The Involvement of Cellular Recombination and Repair Genes in RNA-Mediated Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

We previously demonstrated that a reverse transcript of a cellular reporter gene (*his3-AI*) can serve as the donor for gene conversion of a chromosomal *his3-ΔMscI* target sequence, and that this process requires the yeast recombination gene *RAD52*. In this study, we examine the involvement of other recombination and repair genes in RNA-mediated recombination, and gain insight into the nature of the recombination intermediate. We find that mutation of the mitotic RecA homologs *RAD51*, *RAD55*, and *RAD57* increases the rate of RNA-mediated recombination relative to the wild type, and that these gene functions are not required for RNA-mediated gene conversion. Interestingly, *RAD1* is required for RNA-mediated gene conversion of chromosomal *his3-ΔMscI* sequences, suggesting that the cDNA intermediate has a region of nonhomology that must be removed during recombination with target sequences. The observation that both *RAD1* and *RAD52* are required for RNA-mediated gene conversion of chromosomal but not plasmid sequences indicates a clear difference between these two pathways of homologous RNA-mediated recombination.

THE existence of a pathway for conversion of chromosomal alleles by a cDNA intermediate provides an alternative mechanism for the homogenization of dispersed repeated sequences. It also eliminates the potential for translocations and other gross chromosomal rearrangements, possible when chromosomal DNAs interact. Studies in yeast measuring ectopic recombination between artificially repeated sequences have shown that recombination between repeated sequences on the same (intrachromosomal) and nonhomologous chromosomes readily occurs, and that both crossover and conversion events are detected (reviewed in Petes *et al.* 1991). Ty elements, a family of naturally occurring dispersed repeated sequences, also undergo ectopic recombination. Although translocations have been detected, providing evidence for a physical interaction between the Ty elements, most of the conversion events involving Ty are not associated with reciprocal exchange (Kupiec and Petes 1988; reviewed in Liebman and Picologlou 1988). Kupiec and coworkers have shown that Ty cDNA can serve as a donor for gene conversion of chromosomal Ty elements. These cDNA-mediated recombination events are usually very low, but can be stimulated by transposition induction (Melamed *et al.* 1992). We have demonstrated that a cellular cDNA can serve as the donor for gene conversion of a chromosomal allele, a process we refer to as RNA-mediated gene conversion (Derr and Strathern 1993). This observation, forces the consideration that when a gene conversion event is not associated with the exchange of outside markers, the donor of genetic information may have been a cDNA. RNA-mediated gene conversion of chromosomal sequences represents a novel pathway of recombination and little is known about the nature of the recombination intermediate. This study investigates the effect of known cellular recombination genes on RNA-mediated recombination, in order to identify the cellular factors required, as well as to glean insight into the nature of the recombination intermediate utilized in this novel recombination pathway.

Our ability to detect RNA-mediated recombination in yeast, *Saccharomyces cerevisiae*, relies on a plasmid-borne *his3* reporter gene containing an artificial intron, *GAL1-his3-AI*. The formation of His^+ prototrophs requires transcription of the reporter gene from the galactose-inducible *GAL1* promoter, splicing of the resulting antisense *his3* transcript, and reverse transcription of the spliced, antisense transcript. The reverse transcriptase is provided by the yeast, LTR-containing retrotransposon Ty1. The cDNA can then be inserted into the chromosome in the absence of *HIS3* homology or recombine with plasmid *his3-AI* sequences. Physical analysis of chromosomal His^+ prototrophs revealed *HIS3* sequences flanked by Ty1 sequences. This structure suggested that Ty1 may have additional roles in priming reverse transcription of the *HIS3* sequences, and/or inserting the *HIS3* sequences into the chromosome. Because *HIS3* sequences are embedded in Ty1 sequences, chromo-

