Characterization of the Roles of the *Saccharomyces cerevisiae* RAD54 Gene and a Homologue of RAD54, RDH54/TID1, in Mitosis and Meiosis

Miki Shinohara,^{*,†} Emi Shita-Yamaguchi,^{*,1} Jean-Marie Buerstedde,[‡] Hideo Shinagawa,[†] Hideyuki Ogawa^{*} and Akira Shinohara^{*}

^{*}Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560, Japan, [†]Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan and [‡]Basel Institute for Immunology, CH-4005 Basel, Switzerland

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ABSTRACT

The *RAD54* gene, which encodes a protein in the *SWI2/SNF2* family, plays an important role in recombination and DNA repair in *Saccharomyces cerevisiae*. The yeast genome project revealed a homologue of *RAD54*, *RDH54/TID1*. Properties of the *rdh54/tid1* mutant and the *rad54 rdh54/tid1* double mutant are shown for mitosis and meiosis. The *rad54* mutant is sensitive to the alkylating agent, methyl methanesulfonate (MMS), and is defective in interchromosomal and intrachromosomal gene conversion. The *rdh54/tid1* single mutant, on the other hand, does not show any significant deficiency in mitosis. However, the *rad54 rdh54/tid1* mutant is more sensitive to MMS and more defective in interchromosomal gene conversion than is the *rad54* mutant, but shows the same frequency of intrachromosomal gene conversion as the *rad54* mutant. These results suggest that *RDH54/TID1* is involved in a minor pathway of mitotic recombination in the absence of *RAD54*. In meiosis, both single mutants produce viable spores at slightly reduced frequency. However, only the *rdh54/tid1* mutant, but not the *rad54* mutant, shows significant defects in recombination: retardation of the repair of meiosis-specific double-strand breaks (DSBs) and delayed formation of physical recombinants. Furthermore, the *rad54 rdh54/tid1* double mutant is completely defective in meiosis, accumulating DSBs with more recessed ends than the wild type and producing fewer physical recombinants than the wild type. These results suggest that one of the differences between the late stages of mitotic recombination and meiotic recombination might be specified by differential dependency on the Rad54 and Rdh54/Tid1 proteins.

THE *RAD54* gene of *Saccharomyces cerevisiae* belongs to the *RAD52* epistasis group, which is involved in the recombinational repair of double-strand breaks (DSBs) (RESNICK 1987; PETES *et al.* 1991). The *RAD52* group is comprised of at least nine genes: *RAD50*, *-51*, *-52*, *-54*, *-55*, *-57*, *-59*, *MRE11*, and *XRS2*. *RAD51*, *-52*, *-54*, *-55*, *-57*, and *-59* are thought to be involved in a late step(s) of recombination (SHINOHARA and OGAWA 1995; BAI and SYMINGTON 1996), and recent genetic and biochemical studies have shown physical and functional interactions among these proteins (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993; HAYS *et al.* 1995; JOHNSON and SYMINGTON 1995; CLEVER *et al.* 1996; JIANG *et al.* 1996). Such interactions suggest that these proteins may form a large protein complex.

RAD54 encodes a protein very similar to the members of the *SWI2/SNF2* family. Proteins in this family have motifs characteristic of ATPases and DNA/RNA helicases (EISEN *et al.* 1995). Swi2p/Snf2p is a component of a large protein complex that catalyzes chromatin remodeling in an ATP-dependent manner (reviewed

in FELSENFELD 1996; KINGSTON *et al.* 1996; PAZIN and KADONAGA 1997). Rad54p is known to bind to Rad51p, a yeast RecA homologue, both *in vitro* and *in vivo* (CLEVER *et al.* 1996; JIANG *et al.* 1996; SHINOHARA, unpublished results). Rad51p carries out a search for homology and a strand exchange reaction *in vitro* (SUNG 1994). However, the biochemical activity of Rad54p and the functional interaction with Rad51p are not known.

Various genetic analyses have shown that the *rad51* and *rad54* mutants exhibit almost indistinguishable phenotypes in mitosis (LIEFSHILTZ *et al.* 1995; RATTRAY and SYMINGTON 1995), consistent with the idea of a Rad51p-Rad54p complex. However, significant phenotypic differences between the *rad54* and *rad51* mutants have been reported. While the *rad51* mutant is defective in the formation of viable spores (MORRISON and HASTINGS 1979; SHINOHARA *et al.* 1992), the *rad54* mutant forms viable spores, albeit at a reduced frequency, suggesting that *RAD54* plays a minor role in meiotic recombination (GAME 1983; RESNICK 1987). In addition, a double mutant with mutations in the *RAD54* and in the *SRS2* helicase gene, which is involved in DNA repair, is inviable, while a *rad51 srs2* double mutant is not (PALADINO and KLEIN 1992). These results also suggest functional differences between *RAD51* and *RAD54*.

Here, we report the further characteristics of a *rad54*

Corresponding author: Akira Shinohara, Department of Biology, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560, Japan. E-mail: ashino@bio.sci.osaka-u.ac.jp

¹ Present address: Nippon Roche Co. Ltd., Kamakura, Kanagawa 247, Japan.

