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Role of the *Saccharomyces cerevisiae* Rad51 Paralogs in Sister Chromatid Recombination

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ABSTRACT

Rad51 requires a number of other proteins, including the Rad51 paralogs, for efficient recombination *in vivo*. Current evidence suggests that the yeast Rad51 paralogs, Rad55 and Rad57, are important in formation or stabilization of the Rad51 nucleoprotein filament. To gain further insights into the function of the Rad51 paralogs, reporters were designed to measure spontaneous or double-strand break (DSB)-induced sister or nonsister recombination. Spontaneous sister chromatid recombination (SCR) was reduced 6000-fold in the *rad57* mutant, significantly more than in the *rad51* mutant. Although the DSB-induced recombination defect of *rad57* was suppressed by overexpression of Rad51, elevated temperature, or expression of both mating-type alleles, the *rad57* defect in spontaneous SCR was not strongly suppressed by these same factors. In addition, the UV sensitivity of the *rad57* mutant was not strongly suppressed by *MAT* heterozygosity, even though Rad51 foci were restored under these conditions. This lack of suppression suggests that Rad55 and Rad57 have different roles in the recombinational repair of stalled replication forks compared with DSB repair. Furthermore, these data suggest that most spontaneous SCR initiates from single-stranded gaps formed at stalled replication forks rather than DSBs.

HOMOLOGOUS recombination is an important DNA repair mechanism to maintain genome integrity. Central to the process of homologous recombination is the pairing of DNA molecules and exchange of single strands to form heteroduplex DNA, a reaction catalyzed by members of the RecA/Rad51 family of proteins. Yeast and humans encode two RecA homologs, Rad51 and Dmc1, as well as Rad51-related proteins, referred to as Rad51 paralogs (Gasior *et al.* 2001; Thompson and Schild 2001). Yeast *RAD51* is required for resistance to ionizing radiation, for spontaneous and induced mitotic recombination, and for meiotic recombination (Symington 2002). The Rad51 paralogs of *Saccharomyces cerevisiae* are encoded by the *RAD55* and *RAD57* genes and are determined by genetic studies to function in the same pathway for DNA repair and recombination as *RAD51* (Kans and Mortimer 1991; Lovett 1994; Rattray and Symington 1995). The vertebrate Rad51 paralogs are encoded by the *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2*, and *XRCC3* genes (Thompson and Schild 2001). Mutation of any of these genes in the chicken DT40 cell line results in high sensitivity to DNA cross-linking agents, decreased frequencies of gene targeting, and increased frequencies of spontaneous chromosome aberrations (Takata *et al.* 2001).

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Purified Rad51 forms right-handed helical filaments on single-stranded (ss) and double-stranded (ds) DNA (Ogawa *et al.* 1993; Sung and Robberson 1995). The Rad51-ssDNA nucleoprotein filament is active for homologous pairing and strand exchange with dsDNA. Formation of filaments on ssDNA is stimulated in the presence of the replication protein A (RPA) (Sung and Robberson 1995; Sugiyama *et al.* 1997), which is thought to allow the formation of continuous filaments by removal of secondary structures from ssDNA (Sugiyama *et al.* 1997). However, addition of RPA prior to or simultaneously with Rad51 is inhibitory to DNA binding and strand exchange by Rad51.

The Rad55 and Rad57 proteins, which form a stable heterodimer, can overcome the inhibition to Rad51-promoted strand exchange imposed by RPA, but the mechanism of mediation is unknown (Sung 1997). Consistent with a role in Rad51 recruitment, Rad51 foci are not observed in *rad55* or *rad57* mutants during meiosis (Gasior *et al.* 1998). However, Rad51 is still able to associate with double-strand breaks (DSBs) in *rad55* mutants during vegetative growth although recruitment of Rad51 is slower and less extensive in *rad55* mutants than in wild type (Sugawara *et al.* 2003; Lisby *et al.* 2004; Fung *et al.* 2006). The role of the Rad51 paralogs as accessory proteins for Rad51 is also supported by the observation that overexpression of *RAD51* partially suppresses the radiation or mitomycin C sensitivity of cell lines with mutations in any of the Rad51 paralog-encoding genes (Hays *et al.* 1995; Johnson and

Symington 1995; Takata *et al.* 2001). Furthermore, gain-of-function alleles of yeast *RAD51* that encode proteins with higher affinity for DNA than wild-type Rad51 partially suppress the ionizing radiation (IR) sensitivity of *rad55* or *rad57* mutants (Fortin and Symington 2002). The IR sensitivity of *rad55* or *rad57* mutants is also suppressed by expression of both mating-type alleles in haploids. It has been suggested that this suppression acts at the level of Rad51 activity because *MAT* heterozygosity, deletion of *SRS2*, or overexpression of Rad51 also suppresses other mutants known to have mediator defects, such as *rad51-K191R* (Fung *et al.* 2006) and *rad52-20* (Schild 1995), but does not suppress *rad51* or *rad52* null mutants. In budding and fission yeasts, *rad55* or *rad57* null mutants exhibit cold sensitivity for DSB repair (DSBR) (Symington 2002). Cold sensitivity is a property often associated with proteins composed of multiple subunits or large multiprotein complexes (Scheraga *et al.* 1962), consistent with a role for the Rad51 paralogs in stabilizing Rad51 nucleoprotein filaments.

While the biochemical and cytological studies support a role for the Rad51 paralogs in promoting assembly or stability of the Rad51 nucleoprotein filament (Gasior *et al.* 1998; Van Veelen *et al.* 2005), recent studies suggest the possibility of an additional late function in recombination. Rad51B and the BCDX2 complex have been shown to preferentially bind synthetic Holliday junctions (HJs) over other types of DNA substrates (Yokoyama *et al.* 2004). Furthermore, extracts made from *XRCC3*$^{-/-}$ or *RAD51C*$^{-/-}$ hamster cells lacked normal levels of HJ resolvase activity, suggesting that the Rad51C-Xrcc3 complex may contribute to the resolution of recombination intermediates (Liu *et al.* 2004). Increased evidence for this postulate comes from the report that Rad51C localizes to paired bivalents during the late stages of prophase during meiosis I when crossovers are thought to occur (Liu *et al.* 2007).

Mammalian *XRCC3*$^{-/-}$ or *RAD51C*$^{-/-}$ cell lines, and *Schizosaccharomyces pombe rad57* mutants, which show reduced frequencies of DSB-induced recombination, also show alterations in the products recovered with an increase in long-tract gene conversion events (Brenneman *et al.* 2002; Nagaraju *et al.* 2006; Akamatsu *et al.* 2007; Hope *et al.* 2007). The long-tract events could be due to altered processing of recombination intermediates, perhaps in displacement of the invading strand.

Understanding the role of the Rad51 paralogs in homologous recombination in yeast has been difficult because the *rad55* and *rad57* mutants show minimal phenotypes in assays that measure spontaneous mitotic recombination between heteroalleles located on homologous chromosomes in diploids or positioned on nonhomologous chromosomes in haploids (Lovett and Mortimer 1987; Freedman and Jinks-Robertson 2002). This weak phenotype in recombination assays is in contrast to the high IR sensitivity displayed by *rad55* and *rad57* mutants (Symington 2002). A common feature of the recombination assays is selection for spontaneous recombination between nonsister chromatids, whereas IR-induced damage (DSBs) is thought to be repaired primarily by sister chromatid recombination (Kadyk and Hartwell 1992). In addition, *rad55* or *rad57* mutants that express both *MAT***a** and *MAT*α alleles, as is typically the case in diploids, show much higher resistance to IR, suggesting that the failure to detect a recombination defect in diploids could be a consequence of suppression by *MAT* heterozygosity.

To determine whether the weak recombination defect of *rad55* and *rad57* mutants relates to *MAT* heterozygosity, the type of recombination reporter used, or the source of DNA damage, we designed two recombination systems to measure spontaneous or DSB-induced gene conversion. We show that *rad57* mutants are highly defective for spontaneous gene conversion between direct repeats and that this defect is not suppressed by factors that suppress the IR sensitivity of the *rad57* mutants, suggesting a specialized function for Rad55-Rad57 in the repair of spontaneous lesions. In addition, these studies suggest that the substrate for spontaneous sister chromatid recombination is more likely to be a ssDNA gap (SSG) formed at a stalled replication fork than a DSB.