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somal insertion could be mediated by Ty1 integrase acting on the LTR sequences or by the host recombination machinery, using the homology provided by the flanking Ty1 sequences for recombination with endogenous Ty1 elements. It is important to note that all of the chromosomal *HIS3* insertions analyzed (inserted in the absence of *HIS3* homology) were flanked by Ty1 sequences (Derr *et al.* 1991). No illegitimate recombination events (Schiestl and Petes 1991; Schiestl *et al.* 1993) were detected. To directly measure RNA-mediated gene conversion of a chromosomal allele, *his3-ΔMscI* sequences were placed at the *MAT* locus on chromosome III and the assay was further refined by using a *GAL1-his3-ΔATG* plasmid as the source of cDNA. Because the promoter and initiation codon of the *HIS3* gene are deleted from the *GAL1-his3-ΔATG* plasmid, His^+ prototrophs resulting from gene conversion of plasmid *his3-AI* sequences are eliminated, as are chromosomal events not linked to *MAT*. Now all of the His^+ prototrophs are chromosomal and linked to *MAT* (Derr and Strathern 1993). By using two different sources of cDNA, the *GAL1-his3-AI* and *GAL1-his3-ΔATG* plasmids (Figure 1), we can identify genes involved in RNA-mediated recombination in general, and determine the effect of mutation of these genes on the overall process of RNA-mediated recombination (*GAL1-his3-AI*), as well as determine those genes specifically involved in RNA-mediated gene conversion of chromosomal alleles (*GAL1-his3-ΔATG*).

Many yeast strains with reduced levels of recombination were first defined as radiation-sensitive mutants, defective in the repair of DNA damage induced by ionizing radiation. The radiation-sensitive mutants were grouped based on their sensitivity to UV or ionizing radiation. The *RAD52* epistasis group is involved in the repair of double strand breaks, and represents the major group of genes involved in mitotic recombination in yeast (Resnick 1969; Malone *et al.* 1988). We have reported that *rad52* strains show wild-type levels of RNA-mediated recombination. However, our assays have allowed us to distinguish between pathways that are dependent and independent of *RAD52*. *RAD52* is specifically required for recombination between a diffusible cDNA and homologous, chromosomal sequences. Interestingly, although plasmid events are reduced in *rad52* strains, a *RAD52*-independent mechanism exists for RNA-mediated gene conversion of plasmid *his3-AI* sequences (Derr and Strathern 1993). In this study, I investigate the role of other members of the *RAD52* epistasis group, as well as the role of *RAD1*, a member of the *RAD3* epistasis group, in RNA-mediated recombination and gain insight into the nature of the recombination intermediate.

MATERIALS AND METHODS

Media and yeast strains: Standard yeast media was prepared as described in Sherman *et al.* (1986). The yeast strains used in this study are described in Table 1. All strains are isogenic and were derived from L1890 (*MAT***a** *his3-Δ200 ura3-52 trp1-289 lys*) obtained from G. R. Fink. Strains were constructed by gene disruption (Rothstein 1983; Alani *et al.* 1987). Strain YLD125 was transformed with a 6.6 kb *Bgl* II *leu2::hisG-URA3-hisG* fragment from plasmid pNKY85, resulting in the *leu2::hisG-URA3-hisG* disruption strain YLD615. Excision of the *URA3* gene from the *leu2::hisG-URA3-hisG* disruption by intramolecular recombination between the *hisG* repeats was accomplished by plating on 5-FOA and isolating Ura^- cells (Boeke *et al.* 1984), and was confirmed by Southern blot analysis. YLD615 was subsequently transformed with the appropriate plasmid DNA fragments to generate disruption alleles of the indicated *RAD* genes: with a *Hin*dIII *rad1::LEU2* disruption fragment from plasmid pL962 (R. Keil), a *Xba*I-*Pst*I *rad51::LEU2* disruption fragment from plasmid pAM28 (M. Aker), a *Hin*dIII *rad55::LEU2* disruption fragment from plasmid pSTL11 (S. Lovett), or a *Sac*I *rad57::LEU2* disruption fragment from plasmid pSM51 (D. Schild). The presence of the *rad* mutations was monitored by sensitivity to ionizing radiation (*rad51*, *rad55*, and *rad57*) or sensitivity to UV-irradiation (*rad1*) and further verified by Southern blot analysis. The gamma radiation sensitivity of strains was scored by replica plating test strains to YEPD and irradiating with 50krad from a Gammacell 40 irradiator (Nordion International Inc., Ontario, Canada) containing ^{60}Co. The UV-sensitivity of strains was scored by replica plating test strains to YEPD medium and irradiating with 160 J/m^2 at 254 nm using a UV Stratalinker 1800 (Stratagene, La Jolla, CA).