TABLE 1

Strain list

Strain	Genotype
MSY142	<i>MATa ho::LYS2 lys2 ura3 leu2::hisG</i>
MSY099	<i>MATa rad54::hisG-URA3-hisG ho::LYS2 lys2 ura3 leu2::hisG</i>
MSY084	<i>MATa rdh54/tid1::LEU2 ho::LYS2 lys2 ura3 leu2::hisG</i>
MSY121	<i>MATa rad54::hisG-URA3-hisG rdh54::LEU2 ho::LYS2 lys2 ura3 leu2::hisG</i>
MSY186	<i>MATa rad52::hisG-URA3-hisG ho::LYS2 lys2 ura3 leu2::hisG trp1</i>
MSY197	<i>MATa rad52::hisG-URA3-hisG rad54::hisG-URA3-hisG ho::LYS2 lys2 ura3 leu2::hisG</i>
MSY199	<i>MATa rad52::hisG-URA3-hisG rad54::hisG-URA3-hisG rdh54/tid1::LEU2 ho::LYS2 lys2 ura3 leu2::hisG</i>
NKY1068	<i>MATa ho::LYS2 his4X-ADE2-his4B lys2 ura3 leu2::hisG ade2::LK</i>
MSY038	<i>MATa rad54::hisG-URA3-hisG ho::LYS2 his4X-ADE2-his4B lys2 ura3 leu2::hisG ade2::LK</i>
MSY117	<i>MATa rad54/tid1::LEU2 ho::LYS2 his4X-ADE2-his4B lys2 ura3 leu2::hisG ade2::LK</i>
MSY131	<i>MATa rad54::hisG-URA3-hisG rdh54/tid1::LEU2 ho::LYS2 his4X-ADE2-his4B lys2 ura3 leu2::hisG ade2::LK</i>
NKY1551	<i>MATa/α ho::LYS2/ ho::LYS2 arg4Bgl/ arg4Nsp his4B-LEU2-MluI/ his4X-LEU2-MluI::BamHI-URA3 lys2/ lys2 ura3/ ura3 leu2::hisG/ leu2::hisG</i>
MSY241	<i>MATa/α rad54::hisG-URA3-hisG/ rad54::hisG-URA3-hisG ho::LYS2/ ho::LYS2 arg4Bgl/ arg4Nsp his4B-LEU2-MluI/ his4X-LEU2-MluI::BamHI-URA3 lys2/ lys2 ura3/ ura3 leu2::hisG/ leu2::hisG</i>
MSY134	<i>MATa/α rdh54/tid1::LEU2/ rdh54/tid1::LEU2 ho::LYS2/ ho::LYS2 arg4Bgl/ arg4Nsp his4B-LEU2-MluI/ his4X-LEU2-MluI::BamHI-URA3 lys2/ lys2 ura3/ ura3 leu2::hisG/ leu2::hisG</i>
MSY147	<i>MATa/α rad54::hisG-URA3-hisG/ rad54::hisG-URA3-hisG rdh54/tid1::LEU2 rdh54/tid1::LEU2 ho::LYS2/ ho::LYS2 arg4Bgl/ arg4Nsp his4B-LEU2-MluI/ his4X-LEU2-MluI::BamHI-URA3 lys2/ lys2 ura3/ ura3 leu2::hisG/ leu2::hisG</i>

deletion mutant and the mutant of a yeast *RAD54* homologue, *RDH54/TID1*, which was found during the yeast genome sequencing project. We also examine characteristics of the corresponding double mutant. The results suggest that *RAD54* plays a major role in mitotic DNA repair, while *RDH54/TID1* has a significant role in the repair of meiotic DSBs.

MATERIALS AND METHODS

Media: YPD and dropout media were as described in SHERMAN *et al.* (1983). MYPD was described by SHINOHARA *et al.* (1992). YPA and SPM were described in CAO *et al.* (1990).

Plasmids: pUC118-RAD54 was constructed by ligating the *Pst*I-*Eco*RI fragment containing the *RAD54* gene (EMERY *et al.* 1991) into a *Sma*I site of pUC118 after filling in the enzyme-digested cohesive ends with T4 DNA polymerase. pUC118-RAD54d was constructed by inserting the *Bam*HI-*Bgl*II fragment containing *hisG-URA3-hisG* from pNKY51 (ALANI *et al.* 1987) into *Xba*I-*Bam*HI-digested pUC118-RAD54. A fragment with the *RDH54/TID1* (YBR0715/YBR073w) open reading frame was amplified from the yeast genomic DNA of SK1 by PCR (SAIKI *et al.* 1988) using 5'-GCGGATCCATATGCGCGT AATAAGCGTTAAACC-3' and 5'-GCGGATCCTCATTTGTTCTCTGAGACATATCTCG-3' (*Bam*HI site underlined) as primers. The amplified fragment was inserted into the *Bam*HI site of pBluescript II SK+ (Stratagene), designated pBS-RDH54. pBS-RDH54d was constructed by ligating a *LEU2* fragment into the *Sna*BI site of pBS-RDH54. The *rad52* disruptant of the SK1 derivative, in which a *Clal*-*Pst*I fragment of the *RAD52* gene was replaced by the *hisG-URA3-hisG* fragment, was kindly provided by Dr. A. NABETANI. Wild-type *RDH54/TID1* was cloned from a cosmid, α346 (a generous gift of Dr. H. Y. STEENSMAN).

Strains: All yeast strains described in this study (Table 1) were derived from SK1 (KANE and ROTH 1974). The *rdh54/tid1* disruption and *rad54* deletion were constructed by one-step replacement (ROTHSTEIN 1983) using the *Bam*HI fragment of plasmid pBS-RDH54d and the *Sph*I-*Eco*RI fragment of

plasmid pUC118-RAD54d, respectively. Correct replacement was verified by Southern blot analysis.

Yeast genetics: All yeast manipulations were as described in SHERMAN *et al.* (1983). The "return-to-growth assay" (SHERMAN and ROMAN 1963) was carried out as described in SHINOHARA *et al.* (1992).

MMS sensitivity: For solid plate assays, yeast cells grown to the stationary phase in YPD were plated on YPD plates containing various concentrations of methylmethane sulfonate (MMS). The plates were incubated in the dark at 30°. The colonies were counted after 3 days of incubation for the wild-type and the *rdh54/tid1* mutant, and after 5 days of incubation for MMS-sensitive strains such as *rad54* and *rad54 rdh54/tid1*. Relative survival is expressed as a fraction with the number of MMS-resistant colony-forming units as the numerator and the number of colony-forming units on YPD plates without MMS as the denominator. At least three independent experiments were carried out, and the values obtained from each experiment were averaged.