MATERIALS AND METHODS

Media, growth conditions, and genetic methods: Rich medium (yeast extract–peptone–dextrose, YPD), synthetic complete (SC) medium lacking the appropriate amino acids or nucleic acid bases, sporulation medium, and genetic methods were as described previously (Sherman *et al.* 1986). Synthetic minimal medium containing 2% lactate (pH 5.5) and supplemented with adenine, uracil, and leucine was used for the galactose induction of *I-SceI* in the direct-repeat double-strand break-induced recombination assays. Minimal medium containing 2% lactate (pH 5.5) and supplemented with adenine, uracil, leucine, and tryptophan was used for the galactose induction of *I-SceI* in the heteroallelic double-strand break-induced recombination assays. Transformation of yeast cells was performed by the lithium acetate method (Ito *et al.* 1983).

Yeast strains and plasmids: *S. cerevisiae* strains used in this study are listed in Table 1. All strains are in the *RAD5*-corrected W303 background (*his3-11, 15 leu2-3, 112 trp1-1 ura3-1 ade2-1 can1-100 RAD5*) except those listed specifically as *rad5-535* and BY4742. To create a haploid strain with the *ade2-n::TRP1::ade2-I* direct repeat, pLS189 was digested with *Bgl*II, which cuts between the *Nde*I site mutation and the I-SceI site insertion within *ade2*, to target integration at the *ADE2* locus of LSY697. Trp$^+$ transformants that were red (*ade2*) were analyzed by Southern hybridization to determine the structure of the integrated plasmid. LSY1429#2 contains a direct repeat of *ade2-nde*$^-$ and *ade2-I-SceI*$^+$ alleles separated by vector sequences and *TRP1* (referred to as *ade2-n::TRP1::ade2-I*). Strain LSY1430 was made by the same method to create the *ade2-n::TRP1::ade2-I* reporter in the *exo1* background. Strain LSY1309 was made by gene replacement of the *MAT*α locus with *URA3* using pFP19. Transformants deleted for the *MAT* locus become "**a** fakers" and are competent for mating with a *MAT*α test strain.

TABLE 1

Yeast strains

Strain	Genotype	Source or reference
W1588-4C	*MAT***a**	R. Rothstein
W1588-4A	*MAT*α	R. Rothstein
W3770-4D	*MAT***a** *leu2ΔEcoRI::URA3-HO::leu2ΔBstEII*	Smith and Rothstein (1999)
W4121-20D	*MAT***a** *ADE2 bar1::LEU2 YFP-RAD51*	Lisby *et al.* (2004)
W5857-15A	*MAT***a** *ADE2 bar1::LEU2 YFP-RAD51 RAD54-CFP rad55*	Lisby *et al.* (2004)
B366-6A	*MAT***a** *ade2-n rad5-535*	Bai and Symington (1996)
yKH12α	*MAT*α *ade2-a::URA3::ade2-n rad5-535*	Huang and Symington (1994)
LSY383	*MAT***a** *rad51::LEU2 rad5-535*	Mcdonald and Rothstein (1994)
LSY401	*MAT*α *rad51::LEU2*	H. Klein
LSY408	*MAT*α *rad57::LEU2*	H. Klein
LSY410-1	*MAT***a** *rad51::URA3 rad5-535*	Rattray and Symington (1994)
LSY536	*MAT*α *rad57::URA3 rad5-535*	Johnson and Symington (1995)
LSY697	*MAT***a** *met17-sna ADE2*	Bartsch *et al.* (2000)
LSY1309-1	*MAT*Δ::*URA3 ade2-n rad5-535*	Fung *et al.* (2006)
LSY1390	*MAT*α *rad55::LEU2 srs2::HIS3*	Fung *et al.* (2006)
LSY1392	*MAT*α *rad57::LEU2 srs2::HIS3*	Fung *et al.* (2006)
LSY1421-2A	*MAT***a** *ade2-n::URA3::ade2-a rad55::LEU2*	This study
LSY1421-5B	*MAT*α *ade2-n::URA3::ade2-a rad55::LEU2 rad5-535*	This study
LSY1422-3A	*MAT***a** *ade2-n::URA3::ade2-a rad57::LEU2 rad5-535*	This study
LSY1430	*MAT***a** *ade2-n::TRP1::ade2-I exo1::HIS3*	This study
LSY1429#2	*MAT***a** *ade2-n::TRP1::ade2-I*	This study
LSY1516-10C	*MAT*α *ade2-n::TRP1::ade2-I rad57::URA3 rad5-535*	This study
LSY1518	*MAT***a** *rad55::His3MX6*	This study
LSY1519-1D	*MAT*α *ade2-n::TRP1::ade2-I*	This study
LSY1538-8B	*MAT*α *ade2-n::TRP1::ade2-I rad55::His3MX6*	This study
LSY1566	*MAT*α *ade2-n rad57::LEU2 rad5-535*	This study
LSY1567	*MAT***a** *ade2-n rad55::LEU2*	This study
LSY1568	*MAT***a** *ade2-n rad57::LEU2 rad5-535*	This study
LSY1569	*MAT*α *ade2-I*	This study
LSY1739-6B		
LSY1661	*MAT*α *ade2-I rad55::His3MX6*	This study
LSY1667	*MAT***a***/MAT*α *ade2-n/ade2-I RAD5/rad5-535*	This study
LSY1668	*MAT***a***/MAT*α *ade2-n/ade2-I rad55::LEU2/rad55:: His3MX6*	This study
LSY1693-3A	*MAT*α *ade2-n::TRP1::ade2-I rad51::URA3*	This study
LSY1699	*MAT*Δ::*URA3 ade2-I rad55::His3MX6 rad5-535*	This study
LSY1700	*MAT*α *ade2-n rad55::LEU2 rad5-535*	This study
LSY1703	*MAT*α *ade2-I rad51::URA3 rad5-535*	This study
LSY1708-5C	*MAT*α *ade2-n::TRP1::ade2-I rad51::LEU2*	This study
LSY1721-2D	*MAT***a** *ade2-n::URA3::ade2-I rad5-535*	This study
LSY1722	*MAT***a** *ade2-n rad51::LEU2 rad5-535*	This study
LSY1734	*MAT*α *ade2-n::TRP1::ade2-I sir4::KanMX4*	This study
LSY1736	*MAT*α *ade2-n::TRP1::ade2-I rad51::LEU2 sir4::KanMX4*	This study
LSY1740-1D	*MAT*Δ::*URA3 ade2-n rad51::LEU2*	This study
LSY1759	*MAT*Δ::*URA3/MAT*α *ade2-n/ade2-I RAD5/rad5-535*	This study
LSY1761-2D	*MAT*Δ::*URA3 ade2-n rad57::LEU2 rad5-535*	This study
LSY1767	*MAT*Δ::*URA3/MAT*α *ade2-n/ade2-I rad51::URA3/rad51::LEU2*	This study
LSY1784	*MATa ade2-I dnl4::KanMX4*	
LSY1785	*MAT*Δ::*URA3 ade2-n dnl4::KanMX4*	
LSY1788	*MAT*α *ade2-n::TRP1::ade2-I rad57::URA3*	This study
LSY1789	*MAT*α *ade2-n::TRP1::ade2-I rad57::URA3 sir4::KanMX4*	This study
LSY1790	*MAT*Δ::*URA3/MAT*α *ade2-n/ade2-I dnl4::kanMX4/ dnl4::kanMX4*	This study
LSY1876	*MAT*α *ade2-I rad57::URA3*	This study
LSY1877	*MAT*α *ade2-n rad55::LEU2*	This study
LSY1878	*MAT*α *ade2-I rad51::URA3*	This study
LSY1881	*MAT***a***/MAT*α *ade2-n/ade2-I rad57::LEU2/rad57::URA3 RAD5/rad5-535*	This study

(*continued*)

TABLE 1

(Continued)

Strain	Genotype	Source or reference
LSY1882	*MAT***a**/*MAT*α *ade2-n/ade2-I rad51::URA3/rad51::LEU2 RAD5/rad5-535*	This study
LSY1883	*MAT*Δ::*URA3/MAT*α *ade2-n/ade2-I rad55::LEU2/rad55::His3MX6 RAD5/rad5-535*	This study
LSY1884	*MAT*Δ::*URA3/MAT*α *ade2-n/ade2-I rad57::LEU2/rad57::URA3 RAD5/rad5-535*	This study
LSY1973-1B	*MAT***a** *leu2*Δ*EcoRI::URA3-HO::leu2*Δ*BstEII rad51::HIS3*	This study
LSY1974	*MAT***a** *leu2*Δ*EcoRI::URA3-HO::leu2*Δ*BstEII rad55::HIS3*	This study
BY4742 *sir4::KanMX4*	*MAT*α *sir4::KanMX4 his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*	Winzeler *et al.* (1999)

