to interact with the Rad51 protein (JIANG *et al.* 1996). However, the phenotypes of a *rad54Δ* strain are not identical to a *rad51Δ* strain as regards the *srs2Δ* and *rdh54Δ* mutations. The diploid *rdh54Δ rad51Δ* shows normal growth while the *rdh54Δ rad54Δ* diploid does not. The *srs2Δ rad51Δ* haploid grows normally while the *srs2Δ rad54Δ* haploid is inviable. The Rad51 protein and Rad54 protein may not always function in the same mitotic recombination intermediate.

***RDH54* is required for successful meiosis:** We observed that the *rdh54Δ* diploid sporulated to very low levels and that spore viability among the few asci was reduced. The meiotic defect was more severe than that observed with *rad54Δ* or *srs2Δ* diploids. These strains also gave reduced spore viability of ~50%, but sporulation levels were normal. We have not examined the fate of meiotic DSBs or the recovery of crossover molecules in the *rdh54Δ* diploid, but the low sporulation and spore viability suggests that meiotic progression and recombination is altered in the mutant. SHINOHARA and colleagues (A. SHINOHARA, personal communication) have examined this and find that the *rdh54Δ* diploid has defects in meiotic progression and the processing of meiotic DSBs. Our studies on the overexpression of *RAD54* in the *rdh54Δ* diploid shows that a small relief in meiotic inviability is observed, but that *RAD54* and *RDH54* are not fully interchangeable. This is corroborated by the observation that the *rdh54Δ rad54Δ* diploid fails to sporulate at all, although this may reflect in part the inability of a strain with reduced mitotic growth to undergo the meiotic process.

The meiotic phenotype of the *rdh54Δ* diploid, although severe, is not identical to that of the *dmc1Δ* mutant (BISHOP *et al.* 1992). This again suggests that the two gene products do not have identical roles in recombination, even if they do interact. The *rad51Δ* mutation exacerbated the sporulation defect of the *rdh54Δ* diploid such that the double mutant failed to sporulate. However, vegetative growth was not affected. This suggests that the two mutations are synergistic in their effect on meiosis.

We used the *spo13Δ* mutant to place the action of

RDH54 in meiosis. The *spo13Δ* mutation fails to rescue the reduction in sporulation and spore viability of the *rdh54Δ* diploid, indicating that *RDH54* is required in later stages of meiosis. We suggest that the Rdh54 protein is required in recombination after the appearance of DSBs. Why then is the gene product not absolutely required? One possibility is that the Rad54 protein can substitute for Rdh54 protein in meiosis, although that is not the normal meiotic role of *RAD54*, as the sporulation level and spore viability of the *rdh54Δ* diploid is worse than that of the *rad54Δ* diploid. It is difficult to test this hypothesis as the homozygous double mutant diploid *rdh54Δ/rdh54Δ rad54Δ/rad54Δ* displays a vegetative poor growth phenotype that may impact on the ability of the strains to undergo meiosis. Alternatively, *RDH54* may function in a subset of recombination events such that in its absence most of the meiotic chromosomes still complete recombination. The observation that the early exchange mutant *mei4Δ* suppresses the *rdh54Δ* defect in a *spo13Δ* suggests that the meiotic phenotype of the *rdh54Δ* diploid is related to a failure in recombination. However, the return-to-growth experiments show that the *rdh54Δ* diploid is proficient for a reasonable level of meiotic gene conversion, suggesting that the Rdh54 protein acts at a late step in meiotic recombination or that it is required for only a subset of meiotic recombination events.

The Red1 protein localizes to the synaptonemal complex (SMITH and ROEDER 1997). *red1Δ* mutants undergo a small amount of recombination, but this is not sufficient for spore viability. The *RED1* gene has been proposed to block sister chromatid recombination in meiosis. Relief of this block by a *red1* mutation results in suppression of some of the *dmc1* mutant defects (XU *et al.* 1997; D. BISHOP, personal communication). It should be noted that the role of *RED1* in meiosis is complex (XU *et al.* 1997). Nonetheless, we found that the *red1Δ* mutation was epistatic to the *rdh54Δ* mutation in meiosis. If the *red1Δ* mutation allows a sister chromatid recombination pathway to occur in meiosis, then this bypass pathway is sufficient to relieve the *rdh54Δ* meiotic defect.

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