Genetic Requirements for Spontaneous and Transcription-Stimulated Mitotic Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

The genetic requirements for spontaneous and transcription-stimulated mitotic recombination were determined using a recombination system that employs heterochromosomal *lys2* substrates that can recombine only by crossover or only by gene conversion. The substrates were fused either to a constitutive low-level promoter (*pLYS*) or to a highly inducible promoter (*pGAL*). In the case of the "conversion-only" substrates the use of heterologous promoters allowed either the donor or the recipient allele to be highly transcribed. Transcription of the donor allele stimulated gene conversions in *rad50*, *rad51*, *rad54*, and *rad59* mutants, but not in *rad52*, *rad55*, and *rad57* mutants. In contrast, transcription of the recipient allele stimulated gene conversions in *rad50*, *rad51*, *rad54*, *rad55*, *rad57*, and *rad59* mutants, but not in *rad52* mutants. Finally, transcription stimulated crossovers in *rad50*, *rad54*, and *rad59* mutants, but not in *rad51*, *rad52*, *rad55*, and *rad57* mutants. These data are considered in relation to previously proposed molecular mechanisms of transcription-stimulated recombination and in relation to the roles of the recombination proteins.

HOMOLOGOUS recombination repairs single-strand interruptions and double-strand breaks (DSBs) caused by DNA-damaging agents and provides a mechanism to reestablish collapsed replication forks (reviewed by Kuzminov 1999; Hoeijmakers 2001). Recombination has been extensively studied using the yeast *Saccharomyces cerevisiae* and can be detected experimentally as a gene conversion and/or a crossover event (reviewed by Pâques and Haber 1999). According to the widely accepted DSB repair model of recombination, broken ends are resected to give 3′ single-stranded tails that invade an intact duplex molecule and are used to prime DNA synthesis. The invasion process yields regions of heteroduplex DNA that may contain mismatches, the repair of which results in gene conversion events. The interacting duplexes are ultimately joined by two Holliday junctions, which are resolved to yield either crossover or noncrossover products. In addition to the DSB repair model, recombination also has been proposed to occur by the alternative mechanisms of synthesis-dependent strand annealing (SDSA), break-induced replication (BIR), or single-strand annealing (SSA). Whereas SDSA can give rise to crossover and noncrossover products, BIR and SSA yield only crossovers.

Many proteins that participate in mitotic recombination in yeast have been identified, with most of the corresponding genes falling into the *RAD52* epistasis group (reviewed by Pâques and Haber 1999; Sung *et al.* 2000). Rad51p is the functional homolog of the bacterial RecA strand-exchange protein (Shinohara *et al.* 1992; Ogawa *et al.* 1993; Sung 1994); Rad55p and Rad57p form a stable heterodimer that stimulates the *in vitro* strand-exchange activity of Rad51p when the single-stranded binding protein Rpa is present (Sung 1997b); Rad52p likewise stimulates the strand-exchange activity of Rad51p when Rpa is present (Sung 1997a; New *et al.* 1998; Shinohara and Ogawa 1998; Song and Sung 2000), but also exhibits strand-annealing activity (Mortensen *et al.* 1996; Sugiyama *et al.* 1998); Rad54p has homology to the Swi2p/Mot1p family of chromatin remodeling helicases and stimulates homologous pairing, heteroduplex extension, and DNA remodeling *in vitro* (Eisen *et al.* 1995; Petukhova *et al.* 1998, 1999b; Mazin *et al.* 2000; Van Komen *et al.* 2000; Solinger and Heyer 2001); Rad59p has homology to Rad52p, possesses strand-annealing activity, and stimulates the strand-annealing activity of Rad52p (Bai and Symington 1996; Petukhova *et al.* 1999a; Davis and Symington 2001); and Rad50p, Mre11p, and Xrs2p form a complex that processes the ends of DSBs and promotes sister chromatid interactions (Malone and Esposito 1981; Gottlieb *et al.* 1989; Malone *et al.* 1990; Sugawara and Haber 1992; Ivanov *et al.* 1994; Trujillo *et al.* 1998; Tsubouchi and Ogawa 1998; Usui *et al.* 1998). Rad52p appears to be required for all types of recombination in yeast, whereas the *in vivo* roles of the other proteins vary depending on the assay system used and the types of recombination mechanisms that can potentially occur (Rattray and Symington 1994, 1995; Liefshitz *et al.* 1995; Sugawara *et al.* 1995; Jablonovich

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et al. 1999; Bartsch *et al.* 2000). Both physical and genetic interactions have been detected between members of the *RAD52* epistasis group, leading to the idea that the proteins form a multiprotein complex for recombination (reviewed by Pâques and Haber 1999; see also Krejci *et al.* 2001; Essers *et al.* 2002).

Recombination potentially can be influenced by other DNA metabolic processes and, in particular, transcription has been shown to have a stimulatory effect on mitotic recombination in yeast (reviewed by Aguilera 2001). The link between transcription and recombination was first established in studies of the recombination hotspot *HOT1*, which corresponds to the transcription initiation site of the 35S ribosomal RNA precursor and an enhancer of RNA polymerase I transcription (Keil and Roeder 1984; Voekel-Meiman *et al.* 1987; Voelkel-Meiman and Roeder 1990; Zehfus *et al.* 1990). A stimulatory effect of RNA polymerase II transcription on mitotic recombination was subsequently documented using *gal10* chromosomal direct repeats constructed by integrating a plasmid at *GAL10* (Thomas and Rothstein 1989a,b). Although recombination leading to plasmid loss was found to be stimulated when *GAL10* was constitutively expressed, gene conversion was not elevated, indicating that transcription in this system primarily stimulated SSA rather than repair via the DSB repair model. A second demonstration of a relationship between RNA polymerase II transcription and mitotic recombination was provided by studies of recombination between Ty retrotransposons. Both deletional recombination events involving the Ty long terminal repeats (LTRs) as well as gene conversions between Ty's were stimulated when one of the elements was highly transcribed (Nevo-Caspi and Kupiec 1994). Although the increased level of Ty recombination was shown to be dependent on Rad52p and Rad1p and independent of Rad50p, the high frequency of LTR/LTR recombination prevented determination of the requirements for other Rad proteins in transcription-associated gene conversion events (Nevo-Caspi and Kupiec 1994, 1996).

A recent study from this laboratory systematically examined RNA polymerase II transcription-stimulated mitotic recombination between *lys2* repeats (Saxe *et al.* 2000). Significantly, transcription was found to stimulate recombination between substrates positioned on nonhomologous chromosomes as well as substrates positioned as direct repeats. With either type of repeat, both crossover and gene conversion events were elevated by transcription. In the current study, substrates positioned on nonhomologous chromosomes have been used to determine the genetic requirements for spontaneous *vs.* transcription-stimulated crossovers and gene conversions. These studies reveal distinct genetic requirements for spontaneous *vs.* transcription-stimulated recombination and provide novel insight into the roles of recombination proteins in the *RAD52* epistasis group.