Haploid strains expressing both mating-type alleles were made by transforming *MAT***a** haploids with pRS414-*MAT*α or by deleting *SIR4* using a PCR fragment containing homologous 5′- and 3′-flanking sequences from BY4742 *sir4::KanMX4*, resulting in the integration of the *KanMX4* marker and the loss of the wild-type *SIR4* allele. LSY1788 was made by the same method, replacing the *RAD57* locus in LSY1519-1D with a PCR fragment from LSY536, resulting in integration of the *URA3* marker and loss of the native *RAD57* allele. LSY1518 was made by one-step gene replacement of the *RAD55* locus in W1588-4C with a PCR fragment containing 50 bp of homologous 5′ and 3′ sequences flanking the *His3MX6* module amplified from pFA6a-His3MX6 (Longtine *et al.* 1998). To construct LSY1567, LSY1568, LSY1700, and LSY1722, corresponding strains LSY1421-2A, LSY1422-3A, LSY1421-5B, and LSY1721-2D were patched onto synthetic medium containing 5-fluoroorotic acid (5-FOA) to select for pop-out events (Boeke *et al.* 1987), and then red colonies were screened by PCR of the *ADE2* locus followed by restriction enzyme analysis to obtain clones with the desired *ade2* allele. To construct LSY1566, LSY1569, LSY1661, and LSY1703, respective strains LSY1516-10C, LSY1519-1D, LSY1538-8B, and LSY1693-3A were patched onto synthetic medium containing 5-fluoroanthranilic acid (5-FAA) to select for pop-out events (Toyn *et al.* 2000), and red colonies were then screened by PCR and restriction enzyme analysis to find clones with the desired *ade2* allele. Most other haploid strains were made by mating appropriate haploid strains, sporulating the resulting diploids, and screening the haploid segregants for the desired genotype. Diploids used for recombination assays were made by crossing the appropriate haploid strains.

To create an *ade2* allele with an I-SceI site, two 29-mer oligonucleotides containing the I-SceI recognition sequence were annealed and ligated to *Aat*II-digested pAL78, which contains the *ade2-n* allele (Rattray and Symington 1994), creating pLS188. A 3.6-kb *Bam*HI fragment from pLS188 containing the *ade2-n, I-SceI* allele was cloned into the multiple-cloning site of pRS404 to generate pLS189. The plasmid for expression of I-SceI (p373) was a gift from S. Marcand (Frank-Vaillant and Marcand 2001), pFP19 was a gift from J. Haber, and pRS414-*MAT*α was a gift from R. Rothstein. The high-copy-number plasmid expressing *RAD51* from the native promoter, YEp24::*RAD51*, was described previously (Bai and Symington 1996).

Determination of mitotic recombination frequencies and rates: Mitotic recombination rates were determined by the method of the median (Lea and Coulson 1948). Yeast strains were grown on YPD plates for 2–3 days at 30° or for 3–4 days at 23°, nine independent colonies were inoculated into 5 ml of YPD, and cultures were grown overnight at either 30° or 23°. Cells were pelleted and resuspended in 1 ml of sterile H_2O. Aliquots of appropriate dilutions were plated onto SC medium to determine the number of viable cells in each culture and onto SC medium minus adenine and tryptophan for the direct-repeat assay, or SC −Ade for interhomolog, to determine the total number of recombinants in each culture. Plates were incubated for 3–5 days, after which colonies were counted. For each strain, recombination rates were measured three times on independent isolates and the mean values are presented. *t*-Tests were used to determine the statistical significance of differences in recombination rates between given strains.

To determine *I-SceI*-induced recombination frequencies, strains with genetic recombination reporters were transformed with the *HIS3*-containing I-SceI expression plasmid (p373) and were grown to saturation in selective medium at either 30° or 23°. Cultures were diluted 1:100 into minimal medium containing 2% lactate (pH 5.5) supplemented with the appropriate amino or nucleic acids and cultured overnight at either 30° or 23° to a cell density of 3×10^7 cells/ml. Galactose was added to a final concentration of 2% (w/v). For strains containing the *ade2* direct-repeat reporter, tryptophan was also added upon galactose induction. Cells were removed prior to and 3 hr after galactose induction, and aliquots (50 μl) of appropriate dilutions were plated onto SC medium to determine the number of viable cells in each culture and onto SC −Ade (for *ade2* heteroallelic reporter) or SC −Ade −Trp (for *ade2* direct-repeat reporter) to determine the total number of recombinants in each culture. Plates were incubated for 3 or 5 days at 30° or 23°, respectively, after which colonies were counted. For each strain, recombination frequencies were measured three times on independent His^+ transformants and the mean values are presented.

Clastogen sensitivity tests: For ultraviolent (UV) and γ-irradiation (IR) sensitivity tests, cells were grown in liquid YPD medium at 30° to midlog. The cultures were serially diluted and aliquots of each dilution were spotted onto YPD plates. The plates were irradiated either in a Gammacell-220 irradiator containing ^{60}Co or in a Stratagene (La Jolla, CA) UV Stratalinker 2400 and incubated at 30° for 3 days. For camptothecin (CPT) sensitivity tests, cells were grown in YPD overnight at 30°. Strains were diluted to a concentration of 0.7×10^7 cells/ml, and five additional 10-fold serial dilutions were made. Aliquots of each dilution were spotted onto the indicated media and incubated at 30° for 3 days. Strains were spotted onto YPD plates containing 0.5 or 1 μg/ml CPT and 2% dimethyl sulfoxide (DMSO). Control plates contained 2% DMSO.

Microscopy: Cells were grown in SC medium or SC media minus tryptophan to an optical density at 600 nm (OD_{600}) of 0.2, at which time the liquid cultures were exposed to IR or UV radiation or were left unirradiated. For IR, 1 ml of cells was placed in an Eppendorf tube and exposed to defined doses of γ-rays in a Gammacell-220 ^{60}Co irradiator. For UV irradiation,

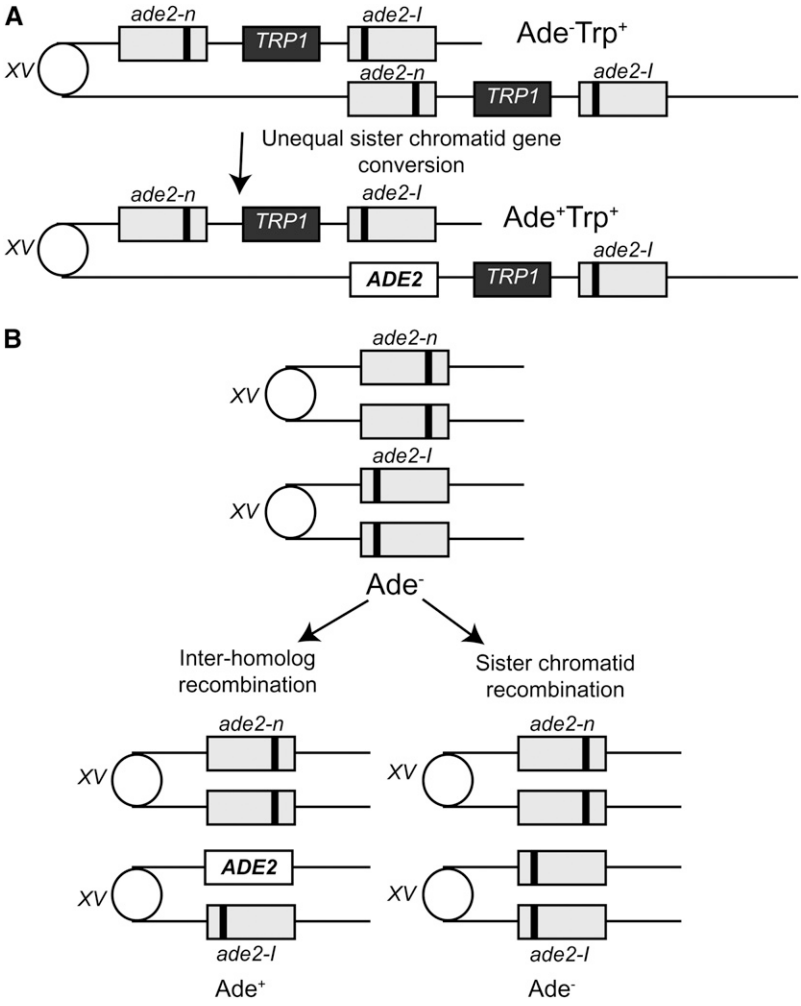

FIGURE 1.—Recombination substrates and products. (A) The direct-repeat recombination substrate contains 3.6-kb repeats with different *ade2* alleles integrated at the endogenous locus on chromosome *XV* separated by plasmid sequences and the *TRP1* gene. One allele contains a 2-bp fill-in mutation of the *Nde*I site resulting in a frameshift while the other allele has an I-SceI cut site insertion disrupting the wild-type *Aat*II site. Unequal sister chromatid or intrachromatid gene conversion between the two *ade2* repeats can generate Ade$^+$ Trp$^+$ recombinants that retain the duplication. Either allele could be converted; only one type of conversion is shown here. (B) The heteroallelic recombination substrate contains the different *ade2* alleles at the native chromosomal loci in diploid strains. Interhomolog recombination between the mutant alleles can generate Ade$^+$ recombinants. Sister chromatid recombination is phenotypically silent in this assay.

