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Homologous recombination is required for the viability of *rad27* mutants

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ABSTRACT

The *RAD27/RTH1* gene of *Saccharomyces cerevisiae* encodes a structural and functional homolog of the 5′–3′ exonuclease function of *Escherichia coli* DNA polymerase I. Four alleles of *RAD27* were recovered in a screen for hyper-recombination, a phenotype also displayed by *polA* mutants of *E.coli*. All four *rad27* mutants showed similar high levels of mitotic recombination, but varied in their growth rate at various temperatures, and sensitivity to the DNA damaging agent methyl methane sulfonate. Dependence of viability of *rad27* strains on recombination was determined by crossing a strain containing a null allele of *RAD27* to strains containing a mutation in either the *RAD1*, *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *MRE11*, *XRS2* or *RAD59* gene. In no case were viable spore products recovered that contained both mutations. Elimination of the non-homologous end-joining pathway did not affect the viability of a *rad27* strain. This suggests that lesions generated in the absence of *RAD27* must be processed by homologous recombination.

INTRODUCTION

The completion of DNA synthesis requires the activity of an exonuclease to remove RNA primers from Okazaki fragments prior to ligation of the nascent strands. In prokaryotes, this function is fulfilled by the combined activities of RNase H and DNA polymerase I (Pol I). Mutation of the gene encoding Pol I, *polA*, confers a pleiotropic phenotype that includes temperature-sensitive (ts) growth, UV sensitivity, sensitivity to DNA methylating agents, elevated rates of recombination and synthetic lethality when combined with mutations in recombination genes, such as *recA* (1–3).

In mammalian cells, a 5′–3′ exonuclease activity required for the completion of lagging strand DNA synthesis *in vitro* has been identified in several laboratories (4–7). This activity, like the 5′–3′ exonuclease activity of Pol I, acts to remove the terminal ribonucleotide of the RNA primer of Okazaki fragments (5,6). The same activity was discovered as a flap endonuclease, FEN-1, capable of removing a non-base paired single-stranded region from a branched duplex structure (8,9). The sequence of the mouse FEN-1 protein revealed significant homology to the *Schizosaccharomyces pombe* rad2 protein (10) and to the predicted product of an open reading frame on chromosome *XI* of *Saccharomyces cerevisiae* that has been named *RAD27*, *RTH1* or *ERC11* (11–13). The FEN-1/Rad27 family of proteins is also related to *S.cerevisiae* Rad2 (*S.pombe* rad13; human XPG), an endonuclease essential for nucleotide excision repair (9,11,12), and ExoI, a 5′–3′ exonuclease implicated in mismatch repair and genetic recombination (14–17). The *RAD27* gene product was purified and shown to exhibit flap endonuclease and 5′–3′ exonuclease activities *in vitro* (9). Genetic analysis of yeast strains deleted for *RAD27* has revealed a pleiotropic phenotype similar to that exhibited by *Escherichia coli polA* mutants (11,12). This includes a reduced growth rate at 30°C, inability to grow at 37°C (ts growth defect), sensitivity to methyl methane sulfonate (MMS), and elevated rates of mitotic recombination and spontaneous mutation. The spectrum of spontaneous mutations generated in *rad27* strains is unusual, resulting from duplications between short repeated sequences (18). Because of the enhanced rate of duplicative mutations, *rad27* mutants also show greatly elevated rates of di- and trinucleotide tract instability (19–21). Several models have been proposed to account for the formation of duplications in *rad27* strains. These include slip mispairing by displacement of the unrepaired primer of one Okazaki fragment by displacement synthesis from the preceding one, and strand breakage to channel lesions into the recombination pathway (18,21). The latter hypothesis is consistent with the observed hyper-recombination phenotype of *rad27* mutants and also with the synthetic lethality of *rad27 rad51* double mutants (10,12,13,18).