MATERIALS AND METHODS

Media and growth conditions: All yeast strains were grown at 30° unless otherwise indicated. Yeast strains were grown nonselectively in YEP medium (1% yeast extract, 2% Bactopeptone with 2% agar for plates) supplemented with 2% dextrose (YEPD) or 2% glycerol/2% ethanol (YEPGE). For selective growth, SC medium (Sherman 1991) missing the relevant nutrient was supplemented with 2% dextrose (SCD) or 2% glycerol/2% galactose/2% ethanol (SCGGE). Ura$^-$ derivatives of yeast strains were selected on SCD plates containing 1 g/liter of 5-fluoroorotic acid (5-FOA; Boeke *et al.* 1987). Sensitivity of yeast transformants to methylmethanesulfonate (MMS) was determined using YEPD plates containing 0.016% MMS (Kodak). Kanamycin-resistant transformants were selected by growing cells overnight on YEPD and then replica plating to YEPD containing 200 mg/liter of geneticin (Sigma).

Plasmid constructions: Plasmids pSR183 and pSR234 contain the *lys2Δ3′* allele fused to the *LYS2* (*pLYS*) and the *GAL1-10* (*pGAL*) promoters, respectively. In both plasmids the *URA3* gene is positioned at the 3′ end of the *lys2Δ3′* allele and is transcribed in the same direction as the *LYS2* sequences. Plasmids pSR184 and pSR235 are identical to plasmids pSR183 and pSR234, respectively, but *URA3* and *LYS2* sequences are transcribed in opposite directions. Plasmid pSR136 contains the *rad52::hisG-URA3-hisG* allele and was constructed by ligating a 2-kb *Eco*RI-*Bam*HI *RAD52* fragment from plasmid YRp7-*RAD52*-A4-*Sal* (Schild *et al.* 1983) to *Eco*RI-*Bam*HI-digested pUC9. The 3.8-kb *Bam*HI-*Bgl*II *hisG-URA3-hisG* cassette from plasmid pNKY51 (Alani *et al.* 1987) was then inserted into the *Bgl*II site within the *RAD52* sequences. Plasmid pRDK713 was constructed by inserting 25 bp (5′-GATCTGTCCCTTAC TAGCTAGGTAG-3′ on the coding strand) into the unique *Bgl*II site within *LYS2* (A. Shoemaker and R. D. Kolodner, unpublished results). The 25-bp insertion causes a frameshift mutation and creates stop codons in all three reading frames.

Yeast strain constructions: All yeast strains used in this study are isogenic derivatives of SJR195 (*MATα ade2-101$_{oc}$ his3Δ200 ura3ΔNco*) and were constructed using a lithium acetate transformation protocol (Gietz and Schiestl 1995). SJR733 and SJR734 are Gal80$^+$ and Gal80$^-$ strains, respectively, that contain the *pLYS-lys2Δ5′/pGAL-lys2Δ3′* crossover-only (C/O) recombination substrates (Figure 1; for construction details see Saxe *et al.* 2000). SJR1305 and SJR1306 are Ura$^-$ Leu$^-$ derivatives of SJR733 and SJR734, respectively, constructed by first selecting a Ura$^-$ derivative on 5-FOA medium and then introducing the *leu2-R* allele by two-step allele replacement using *Kpn*I-digested pJH189 (Lichten *et al.* 1987).

Yeast strains SJR1599 and SJR1583 (Gal80$^+$ and Gal80$^-$, respectively) contain the GCD (*g*ene *c*onversion with *d*onor highly transcribed) gene conversion substrates with the *pGAL* donor and *pLYS* recipient alleles and were constructed as follows. First SJR1503 and SJR1504, Lys$^+$ derivatives of SJR357 (*MATα ade2-101$_{oc}$ his3Δ200 ura3ΔNco lys2ΔBgl*) and SJR358 (*MATα ade2-101$_{oc}$ his3Δ200 ura3ΔNco lys2ΔBgl gal80::HIS3*), respectively, were made by transformation with *Eco*RV-digested pDP6 (Fleig *et al.* 1986). SJR1516 and SJR1517, *leu2-R* derivatives of SJR1503 and SJR1504, respectively, were then made by two-step allele replacement using *Kpn*I-digested pJH189 (Lichten *et al.* 1987). The *LYS2* allele of SJR1516 and SJR1517 was replaced with the *lys2-oligo* allele by two-step allele replacement using *Xho*I-digested pRDK713. The *pGAL-lys2Δ3′* allele was next introduced at the *URA3* locus by transformation with *Sma*I-digested pSR235. Following selection of Ura$^+$ transformants, integration of a single copy of the plasmid at the *URA3* locus was confirmed by Southern blot analysis or PCR. Finally, Ura$^-$ derivatives were selected on 5-FOA medium.

Yeast strains SJR1584 and SJR1582 (Gal80$^+$ and Gal80$^-$,

respectively) contain the GCR (*g*ene *c*onversion substrates with *r*ecipient highly transcribed) gene conversion substrates with the *pLYS-lys2Δ3′* donor and *pGAL-lys2-oligo* recipient alleles. These strains were constructed as described above, except for starting with strains containing the *pGAL-LYS2* allele (for a description see Saxe *et al.* 2000) and using *Sma*I-digested pSR184 to introduce the *pLYS-lys2Δ3′* allele.

rad50, *rad55*, and *rad59* derivatives of SJR1305, SJR1306, SJR1599, SJR1583, SJR1584, and SJR1582 were constructed using a PCR-based gene disruption methodology (Wach *et al.* 1994). PCR fragments containing the bacterial *kan* gene or the yeast *LEU2* gene flanked by ~60 bp of appropriate yeast sequences were obtained using either pFA6-kanMX2 or pUC7-*LEU2* as a template, respectively. Following transformation, geneticin-resistant or Leu^+ yeast transformants were selected as appropriate and the disruption was confirmed by MMS sensitivity and by PCR.

rad51, *rad52*, *rad54*, and *rad57* derivatives of SJR1305, SJR1306, SJR1599, SJR1583, SJR1584, and SJR1582 were constructed by transformation with *Bam*HI-digested pSR464 (*rad51::URA3*; Aboussekhra *et al.* 1992), *Bam*HI-*Eco*RI-digested pSR136 (*rad52::hisG-URA3-hisG*), *Bgl*II-digested pSR465 (*rad54::URA3*; obtained from L. Symington), and *Sac*I-digested pSR478 (*rad57::LEU2*; obtained from D. Schild), respectively. Following selection of Ura^+ or Leu^+ transformants as appropriate, disruptions were confirmed by MMS sensitivity and PCR. A complete list of the wild-type and *rad* mutant strains used to measure recombination rates is given in Table 1.

Analysis of recombinants: Approximately 10 recombinants from each strain were analyzed to confirm the nature of the underlying recombination event. For the C/O substrates, presence of the *lys2Δ5′Δ3′* allele in Lys^+ recombinants was considered diagnostic of a true reciprocal exchange, and PCR was used to detect this allele. Under high-transcription conditions, all recombinants were generated by a reciprocal exchange event; under low-transcription conditions, at least 8/10 recombinants were generated by a reciprocal exchange event. In contrast to the results obtained with the other mutants, a significant difference in the nature of the recombinants under low- *vs.* high-transcription conditions was detected in the *rad54* strain. In this strain only 56% (14/25) of the recombinants under low-transcription conditions result from a reciprocal exchange event, while 90% (28/31) of the recombinants under high-transcription conditions result from a reciprocal exchange event ($P = 0.008$ by contingency chi-square analysis). Recombinants generated by a reciprocal exchange event also could be detected in *rad52* strains, as 2/6 low-transcription recombinants and 3/3 high-transcription recombinants resulted from a reciprocal exchange event. Because a disruption rather than a deletion allele was used, it is possible that there is residual Rad52p activity in these strains.