Rate determination of His^+ prototroph formation: Rates were computed by the median method of Lea and Coulson (1949) or by the fraction of tubes with no mutants as described by the Poisson distribution. Sixty (*GAL1-his3-AI*: *RAD*, *rad51*, *rad52*, *rad55*, and *rad57*; *GAL1-his3-ΔATG*: *rad51*, *rad55*, and *rad57*) or 100 (*GAL1-his3-AI*: *rad1*, and *rad1rad52*; *GAL1-his3-ΔATG*: *RAD*, *rad52*, *rad1*, and *rad1rad52*) independent cultures were tested from each strain. 3-ml cultures were inoculated with single colonies and grown for 4–5 days in media lacking uracil and containing galactose at 20°. A sample from three or five cultures (from 60 or 100 independent cultures, respectively) was removed for titering and plated on YEPD in order to determine the total number of cells assayed. All cultures were then concentrated and plated on plates lacking histidine and containing glucose, to stop the induction and to determine the number of His^+ prototrophs, a measure of RNA-mediated recombination, with incubation at 30°.

Chromosomal versus plasmid events: One His^+ prototroph was picked randomly from each culture and patched to SC-his glucose plates. Chromosomal events were distinguished from plasmid events by first replica plating on 5-fluoroorotic acid which selects for loss of the URA-marked *his3* reporter plasmid (Boeke *et al.* 1984) and then replica plating to SC-ura and SC-his plates. Chromosomal events have a Ura^- His^+ phenotype.

Recovery of plasmids from yeast to *E. coli*: Quick yeast DNA preps were prepared by the method of Polaina and Adam (1991) optimizing for plasmid recovery. Five ul of DNA was transformed into 25 ul ElectroMAX DH10B cells (Life Technologies, Gaithersburg, MD) by electroporation at 200 ohms, 25 uFD and 2.5 kV, incubated at room temperature in 500 ul SOC for 30 min and plated on L-amp plates to select for recovery of the plasmid. Plasmid mini preps were prepared by alkaline lysis and digested with the restriction endonuclease *Pst*I. Standard molecular techniques were performed as described in Maniatis *et al.* (1982).

PCR analysis: Linkage between *HIS3* and *MAT* was determined by polymerase chain reaction (PCR). Primer a: 5′-CAT GCTCTGGCCAAGCATTCC (nucleotides 222–242; Struhl 1985) is complementary to sequences spanning the *MscI* site,

deleted in *his3-ΔMscI* and interrupted in plasmid *his3-AI* sequences. Primer b: 5′-CTGGGTAGAGTCTTATTGGCA (nucleotides 197379–197399; Oliver *et al.* 1992) is complementary to *MAT* sequences. If *HIS3* is linked to *MAT* and no additional sequences are present, then a 1-kb PCR product is expected. Primer c: 5′-CCCCGGCCGAATTCAGAGCAGAAAGCCCTAGTA (*HIS3* nucleotides 27–47; Struhl 1985) was used in combination with primer b as a positive control. The conditions for PCR were 2 ul yeast DNA, 300 ng each primer, 200 μM dNTPs, and 2.5 units *Taq* polymerase (Perkin Elmer, Foster City, CA); with denaturation at 94° for 1 min, annealing at 55° for 1 min and extension at 72° for 2 min, for 25 cycles, followed by a 7 min extension at 72°. Primer e: 5′-GGTGTGCGTTTG CTGCAAAGT (nucleotides 196869–196899; Oliver *et al.* 1992) is complementary to *MAT* sequences upstream of the inserted *his3-ΔMscI* target. Primer d: 5′-GGAATGCTTGGCCA-GAGCATG (nucleotides 242–222; Struhl 1985) is complementary to sequences spanning the *MscI* site and is the antisense of primer a. If no additional sequences are present, then primers e and d should yield a PCR product of 0.5 kb. The conditions for PCR were the same as above with the exception that annealing was at 64°.