In bacteria, yeast and humans, a number of genes have been identified that when mutated confer a hyper-recombination phenotype (3,22–28). These generally define proteins directly involved in DNA metabolism, e.g. DNA ligase (29), DNA polymerases (3,22,23), DNA helicases (28,30–32) and DNA topoisomerases (24,33). It is assumed that defects in DNA metabolism lead to the accumulation of recombinogenic lesions, hence the hyper-recombination phenotype, and, in some cases, lethality when combined with mutations in recombination genes (10,18,30,34). In this report we describe a yeast strain that was used to identify hyper-recombination mutants by a simple colony color-sectoring assay. Of the mutants identified, four were shown to contain alleles of *RAD27*. Characterization of the different alleles is presented.

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MATERIALS AND METHODS

Media, growth conditions and genetic methods

Rich medium (YPD), synthetic complete (SC) medium lacking the appropriate amino acid or nucleic acid base, and sporulation medium were prepared as described previously (35). Rich medium plates containing MMS were used within 1 day of preparation. Yeast cells were grown at 30°C unless otherwise stated. Transformations were performed by the lithium acetate method (36). Sporulation and tetrad dissection was carried out as described previously (35). For crosses of *rad27* to recombination mutants, at least two independently derived diploids were used for tetrad dissection.

Yeast strains and plasmids

All of the strains used for this study are derivatives of W303-1A or W303-1B and are listed in Table 1. The strain used to identify recombination mutants contains an inverted repeat of two mutant copies of the *ade2* gene (37) and a direct repeat of two mutant copies of *leu2* separated by *LYS2* sequences (Fig. 1). This strain was constructed as follows. Strain LSY114-6C was transformed with *Eco*RI-digested pLS119 to target integration at the *LEU2* locus. Lys$^+$ transformants that were also Leu$^-$ were analyzed by minifluctuation tests to ensure that direct repeat recombination could occur to generate Leu$^+$ prototrophs. The resulting strain, LYS409, was crossed to yAR91 and diploids were dissected to yield haploid progeny containing both the *leu2* and *ade2* recombination reporters. Strain LSY412-7C was used for mutagenesis, and a 5-fluoroorotic acid (5-FOA)-resistant derivative of strain LSY412-19A, LSY459, was used for backcrossing putative mutants. Strains YBL2 and YBL3 were constructed by one-step gene replacement (38) of W303-1B and W303-1A, respectively, using plasmid pMR*rad27*Δ*::TRP1* (11). To measure MMS sensitivity, each of the *rad27* mutants was crossed to a *RAD5* derivative of W303, HKY579-9A (39).

Table 1. Yeast strains

Name	Relevant genotype or description[a]	Source
W303-1A	*MAT***a** *leu2-3, 112 trp1-1 can1-100 ade2-1 his3-11, 15 rad5-535*	R. Rothstein
W303-1B	*MAT*α *leu2-3, 112 trp1-1 can1-100 ade2-1 his3-11, 15 rad5-535*	R. Rothstein
W838-24D	*MAT***a** *rad1::LEU2 rad52::TRP1*	R. Rothstein
R877	*MAT*α *hdf1::LEU2*	R. Rothstein
YAR71	*MAT***a** *ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n*	37
yAR91	*MAT*α *ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n*	37
B360-1A	*MAT***a** *rad59::LEU2*	49
HKY579-9A	*MAT*α *RAD5 LEU2*	39
HKY595-1C	*MAT*α *RAD5 rad51::LEU2*	H. Klein
HKY604-17A	*MAT*α *RAD5 rad50::hisG-URA3-hisG*	H. Klein
YBL2	*MAT*α *rad27::TRP1*	This study
YBL3	*MAT***a** *rad27::TRP1*	This study
LSY114-6C	*MAT***a** *LEU2 lys2*	This study
LSY409	*MAT***a** *leu2-bst::LYS2::leu2-112 derivative of LSY114-6C* (integration of pLS119)	This study
LSY412-7C	*MAT*α *leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n* (spore from LSY409 X yAR91 diploid)	This study
LSY412-19A	*MAT***a** *leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG ade2-5′Δ-TRP1-ade2-n* (spore from LSY409 X yAR91 diploid)	This study
LSY459	*MAT***a** *leu2-bst::LYS2::leu2-112 ade2::hisG ade2-5′Δ-TRP1-ade2-n* (5-FOA resistant derivative of LSY412-19A)	This study
LSY470-2C	*MAT***a** *rad27-3 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY470-4A	*MAT*α *rad27-3 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY471-1D	*MAT*α *rad27-4 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY471-2D	*MAT***a** *rad27-4 leu2-bst::LYS2::leu2-112 ade2::hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY472-2C	*MAT***a** *rad27-5 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY472-7A	*MAT*α *rad27-5 leu2-bst::LYS2::leu2-112 ade2::hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY473-1B	*MAT***a** *rad27-6 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY473-2D	*MAT*α *rad27-6 leu2-bst::LYS2::leu2-112 ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n*	This study
LSY486-2A	*MAT*α *rad27::URA3*	16
LSY502-15C	*MAT***a** *rad27::URA3 ade2::hisG-URA3-hisG spo13::hisG ade2-5′Δ-TRP1-ade2-n* (derived from yAR71 X LSY486-2A)	This study
LSY702-6B	*MAT*α *rad27::TRP1 RAD5* (derived from YBL3 X HKY579-9A)	This study
LSY703-9D	*MAT***a** *rad27-3 LEU2 RAD5* (derived from LSY470-2C X HKY579-9A)	This study
LSY704-6C	*MAT*α *rad27-4 LEU2 RAD5* (derived from LSY471-2D X HKY579-9A)	This study
LSY705-4B	*MAT***a** *rad27-5 LEU2 RAD5* (derived from LSY472-2C X HKY579-9A)	This study
LSY706-3A	*MAT***a** *rad27-6 LEU2 RAD5* (derived from LSY473-1B X HKY579-9A)	This study