For liquid assays, strains were grown in YPD medium to saturation at 30°. Cells were collected and washed twice in sterile water. Cells were resuspended in 50 mM KH_2PO_4 (pH 7.0) at a concentration of 1×10^7 cells/ml and MMS added to a concentration of 0.1%. At 10-min intervals, 0.5 ml MMS-treated cells were mixed with 0.5 ml of 10% $\text{Na}_2\text{S}_2\text{O}_3$ to inactivate the MMS. The cells were then plated on YPD medium at appropriate dilutions and incubated at 30°. Survival was determined after 3 days for the wild-type and the *rdh54/tid1* mutant cells, and after 4 days for the *rad54* and *rad54 rdh54/tid1* mutant cells.

Determination of mitotic recombination frequencies: Recombination rates were calculated according to the median method of LEA and COULSON (1948). Strains were streaked out on solid YPD medium from -80° stock and grown at 30° for 3 days. Nine colonies from each strain were resuspended in YPD, grown for 12 hr, and plated at the appropriate dilutions to determine the total cell number (YPD medium) and the number of recombinants (SC-His or SC-His-Ade medium).

Physical analysis of meiosis: The methods of identification

of DSBs and physical recombinants have been described in CAO *et al.* (1990) and XU and KLECKNER (1995). In the meiotic time course analysis of the *rad54 rdh54/tid1* mutant strain, a near stationary culture of the mutant cells in YPD was inoculated into YPA at a 1:20 dilution, whereas the wild type and other strains were inoculated at a 1:100 dilution. The radio-labeled DNAs were prepared by a random primer method (FEINBERG and VOGELSTEIN 1984) using pNKY291 (CAO *et al.* 1990) as the template.

DAPI staining: Meiotic cells were collected at each time point, fixed with 50% ethanol and stained with 4',6-diamino-2-phenylindole pyrrolindone (DAPI). The cells were examined under a fluorescence microscope (Zeiss, Axiovert 135M). At least 100 cells were observed in each sample to determine the percentage of cells with one, two, three, and four DAPI staining bodies.

RESULTS

Construction of the *rdh54/tid1* mutant and its mitotic phenotypes: In a search of the *S. cerevisiae* protein database for homologous open reading frames (ORF) to the Rad54 protein, one ORF on chromosome II (YBR0715/YBR073w) was found that had the highest homology to Rad54p among the *SWI2/SNF2* helicase family in the yeast. The ORF shares 34% identity with the Rad54 protein (Figure 1; VAN DER AART *et al.* 1994; EISEN *et al.* 1995). Interestingly, the gene has three degenerate MCB sequences (*MfuI* cell cycle box) in the upstream region, which are responsible for transcriptional induction in G1/S phase (reviewed in JOHNSTON and LOWNDES 1992). *RAD54* also has two MCBs in its upstream region (COLE *et al.* 1989) and its expression is induced at the G1/S boundary (JOHNSTON and JOHNSON 1995). The gene has been named *RDH54* (*RAD* homologue 54; KLEIN 1997) and *TID1* (two-hybrid interaction with *DMC1*; DRESSER *et al.* 1997). We refer to this gene as *RDH54/TID1*. To determine the function of the *RDH54/TID1* gene, the disruption strain in which *LEU2* was inserted into the gene was constructed in the SK1 background. In addition, a double mutant with a *rad54* deletion was also constructed in the same strain background to determine the functional relationship between the two genes.

The *RDH54/TID1* gene is not essential for the growth of yeast cells. The doubling times of the wild type, *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* haploids were 2, 2.5, 2, and 3 hr, respectively, while those of wild type, *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* homozygous diploids were 2, 2.5, 2, and 5 hr, respectively. Thus, the *rad54 rdh54/tid1* diploid cells showed poorer growth than the haploid cells with the same genotype. Microscopic examination revealed that the *rad54 rdh54/tid1* diploid cells were two- to threefold larger than the wild-type diploid cells and that some cells had an elongated bud, although DAPI staining failed to reveal any defects in nuclear morphology (data not shown).

Properties of the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* mutants during vegetative growth: In mitosis, the *rdh54/tid1* mutant haploid did not show any dramatic

defects in the repair of MMS-induced DNA damage, whereas the *rad54* mutant was sensitive to MMS (Figure 2), as previously reported (GAME 1983). However, the *rad54 rdh54/tid1* double mutant was slightly, but significantly, more sensitive to MMS than the *rad54* single mutant (Figure 2), when measured by solid plate assay (Figure 2A) and by liquid assay (Figure 2B). This suggests that *RDH54/TID1* is required for repair of MMS-induced DNA damage in the absence of *RAD54*.

Next, the effect of the *rdh54/tid1* mutation on recombination was examined. The *rad54* single mutant showed a hyper-recombinational phenotype for intrachromosomal deletions of a direct repeat. These deletions are often generated by a distinct single-strand annealing recombination pathway (reviewed in KLEIN 1995). Recombination between the *his4B-ADE2-his4X* allele on chromosome III (BISHOP *et al.* 1992) was examined. The His⁺Ade⁻ prototrophs arise by intrachromatid "pop-out" or unequal sister chromatid exchanges while His⁺Ade⁺ prototrophs arise in many ways, e.g., intrachromosomal gene conversion (Figure 3A). The frequency of His⁺Ade⁺ prototrophs in the *rdh54/tid1* mutant was found to be the same as that in the wild type (Figure 3B). In the *rad54* mutant, the frequency of formation of His⁺Ade⁺ prototrophs was reduced 9.1-fold, but formation of His⁺Ade⁻ prototrophs was increased 1.9-fold. The *rad54 rdh54/tid1* double mutant was almost similar to the *rad54* single mutant.