1 ml of cells was briefly centrifuged and resuspended in 20 μl of media, and 10 μl of the concentrated cell culture was spotted onto a microscope slide, left uncovered, and irradiated at 20 J/m^2 in a Stratagene Stratalinker 2400. Aliquots of the cultures were processed immediately for imaging as described previously (LISBY *et al.* 2004). Yellow fluorescent protein (YFP) fluorescence was acquired using Openlab software (Improvision).

RESULTS

Experimental design: To measure sister and intrachromatid recombination events, we constructed a substrate that contains a direct repeat of alleles of the *ade2* gene separated by plasmid sequences and a copy of the *TRP1* gene integrated at the *ADE2* locus on chromosome *XV* (Figure 1A). One allele contains a 2-bp fill-in mutation of the *Nde*I site resulting in a frameshift (HUANG and SYMINGTON 1994); the other allele contains a 29-bp insertion of the I-SceI recognition site at the *Aat*II site. Recombination between the *ade2* repeats to generate a wild-type copy of the *ADE2* gene results in two phenotypic classes. Events that retain the duplication are Ade$^+$ Trp$^+$; these could occur by intrachromatid or unequal sister chromatid gene conversion. Recombination events that result in the loss of one of the repeats and the *TRP1* marker are referred to as pop-outs. These could occur by any one of an array of mechanisms, including intrachromatid crossing over, unequal sister chromatid exchange, unequal sister chromatid conversion, single-strand annealing, or replication mispairing (SYMINGTON 2002). Recombination between the *ade2* alleles could occur spontaneously or be induced following induction of the I-SceI nuclease, which makes a DSB at the artificially inserted cut site within the *ade2-I* allele. This DSB will induce recombination with the *ade2-n* repeat.

To measure mitotic interchromosomal recombination events, we introduced the two different *ade2* alleles in diploid strains (Figure 1B). Analogous to the direct-repeat recombination substrate, recombination between the *ade2* heteroalleles can occur spontaneously or be induced by a site-specific DSB. Using these assay systems with the same pair of heteroalleles, spontaneous or DSB-induced direct-repeat or interchromosomal recombination can be compared in various mutant strains.

Spontaneous gene conversion between direct repeats requires *RAD55* and *RAD57*: To determine the role of *RAD57* in spontaneous sister chromatid recombination

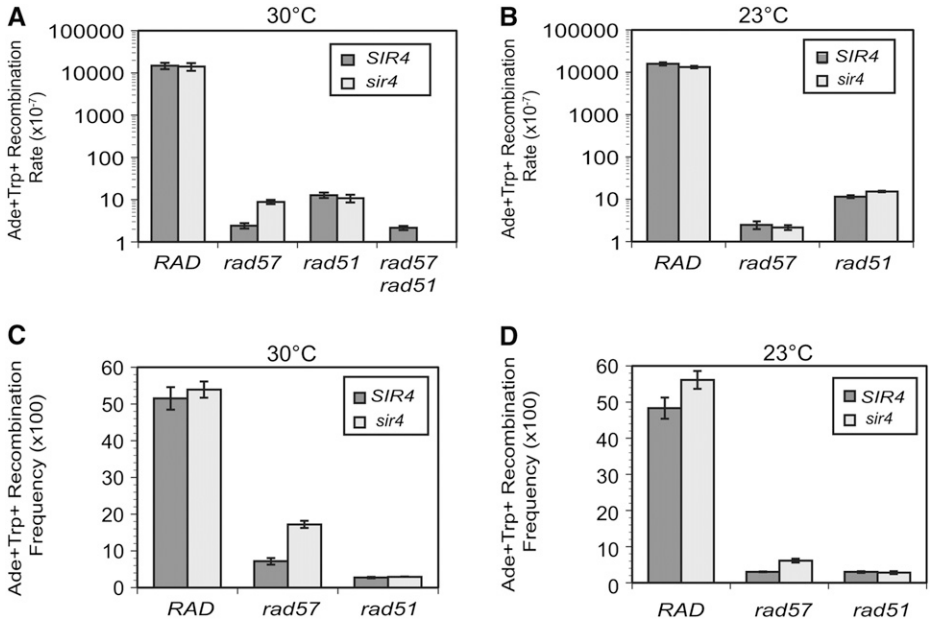

FIGURE 2.—*RAD57* is required for spontaneous sister chromatid recombination. (A) Spontaneous sister chromatid recombination rates at 30°. *SIR4* strains used were LSY1519-1D (Rad$^+$), LSY1788 (*rad57::URA3*), LSY1708-5C (*rad51::LEU2*), and LSY1933-5C (*rad57::URA3 rad51::LEU2*); *sir4* strains used were LSY1734 (Rad$^+$), LSY1789 (*rad57::URA3*), and LSY1736 (*rad51::LEU2*). (B) Spontaneous sister chromatid recombination rates at 23°. (C) DSB-induced recombination frequencies at 30°. (D) DSB-induced recombination frequencies at 23°.

(SCR), we measured the rate of Ade$^+$ prototroph formation in *rad57* and wild-type strains using the direct-repeat substrate (Figure 1A). Consistent with previous reports (McDONALD and ROTHSTEIN 1994; LIEFSHITZ *et al.* 1995), the rate of direct-repeat recombination was the same for wild-type and *rad57* strains, but closer analysis revealed that the classes of recombinants recovered from the two strains were markedly different. Eighty-five percent of the Ade$^+$ recombinants in the wild-type strain were also Trp$^+$, whereas only 0.2% of the Ade$^+$ recombinants in the *rad57* mutant were Trp$^+$ (supplemental Figure 1 at http://www.genetics.org/supplemental/). Thus most of the Ade$^+$ recombinants in the wild-type strain occur by gene conversion, whereas pop-outs account for most of the events in the *rad57* mutant. The pop-out events most likely occur by single-strand annealing because this mechanism is known to be independent of *RAD51* and *RAD57* (IVANOV *et al.* 1996). These results suggest that *RAD57* is important for gene conversion between direct repeats and consequently all further assays with this substrate measured formation of Ade$^+$ Trp$^+$ recombinants.

The rate of spontaneous Ade$^+$ Trp$^+$ recombinants in the wild-type strain, 1.48×10^{-3}/cell/generation, is artificially high due to the slight toxicity of the red pigment that accumulates in *ade2* mutants, resulting in a selective advantage for Ade$^+$ recombinants. Because of the low rates of recombination observed for some mutants it was necessary to expand the cultures for an additional day, resulting in the wild-type rates being higher than reported previously using similar substrates (HUANG and SYMINGTON 1994; RATTRAY and SYMINGTON 1994). The *rad57* mutant was severely defective in this assay system, showing a 6000-fold reduction in the rate of recombination compared to wild type (Figure 2A). Because isogenic strains containing a disruption of the *RAD55* gene behaved equivalently to *rad57* mutants in all of the recombination and cell survival assays (data not shown), for brevity only data from the *rad57* mutant are shown. Restriction enzyme mapping and Southern blot analysis of DNA from 20 independently derived Ade$^+$ Trp$^+$ recombinants from wild type and the *rad57* mutant confirmed retention of the duplication, as expected for a simple gene conversion event (data not shown). Unexpectedly, the *rad51* mutant had a recombination rate 4-fold higher than that of the *rad57* mutant ($P < 0.01$) and the *rad51 rad57* double mutant behaved the same as the *rad57* mutant (Figure 2A). Although the wild-type rate in this system may be artificially high, the *rad55* mutant showed a 50-fold reduction in the rate of spontaneous gene conversion using a *leu2* direct-repeat construct, similar to the rate observed for the *rad51* mutant (supplemental Figure 2 at http://www.genetics.org/supplemental/). In contrast to the *ade2* assay, there was no significant difference in the rate of Leu$^+$ Ura$^+$ recombinants between *rad51* and *rad55* mutants. This could be due to the greater range observed with the *ade2* assay, allowing detection of small, but significant differences between mutants. These findings support the notion that Rad55 and Rad57 are extremely important in the loading of Rad51 onto single-stranded DNA in the context of the replication fork.