[a]All strains are derivative of W303-1A or W303-1B; only differences in genotype from the parental strains are listed.

Plasmid pLS119 that was used to construct the chromosomal *leu2* recombination reporter was made as follows. A *Spe*I/*Eco*RI fragment from plasmid pLS32, containing the *leu2-bst* allele (40), was substituted for the *Spe*I/*Eco*RI fragment of pUC19::*leu2-112* to generate a *leu2* gene containing two mutant alleles (pLS97). A 1.1 kb *Hin*dIII fragment containing the *URA3* gene was cloned into the *Hin*dIII site within the multiple cloning site of pLS97, generating plasmid pLS98. A 5 kb *Eco*RI/*Hin*dIII fragment containing the *LYS2* gene was inserted into the *Stu*I site within the *URA3* gene of pLS98 to generate plasmid pLS119.

Mutagenesis

Mutagenesis of strain LSY412-7C was as described (41). In brief, cells were grown to saturation in rich medium, harvested and resuspended at a density of 2×10^7 ml in 50 mM potassium phosphate, pH 7.0. Ethyl methane sulfonate (EMS) was added to 3% and cells were incubated at 30°C, with shaking, for 30 min. An equal volume of 10% sodium thiosulfate was added to the culture. The cells were harvested, washed twice with water and then suspended in rich medium. The culture was incubated at 30°C, with shaking, for 3 h and then harvested, washed with water and stored at 4°C for up to 1 week. Cells were diluted with water and plated on YPD plates at a density of ~200 colonies/plate to screen for recombination mutants.

MMS sensitivity assays

Stationary phase cultures were harvested and resuspended in 0.1 M potassium phosphate, pH 7.0, at a density of 4×10^7/ml. MMS was added to a final concentration of 0.2% and samples were incubated at 30°C with shaking. Samples (0.1 ml) were removed at various times after MMS addition, mixed with an equal volume of 10% sodium thiosulfate, and then serially diluted with water. Dilutions were plated on solid YPD medium and counted after 3 days at 30°C. To assay for MMS sensitivity on solid medium, serial dilutions of late log-phase cultures were spotted onto YPD medium containing 0, 0.01 or 0.015% MMS. The plates were incubated at 23, 30 or 35°C for 3–4 days.