When heteroallelic interchromosomal recombination between *his4* heteroalleles in a diploid cell was analyzed (Table 2), the *rdh54/tid1* single mutant showed the same rate of formation of His⁺ prototrophs as the wild type. The *rad54* single mutant reduced recombination at the *HIS4-LEU2* locus 12.5-fold. Recombination in the *rad54 rdh54/tid1* double mutant was further decreased 10-fold compared to the *rad54* mutant, indicating that *RDH54/TID1* is required for interchromosomal recombination in the absence of *RAD54*.

***rad52* is epistatic to *rad54* and *rdh54/tid1* in repair of MMS-induced DNA damage:** To investigate the epistatic relationship between *RDH54/TID1* and *RAD52*, a gene that plays a crucial role in most recombination events in *S. cerevisiae*, *rad52 rdh54/tid1* and *rad52 rad54* double mutants, and a *rad52 rad54 rdh54/tid1* triple mutant were constructed and tested for their sensitivity to MMS (Figure 4). The *rad52* mutant was more sensitive to MMS than the *rad54* mutant. The *rad52 rad54* double mutant yielded the same MMS sensitivity curve as the *rad52* single mutant, confirming an earlier finding that the *rad52* mutation is epistatic to the *rad54* mutation in repair of damaged DNA (GAME 1983). The *rad54 rdh54/tid1* double mutant was slightly more resistant to MMS than the *rad52* single mutant. The *rad52 rdh54/tid1* double mutant and *rad52 rad54 rdh54/tid1* triple mutant were as sensitive to MMS as the *rad52* mutant. These findings indicate that the *rad52* mutation is epistatic to the *rdh54/tid1* mutation in the repair of

Radh54/Tid1 MAVISVKKPRR---REK--I-LQE---VK---NSSVYQTVFDSGT---TQMQLPKYENKPF---K---PFR--RV--GS-NKYTQ--I-KPTATAMTTA 69
 Rad54 MA-----R-RRLPDRPPNGIGAGERPLVPRPIN--V-Q---DSVRLI---K---PFRVPYKNTHTIPEAAGRATGSPNIVGGRSRPRRSAT-VCYS 79

 P--ISKAK-VTVN-----I-KRSI---SAGPTINLAKKPNLSSNENI-RY-ET--IM-Y--RK--PTI---KRHK-TWSG---DGYATLKASSDK 137
 GLDIN-ADEAEYNSQDISFSQITKRKDALSAQR-I--AKDPRLSHIQYTHRSFTVPKGVQVQHSIEILGMKK-KLTPPRPLHDP--I-----DE 167

 LOFYNECKFLGS-S---MLPSD-S-DSLFEFLFKAG-SNEVLDYELKEAEIRSAKEALSQNMGNFSPTTSTTETVES--TKNDG--KYQMPISQ 225
 --F---AIV-LYDFSVGEMIVHDTSMDNKEESKKMIKST--Q---E-RDN--IN--KERNSOEEF-F---TORIGRH-EALMT-N-CVRNK---PIRE 241

 LFSNTVVKRFKSVTKQTNEHMTTVPKTSQ--NSAKKYYYPVD--VNKIDNPIMNKNAAAEVDVLDPLICKELRPHQREGVKEMYDC---I-MG--L-AR 316
 I-L-LGD-----SE---N-----SAEN-K-KK---EASV-----P-V-----V--I-DPKLAKILRPHQVEGVRELYRCVTCVVKDYIEA- 297

 PTIE--NPDIDOTWSELVLEND---SDI-----S-----C---CLIADEMGLGKTLMSLTILWTLIRQTEFASKVSCSCSGIPTGLTKKILVCPV 395
 ---EAFN-----IS-S---E-DPLKSDEKALTESQKTEQNNRGAYGCIADFMGLGKTLQCIATLWTLIRQTE-----Q-GKRLIDKCI-C-V-CPS 372

 TLICNMKREFGKWINLSRICV--LI-L-----SSR--N---SPDMDKMA-V--RNEIKVORTYQVLIIGVEKILS-VSELEKKNKH--LIDMIVCDEC 474
 SIVANMANELIKWI-----CPNTLTPAVDGKSSMGCGNTTVSQAIHAWAQGRNIVKP-----VLIISVETLRRAVDQ--L-KNCNVG--I-MIA-DEG 457

 HRLKNGASKILNLLKSLD-I---RRKILLTGTPIONDINEEFTIIDFINPGILGSGFASFKRR-FIIPITRAQDTANRYNEELLEKEGEERS-KEMIEIT-K 567
 HRLKNGDS--I-LFTALDSISCFRRVIL-SGTPIONDISEFTALLSFNSPGLGSRPEF-RNFENPIIRCD-ADATDKEIT-KGEAQQLKST-IVSK 549

 RFTIERTNAILEKYDLPKTDI--LIFC--KPYSGOILAFKDILOGAR-LDF---QLTFSST---GLITIPKKVCNSPGIVGS-D-----PY-VK 645
 -FILERTNDILAKYLECKYEYHVI-EVNLKPL--QNELYNKLIK--REVKKVVKCVGG-SQPRAGI-I-L-KKICNHNLENFEDEFDDDELDLEIPDDN 640

 ---SHIK--DTQSQDSYS-R-S-INSGLI-KVLMTLLEGIRCKEKE---KVVVVSNTOTLDTIENIMNMAGM-SHORLDC--SIPAKORDSIVTSFNRN 730
 MPGS--KARDVQTK--YSAKFSI-ERF-LHK-----I-R-I-ESDDKIIVLISNYTOTLDTLEK-MCRYKHYSAVRLDCTWSIN-R-ROKLVDRFND- 720