In the GCD and GCR gene conversion systems, phenotypic analysis was used to determine which promoter the *LYS2* allele was fused to. Strains with the *pLYS-LYS2* allele grow on lysine-deficient medium containing either glucose or galactose as a carbon source, while strains containing the *pGAL-LYS2* allele grow only on galactose-containing medium. A gene conversion event between the GCD substrates should result in the *LYS2* allele fused to *pLYS* whereas either a crossover event or a break-induced replication event should result in the *LYS2* allele fused to *pGAL* (see Figure 1). In strains containing the GCR substrates, the opposite promoter configuration would be expected. With the GCD strains, results were consistent with a gene conversion mechanism in all recombinants under both low- and high-transcription conditions. In the strains containing the GCR system, under both low- and high-transcription conditions, the results were consistent with a gene conversion event generating at least 8/10 of the recombinants analyzed.

TABLE 1

Yeast strains

Assay	Strain	*RAD* genotype	*GAL80* genotype
C/O	SJR1305	+	+
C/O	SJR1306	+	−
C/O	SJR1440	*rad50*	+
C/O	SJR1448	*rad50*	−
C/O	SJR1312	*rad51*	+
C/O	SJR1427	*rad51*	−
C/O	SJR1310	*rad52*	+
C/O	SJR1362	*rad52*	−
C/O	SJR1311	*rad54*	+
C/O	SJR1361	*rad54*	−
C/O	SJR1446	*rad55*	+
C/O	SJR1439	*rad55*	−
C/O	SJR1366	*rad57*	+
C/O	SJR1438	*rad57*	−
C/O	SJR1445	*rad59*	+
C/O	SJR1444	*rad59*	−
GCD	SJR1599	+	+
GCD	SJR1583	+	−
GCD	SJR1610	*rad50*	+
GCD	SJR1620	*rad50*	−
GCD	SJR1611	*rad51*	+
GCD	SJR1600	*rad51*	−
GCD	SJR1612	*rad52*	+
GCD	SJR1595	*rad52*	−
GCD	SJR1613	*rad54*	+
GCD	SJR1597	*rad54*	−
GCD	SJR1614	*rad55*	+
GCD	SJR1588	*rad55*	−
GCD	SJR1615	*rad57*	+
GCD	SJR1592	*rad57*	−
GCD	SJR1616	*rad59*	+
GCD	SJR1602	*rad59*	−
GCR	SJR1584	+	+
GCR	SJR1582	+	−
GCR	SJR1590	*rad50*	+
GCR	SJR1619	*rad50*	−
GCR	SJR1601	*rad51*	+
GCR	SJR1593	*rad51*	−
GCR	SJR1585	*rad52*	+
GCR	SJR1594	*rad52*	−
GCR	SJR1586	*rad54*	+
GCR	SJR1596	*rad54*	−
GCR	SJR1617	*rad55*	+
GCR	SJR1589	*rad55*	−
GCR	SJR1587	*rad57*	+
GCR	SJR1591	*rad57*	−
GCR	SJR1603	*rad59*	+
GCR	SJR1618	*rad59*	−

C/O, *pLYS-lys2Δ5′/pGAL-lys2Δ3′* crossover-only substrates; GCD, *pLYS-lys2-oligo/pGAL-lys2Δ3′* gene conversion-only substrates; GCR, *pGAL-lys2-oligo/pLYS-lys2Δ3′* gene conversion-only substrates.

Determination of recombination rates: Recombination rates were determined by the method of the median (Lea and Coulson 1949). Yeast strains were grown on YEPD plates for 3 days, independent colonies were inoculated into 5 ml of

YEPGE, and cultures were grown for 3 days on a roller drum. Cells were washed with 5 ml of sterile H_2O and resuspended in 1 ml of sterile H_2O. Aliquots (100 μl) of appropriate dilutions were plated onto YEPD plates to determine the number of viable cells in each culture and onto SCGGE-Lys plates to determine the total number of recombinants in each culture. Colonies on YEPD plates were counted after 2 days and those on SCGGE-Lys plates after 3 days. The average number of viable cells and the median number of recombinants for each yeast strain were determined from a minimum of 12 cultures. Recombination rates are considered to be statistically different if the 95% confidence intervals do not overlap (Dixon and Massey 1969).

RESULTS

The recombination systems: The recombination systems used in this study consist of RNA polymerase II-transcribed substrates positioned on nonhomologous chromosomes in haploid strains (Figure 1). In all systems, one substrate was fused to the constitutive low-level *LYS2* promoter (*pLYS*) while the other substrate was fused to the highly inducible *GAL1-10* promoter (*pGAL*). The crossover-only recombination substrates (C/O substrates) consist of 5′ and 3′ truncated *lys2* alleles, with the promoter configuration being the one that was reported previously to yield the highest level of transcription-stimulated recombination (Saxe *et al.* 2000). The gene conversion-only recombination substrates consist of full-length and truncated *lys2* alleles, with the alleles positioned in opposite orientations relative to the centromeres of their chromosomes to preclude the production of viable crossover Lys^+ recombinants. Because only the full-length *lys2* allele (*lys2-oligo*) can become prototrophic in this conversion-specific assay, this allele will hereafter be referred to as the recipient allele and the truncated *lys2* allele as the donor allele. Fusion of these substrates to different promoters allows the genetic requirements for transcription-stimulated gene conversion to be analyzed when the donor (GCD substrates) *vs.* the recipient (GCR substrates) allele is highly transcribed.

Transcriptional activity of *pGAL* was regulated by growing isogenic $Gal80^+$ and $Gal80^-$ strains under non-inducing, nonrepressing conditions (glycerol/ethanol as carbon sources in the absence of galactose). Under these conditions the $Gal80^-$ strains highly transcribe the recombination substrate fused to *pGAL* (high-transcription conditions) while the $Gal80^+$ strains, which contain the negative regulatory protein Gal80p, transcribe the recombination substrate that is fused to *pGAL* at a very low level (low-transcription conditions). The transcriptional activity of *pGAL* has been shown to increase 1000-fold under high-transcription conditions relative to low-transcription conditions (Saxe *et al.* 2000). Spontaneous and transcription-stimulated crossovers and gene conversions were examined in wild-type (*RAD*), *rad50*, *rad51*, *rad52*, *rad54*, *rad55*, *rad57*, and *rad59* strains grown at 30°. Physical and phenotypic analyses of recombinants were consistent with the vast majority of crossovers corresponding to reciprocal exchange events and the vast majority of gene conversions resulting from the transfer of genetic information from the truncated to the full-length allele (see materials and methods for details).