Measurement of recombination rates

For rapid rescreening of putative mutants, 1 cm patches of cells grown on YPD were replica plated to SC medium lacking adenine and leucine. The double selection for recombination events at both reporters provided a more stringent test for hyper-recombination. Strain LSY412-7C yielded 1–10 papillae by this assay whereas hyper-recombination mutants exhibited >40 papillae/patch of cells. Minifluctuation tests were performed by suspending individual colonies that had been grown on YPD plates for 3 days in 1 ml water and then plating 0.1 ml on SC-Ade or SC-Leu plates. Recombination rates were determined as described previously (37). For each genotype rates were determined for at least two independent spores.

RESULTS

Identification of four alleles of *RAD27* as hyper-recombination mutants

A colony color sectoring assay based on recombination between inverted repeats of mutant alleles of the *ade2* gene was developed to identify recombination mutants in *S.cerevisiae* (37). Recombination events are detected by the formation of a white sector within a red colony providing a simple visual screen for mutants with elevated or reduced levels of recombination. This strain was further modified to include a direct repeat of mutant alleles of the *leu2* gene separated by a copy of the *LYS2* gene. Thus the strain used for mutagenesis contained two recombination reporters to monitor the rate of mitotic recombination (Fig. 1). Following mutagenesis of strain LSY412-7C with EMS, 27 000 survivors were screened for colonies that showed elevated levels of white sectors. From the primary screen, >100 putative hyper-recombination mutants were identified. These were rescreened by replica plating patches of cells pregrown on rich medium onto synthetic medium lacking adenine and leucine to detect the formation of Ade$^+$ Leu$^+$ papillae. From the secondary screen, eight mutants that showed at least a 10-fold increase in the number of Ade$^+$ Leu$^+$ papillae were recovered and were analyzed further (U46, U47, U50, U68, U70, U92, U99 and U101). When backcrossed, six of the eight mutants showed clear 2:2 segregation of the elevated sectoring phenotype in all tetrads examined. Five of these six mutants also showed a ts growth defect at 37°C, which segregated with the hyper-recombination phenotype. Because many *cdc* mutants show a hyper-recombination phenotype, the five ts mutants were crossed to strains containing *cdc2*, *cdc4*, *cdc5*, *cdc6*, *cdc7*, *cdc8*, *cdc9*, *cdc13*, *cdc15*, *cdc17*, *mcm2*, *mcm3* and *pkc1* mutations to test for complementation of the ts growth defect. Based on the inability of diploids to grow at 37°C, one mutant was classified as a *cdc2* mutant, and another as *cdc17*. The ts growth defect of the other three mutants, U50, U70 and U99, was complemented by all of these strains. When crossed to each other, no complementation of the hyper-recombination and ts growth defects was observed among these three mutants. Allelism was verified by dissecting tetrads from each pairwise cross and demonstrating that all four spores were ts for growth and had elevated levels of recombination. The non-ts hyper-recombination mutant, U47, when crossed to U50, U70 and U99 failed to complement the hyper-recombination phenotype. Thus four alleles of the same gene were recovered from the screen. Because deletions of *RAD27/RTH1* cause a hyper-recombination phenotype

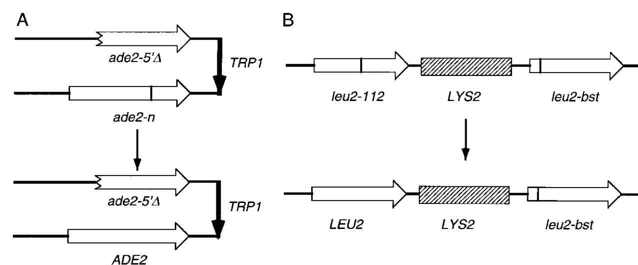

Figure 1. Recombination reporters used to detect mitotic recombination. (**A**) The *ade2* inverted repeat reporter contains one copy of *ade2* with a deletion of the 5′ coding region; the other copy of *ade2* contains a frameshift mutation at the unique *Nde*I site. Recombination events can occur by gene conversion (shown) and/or crossing over (not shown) to generate a wild type copy of the *ADE2* gene, which in turn results in the formation of white-colored Ade$^+$ sectors among red-colored Ade$^-$ cells (37). (**B**) The *leu2* recombination reporter contains heteroalleles of *leu2* separated by plasmid sequences and the *LYS2* gene. Recombination events can occur by a variety of mechanisms to produce a functional copy of *LEU2*. Only one type of recombination event, gene conversion, is shown.

and conditional lethality, we considered the possibility that U47, U50, U70 and U99 are alleles of *RAD27*. This was confirmed by complementation and allelism tests using strain LSY486-2A, which contains a deletion of the *RAD27* gene. The *rad27* alleles in the four mutants were named *rad27-3* to *rad27-6*.