 PAI--FGFLLSAKSGCGVGINLVRSRLILFNDWNPSVDLQMSRIHRDGOKPCFIVRLVATGCIIDEKILQRLMKNLSQ-----KFLGDSMRNKS 823
 PEGQBEIELSSKAGCCGINLIGANRLIILMDPWNPAADQAPARVORDGOKKDCFIYRFISCTIEEKIFQRQSKMKSLSVCVDAKE--DVE-RLF-S 816

 SNDDLFNKEDIKDLFSVHTDKS-NTHDLICSCDGLGEEIE-YPET--NQ--QNTVELRKSTTTWTSI-I--DLQKKMNEAAT-NDDAKKSQYIROQI 913
 S-D--N--LRQLFQ-----KNENT--IC-----ETHEIVHCKRONAAGKQ-----I-KRAP-----AWTYGD-----ATTWN-----H 867

 VHYKHIDPARQDEIFDEVIT--DSFTELK-----DSIAFAFVKPGEICLREQ* 958
 NH-----D-A-----I--EK-TND-HL-LKNEHHYND-ISFAFOY---ISH---* 898

FIGURE 1.—Amino acid comparison of Rad54 and Radh54/Tid1 proteins. The upper and lower sequences represent the amino acid sequences of the *Radh54/Tid1* and the *Rad54* proteins, respectively. The gaps were introduced to maximize homology. Identical amino acids are shaded, and homologous amino acids are lightly shaded. The following groups of amino acids were taken as homologous: D and E; N and Q; S and T; K and R; A, L, M and V; F, H, I, Y, and W.

FIGURE 2.—MMS sensitivity of the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* mutants. The survival curve for each haploid strain was obtained as described in MATERIALS AND METHODS. (A) Solid plate assay. (B) Liquid assay. Symbols are as follows: wild type (○), *rad54* (●), *rdh54/tid1* (▲), and *rad54 rdh54/tid1* (□).

MMS-induced DNA damage. Therefore, it is likely that a putative repair pathway involving *RDH54/TID1* depends on *RAD52* function.

Properties of *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1*

mutants in meiosis: Meiotic phenotypes of the mutants were also analyzed. All analyses were carried out in the SK1 background, which enters meiosis in a very rapid and synchronous manner (KANE and ROTH 1974). The

FIGURE 3.—Recombination between intrachromosomal direct repeats. (A) Configuration of parental and resultant chromosomes. (B) Intrachromosomal recombination was assayed between tandemly duplicated *his4* heteroalleles (BISHOP *et al.* 1992) as described in MATERIALS AND METHODS. The recombination rates were calculated as described in MATERIALS AND METHODS. His⁺ recombinants were assayed for adenine prototrophy by replica-plating onto an SD plate without adenine.

Strain	Genotype	His ⁺ Ade ⁻ (Relative rec.)	His ⁺ Ade ⁺ (Relative rec.)
NKY1068	<i>RAD54 RDH54/TID1</i>	9.26 × 10 ⁻⁶ (1.00)	6.85 × 10 ⁻⁶ (1.00)
MSY038	<i>rad54Δ RDH54/TID1</i>	1.76 × 10 ⁻⁵ (1.92)	7.73 × 10 ⁻⁷ (0.11)
MSY117	<i>RAD54 rdh54/tid1Δ</i>	8.79 × 10 ⁻⁶ (0.95)	9.08 × 10 ⁻⁶ (1.32)
MSY131	<i>rad54Δ rdh54/tid1Δ</i>	5.03 × 10 ⁻⁵ (5.44)	10.6 × 10 ⁻⁷ (0.15)

TABLE 2

Interchromosomal recombination in diploid strains

Strain	Genotype	<i>HIS</i> ⁺ (relative rec.)
NKY1551	<i>RAD54 RDH54/TID1</i>	9.22×10^{-7} (1.0)
MSY241	<i>rad54Δ RDH54/TID1</i>	7.52×10^{-8} (0.08)
MSY134	<i>RAD54 rdh54/tid1Δ</i>	7.27×10^{-7} (0.79)
MSY147	<i>rad54Δ rdh54/tid1Δ</i>	7.01×10^{-9} (0.008)

Mitotic heteroallelic interchromosomal recombination in the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* mutants. The recombination rate between *his4X* and *his4B* heteroalleles at *HIS4-LEU2* was measured as described in MATERIALS AND METHODS.

following assays were used for meiotic time course analyses: DAPI staining to monitor the meiotic cell cycle progression, return-to-growth experiment to assay the formation of prototrophs, DNA analysis of DSBs, and DNA analysis of physical recombinants. When incubated in sporulation medium (SPM), wild-type, *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* cells formed asci at a frequency of 88, 30, 44, and 0.5%, respectively, after a 24-hr incubation (Table 3). The spore viability of wild-type, *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* cells was 98, 53, 82, and 1.6%, respectively. The high spore viability of either single mutant was consistent with the previous reports in other strain backgrounds (GAME 1983; RESNICK 1987; KLEIN 1997). The distribution of viable spores per ascus differed in the *rad54* and *rdh54/tid1* mutants (Table 3).

Progression of the first (MI) and second (MII) meiotic divisions in the mutants was examined in a time course analysis of DAPI stained cells incubated in SPM (Figure 5). In the wild type, cells finishing MI began to appear at 3 hr, peaked at 7 hr, and then decreased. Cells finishing MII appeared at 5 hr and then accumulated. By 12 hr, >90% of wild-type cells had completed both meiotic divisions. The *rad54* deletion mutant showed an aberrant progression of meiotic divisions; only 30% of the *rad54* cells had completed MII by 24 hr. The *rdh54/tid1* mutants also showed impaired kinetics of meiotic progression; only 34% cells had finished MII after 24-hr incubation in SPM medium. In contrast to the single mutants, the *rad54 rdh54/tid1* double mutant was almost completely defective in the progression of meiotic divisions, suggesting a meiosis-specific arrest in the mutant.