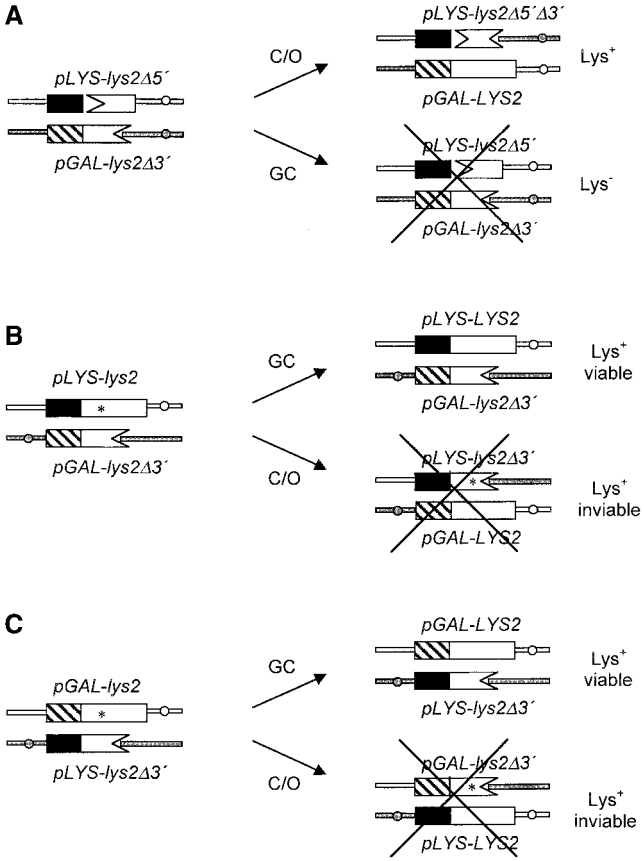

Figure 1.—The recombination substrates. Open boxes depict *LYS2* sequences; asterisks depict the *lys2-oligo* allele; solid and hatched boxes depict *pLYS* and *pGAL*, respectively; open and shaded lines depict chromosome *II* and *V* sequences, respectively; and circles depict centromeres. C/O, crossover; GC, gene conversion. (A) Crossover-only substrates, C/O system, are shown. Because experiments were done in haploid strains, recombinants containing only one translocation chromosome would be inviable due to a deficiency in genetic material. (B) Gene conversion-only substrates with donor highly transcribed, GCD system, are shown. (C) Gene conversion-only substrates with recipient highly transcribed, GCR system, are shown. In the GCD and GCR systems, a crossover between chromosomes *II* and *V* will yield dicentric and acentric translocation chromosomes, resulting in cell death.

Genetic requirements for spontaneous and transcription-stimulated crossovers: The crossover rate data for the *RAD* and *rad* mutant strains containing the C/O system are presented in Table 2. Under low-transcription conditions the crossover rate is reduced ~10-fold in the *rad51*, *rad52*, and *rad54* strains relative to the *RAD* strain. A less severe reduction is observed in *rad55*, *rad57*, and *rad59* strains, with the crossover rate decreasing only 3-fold relative to the *RAD* strain. In contrast to

TABLE 2

Crossover rates in wild-type and *rad* strains containing the crossover-only (C/O) recombination system

	Low txn (Gal80$^+$)		High txn (Gal80$^-$)		
RAD genotype	Rate ($\times 10^{-8}$)	Normalized[a]	Rate ($\times 10^{-8}$)	Normalized[a]	High txn/low txn
Wild type	0.96 (0.86–1.09)	1.0	12.0 (10.8–12.9)	12.5	12.5
rad50	3.37 (2.97–3.78)	3.51	15.4 (10.7–20.8)	16.0	4.56
rad51	0.09 (0.06–0.21)	0.09	0.06 (0–0.07)	0.06	0.67
rad52	0.08 (0–0.09)	0.08	0.07 (0–0.20)	0.07	0.88
rad54	0.10 (0.09–0.11)	0.10	0.23 (0.17–0.29)	0.24	2.30
rad55	0.29 (0.26–0.36)	0.30	0.35 (0.26–0.43)	0.36	1.21
rad57	0.30 (0.19–0.36)	0.31	0.49 (0.32–0.60)	0.51	1.63
rad59	0.34 (0.30–0.53)	0.35	8.08 (5.24–10.4)	8.42	23.8

The 95% confidence interval is given in parentheses below each rate. txn, transcription.
[a] Normalized to wild type, low transcription.

the other mutants, a *rad50* strain exhibits a hyperrecombinational phenotype under low-transcription conditions, with the crossover rate increasing 3.5-fold.

In the *RAD* strains, the crossover rate under high-transcription conditions is elevated 13-fold relative to the rate under low-transcription conditions. In contrast, the crossover rates in the *rad51*, *rad52*, *rad55*, and *rad57* strains under high-transcription conditions do not increase significantly over the rates observed under low-transcription conditions. Although the *rad54* strain exhibits a small (2.3-fold) increase in the crossover rate under high-transcription conditions, this increase is much less than the 13-fold increase observed in the *RAD* strain. The *rad59* strain also exhibits a significant increase in crossover rate under high-transcription conditions, with crossovers increasing 24-fold. In this case the magnitude of the increase is greater than that observed in the *RAD* strain, suggesting either that transcription-stimulated crossovers are less dependent than spontaneous crossovers on *RAD59* or that transcription partially compensates for loss of *RAD59*. In the *rad50* mutant under high-transcription conditions, the crossover rate increases 4.6-fold relative to low-transcription conditions. The recombination rate under high-transcription conditions in the *rad50* mutant is not statistically different from the recombination rate under high-transcription conditions in the *RAD50* control strain.

Genetic requirements for spontaneous and transcription-stimulated gene conversions when the donor allele is highly transcribed (GCD system): The spontaneous and transcription-stimulated gene conversion rates in *RAD* and *rad* mutant strains containing the GCD substrates are presented in Table 3. The relative phenotypes of the mutants under low-transcription conditions with respect to gene conversions are similar to those with respect to crossovers, with *rad51*, *rad52*, and *rad54* mutants exhibiting the most severe phenotypes (at least a 30-fold decrease in recombination rate); *rad55*, *rad57*, and *rad59* exhibiting weaker phenotypes (an ~2-fold decrease); and the *rad50* mutant exhibiting a hyperrecombinational phenotype (a 17-fold increase). We were unable to obtain an accurate estimate of the gene conversion rate in *rad51*, *rad52*, and *rad54* strains because the Lys$^+$ rates are not above the background reversion rate of the *lys2-oligo* allele (see Table 3 legend).