Characterization of the *rad27* mutants

The rate of recombination for each of the strains, including a *rad27*Δ mutant, was determined (Table 2). Each of the mutants showed a >10-fold higher rate of recombination than the wild type strain, but among the *rad27* mutant strains there was only a 2-fold difference. The mutants were tested for defects in meiotic recombination by generation of homozygous diploids and determination of sporulation efficiency and spore viability. Defects in meiotic recombination are manifested by sporulation deficiency or reduced spore viability. The *rad27*Δ diploid exhibited 70% spore viability, whereas the other diploids showed between 80 and 96% spore viability, compared with 98% for the wild type strain. The reduction in spore viability observed for the *rad27*Δ strain is most likely due to the accumulation of recessive mutations during vegetative growth of the diploids prior to sporulation rather than a defect in meiotic recombination, because extended vegetative growth led to much higher spore inviability.

Table 2. Recombination rates of the *ade2* inverted-repeat substrate

Strain	Rate $Ade^+ \times 10^{-3}$	Relative rate
RAD27	0.2	1.0
*rad27*Δ	3.9	21.0
rad27-3	2.4	12.7
rad27-4	3.2	16.9
rad27-5	2.4	12.4
rad27-6	2.1	10.9

The doubling time in rich medium was determined for each of the mutants at both 30 and 37°C (Table 3). The growth rate of the *rad27-4* strain was similar to the *rad27*Δ strain, whereas the other strains had less severe growth defects. The strain containing the *rad27-6* allele showed the least severe growth defect at 30°C, but had a doubling time of 4.5 h, similar to the *rad27-4* strain, at 37°C. The *rad27-3* mutant, which was originally characterized as non-ts for growth, had the least severe growth defect at 37°C.

Table 3. Doubling time of *rad27* mutants

Strain genotype	Doubling time (min) at 30°C	Doubling time (min) at 37°C
RAD27	105	138
*rad27*Δ	173	318
rad27-3	132	186
rad27-4	165	306
rad27-5	132	207
rad27-6	123	270

Because deletion alleles of *RAD27* confer sensitivity to the DNA damaging agent MMS, each of the *rad27* mutants was tested for survival following exposure to MMS. W303, the common laboratory strain that was used for mutagenesis, was discovered later to contain a weak allele of *RAD5*, *rad5-535*, that confers partial sensitivity to high concentrations of MMS (39). To eliminate effects of the *rad5-535* allele, all of the strains were crossed to a *RAD5* derivative of W303 to obtain haploid segregants that were *RAD5 rad27*. Initially, the tests for MMS sensitivity were done in liquid using stationary phase cultures. Under these conditions, mutants *rad27-3* and *rad27-4* were almost as sensitive to MMS as the *rad27* null mutant, and *rad27-6* was the most resistant (Fig. 2). Sensitivity to MMS was also tested by plating cells on solid medium containing different concentrations of MMS, followed by incubation at different temperatures (Fig. 3). By this assay the *rad27-4* strain was most like the *rad27*Δ strain; however, the other mutants had quite different properties. Surprisingly, the *rad27-3* strain was found to be more sensitive to MMS than the *rad27*Δ strain at all temperatures tested. The increased sensitivity conferred by the *rad27-3* allele was observed for all spores tested from the second and third backcrosses, suggesting that it is unlikely to be due to an additional mutation. The *rad27-6* strain was almost as resistant to MMS as the wild type strain at 23°C, but at 35°C showed high sensitivity. Thus, based on MMS sensitivity and growth rate, the *rad27-6* mutation appears to cause conditional defects.