The spontaneous direct-repeat gene conversion defect conferred by *rad57* is not suppressed by *MAT* heterozygosity and is temperature independent: It has been reported previously that *MAT* heterozygosity, overexpressing Rad51, or high temperature suppress the ionizing radiation sensitivity and DSB-induced recombination defects of *rad55* and *rad57* mutants (LOVETT and MORTIMER 1987; HAYS *et al.* 1995; JOHNSON and SYMINGTON 1995). Therefore, it was of interest to know if these suppressors of Rad51 mediator defects would

suppress the defect in spontaneous direct-repeat recombination observed for the *rad57* mutant.

To make haploid strains express both mating-type alleles, we made a *sir4* mutation in these strains. The *SIR* genes are required for transcriptional silencing of the *HMR***a** and *HML*α loci (Rine and Herskowitz 1987). The *sir4* mutation suppressed the spontaneous recombination defect of the *rad57* mutant by only 4-fold compared to the *rad57* single mutant. The *rad57 sir4* double mutant still showed a 1500-fold decrease in the recombination rate compared to the Rad$^+$ *sir4* strain and had the same recombination rate as a *rad51* mutant (Figure 2A). As described below, the IR sensitivity of the *rad57* mutant was suppressed >100-fold by the *sir4* mutation. To ensure that the *sir4* mutation is equivalent to simply expressing both *MAT* alleles, we transformed a *rad57 MAT***a** haploid with a plasmid expressing the *MAT*α allele and determined recombination rates. The rate was 4-fold higher than that of the *rad57* strain expressing only one *MAT* allele (data not shown), consistent with the *sir4* effect being due to *MAT* heterozygosity. In accordance with *MAT* heterozygosity upregulating Rad51 filament formation or function, *MAT* heterozygosity does not suppress the spontaneous recombination defect of a *rad51* mutant (Figure 2A). It was also found that overexpressing Rad51 suppresses the spontaneous direct-repeat recombination defect of the *rad55* mutant by only 4-fold (supplemental Figure 3 at http://www.genetics.org/supplemental/). When recombination rates were determined at 23°, the *rad57* mutant did not display a further decrease ($P > 0.5$) (Figure 2B). This was surprising since *rad55* and *rad57* mutants have been reported to exhibit cold sensitivity to ionizing radiation and for DSB-induced recombination (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995).

The DSB-induced direct-repeat gene conversion defect of the *rad57* mutant is suppressed by *MAT* heterozygosity and is temperature dependent: Earlier studies have reported that *rad55* and *rad57* haploid strains are defective for DSB-induced recombination, but are not as defective as a *rad51* mutant (Firmenich *et al.* 1995). It has also been shown that the DSBR defect of *rad55* or *rad57* mutants can be suppressed 25-fold by overexpression of Rad51 (Hays *et al.* 1995). We wanted to verify these past results with the *ade2* direct-repeat reporter and investigate whether other suppressors of Rad51 filament formation, specifically *MAT* heterozygosity and temperature, suppress the *rad57* DSB-induced recombination defect. At 30° the *rad57* mutant showed a 6-fold reduction in DSB-induced direct-repeat gene conversion compared to wild type ($P < 0.001$), whereas recombination was decreased by 18-fold in the *rad51* mutant, significantly less than in wild-type ($P < 0.0005$) or *rad57* ($P < 0.01$) strains (Figure 2C). This differs from what was observed in the spontaneous recombination assay, in which *rad57* had a more severe phenotype than the *rad51* mutant. This distinction between spontaneous and DSB-induced recombination is furthered by the observation that *MAT* heterozygosity effectively suppressed the *rad57* mutant; DSB-induced recombination in the *rad57 sir4* double mutant was significantly increased compared with *rad57* ($P < 0.005$) and the double mutant now had only a 3-fold defect in relation to wild type. More support for the incongruity in *RAD57*'s role between spontaneous and DSB-induced recombination was garnered by the observation that the DSB-induced gene conversion defect of the *rad57* mutant is cold sensitive. At 23°, the *rad57* mutant showed a 2.3-fold reduced recombination frequency compared to *rad57* at 30° ($P = 0.01$) and was as defective as *rad51*. Suppression by *MAT* heterozygosity also appears to be temperature dependent because the *rad57 sir4* double-mutant recombination frequency was reduced 2.8-fold at 23° compared with *rad57 sir4* at 30° ($P = 0.005$) (Figure 2D). The strong suppression of the *rad57* DSB-induced recombination defect by suppressors of Rad51 filament formation suggests that the function of Rad55-Rad57 in DSBR is primarily in mediation of the Rad51 filament. This is in contrast to the role of Rad55-Rad57 in the repair of spontaneous lesions, which is not cold sensitive and not strongly suppressed by *MAT* heterozygosity or by overexpression of Rad51.

***MAT* heterozygosity strongly suppresses the sensitivity of the *rad57* mutant to genotoxic agents that cause DSBs but not single-stranded gaps:** A prediction from the above findings is that *MAT* heterozygosity should suppress the sensitivity of the *rad57* mutant to agents that create DSBs. The *sir4* mutation (*MAT* heterozygous) suppressed the IR sensitivity of the *rad57* mutant by >100-fold to 200 Gy IR (Figure 3). As described previously, the suppression by *MAT* heterozygosity is not observed for the *rad51* mutant. CPT stabilizes the covalent DNA-Top1 intermediate that forms during the catalytic DNA nicking-closing cycle of Top1 and these stable nicks can then be converted into recombinogenic DSBs during replication (Hsiang *et al.* 1989). Similar to the response to IR, the *rad57* mutant is extremely sensitive to CPT and this sensitivity is strongly suppressed by *MAT* heterozygosity (Figure 3). Thus the *rad57* defect in repair of DSBs made in the context of the replication fork is the same as DSBs made by IR.

UV irradiation is thought to cause replication fork stalling and consequently, single-stranded gaps (Lopes *et al.* 2006). The *rad57* mutant was 1000-fold more UV sensitive than wild type at a UV dose of 60 J/m^2 and had a sensitivity that closely resembled that of the *rad51* mutant. Significantly, the *rad57 sir4* double mutant showed only a 3- to 10-fold increase in UV survival compared to the *rad57* single mutant (Figure 3). Similarly, overexpression of Rad51 suppressed the UV sensitivity of the *rad55* mutant only slightly while sensitivity to CPT was strongly suppressed (supplemental Figure 3 at http://www.genetics.org/supplemental/). The weak suppression

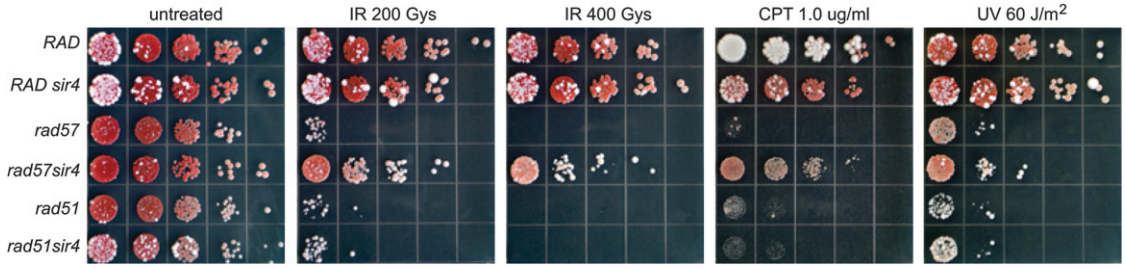

FIGURE 3.—*MAT* heterozygosity strongly suppresses the sensitivity of *rad57* to DSB-inducing genotoxic agents but not those forming single-stranded gaps. Tenfold serial dilutions of log-phase cultures of the strains were spotted onto YPD plates and left unirradiated or irradiated with 200 or 400 Gy or were UV irradiated at 75 J/m². Survival was assessed following growth for 3 days at 30°. Serial dilutions of saturated cultures of the same strains as above were spotted onto YPD only or YPD containing 2% DMSO and 1.0 μg/ml camptothecin (CPT). Survival was assessed following growth for 3 days at 30°. Strains used were LSY1519-1D (*RAD*), LSY1734 (*RAD sir4::kanMX6*), LSY1788 (*rad57::URA3*), LSY1789 (*rad57::URA3 sir4::kanMX6*), LSY1708-5C (*rad51::LEU2*), and LSY1736 (*rad51::LEU2 sir4::kanMX6*).

of the UV sensitivity and spontaneous recombination defect of the *rad57* mutant by *MAT* heterozygosity and overexpression of Rad51, in contrast to the strong suppression seen in assays for DSBR, suggests that SSGs are the primary lesion initiating spontaneous SCR.