Under high-transcription conditions the gene conversion rate in the *RAD* strain increases 6.8-fold relative to the rate under low-transcription conditions. Similarly, *rad51*, *rad54*, and *rad59* strains exhibit significant increases in gene conversions under high-transcription conditions relative to low-transcription conditions. With respect to *rad51* mutants, it should be noted that transcription has a different impact on gene conversions *vs.* crossovers, with transcription stimulating only gene conversion events. In contrast to the *rad51*, *rad54*, and *rad59* mutants, *rad55* and *rad57* strains exhibit a significant 2-fold decrease in gene conversion rate under high-transcription conditions relative to low-transcription conditions. No increase in the gene conversion rate in the *rad52* strain under high-transcription conditions relative to low-transcription conditions is evident. In a *rad50* mutant, a slight but significant rate increase under high-transcription relative to low-transcription conditions is observed. This increase is consistent with an

TABLE 3

Gene conversion rates in wild-type and *rad* strains containing the GCD substrates (donor highly transcribed)

	Low txn (Gal80$^+$)		High txn (Gal80$^-$)		
RAD genotype	Rate ($\times 10^{-8}$)	Normalized[a]	Rate ($\times 10^{-8}$)	Normalized[a]	High txn/low txn
Wild type	3.23 (2.70–3.78)	1.0	21.9 (20.9–24.5)	6.78	6.78
rad50	54.8 (50.1–59.0)	17.0	70.5 (66.2–74.7)	21.8	1.29
rad51	0.06 (0-0.12)	NABR	0.47 (0.33–0.64)	0.15	≥7.83
rad52	0.03 (0–0.07)	NABR	0.04 (0–0.13)	NABR	NABR
rad54	0.10 (0.06–0.16)	NABR	0.34 (0.22–0.60)	0.11	≥3.40
rad55	1.95 (1.40–2.48)	0.60	0.96 (0.79–1.32)	0.30	0.49
rad57	2.73 (1.98–3.24)	0.85	1.43 (1.21–1.56)	0.44	0.52
rad59	2.12 (1.86–2.63)	0.66	14.8 (11.9–18.0)	4.58	6.98

Gene conversion rates indicated as being NABR (not above background reversion) correspond to Lys$^+$ rates that are not statistically above the background reversion rates of the *lys2-oligo* allele. The *pGAL-lys2-oligo* allele reverts at a rate of 5.1×10^{-10} in a wild-type Gal80$^-$ background and at a rate of 1.3×10^{-9} in a *rad52* Gal80$^-$ background. The 95% confidence interval is given in parentheses below each rate. txn, transcription.

[a] Normalized to wild type, low transcription.

additive relationship between transcription and disruption of *RAD50* on gene conversions, with disruption of *RAD50* resulting in a 17-fold increase in gene conversions, transcription resulting in a 6.8-fold increase in gene conversions, and both transcription and disruption of *RAD50* resulting in a 22-fold increase in gene conversions.

Genetic requirements for spontaneous and transcription-stimulated gene conversions when the recipient allele is highly transcribed (GCR system): Spontaneous and transcription-stimulated gene conversion rates in the *RAD* and *rad* mutant strains containing the GCR system are presented in Table 4. Again, as in the C/O and the GCD systems, *rad51*, *rad52*, and *rad54* mutants exhibit the most severe phenotypes (at least an 18-fold decrease in recombination rate); *rad55*, *rad57*, and *rad59* exhibit weaker phenotypes (a 2- to 4-fold decrease); and a *rad50* mutant exhibits a hyperrecombinational phenotype (an 8.4-fold increase).

Under high-transcription conditions the Lys$^+$ gene conversion rate in the *RAD* strain increases 18-fold relative to the rate under low-transcription conditions. *rad55*, *rad57*, and *rad59* strains exhibit a transcription-associated increase in gene conversions comparable to that observed in the *RAD* strain. The increase is particularly striking in the *rad55* and *rad57* mutants and directly contrasts with the results obtained with the GCD system where high levels of transcription inhibited recombination. Although the *rad51* and *rad54* strains also exhibit a significant increase in gene conversions under high-transcription conditions (5.5- and 3.5-fold, respectively), the increase is less than that observed in the *RAD*, *rad55*, *rad57*, and *rad59* strains. The Lys$^+$ rate in the *rad52* strain under high-transcription conditions does not increase significantly over the rate in the *rad52* strain under low-transcription conditions and likely reflects reversion of the *lys2-oligo* allele. In the *rad50* mutant, under high-transcription conditions, gene conversions increase 3.6-fold relative to low-transcription conditions. As with the GCD system, the data obtained with the GCR system are consistent with an additive relationship between transcription and disruption of *RAD50*.

DISCUSSION

The genetic requirements for spontaneous and transcription-stimulated mitotic crossover and gene conversion have been determined in order to investigate the basis for transcription-stimulated recombination and to further explore the *in vivo* roles of the recombination proteins. The recombination systems used here have several features that should be noted. First, because each system detects only crossover or only gene conversion events, a differential impact of transcription on crossovers *vs.* gene conversions can be discerned. Second, the positioning of the recombination substrates on nonhomologous chromosomes avoids some of the mechanistic ambiguities associated with other types of recombination systems, such as direct and inverted repeats (reviewed by Pâques and Haber 1999; see also Bartsch

TABLE 4

Gene conversion rates in wild-type and *rad* strains containing the GCR substrates (recipient highly transcribed)

	Low txn (Gal80$^+$)		High txn (Gal80$^-$)		
RAD genotype	Rate ($\times 10^{-8}$)	Normalized[a]	Rate ($\times 10^{-8}$)	Normalized[a]	High txn/low txn
Wild type	5.50 (4.75–6.07)	1.0	96.1 (87.6–109)	17.5	17.5
rad50	46.0 (38.8–50.3)	8.36	167 (149–187)	30.4	3.63
rad51	0.30 (0.24–0.35)	0.05	1.64 (1.31–2.10)	0.30	5.47
rad52	0.02 (0–0.08)	NABR	0.08 (0.07–0.11)	NABR	NABR
rad54	0.23 (0.10–0.24)	0.04	0.81 (0.71–1.12)	0.15	3.52
rad55	1.55 (1.34–1.79)	0.28	36.6 (32.1–38.4)	6.65	23.6
rad57	2.57 (2.10–2.95)	0.47	49.8 (48.1–53.6)	9.05	19.4
rad59	2.77 (2.40–3.22)	0.50	67.7 (62.1–76.7)	12.3	24.4

Gene conversion rates indicated as being NABR (not above background reversion) correspond to Lys$^+$ rates that are not statistically above the background reversion rates of the *lys2-oligo* allele. The *pGAL-lys2-oligo* allele reverts at a rate of 5.1×10^{-10} in a wild-type Gal80$^-$ background and at a rate of 1.3×10^{-9} in a *rad52* Gal80$^-$ background. The 95% confidence interval is given in parentheses below each rate. txn, transcription

[a] Normalized to wild type, low transcription.

et al. 2000; Malagon and Aguilera 2001). Indeed, physical analyses indicated that the majority of recombinants obtained with the C/O system corresponded to true reciprocal exchange events, consistent with these events arising via the resolution of a Holliday junction intermediate rather than by a nonreciprocal mechanism such as BIR. Similarly, recombinants generated in the GCD and GCR systems correspond to gene conversion events, which could, in principle, occur via either the DSB repair or the SDSA model. In the discussion that follows, the recipient and donor alleles in gene conversion events are defined according to current models of recombination. The recipient thus is assumed to be the molecule within which the initiating DSB occurs and the nucleoprotein filament is formed, whereas the donor is assumed to be the molecule that is invaded by the nucleoprotein filament. Although this donor/recipient distinction appears to be correct in the majority of gene conversion events, transformation experiments have demonstrated that a broken DNA molecule can also donate information at a low frequency (Roitgrund *et al.* 1993). Finally, in the systems used here recombination occurs between chromosomes rather than between a plasmid and a chromosome. Plasmid/chromosome *vs.* chromosome/chromosome recombination could proceed by different mechanisms and plasmid *vs.* chromosome chromatin structure may differ, either of which could alter genetic requirements in plasmid/chromosome systems (Sugawara *et al.* 1995).