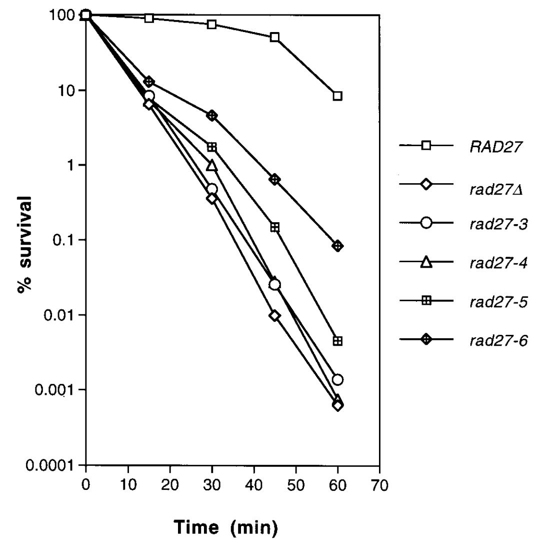

Figure 2. Sensitivity of *rad27* mutants to MMS. Stationary phase cultures were treated with MMS as described in Materials and Methods.

Recombination is required for the viability of *rad27* mutants

To determine whether the viability of *rad27* strains is dependent on recombination, a strain containing a null allele of *RAD27* was crossed to strains containing a mutation in either the *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *MRE11* or *XRS2* gene. For all of the crosses at least 22% of the spores were inviable. The viable spores were genotyped according to the biosynthetic markers used to make the gene disruptions. No viable spore products were recovered that contained both mutations, even when germinated at 23°C (Fig. 4). Mutation of *RAD1* has been shown to reduce the frequency of some mitotic

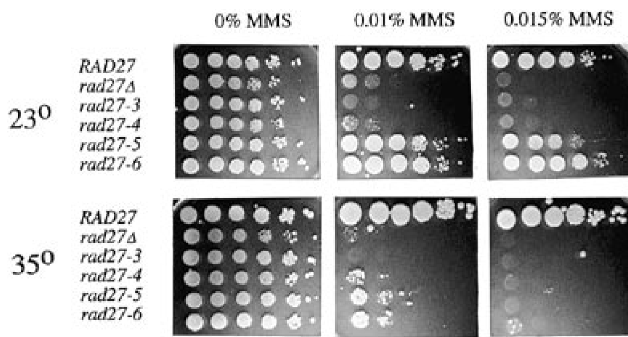

Figure 3. Effects of temperature on the MMS sensitivity of *rad27* mutants. Cultures of each strain were serially diluted and 4 µl aliquots were spotted onto YPD plates containing the indicated amount of MMS. Duplicate plates were incubated at 23 and 35°C.

recombination events, including one-step gene replacement and direct repeat recombination (42–44). A *rad1 rad52* double mutant strain was crossed to *rad27* to determine whether Rad1 is required to process lesions generated in *rad27* strains. From this cross, *rad1 rad27* double mutant spores were recovered showing that *RAD1* does not play a role in the recombination events stimulated in the absence of *RAD27* (Fig. 4A). A *rad1* mutation has also been shown to suppress the lethality of *rad52 rad3-101* double mutants (30). However, the *rad1* mutation was unable to suppress the lethality of the *rad27 rad52* double mutant. The *RAD50*, *MRE11* and *XRS2* genes are required for the non-homologous end-joining (NHEJ) pathway of double-strand break repair (45,46) and this has been suggested to be their primary DNA repair function in mitotic cells. We found that all three mutations cause inviability in combination with *rad27* (Fig. 4C and data not shown). To more directly determine the role of the NHEJ pathway in repair, an *hdf1* strain (deficient in Ku70) was crossed to *rad27*. From this cross double mutant spores were obtained at the expected frequency (24% of spore products) indicating that the Ku-mediated double-strand-break repair pathway is not required to repair lesions generated in *rad27* mutants (Fig. 4D).

In all of the above crosses, strains containing a *rad27*Δ allele were used. Since the *rad27-6* allele confers a ts phenotype for growth and MMS sensitivity it was of interest to determine whether a strain could be made containing the *rad51* and *rad27-6* mutations. When a *rad27-6* strain was crossed to a *rad51*Δ strain and the dissected spores germinated at 23°C, most were found to be viable. The *rad27-6 rad51* double mutants exhibited reduced growth at 23°C and showed no growth at 35°C, whereas the single mutants grew at both temperatures verifying the conditional nature of the *rad27-6* allele.