***MAT* heterozygosity suppresses the defect of *rad57* in the formation of YFP-Rad51 foci following UV-induced damage:** The lack of suppression of the UV sensitivity of *rad55* and *rad57* mutants by known suppressors of Rad51 filament formation could be interpreted as evidence for a late role for Rad55-Rad57 in recombination after Rad51 filament formation or that loading of Rad51 onto single-stranded DNA during UV-induced recombination is qualitatively different from filament formation in DSB-induced recombination. To differentiate between these two possibilities we monitored Rad51 recruitment to DNA-damaged sites by epifluorescence microscopy, using a fusion of YFP to Rad51 (LISBY *et al.* 2004) (Figure 4). Although we assume Rad51 foci formation corresponds to recruitment and filament formation by Rad51 at sites of DNA damage, the foci could also represent Rad51-mediated joint molecules. As expected, the *MAT* homozygous *rad55* mutant was defective in forming YFP-Rad51 foci after UV or IR treatment ($P < 0.001$). In agreement with the clastogen spot assays, *MAT* heterozygosity partially suppressed the *rad55* defect in YFP-Rad51 focus formation following treatment with IR ($P < 0.005$). However, in contrast to the weak suppression of the UV sensitivity of the *rad55* mutant by *MAT* heterozygosity, expression of both mating-type alleles in the *rad55* mutant rescued YFP-Rad51 formation in response to UV ($P = 0.0001$). As reported previously, the Rad51 foci formed in response to UV or IR in the *rad55* mutant were less bright than those observed in wild-type cells (FUNG *et al.* 2006). The dimmer foci suggest Rad51 is still able to nucleate in the absence of the Rad51 paralogs, but is unable to form extensive filaments or the filaments are less stable.

***MAT* heterozygosity and temperature suppress the spontaneous and DSB-induced interhomolog recombination defects of *rad57Δ/rad57Δ* diploids:** In previous studies, *rad55* homozygous diploids were shown to have wild-type frequencies of recombination at 30° and a 10-fold decrease in recombination frequencies at 23° (LOVETT and MORTIMER 1987; SIGNON *et al.* 2001). We confirmed that at 30° *MAT* heterozygous *rad57* or *rad55* diploids do not have a defect in spontaneous interhomolog recombination yet the *rad51* mutant shows a 240-fold reduction compared to wild type ($P < 0.0005$) (Figure 5A, *rad55* data not shown). Akin to the spontaneous interhomolog results, the *MAT* heterozygous *rad57* diploid did not display a DSB-induced recombination defect. This is in contrast to the *rad51* diploid, which showed a 1000-fold reduction in DSB-induced recombination compared with wild type ($P < 0.0005$) (Figure 5C).

These results suggest either that *RAD55* and *RAD57* are not important for interhomolog recombination or that temperature and/or *MAT* heterozygosity suppress

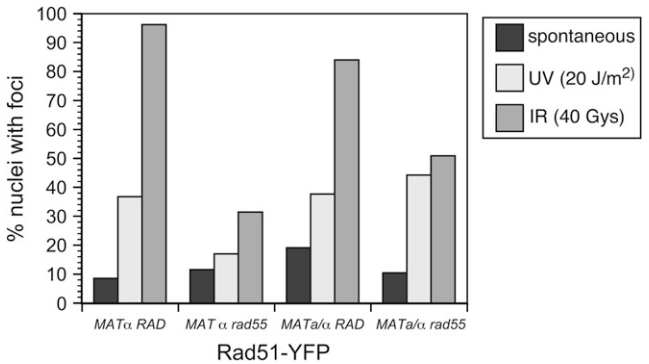

FIGURE 4.—The UV sensitivity of the *rad55* mutant is caused by defects independent of Rad51 recruitment. YFP fusions were made with Rad51 in *RAD* and *rad55* backgrounds (LSY1575 and W5857-15A, respectively). *MAT***a** haploids were transformed with pRS414-*MAT*α to express both mating-type alleles. Log-phase cultures of the strains were exposed to no irradiation, 20 J/m² of UV irradiation, or 40 Gy IR, followed by microscopy to monitor focus formation. For each strain between 77 and 183 cells were counted.

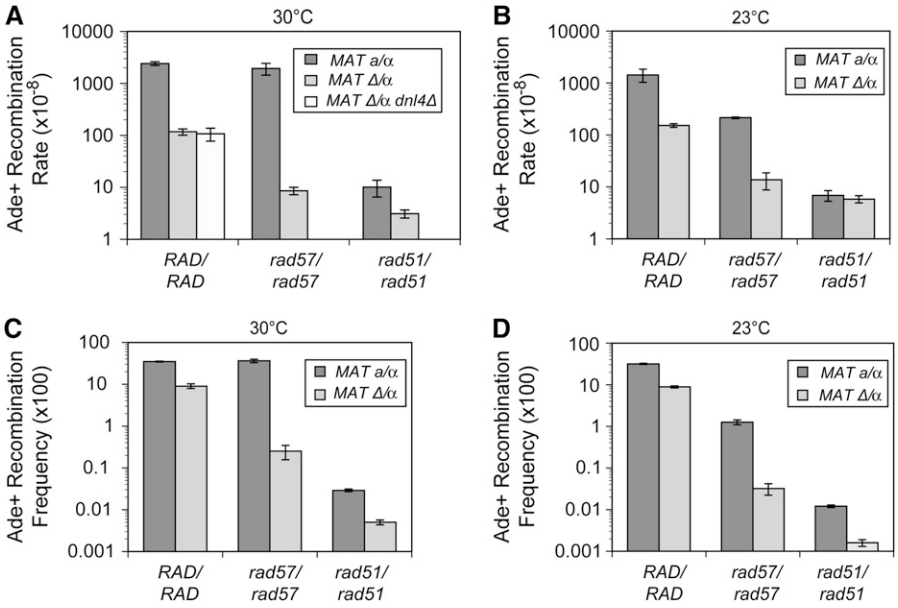

FIGURE 5.—*MAT* heterozygosity and temperature suppress the interhomolog recombination defect of the *rad57* diploid. (A) Spontaneous interhomolog recombination rates at 30°. *MAT* heterozygous (*MAT***a**/α) strains used were LSY1667 (*RAD/RAD*), LSY1881 (*rad57::LEU2/rad57::URA3*), and LSY1882 (*rad51::URA3/rad51::LEU2*). *MAT* homozygous (*MAT*Δ/α) strains used were LSY1759 (*RAD/RAD*), LSY1884 (*rad57::URA3/rad57::LEU2*), and LSY1767 (*rad51::URA3/rad51::LEU2*). (B) Spontaneous interhomolog recombination rates at 23°. (C) DSB-induced interhomolog recombination frequencies at 30°. (D) DSB-induced interhomolog recombination frequencies at 23°.

the phenotype of *rad55* and *rad57* mutants in this assay. To distinguish between these possibilities, we assayed spontaneous and DSB-induced recombination at 23°. At 23°, the *MAT* heterozygous *rad57* mutant had a 9-fold decrease in the rate of spontaneous interhomolog recombination compared to *rad57* at 30° ($P < 0.05$) (Figure 5B). The problem of *MAT* heterozygosity in the diploid reporter strains was circumvented by deleting one of the *MAT* alleles. As reported previously, wild-type strains expressing only one *MAT* allele showed a reduced rate of spontaneous interhomolog recombination (FRIIS and ROMAN 1968). At 30° recombination was reduced 17-fold in the *MAT*α *rad57* diploid compared to a *MAT*α Rad$^+$ diploid ($P = 0.001$) (Figure 5A). Both of these results are in contrast to the *MAT* heterozygous *rad57* mutant that displayed wild-type levels of spontaneous interhomolog recombination at 30°. The *MAT*α *rad57* mutant did not display a further defect in spontaneous recombination at 23° (Figure 5B). The same trends were seen in the DSB-induced interhomolog assay. *MAT* heterozygosity and temperature suppressed the DSB-induced recombination defect of the *rad57* mutant (Figure 5, C and D). DSB-induced interhomolog recombination was also reduced in the *MAT*α *rad51* diploid compared with the *MAT* heterozygous *rad51* diploid. One possible explanation is that nonhomologous end joining (NHEJ) is active in *MAT*α diploids and could compete for repair of the I-SceI-induced break without production of Ade$^+$ recombinants (FRANK-VAILLANT and MARCAND 2001; VALENCIA *et al.* 2001). Activation of NHEJ could explain the difference between *MAT*α and *MAT***a**/α diploids for DSB-induced recombination, but is not responsible for the difference in spontaneous recombination (Figure 5A). These findings support the idea that *MAT* heterozygosity suppresses the role of *RAD55* and *RAD57* in interhomolog recombination and this suppression appears to be temperature dependent. Interestingly, the increased need for *RAD57* over *RAD51* found in the spontaneous direct-repeat recombination assay is not seen in the interhomolog assay. The *rad51* mutant had a more severe defect than the *rad57* mutant under all conditions tested.