A summary of the recombination rate data obtained with the C/O, GCD, and GCR substrates in the wild-type and *RAD52* epistasis group mutants is presented graphically in Figure 2 and the results are discussed in detail below. The major observations are summarized as follows: (i) the effects of *RAD50* disruption and transcription on gene conversion were additive; (ii) transcription stimulated gene conversions, but failed to stimulate crossovers in *rad51* mutants; (iii) like spontaneous events, transcription-associated events were completely dependent on Rad52p; (iv) transcription stimulated all types of recombination in *rad54* mutants, although not to the same extent as in wild-type strains; (v) Rad55p/Rad57p were required for transcription-stimulated crossover and for gene conversion when the donor allele was highly transcribed, but were not required for gene conversion when the recipient allele was highly transcribed; and (vi) transcription stimulated all recombination in *rad59* mutants to at least the same extent as in wild-type strains.

Spontaneous gene conversions and crossovers in wild type *vs. RAD52* epistasis group mutants: Gene conversions occurred at a rate three- to sixfold higher than crossovers in the wild-type strain under low-transcription conditions. This difference is consistent with the general observation that gene conversions are favored in mitosis (Esposito 1978; Haber and Hearn 1985; Kupiec and Petes 1988), but also may reflect the difference in the length of substrate overlap in the C/O *vs.* the GCD/GCR systems (1.9 *vs.* 3.3 kb, respectively; Jinks-Robertson *et al.* 1993). The relative phenotypes of the *RAD52*

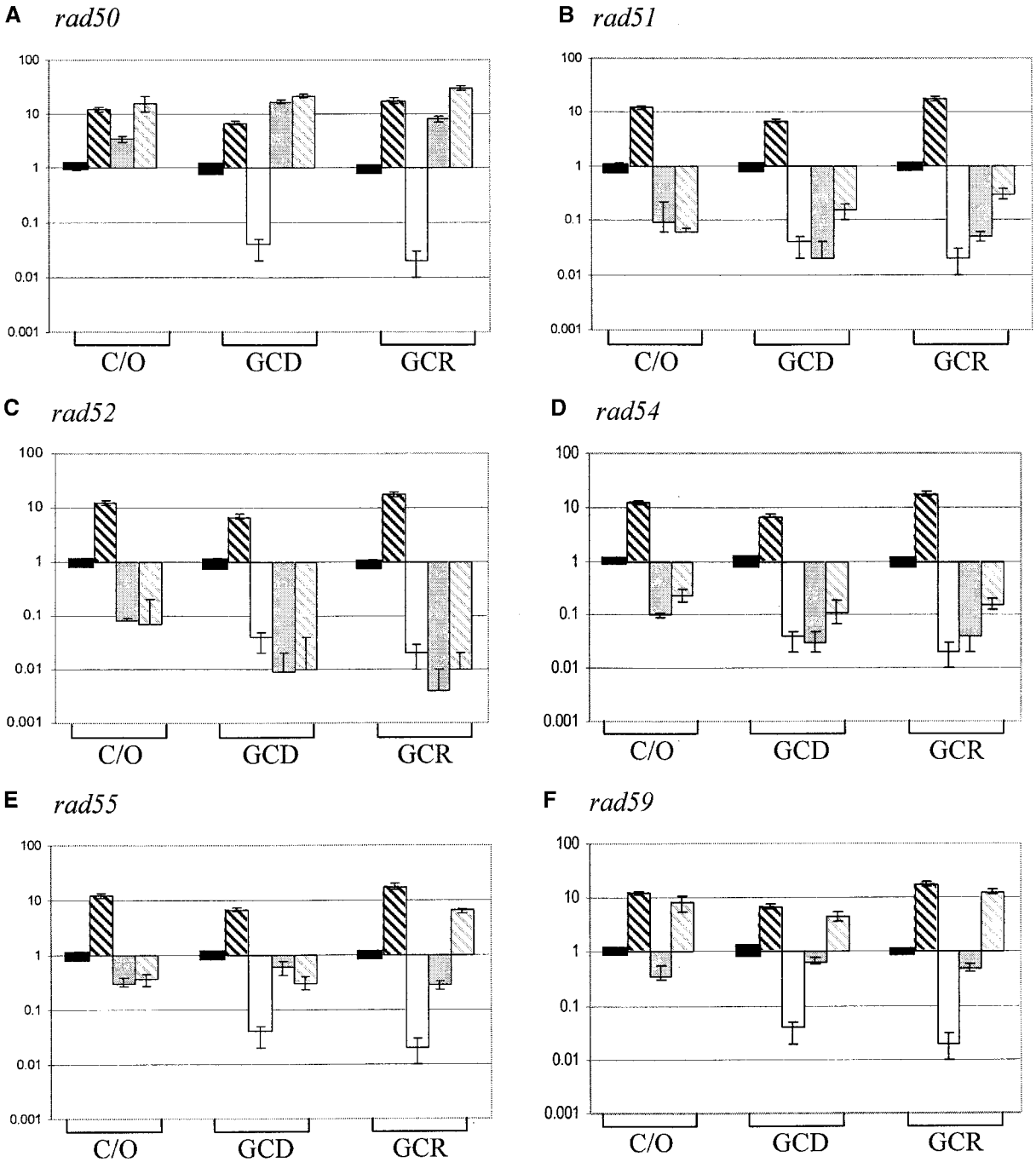

FIGURE 2.—Relative recombination rates in the wild-type and *RAD52* epistasis group mutants containing the C/O system, GCD system, or GCR system. Rates in each substrate system are normalized to the rate of the wild-type, low-transcription strain. The data obtained in the *rad57* mutants are not shown, but are similar to that obtained in the *rad55* mutants. Solid bars: wild-type strains, data obtained under low-transcription conditions. Shaded bars: mutant strains, data obtained under low-transcription conditions. Black hatched bars: wild-type strains, data obtained under high-transcription conditions. Gray hatched bars: mutant strains, data obtained under high-transcription conditions. Open bars: included with the GCR/GCD data, the reversion rate of the *pGAL-lys2-oligo* allele in a *rad52*, high-transcription background normalized to the recombination rate in the wild-type, low-transcription strain. Error bars depict 95% confidence intervals. The width of the solid bars depicts the 95% confidence interval for the wild-type, low-transcription strains.

epistasis group mutants under low-transcription conditions were similar in the C/O and GCD/GCR systems, with *rad51*, *rad52*, and *rad54* mutants exhibiting the most severe recombination defects; *rad55*, *rad57*, and *rad59* mutants exhibiting weaker defects; and *rad50* mutants exhibiting hyperrecombinational phenotypes. As

seen in previous studies, *rad55* and *rad57* mutants exhibited similar phenotypes (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995; Rattray and Symington 1995; Sugawara *et al.* 1995; Ivanov *et al.* 1996; Bartsch *et al.* 2000; Signon *et al.* 2001), which is consistent with these proteins functioning as a complex (Sung 1997b). Although previous studies generally have found *rad55* and *rad57* mutants to be strongly cold sensitive for defects in mitotic recombination (Lovett and Mortimer 1987; Hays *et al.* 1995; Johnson and Symington 1995; Liefshitz *et al.* 1995; Rattray and Symington 1995; Bartsch *et al.* 2000), disruption of *RAD55* or *RAD57* in our strains resulted in little or no decrease in recombination at either 30° or 23° (data not shown). It should be noted that a similar lack of cold sensitivity was reported when spontaneous gene conversion between nonhomologous chromosomes was assayed (Liefshitz *et al.* 1995). The phenotypes of *rad55/rad57* mutants thus could be related to the type of assay system used or could reflect strain background differences.