RESULTS

Isogenic yeast strains were constructed, containing mutations in genes known to be involved in DNA recombination (Table 1). Disruption of the appropriate chromosomal allele was confirmed by Southern blotting and increased sensitivity to gamma-irradiation (*rad51*, *rad55*, and *rad57*) or increased sensitivity to UV-irradiation (*rad1*) (see materials and methods). The involvement of these genes in RNA-mediated recombination was determined in rate experiments employing the *GAL1-his3-AI* and *GAL1-his3-ΔATG* plasmids (see materials and methods). By using the *GAL1-his3-AI* plasmid, at least three pathways of *HIS3* cDNA insertion can be monitored: insertion mediated by Ty1 integrase, a *RAD52*-dependent mechanism of RNA-mediated gene conversion (plasmid and chromosomal events), and a *RAD52*-independent mechanism of RNA-mediated gene conversion (plasmid events only). Thus, this analysis allows us to ascertain the effect of these mutations on the overall process of RNA-mediated recombination. In contrast, by using the *GAL1-his3-ΔATG* plasmid we specifically measure recombination between the *HIS3* cDNA and the chromosomal *his3-ΔMscI* allele, *i.e.*, plasmid events and chromosomal events not linked to *MAT* are eliminated (Figure 1; Derr and Strathern 1993).

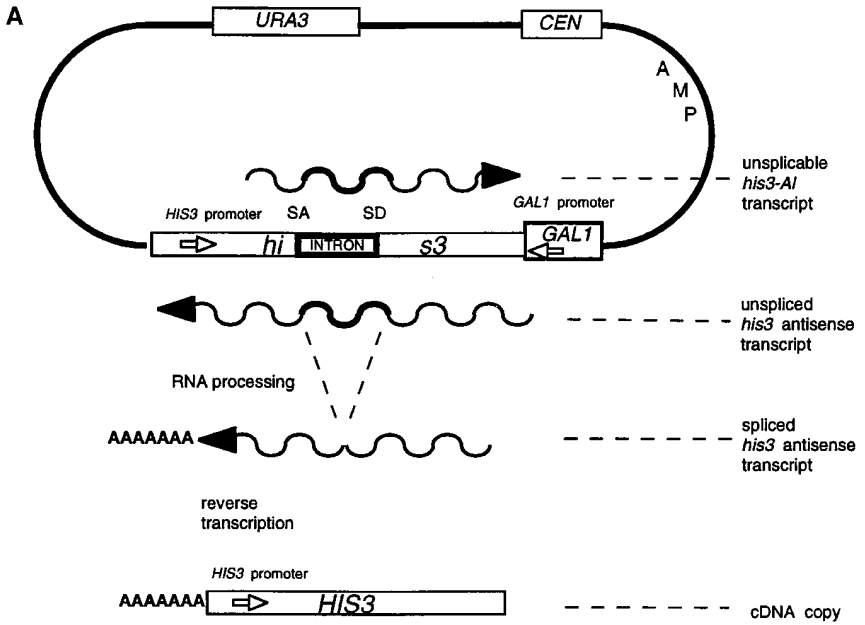
The involvement of recombinational repair genes: *RAD51*, *RAD55*, and *RAD57* are mitotic RecA homologs involved in recombinational repair (Kans and Mortimer 1991; Aboussekhra *et al.* 1992; Basile *et al.* 1992; Shinohara *et al.* 1992; Lovett 1994). Mutation of these genes has been shown to reduce gene conversion unassociated with crossing over (Rattray and Symington 1994; Rattray and Symington 1995). The requirement for these genes in RNA-mediated recombination in general was determined using the *GAL1-his3-AI* plasmid. As shown in Table 2, the overall rate of appearance of His^+ prototrophs was increased by disruption of *RAD51*, *RAD55* or *RAD57*. Both plasmid and chromosomal events were observed in all three mutant strains, although the proportion of chromosomal events was increased relative to the wild type. The *HIS3* plasmid events that were observed in a *rad51*-mutant background revealed no gross rearrangements but appeared to represent simple RNA-mediated gene conversion of plasmid *his3-AI* sequences (data not shown).