DISCUSSION

A yeast strain is described with general utility for the identification of mutants with increased rates of recombination. This strain has several advantages over those described previously: (i) the strain exhibits high rates of spontaneous mitotic recombination; (ii) recombination events can be visualized by a colony color sectoring assay; (iii) a second reporter ensures that defects are general rather than specific to the *ade2* reporter; and (iv) the use of an inverted repeat substrate avoids events that can occur by non-conservative mechanisms, such as single-strand annealing. Using this strain, four alleles of *RAD27* were identified, as well as *cdc2* and *cdc17* mutants. The identification of mutants defective in three different replication functions verifies the utility of this strain in screens for hyper-recombination defects. The

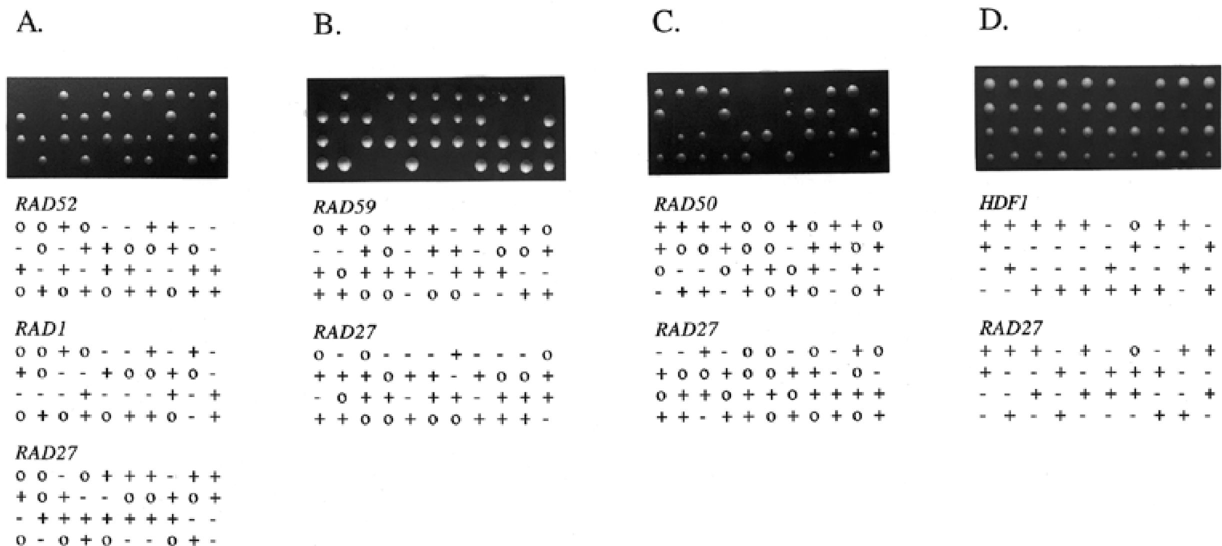

Figure 4. Inviability of *rad27* with mutants of the *rad52* epistasis group. (**A**) Spores derived from a diploid heterozygous for *RAD27*, *RAD1* and *RAD52*. (**B**) Spores derived from a diploid heterozygous for *RAD27* and *RAD59*. (**C**) Spores derived from a diploid heterozygous for *RAD27* and *RAD50*. (**D**) Spores derived from a diploid heterozygous for *RAD27* and *HDF1*. In each case the grid below the tetrad dissection indicates the genotype of each spore. +, wild type locus; –, mutant locus; O, dead spore.

most likely explanation for the recovery of four *rad27* mutants in this screen is that the strains grow quite well at 30°C, whereas most *cdc* mutants that exhibit hyper-recombination grow poorly at the semi-permissive temperature.