Many diverse recombination systems have been used to study the roles of Rad proteins, but there have been few systematic studies that allow direct comparisons between mutants. The most comprehensive study of this sort examined the roles of the *RAD52* epistasis group genes in mitotic gene conversion and crossover between inverted repeats (IRs; Rattray and Symington 1994, 1995; Bai and Symington 1996). In the IR system *rad52* mutants exhibited the most severe recombination defects; *rad54*, *rad55*, and *rad57* mutants exhibited less severe defects; and *rad51*, *rad59*, and *rad50* mutants exhibited the weakest defects. This is in stark contrast to our system, where *rad51* mutants had a greater recombination defect than did *rad55/rad57* mutants and where *rad50* mutants were hyperrecombinational. In addition, gene conversion was more dependent on *RAD51* than was crossover in the IR system. Although we cannot differentiate roles for Rad51p in spontaneous gene conversion *vs.* crossover in our system (gene conversion rates were not above the background reversion rates), this protein seems to be required more for crossover than for gene conversion under high-transcription conditions (see below). Consistent with our results, studies using a gap repair assay also suggest that crossovers are dependent on *RAD51* (Bartsch *et al.* 2000). The conflicting results obtained with the IR system likely reflect the different mechanisms by which recombinants are generated. Specifically, a recent study has indicated that recombination in the IR system occurs predominantly by a *RAD51*-independent pathway involving break-induced replication followed by single-strand annealing (Kang and Symington 2000).

rad50 mutants generally are defective in sister chromatid recombination, but exhibit hyperrecombinational phenotypes with respect to recombination between nonsisters (Malone and Esposito 1981; Gottlieb *et al.* 1989; Malone *et al.* 1990). The conflicting results obtained with the *rad50* mutants in our system *vs.* the IR system thus likely reflect the role of Rad50p in specifically promoting sister chromatid interactions (reviewed by Pâques and Haber 1999). Alternatively, the hyperrecombinational phenotype of *rad50* mutants could be a consequence of the shorter 3′ single-stranded tails in *rad50* mutants (see Pâques and Haber 1999). In recombination assays in which conversion of only one of two mutant sites generates a selectable recombinant, shorter tails would be expected to result in the generation of more selectable recombinants. Our results are more consistent with the idea that the hyperrecombinational phenotype of *rad50* mutants reflects the role of Rad50p in promoting sister chromatid interactions, as *rad50* mutants containing the C/O system (which has no mutant sites that need to undergo gene conversion) also exhibited a hyperrecombinational phenotype.

The relative roles of the *RAD52* epistasis group genes in spontaneous gene conversions between *lys2* heteroalleles positioned on nonhomologous chromosomes has been examined previously (Liefshitz *et al.* 1995; Jablonovich *et al.* 1999). While the low-transcription results reported here agree well with the earlier results, it should be noted that our results extend the previous analysis by examining spontaneous crossovers as well. Interestingly, our results as well as those of Jablonovich *et al.* (1999) suggest a role for Rad59p in recombination between nonhomologous chromosomes in haploids, whereas recombination between homologous chromosomes in diploids has been reported to increase in a *rad59/rad59* mutant (Bai and Symington 1996). These conflicting results may reflect differences between recombination in haploids *vs.* diploids (*e.g.*, ploidy, *MAT* status, or cell-cycle effects), differences between allelic *vs.* ectopic recombination, or strain background differences.

Transcription-stimulated gene conversions and crossovers in wild-type strains: The crossover and gene conversion rates in the *RAD* strains increased 7- to 17-fold under high-transcription conditions. The weak asymmetry reported here in the GCD *vs.* GCR systems is in stark contrast to results obtained in our previous study, which indicated that transcription stimulates recombination only when the recipient allele is highly transcribed (Saxe *et al.* 2000). Much of the asymmetry observed in the earlier study appears to be due to a transcription-associated bias in the mismatch repair system (J. Freedman and S. Jinks-Robertson, unpublished data). The weak asymmetry that does persist, however, is consistent with the idea that one effect of transcription is to increase the number of recombination-initiating lesions. Recent experiments examining plasmid-chromosome gene conversion or recombination between direct/inverted repeats are also consistent with transcription increasing the number of initiating lesions (S. González-Barrera, M. García-Rubio and A. Aguilera, per-

sonal communication). It should be noted, however, that if this were the only effect of transcription, high transcription of the donor allele in our system would be expected to have little, if any, effect on the overall gene conversion rate. The fact that transcription of the donor has such a large effect implicates additional effects of transcription (see discussion of *rad* mutants below).

Transcription-stimulated gene conversions and crossovers in *RAD52* epistasis group mutants: Several general models have been proposed to account for the stimulatory effect of transcription on mitotic recombination (reviewed by Aguilera 2001). The first model proposes that transcription stimulates recombination by increasing the number of recombination-initiating lesions. Such lesions could result from enhanced susceptibility of transcriptionally active DNA to nucleases or other damaging agents, transcription-associated changes in supercoiling, transcription elongation blocks, or transcriptional interference with replication fork passage. Consistent with the notion that high levels of transcription enhance DNA damage, transcription also has been found to stimulate mutation rates in yeast (Datta and Jinks-Robertson 1995; Morey *et al.* 2000). It should be noted that if transcription stimulates mitotic recombination solely by increasing the number of recombination-initiating lesions, one would expect the magnitude of the transcription-stimulated recombination to be the same in wild-type and *rad* mutant strains. A second general model for transcription-stimulated recombination suggests that the disruption of chromatin structure during transcription increases the efficiency of one or more individual steps in the recombination process. According to this model, one might predict that high levels of transcription would relax the genetic requirements for one or more Rad proteins. A previous study examining HO-induced mating-type switching led to the suggestion that Rad51p, Rad54p, Rad55p, and Rad57p indeed specifically play a role in facilitating strand invasion into otherwise inaccessible chromosomal donor sequences (Sugawara *et al.* 1995). The results of a subsequent study have demonstrated, however, that gap repair is similarly dependent on *RAD51*, *RAD52*, *RAD57*, and *RAD59* when the donor is plasmid borne *vs.* chromosomal (Bartsch *et al.* 2000). Finally, recombination can occur by multiple pathways with different genetic requirements, raising the possibility that transcription may stimulate only one of several pathways. This leads to the prediction that the genetic requirements for spontaneous *vs.* transcription-stimulated recombination should differ in a manner reflecting the pathway(s) used. As is evident in Figure 2, the effects of transcription on recombination varied widely depending on the assay system used and the member of the *RAD52* epistasis group that was eliminated. The results obtained in each *rad* mutant will be discussed individually below. Taken together, the data do not conform to the simplistic predictions made above if one assumes that transcription only increases the number of recombination-initiating lesions or that transcription functionally substitutes for only some of the Rad proteins. Instead, it is likely that transcription impacts recombination at several different levels.