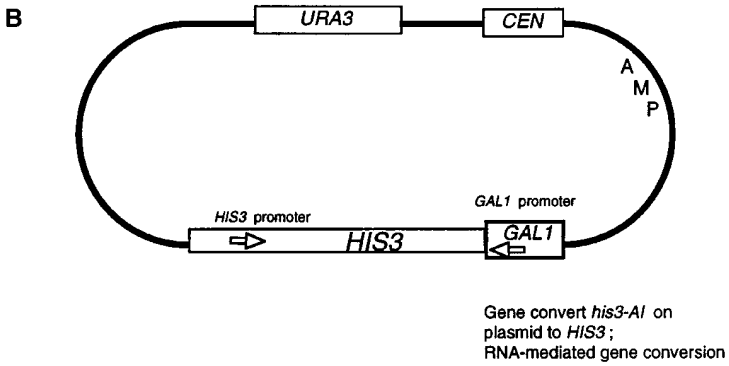
By using the *GAL1-his3-AI* plasmid, we can monitor the overall affect of these mutations on RNA-mediated recombination, however, we cannot determine whether chromosomal insertions were mediated by Ty1 integrase or the host recombination machinery. Therefore, to look specifically at the requirement for these genes in RNA-mediated gene conversion of chromosomal *his3-ΔMscI* sequences, the *GAL1-his3-ΔATG* plasmid was used. We have previously reported that *RAD52* is required for RNA-mediated gene conversion of chromosomal sequences. I therefore asked if other members of the *RAD52* epistasis group were also specifically required for this event. As shown in Table 2, *RAD51*, *RAD55* and *RAD57* are not required for RNA-mediated gene conversion of chromosomal sequences. I asked if the insertions at *MAT* represented simple gene conversion events. To address this question, PCR was used to determine if conversion of the chromosomal target resulted in the expected size fragment, or if additional sequences were present (Figure 2; see materials and methods). All of the His^+ prototrophs analyzed from wild type

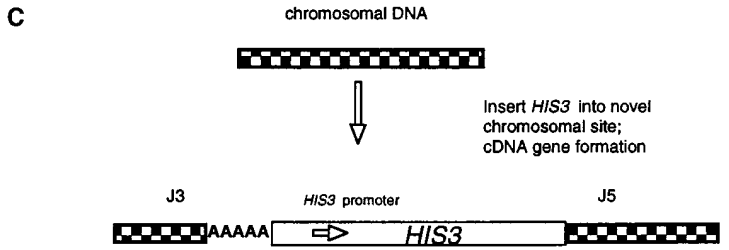
TABLE 1

Description of strains

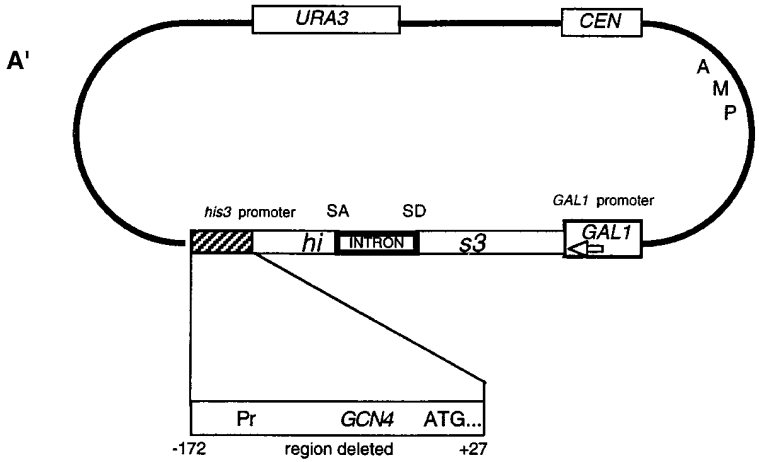
Name	Genotype
YLD125	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys*
YLD157	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys rad52::hisG*
YLD615	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG-URA3-hisG*
YLD855	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad51::LEU2*
YLD658	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad55::LEU2*
YLD659	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad57::LEU2*
YLD655	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad1::LEU2*
YLD746	*MAT***a***::his3-ΔMscI his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad1::LEU2 rad52::hisG*