The *rad27* strains all show a similar elevation of the rate of recombination (Table 2), but differ in the severity of other phenotypes. For example, the *rad27-6* allele shows the least severe growth defect and MMS sensitivity at 30°C, but at 37°C has growth characteristics and MMS sensitivity similar to the null mutant (Figure 3). The viability of a *rad27-6 rad51* double mutant at 23°C confirms the conditional nature of the *rad27-6* mutation. The conditional phenotype of the *rad27-6* allele may be useful for further analysis of the role of Rad27 in replication and mutation avoidance. The *rad27-3* strain shows MMS sensitivity similar to the *rad27* null mutant in stationary phase cultures, but when grown on solid medium containing MMS it appears to be more sensitive than the null mutant. The MMS sensitivity of stationary phase cultures is thought to reflect a defect in base excision repair (47). When cells are grown on plates that contain MMS they must tolerate the drug during all phases of the cell cycle. It has recently been shown that low levels of MMS added to growing cultures causes a delay in S-phase that is dependent on checkpoint functions (48). Thus, it is possible that the *rad27-3* strain is defective in this checkpoint leading to additional sensitivity of growing cultures to MMS.

It has previously been reported that *rad27 rad51* double mutants of *S.pombe* and *S.cerevisiae* are inviable (10,18). However, Sommers *et al*. (12) reported that *rad52 rad27* double mutants are viable, but grow slowly. To determine the requirement for genes of the *RAD52* epistasis group for viability of *rad27* strains, diploids were constructed that were heterozygous for *RAD27* and for each of the members of the *RAD52* group genes and then dissected to determine the percent spore viability. From each cross, 22–32% of the spores were inviable as would be expected for lethality of two unlinked mutations. The observed lethality of the *rad27 rad52* double mutant conflicts with the results of Sommers *et al*. One possible explanation is that the alleles used in the study by Sommers *et al*. are leaky, or that the strains used in their study contain a suppressor mutation.

The finding that *rad27 rad59* double mutants are inviable was surprising because *rad59* mutants show much greater resistance to ionizing radiation than other mutants of the *rad52* group (49). The *RAD59* gene was originally identified by its requirement for *RAD51*-independent mitotic recombination and subsequently shown to encode a homolog of Rad52 (49). Because *rad59* mutants show no defect in recombination between homologs, or in meiosis, but are defective in recombination between inverted repeats, it was suggested that Rad59 might be involved in sister chromatid recombination. The observed lethality with *rad27* is consistent with this hypothesis. Furthermore, it suggests that synthetic lethality with *rad27* is a sensitive screen for recombination defects. Although *rad1* and *rad59* mutants show similar sensitivity to ionizing radiation, *rad1 rad27* double mutants are viable. This result confirms that Rad1 function during mitotic recombination is limited to removal of non-homologous sequences (44).

We were also unable to generate double mutants of *rad27* with *rad50, xrs2* or *mre11*. These three mutants show poor vegetative growth and extreme sensitivity to ionizing radiation. The recent finding that these mutants are defective in the NHEJ pathway for double-strand break repair, show elevated rates of heteroallelic recombination in diploids and are proficient at mating type switching, has led to the suggestion that the primary function of these genes is in the end-joining pathway. The Ku heterodimer is essential for NHEJ and has no known role in homologous recombination. In this study, a *rad27 hdf1* was shown to be viable indicating that the NHEJ pathway is not required for repair of lesions that occur in *rad27* strains. From these experiments one can conclude that Rad50, Mre11 and Xrs2 are required for recombination events between sister chromatids, or that the nuclease activity of the Mre11/Rad50/Xrs2 complex is essential for processing Okazaki fragments in the absence of Rad27 (50).

In this study we have shown that yeast strains containing a null mutation of *RAD27* are dependent on homologous recombination for survival. Homologous recombination is also required to process DNA lesions formed by defects in DNA ligase I and PCNA (34,51). All of these factors participate in lagging strand DNA synthesis. Mutation of *RAD27* also causes inviability in combination with the *pol3-01* mutation (an allele containing a mutation in the 'proofreading' exonuclease domain of DNA polymerase δ), with an *exo1* mutation (defective for a 5′–3′ exonuclease), and with a ts allele of *DNA2*, which encodes a DNA helicase (17,21,52). These defects may define alternate modes of processing Okazaki fragments in the absence of the Rad27 nuclease.

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