RAD50 encodes a protein that plays a role in promoting sister chromatid interactions and in processing the ends of double-strand breaks (reviewed by Pâques and Haber 1999). Although the crossover rate in the *rad50* mutant increased under high-transcription conditions, it did not differ significantly from the rate in the wild-type strain under these same conditions. With respect to gene conversions, however, there appeared to be an additive relationship between disruption of *RAD50* and high levels of transcription. These additive relationships suggest that *RAD50* disruption *vs.* high levels of transcription increase recombination by different and nonoverlapping mechanisms.

RAD51 encodes the functional homolog of the RecA strand-exchange protein (Shinohara *et al.* 1992; Ogawa *et al.* 1993; Sung 1994). Although transcription stimulated gene conversions in *rad51* mutants, it had no effect on the crossover rate. This disparity may reflect the different mechanisms by which reciprocal crossover *vs.* gene conversion events can arise (see Pâques and Haber 1999). While gene conversions can be generated by mechanisms that do not involve the formation of a Holliday junction (*e.g.*, SDSA), reciprocal crossovers require the formation, stabilization, and resolution of a Holliday junction(s). We suggest that the stringent requirement for Rad51p for crossover under high-transcription conditions may reflect a role for Rad51p in stabilizing the single-end invasion intermediate, thus allowing the other end of the DSB to be engaged and a Holliday junction to be formed. It is possible that the stimulation of gene conversions in *rad51* mutants reflects the lack of a requirement for Rad51p for SDSA or reflects a role of transcription in facilitating the pairing and strand-exchange reactions during SDSA. Although the genetic requirements for SDSA have not been reported, they would be expected to be similar to those for BIR, which is *RAD51* independent (Malkova *et al.* 1996; Kraus *et al.* 2001; Signon *et al.* 2001). It should be noted that transcription of the donor allele resulted in a greater stimulation of gene conversions than did transcription of the recipient allele in *rad51* mutants relative to wild-type strains. It is possible that transcription of the donor may give single-stranded character to the invaded duplex, thus increasing the efficiency of gene conversions arising via a *RAD51*-independent, *RAD52*-dependent strand-annealing pathway (Kang and Symington 2000).

Previous studies have shown that *rad52* mutants exhibit the most severe recombination defects (reviewed by Pâques and Haber 1999), which is thought to reflect the requirement for Rad52p in all pathways of recombination in yeast. Consistent with its participation in multiple pathways, Rad52p has strand-annealing activity *in*

vitro (Mortensen *et al.* 1996; Sugiyama *et al.* 1998) and, like Rad55p/Rad57p, it stimulates the strand exchange activity of Rad51p when Rpa is present (Sung 1997a; New *et al.* 1998; Shinohara and Ogawa 1998; Song and Sung 2000). Transcription-stimulated crossovers and gene conversions were dependent on *RAD52*, which may reflect a requirement for Rad52p-mediated strand annealing in DSB repair as well as in SDSA. In DSB repair the protein may be required for capture of the second end of the DSB, which corresponds to a strand-annealing rather than a strand-invasion reaction. The Rad52p homolog, Rad59p, also has strand-annealing activity and recently was reported to stimulate the annealing activity of Rad52p (Bai and Symington 1996; Petukhova *et al.* 1999a; Davis and Symington 2001). Under high-transcription conditions, both crossovers and gene conversions were stimulated in *rad59* mutants to at least the same extent as in the wild-type strains, consistent with the idea of transcription increasing the occurrence of recombination-initiating lesions. Alternatively, it is possible that transcription facilitates the Rad52p-mediated strand-annealing reactions during DSB repair and SDSA, thereby alleviating the requirement for Rad59p in this process.

RAD54 encodes a protein homologous to the Swi2p/Mot1p family of chromatin remodeling helicases (Eisen *et al.* 1995). *In vitro*, Rad54p has DNA remodeling activity and has been shown to stimulate homologous pairing as well as heteroduplex extension (Petukhova *et al.* 1998, 1999b; Mazin *et al.* 2000; Van Komen *et al.* 2000; Solinger and Heyer 2001). Transcription significantly stimulated both crossovers and gene conversions in *rad54* mutants, although not to the same extent as observed in wild-type strains. The stimulation of recombination in *rad54* mutants may reflect a transcription-associated change in chromatin structure or supercoiling, either of which could partially relieve the requirement for Rad54p in creating a favorable chromosomal context within which the homology search as well as later heteroduplex extension can occur. In *rad54* mutants, it should be noted that when the donor allele was highly transcribed the high-transcription recombination rate/low-transcription recombination rate was at least 50% of the wild-type ratio whereas when the recipient allele was highly transcribed the high-transcription recombination rate/low-transcription recombination rate was only 20% of the wild-type ratio. This result is consistent with the suggestion that Rad54p plays a role in strand separation/chromatin remodeling of donor sequences.

The products of the *RAD55* and *RAD57* genes form a stable heterodimer that stimulates the strand-exchange activity of Rad51p when Rpa is present, suggesting a role for Rad55p/Rad57p in displacing Rpa and facilitating the loading of Rad51p onto single-stranded tails during nucleoprotein filament formation (Sung 1997b). Transcription of the recipient allele (GCR system) stimulated gene conversions in *rad55/rad57* mutants to the same extent as in the wild-type strain, consistent with the idea of transcription increasing the occurrence of recombination-initiating lesions. Alternatively, it is possible that the association of Rad51p with the RNA polymerase II holoenzyme complex (Maldonado *et al.* 1996) facilitates targeting of Rad51p to the recipient allele and thereby alleviates the requirement for Rad55p/Rad57p to appropriately load Rad51p. Finally, it is possible that the transcription machinery may participate in the displacement of proteins associated with the recipient locus, thereby facilitating nucleoprotein filament formation in the absence of Rad55p/Rad57p. In striking contrast to the stimulatory effect of transcription observed with the GCR substrates, high transcription of the donor allele failed to stimulate gene conversions in *rad55/rad57* mutants. Although Rad55p/Rad57p has been implicated only in the initial nucleoprotein filament formation, our data suggest that the complex may remain associated with the Rad51p nucleoprotein filament and could play a subsequent role in displacing proteins (*e.g.*, the transcription machinery) associated with the donor duplex molecule, thereby facilitating strand invasion and/or heteroduplex extension. As observed with the GCD substrates, transcription did not stimulate crossovers, which again might reflect a role of Rad55p/Rad57p in displacing the transcription machinery, thereby allowing the other end of the DSB to be engaged and a Holliday junction to be formed.

In summary, the use of highly transcribed recombination substrates has revealed clear differences between *rad* mutants that exhibit similar phenotypes in spontaneous recombination assays. The experiments reported here demonstrate that the genetic requirements for spontaneous *vs.* transcription-stimulated crossovers and gene conversions are different, which may reflect the distinct mechanisms for generating these alternative products of recombination. Furthermore, with respect to transcription-stimulated gene conversions, the genetic requirements differ depending on whether the recipient *vs.* the donor allele is highly transcribed. Finally, the relationship between transcription and recombination appears to be complex, with transcription stimulating recombination not only by increasing the number of recombination-initiating lesions, but also by facilitating subsequent steps of the recombination process.

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