We next examined formation of recombinants at the *HIS4-LEU2* locus on chromosome III by a return-to-growth assay (SHERMAN and ROMAN 1963), which is thought to measure the commitment to meiotic recombination. In the wild type, a 500-fold induction of *His*⁺ prototrophs was observed when cells were incubated in SPM (Figure 6). Both the *rdh54/tid1* and *rad54* single mutants induced formation of *His*⁺ prototrophs, although the frequency at 0 hr, which corresponded to the mitotic cell cycle, was reduced 40-fold in the *rad54*

FIGURE 4.—Epistasis relationship of the *rad54* and *rdh54/tid1* mutations with respect to *rad52* for MMS sensitivity. The survival of each haploid mutant strain with the *rad52* mutation was assessed on YPD plates containing various concentrations of MMS. Symbols are as follows: wild type (○), *rad54* (●), *rad54 rdh54/tid1* (□), *rad52* (Δ), *rad52 rad54* (×), *rad52 rdh54/tid1* (▲), and *rad52 rad54 rdh54/tid1* (◆).

single mutant. There was no obvious delay in the formation of the recombinants in either the *rad54* and *rdh54/tid1* mutants under these conditions, which was in contrast to the delay of the other events in meiosis. The *rad54 rdh54/tid1* double mutant showed a drastic defect in the formation of *His*⁺ recombinants. Although a 100-fold increase from 0 hr was observed after 5 hr of incubation in SPM, the level of the prototrophs peak in the double mutant was nearly 1000-fold lower than in the wild type. In addition, the rate of survival of the double mutant was reduced to 7% after 12 hr of incubation, whereas that of the single mutants was not reduced at all. Therefore, *RAD54* and *RDH54/TID1* are more or less redundant for the formation of recombinants under return-to-growth conditions.

Physical analysis of meiosis-specific DSBs and recombinants in *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* homozygous diploids: To examine *RAD54* and *RDH54/TID1* in meiosis in more detail, we analyzed meiosis-specific DSBs at the well-characterized *HIS4-LEU2* meiotic recombination hot spot on chromosome III (CAO *et al.* 1990; XU and KLECKNER 1995). DSBs are introduced at two sites in the locus and can be identified by

TABLE 3
Spore formation efficiency and spore viability

Strain	Genotype	No. of viable spores ^a					Viable spores/ total spores ^a	Spore formation efficiency (%)
		4	3	2	1	0		
NKY1551	<i>RAD54 RDH54/TID1</i>	37 (92.5)	3 (7.5)	0 (0)	0 (0)	0 (0)	157/160 (98.1)	88.1
MKY241	<i>rad54</i> Δ <i>RDH54/TID1</i>	8 (13.3)	11 (18.3)	24 (40.0)	13 (21.7)	4 (6.7)	126/240 (52.5)	30.1
MKY134	<i>RAD54 rdh54/tid1</i> Δ	45 (56.3)	19 (23.8)	9 (11.3)	6 (7.5)	4 (1.3)	261/320 (81.5)	44.0
MKY147	<i>rad54</i> Δ <i>rdh54/tid1</i> Δ	0 (0)	0 (0)	0 (0)	1 (6.2)	15 (93.8)	1/64 (1.6)	0.5

Spore viability of the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* mutants. Spores formed at 24 hr in SPM were dissected, and their viability was measured. The distribution of viable spores per tetrad is also shown. Frequency of spore formation was determined by observing 24-hr culture under microscope.

^a Percentages are shown in parentheses.

the appearance of specific fragments separated from a parental fragment in Southern blot analysis (Figure 7A). In the wild type, DSBs were introduced as early as 2 hr after incubation in SPM. They reached a maximum at 5 hr and then began to disappear (Figure 7B). The bands representing DSBs in the wild-type cells were smeared because of resection of the 5' ends of DSBs (SUN *et al.* 1991; BISHOP *et al.* 1992). The *rad54* mutant did not show any significant defects in the appearance of DSBs, but a slight 1–2 hr delay in the disappearance

of DSBs was observed in the mutant (Figure 7C; D. BISHOP, personal communication). This is striking because the *rad54* mutant showed a decreased spore viability and aberrant cell cycle progression in meiosis (see above). In the *rdh54/tid1* mutants, DSBs were introduced at $\sim$ 2 hr in meiosis and plateaued at 5 hr, similar to the wild type. However, at later times, disappearance of DSBs was delayed in the *rdh54/tid1* mutant (compare the 10-hr lanes of the wild type and *rdh54/tid1*). As much as a 5-hr delay in the disappearance of DSBs was

FIGURE 5.—Meiotic cell cycle progression in the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* homozygous diploids. Progression of MI and MII was examined by staining the cells with DAPI at the designated times and performing fluorescence microscopy. The percentages of cells that had undergone MI or MI and MII at various times throughout sporulation are plotted. (A) Wild type. (B) *rad54*. (C) *rdh54/tid1*. (D) *rad54 rdh54/tid1*. In each figure, ● represent a cell with two, three, and four DAPI staining bodies. ○ indicate cells with three and four DAPI staining bodies.