As anticipated the *MAT*α *rad57* diploid was extremely sensitive to IR, similar to a *MAT*α *rad57* haploid strain (Figure 6). To determine whether the increased resistance of the *MAT* heterozygous *rad57* diploid is due to increased interhomolog or SCR, we compared survival

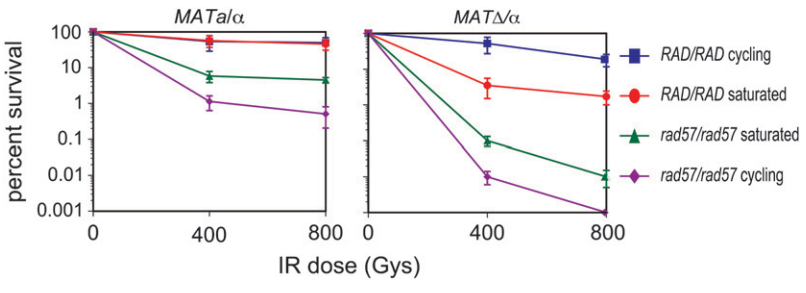

FIGURE 6.—Rad55 and Rad57 are more necessary for sister chromatid recombination than for interhomolog recombination. Strains were either grown to log phase (cycling) or grown 4 days to saturation. Serial dilutions were plated onto solid YPD medium and were unirradiated or exposed to 400 or 800 Gy of γ-irradiation; surviving colonies were counted after 3 days. *MAT* heterozygous (*MAT***a**/α) strains used were LSY1667 (*RAD/RAD*) and LSY1881 (*rad57::URA3/rad57::LEU2*); *MAT*Δ/α strains used were LSY1759 (*RAD/RAD*) and LSY1884 (*rad57::URA3/rad57::LEU2*).

to IR for exponential and stationary phase cultures. Growing cells will preferentially repair IR-induced lesions from sister chromatids whereas stationary phase cultures are predominantly made up of G_1 cells that have only a homolog to template repair. The *RAD MAT* heterozygous strains showed high radiation resistance for both exponential and stationary phase cultures (Figure 6). In contrast, G_1-phase *MAT* heterozygous *rad57* diploids were 10-fold more resistant to IR than their cycling counterparts. These data augment the hypothesis that *MAT* heterozygosity efficiently suppresses the defect of *rad55* and *rad57* mutants in DSB-induced recombination and that this suppression is more effective for interhomolog recombination than SCR.

DISCUSSION

Loss of resistance to IR is generally coupled with decreased proficiency in recombination. Thus the strong IR sensitivity of *rad55* and *rad57* mutants is paradoxical in light of their minimal defects in spontaneous mitotic recombination as noted in earlier studies. IR sensitivity of haploid yeast in active growth results from failure to repair DSBs by sister chromatid recombination. One possible explanation is that previous studies focused on the behavior of *rad55* and *rad57* mutants in nonsister recombination events or direct-repeat recombination assays that select for pop-outs (Lovett and Mortimer 1987; Mcdonald and Rothstein 1994; Freedman and Jinks-Robertson 2002). Alternatively, Rad55-Rad57 could have a more important role in DSBR than in the repair of spontaneous lesions. Further complications in interpretation arise from the observation that *MAT* heterozygosity and temperature suppress the IR sensitivity of *rad55* and *rad57* mutants and these suppressive effects could obviate any phenotype seen in diploids grown at 30° (Lovett and Mortimer 1987). To elucidate the role of the yeast Rad51 paralogs in homologous recombination two recombination reporters were designed to measure recombination proficiency in regard to template choice (sister *vs.* homolog), source of DNA damage (spontaneous or DSB induced), and mating type (*MAT* homozygous *vs. MAT* heterozygous).

Spontaneous sister chromatid recombination requires the Rad51 paralogs and known suppressors of Rad51 filament formation do not strongly suppress the mutant defects: The *rad57* haploid mutant displayed a 6000-fold decrease in the rate of spontaneous gene conversion between direct repeats compared to wild type (Figure 2A). These events are expected to arise by unequal gene conversion between sister chromatids or by intrachromatid gene conversion. Although we cannot distinguish between these mechanisms, previous studies suggest most events are the result of SCR. The similar phenotype conferred by *rad57* in the *ade2* repeat assay and by sensitivity to UV is also consistent with the notion that the Ade^+ Trp^+ recombinants are generated by SCR. An unexpected finding was that the *rad51* mutant was not as defective as *rad57* in the direct-repeat recombination assay. Furthermore, a haploid *rad57* mutant expressing both mating-type alleles or a *rad55* mutant overexpressing Rad51 still showed a 1500-fold reduced recombination rate compared to wild type. The partially suppressed recombination rates of the mutants were equivalent to the rate observed for the *rad51* mutant, whereas in all the other assays the recombination rates of the *MAT* heterozygous *rad57* mutant were significantly higher than that of *rad51*. We also found that the *rad57* mutant was not cold sensitive in the spontaneous direct-repeat assay, in contrast to the phenotype in DSBR. These findings suggest that Rad55 and Rad57 have a unique role in spontaneous SCR that is distinct from their role in DSBR.

The phenotype of the *rad57* mutant in spontaneous recombination between direct repeats of *ade2* is much more severe by comparison than when the alleles are in different configurations. Gene conversion between *ade2* repeats oriented as an inverted repeat was previously shown to be decreased 20- to 30-fold in *rad55* and *rad57* mutants and the mutant defects were temperature dependent. It is possible that events initiate or are resolved by different mechanisms during recombination between inverted repeats, compared with direct repeats. DSBs have been detected at some inverted repeats in yeast and may form at low frequency within the *ade2*-inverted repeat (Lobachev *et al.* 2002; Lemoine *et al.* 2005). In an assay to measure spontaneous unequal sister chromatid exchange, *rad51*, *rad55*, and *rad57* mutants were found to have the same rate as wild type (Dong and Fasullo 2003). The failure to detect Rad51-dependent events in this system could be due to the selection for exchange events, the short homology between the repeats (0.3 kb), or use of an alternate post-replication repair pathway, such as template switching, to generate recombinants.

Repair of a single HO-induced DSB by ectopic recombination in haploid cells requires *RAD55* and *RAD57*, even at 30° (Hays *et al.* 1995; Sugawara *et al.* 1995; Aylon *et al.* 2003). As measured with the direct-repeat reporter, the *rad57* mutant was moderately defective in DSB-induced recombination compared to wild type, although the phenotype was not as severe as the *rad51* mutant (Figure 2C) or as reported previously for DSB-induced ectopic recombination. Additionally, *MAT* heterozygosity, overexpression of Rad51, or elevated temperature effectively suppressed the DSBR defect of the *rad57* mutant (Figures 2 and 3). The cold sensitivity and the strong suppression by factors acting in Rad51 filament formation of the *rad57* mutant phenotype in DSBR contrast with the behavior of the *rad57* mutant in the spontaneous direct-repeat recombination or UV-sensitivity assays.