FIGURE 6.—Commitment to meiotic recombination in the *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* mutants. (A) Fractions of surviving cells were measured as the ratio of colony-forming units at the time indicated over colony-forming units at time 0. (B) Commitment to gene conversion at the *HIS4-LEU2* locus in the wild-type (○), *rad54* (●), *rdh54/tid1* (▲) and *rad54 rdh54/tid1* (□) homozygous diploid was analyzed in a return-to-growth experiment, as described in MATERIALS AND METHODS. The ratio of His⁺ recombinant colony forming units to total colony-forming units at each time is indicated.

found in the mutant, and DSBs were more smeared at 7 and 10 hr than at 5 hr, suggesting more resection of the ends of the DSBs in the *rdh54/tid1* mutant. At later times, such as 12 hr, DSBs had disappeared completely in the *rdh54/tid1* mutant. In contrast, the *rad54 rdh54/tid1* double mutant accumulated DSBs with more resection than the wild type or either single mutant. The DSBs remained unrepaired in the double mutant even at 12 hr. At 24 hr of incubation, the DSBs seemed to disappear; however, this may have been due to greater resection or degradation of the DSBs.

Formation of physical recombinants was also examined in the mutants. The bands that corresponded to

crossover recombinants were separated from parental fragments by restriction site polymorphisms of *XhoI* sites between homologous chromosomes (Figure 8A). In the wild type, recombinant bands began to accumulate after 5 hr in SPM and reached to 25% of the intensity of the parental bands by 12 hr. The *rad54* mutant exhibited a slight 1–2 hr delay in the formation of physical recombinants (Figure 8C). However, in the *rdh54/tid1* single mutant, formation of the recombinants was clearly retarded (Figure 8B); the recombinants could not be detected until 10 hr after transfer to SPM, which is 5 hr later than in the wild type. The recombinant bands accumulated to 20% of the intensity of the parental bands at 24 hr. The *rad54 rdh54/tid1* double mutant hardly formed any recombinants. As few as 2% of parental DNAs had been converted into recombinants in the *rad54 rdh54/tid1* double mutants at 24 hr (Figure 8B). Taken together, these results indicate that the *RAD54* gene is required for efficient repair of DSBs and formation of crossover products during meiosis in the absence of *RDH54/TID1*.

DISCUSSION

In this study we show that a Rad54p homologue, Rdh54p/Tid1p, participates in recombination and DNA repair. The *rad54 rdh54/tid1* double mutant shows a more severe defect both in the repair of MMS-induced DNA damage and in interchromosomal gene conversion in mitosis than the *rad54* mutant. In meiosis, the double mutant exhibits severe deficiencies compared to the individual single mutants. Despite the apparent functional redundancy, mitotic recombination and DNA repair depend heavily on *RAD54*, while meiotic recombination depends more on *RDH54/TID1* than on *RAD54*.

Roles of *RAD54* and *RDH54/TID1* in mitosis: Although the *rdh54/tid1* mutant was as resistant to MMS as the wild type, the *rad54* single mutant was sensitive to MMS; the *rad54 rdh54/tid1* double mutant was more sensitive than the *rad54* single mutant. Thus, Rad54p plays a major role in the repair of MMS-induced DNA damage in vegetative growth, and Rdh54p/Tid1p has little role in repair in the presence of Rad54p. *RDH54/TID1* plays a minor role in DNA repair and probably is involved in an alternative repair pathway in the absence of *RAD54*. Alternatively, the activity of the Rdh54/Tid1 protein may be too weak to suppress the *rad54* defects, or the amount of Rdh54/Tid1 protein might not be sufficient to substitute for the Rad54 protein in a mitotic cell.

The *RDH54/TID1*-dependent DNA repair pathway seems to involve homologous recombination, since the *rad54 rdh54/tid1* mutant was also more deficient in interchromosomal recombination than the *rad54* single mutant. In addition, both the *RAD54*-dependent major recombination pathway and the *RDH54/TID1*-depend-

FIGURE 7.—Meiosis-specific DSBs in *rad54*, *rdh54/tid1*, and *rad54 rdh54/tid1* homozygous diploids. (A) A map of the *HIS4-LEU2* locus on chromosome III is shown. DSBs were analyzed by *Pst*I digestion. (B and C) DNA was isolated from cells at the indicated times after incubation in SPM and assayed as described in MATERIALS AND METHODS. The probe used was pNKY291. (B) Wild type, *rdh54/tid1*, and *rad54 rdh54/tid1*. (C) *rad54*.

dent pathway require *RAD52*. However, our results did not allow us to distinguish whether the *RDH54/TID1* simply replaces *RAD54* in a major mitotic recombination pathway or plays a role in a distinct recombination pathway. SYMINGTON and colleagues recently showed

that intrachromosomal recombination between inverted repeats is governed by two distinct recombination pathways: a major *RAD51(RAD54)*-dependent and a minor *RAD51(RAD54)*-independent (RATTRAY and SYMINGTON 1995; BAI and SYMINGTON 1996). Both path-

FIGURE 8.—Physical recombinants in meiosis. (A) A map of the *HIS4-LEU2* locus on chromosome III is shown. Crossover recombinants were analyzed by *XhoI* digestion. DNA was isolated from cells at the indicated times after incubation in SPM and assayed as described in MATERIALS AND METHODS. The probe used was pNKY291. (B) Wild type, *rdh54/tid1*, and *rad54rdh54/tid1*. (C) *rad54*. P1 and P2 indicate 19.9- and 12.4-kb parental bands, and R1 and R2 indicate 18.5- and 13.8-kb crossover products.

ways are known to require the *RAD52* function. Thus, *Rdh54/Tid1* protein might be involved in the minor *RAD51(RAD54)*-independent pathway.

It is interesting that the *rad54rdh54/tid1* diploid cells were slower growing compared to haploid cells with the same genotype, suggesting a diploid-specific function for *RDH54/TID1*. In addition, the *rdh54/tid1* mutant did not exhibit any synergistic defect in intrachromosomal gene conversion when combined with the *rad54* mutation, but showed a reduction in interchromosomal gene conversion. These findings suggest a role of the

RDH54/TID1 in recombination between the chromosomes, sisters, and/or homologues. KLEIN reported diploid specific phenotypes of the *rdh54/tid1* single mutant in a strain with a different background (KLEIN 1997), supporting the notion of an interchromosome-specific function of *Rdh54/Tid1* protein.

Roles of *RAD54* and *RDH54/TID1* in meiosis: The *rad54rdh54/tid1* double mutant exhibited synergistic defects in meiosis compared to both single mutants. The meiotic specific functions that showed the synergistic defects were the formation of prototrophs, the

repair of meiosis-specific DSBs, the formation of physical recombinants, and cell cycle progression of meiosis. These findings indicate that *RDH54/TID1* is necessary for the repair of meiotic DSBs, which leads to the formation of recombinants, in the absence of *RAD54*. The presence of such a functional homologue of *RAD54* helps explain previous reports of the absence of meiotic phenotypes of *rad54* mutants. Other *RAD52* group mutants, e.g., *rad51*, have strong meiotic defects.

Why do meiotic cells require both Rad54 and Rdh54/Tid1 proteins to repair DSBs, while Rad54p alone is sufficient for mitotic cells to repair DSBs? Only the *rdh54/tid1* mutant, not the *rad54*, showed a clear deficiency in meiotic DNA events such as the delay in DSB repair and a delay in the formation of recombinants. *rad54* did not show any drastic defects in either the repair of meiosis-specific DSBs or the production of crossover recombinants, despite a defect in viable spore formation. The defect of the *rad54* cells in production of viable spores might be due to aberrant meiotic recombination, which cannot serve for the proper segregation of chromosomes in meiotic divisions. Alternatively, *RAD54* might be involved in a meiotic event other than recombination. Taken together, these findings suggest that the Rad54 and Rdh54/Tid1 proteins play different roles in meiosis. There might be two recombination pathways during meiosis, both of which can repair meiosis-specific DSBs: one dependent on *RAD54* and the other on *RDH54/TID1*. The *RDH54/TID1*-dependent pathway seems to be more efficient than the *RAD54*-dependent pathway. Alternatively, both Rad54 and Rdh54/Tid1 proteins may perform different roles in a single pathway. In either case, *RDH54/TID1* function is likely to confer meiosis specificity on the repair of DSBs. Consistent with this, the *rad54* cells that underwent meiosis under return-to-growth conditions can repair DSBs, judging from the proficiency of prototroph formation, while mitotic *rad54* cells cannot. This suggests that *RDH54/TID1* acquires the ability to repair DSBs in a very efficient way through incubation in SPM. *RDH54/TID1* function may be modified during meiosis (see below). However, we cannot exclude the possibility that the threshold amounts of the Rad54 and Rdh54/Tid1 proteins required for meiosis differ from the thresholds for vegetative growth.

During meiosis, recombination occurs in a specialized chromosome context (reviewed in ROEDER 1995; KLECKNER 1996). The meiosis-specific chromosome structure is thought to create a constraint on recombination and force recombination to occur between homologous chromosomes (XU *et al.* 1997; D. BISHOP, personal communication). In particular, the presence of the proposed meiotic constraints may confer a requirement for *RDH54/TID1* on meiotic cells for efficient repair of DSBs between homologues. This is consistent with a role of *RDH54/TID1* in recombination

between chromosomes during vegetative growth described above and reported by KLEIN (1997).

The meiotic cell cycle defects observed in the *rad54rdh54/tid1* double mutant may be a secondary effect induced by the inability of the mutant cells to repair DSBs, as described in other mutants that undergo arrest in meiotic prophase (BISHOP *et al.* 1992), since the status of recombination is monitored by a meiosis-specific surveillance mechanism (LYDALL *et al.* 1996; XU *et al.* 1997). In addition, cell cycle progression of the *rdh54/tid1* mutant cells was more severely perturbed than expected from the delay in repair of DSBs. At 10 hr of incubation in SPM, most DSBs in the *rdh54/tid1* mutant remained unrepaired, while 50% of the cells had completed MI and MII. Therefore, a checkpoint function might be partially abolished in the *rdh54/tid1* mutant. A more sensitive assay is required to investigate a role of *RDH54/TID1* in a meiotic checkpoint.

Possible functions of Rad54 and Rdh54/Tid1 proteins in recombination: Physical analysis of meiotic DSBs showed that the *rad54rdh54/tid1* mutant accumulated meiotic DSBs with more extensively resected ends than the wild type, indicating that Rad54 and Rdh54/Tid1 proteins are involved in a late stage in repair of DSBs, after the processing of DSB ends. A similar inability to repair DSBs in the *rad54* single mutant in vegetative growth was reported for HO endonuclease-induced DSBs (SUGAWARA *et al.* 1995). It remains to be resolved whether Rdh54p/Tid1p can bind to Rad51p, as observed for Rad54p (CLEAVER *et al.* 1996; JIANG *et al.* 1996). Given the phenotypic similarity of the *rad54rdh54/tid1* mutant to a *dmc1* mutant, we propose that the Rad54p and Rdh54p/Tid1p proteins are engaged in a process in which the Dmc1 protein acts, probably after the action of Rad51p (BISHOP 1994). Consistent with this idea, we observed the accumulation of Rad51p foci in meiotic nuclear spreads of the *rad54rdh54/tid1* double mutant (our unpublished results). The meiosis-specific RecA homologue Dmc1p may interact with Rdh54p/Tid1p as well as with Rad54p. The interaction of Rdh54p/Tid1p with a meiosis-specific protein might modify the *RDH54/TID1* function in meiosis. This would account for the more severe meiotic phenotypes than mitotic phenotypes of the *rdh54/tid1* mutant. Indeed, an interaction between the Dmc1 and the Rdh54/Tid1 proteins has been shown by a two hybrid assay (DRESSER *et al.* 1997).

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