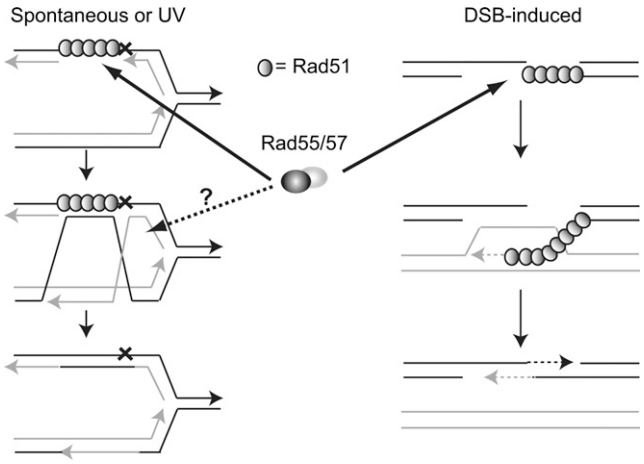

FIGURE 7.—The role of Rad55-Rad57 in spontaneous *vs.* DSB-induced recombination. The Rad51 paralogs are important for mediation of the Rad51 filament in the repair of single-stranded gaps as well as DSBs. In SSGR the Rad51 paralogs may have an additional function subsequent to Rad51 filament formation. Possible functions include pairing of topologically constrained ssDNA with dsDNA or the processing of branched recombination intermediates formed in the repair of single-stranded gaps but not DSBs.

Initiation of spontaneous sister chromatid recombination at single-stranded DNA gaps? The phenotype of *rad55* and *rad57* mutants in the spontaneous SCR assay was remarkably similar to that observed for the repair of UV-induced lesions in that both defects were only weakly suppressed by *MAT* heterozygosity or overexpression of Rad51. In excision repair-deficient yeast cells, treatment with UV leads to the uncoupling of leading- and lagging-strand synthesis and results in long stretches of single-stranded DNA specifically on the leading strand as well as smaller single-stranded DNA gaps on both strands (LOPES *et al.* 2006). Therefore, it seems likely that most spontaneous recombination events initiate at SSGs in the context of the replication fork. In support of this idea, the mammalian Rad51 paralogs, Xrcc2 and Xrcc3, were shown to be required for replication fork slowing following treatment with cisplatin or UV, presumably because homologous recombination is necessary for bypass of the lesions and this process is slower than lesion bypass by other mechanisms (HENRY-MOWATT *et al.* 2003). These data implicate the Rad51 paralogs in homologous recombination in the context of the replication fork. LETTIER *et al.* (2006) characterized an unusual class of *rad52* mutants that are defective for DSBR, but show normal rates of spontaneous and UV-induced interhomolog recombination, providing further support for the proposal that spontaneous recombination initiates at lesions other than DSBs. The postreplication repair (PRR) pathway provides alternate mechanisms to bypass damage at stalled replication forks. *Rad57* mutants show increased spontaneous mutagenesis that is *REV3* dependent (RATTRAY *et al.* 2002), consistent with competition between homologous recombination and PRR at SSGs.

There are some parallels between the Rad51 paralogs in eukaryotes and RecF, RecO, and RecR (RecFOR) functions in bacteria. RecFOR are important for recombinational repair in response to UV and it has been suggested that RecFOR function during the recovery of replication after UV-induced DNA damage (COURCELLE *et al.* 1997; MORIMATSU and KOWALCZYKOWSKI 2003). The UV sensitivity of *recFOR* mutants can be suppressed by overexpression of *recA* or by gain-of-function *recA* alleles that encode proteins with higher DNA affinity than wild-type RecA (WANG *et al.* 1993; KOWALCZYKOWSKI *et al.* 1994). Thus, Rad55 and Rad57 appear to behave somewhat analogously to RecFOR in the repair of single-stranded gaps in the context of DNA replication. In contrast to *rad55* and *rad57*, *recFOR* mutants are not sensitive to agents that create DSBs unless the *sbcB* and *sbcCD* nucleases are inactivated in the *recBC* background (KOWALCZYKOWSKI *et al.* 1994).

Role of the Rad51 paralogs in single-strand gap repair: On the basis of the weak suppression of the *rad57* defect in spontaneous SCR by suppressors of the DSBR defect, we suggest the role for Rad55-Rad57 in the repair of SSGs formed at stalled replication forks is distinct from its role in DSBR. The increased need for Rad55-Rad57 in the repair of SSGs compared to DSBs could be due to one of two possibilities that are not mutually exclusive: either there is a greater need for the paralogs in Rad51 filament extension or stabilization in SSG repair or the paralogs are needed for a subsequent step in the repair of SSGs that is not essential during DSBR (Figure 7). Both types of DNA repair appear to require the mediator function of the Rad51 paralogs because Rad51 recruitment in response to UV or IR is reduced in the *rad55* mutant (Figure 4). However, Rad51 recruitment to UV- or IR-induced lesions in the *rad55* mutant was partially suppressed by *MAT* heterozygosity even though expression of both mating-type alleles only weakly suppressed cell survival after UV irradiation. Although Rad51 foci formation is partially suppressed by *MAT* heterozygosity it seems likely that the filaments formed are not fully functional to account for the incomplete suppression of the DSBR defects and these partial filaments formed at SSGs are unable to promote pairing with a sister chromatid. One difference between SSG repair (SSGR) and DSBR is that pairing between ssDNA formed at a gap on the template strand and the sister chromatid creates a topological problem compared with invasion of a ssDNA tail formed at a DSB, which has a free end and presumably no restraint on rotation. It is possible that Rad55-Rad57 is required at this step. A defect in pairing with duplex DNA is consistent with the failure to recombine despite partial suppression of Rad51 recruitment by *MAT* heterozygosity. Finally, the branched structures formed during SSGR may require junction-processing activities. In this

regard it is notable that a resolvase activity has been identified that is associated with the human Rad51 paralogs, Rad51C and Xrcc3 (Liu *et al.* 2004, 2007).

Temperature and *MAT* heterozygosity suppress the role of *RAD55* and *RAD57* in interhomolog recombination: Consistent with previous reports (Lovett and Mortimer 1987; Signon *et al.* 2001), we found that *MAT* heterozygous *rad57/rad57* diploids displayed no defect in spontaneous or DSB-induced interhomolog recombination at 30° (Figure 5). However, a defect in interhomolog recombination was observed for the *rad57* diploid expressing only one *MAT* allele and/or at 23°. The full suppression observed by *MAT* heterozygosity and temperature in the spontaneous interhomolog recombination assay, but not in the spontaneous direct-repeat system, suggests the primary role for Rad55-Rad57 in interhomolog recombination is in mediating Rad51 filament formation whereas in SCR the paralogs might have ancillary role(s) apart from Rad51 mediation. Furthermore, the similar behavior of the *rad57* diploid for both spontaneous and DSB-induced events raises the possibility that spontaneous lesions channeled to the homolog are DSBs. Spontaneous interhomolog recombination is unlikely to occur in the context of the replication fork and Rad55-Rad57 appear more important for sister chromatid than nonsister recombination as evidenced by the diploid IR survival data (Figure 6). The *MAT* heterozygous *rad57* mutant was more proficient in repair when cultures were not cycling, presumably due to interhomolog recombination in the G_1 phase, than in repair during exponential growth. In contrast, Rad^+ diploids grown to exponential or stationary phase show equivalent sensitivity to IR. The increased survival of *rad57* diploids in stationary phase is unlikely to be due to nonhomologous end joining because this pathway is suppressed by mating-type heterozygosity (Frank-Vaillant and Marcand 2001; Valencia *et al.* 2001) and supports the hypothesis that Rad55-Rad57 are more important for sister chromatid than nonsister recombination.

The sporulation and spore viability defects of *MAT***a**/α *rad57/rad57* diploids seem at odds with the suppression of the mitotic DSBR defect of *rad57* mutants by *MAT* heterozygosity. Meiotic DSBs persist and are hyperresected in *rad55* and *rad57* mutant diploids, but recombinant products are still detected by RFLP analysis and by the return-to-growth protocol (Game *et al.* 1980; Borts *et al.* 1986; Soustelle *et al.* 2002). In mitotic growth, the *MAT* heterozygous *rad57* diploid is 10- to 100-fold more sensitive to 800 Gy IR than the corresponding *RAD57* diploid even though repair of a single DSB appears to be normal. We suggest the kinetics of DSBR are considerably slower in the absence of Rad55-Rad57. Slower or inefficient processing may suffice for repair of a single DSB (Aylon *et al.* 2003), but not for multiple lesions. This decrease in the efficiency of repair of multiple lesions could account for the IR sensitivity and the sporulation defect of *rad57 MAT* heterozygous diploids. Although failure to repair some DSBs might be tolerated in diploids, the haploid products of meiosis would not be viable if half of the 150 or so meiotic DSBs were unrepaired.

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