GAL1-his3-ΔATG



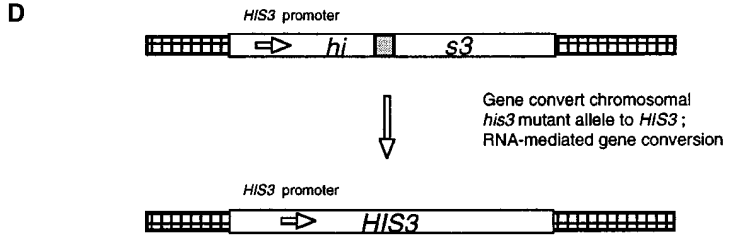


TABLE 2

Rate of His$^+$ prototroph formation in various *rad* strains

Strain (*rad* mutation)	Rate ($\times 10^{-8}$)[a] *GAL1-his3-AI* (% chromosomal)	Rate ($\times 10^{-8}$)[b] *GAL1-his3-ΔATG*
YLD125 (*RAD*)	6.5 (33)	0.2
YLD157 (*rad52*)	6.7 (87)	0 ($0/3.4 \times 10^9$)
YLD855 (*rad51*)	31.6 (79)	1.2
YLD658 (*rad55*)	17.9 (66)	1.1
YLD659 (*rad57*)	19.0 (68)	0.9
YLD655 (*rad1*)	1.1 (33)	0.01 ($2/1.3 \times 10^{10}$)
YLD746 (*rad1rad52*)	6.1[b] (63)	0 ($0/3.7 \times 10^9$)

[a] Rates were computed by the method of the median Lea and Coulson (1949).

[b] Rates were computed by the Poisson distribution as the fraction of the tubes with no mutants.

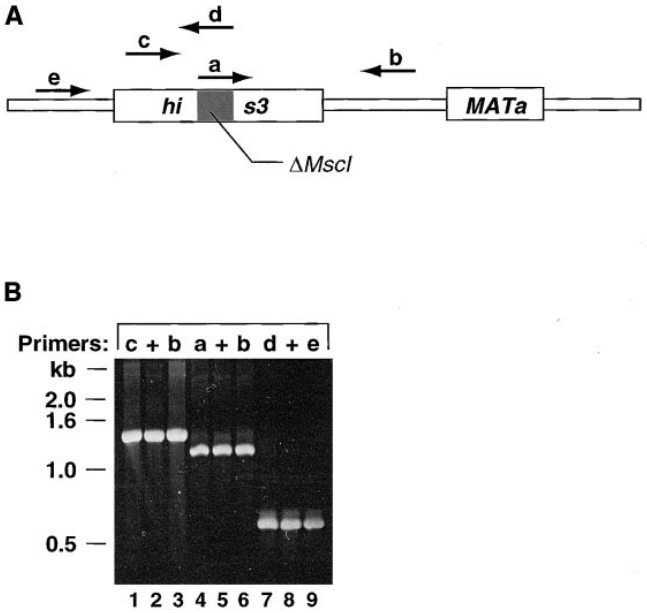


Figure 2.—PCR analysis to determine if conversion of the chromosomal *his3-ΔMscI* target represents a simple RNA-mediated gene conversion event. (A) Structure of *his3-ΔMscI* on chromosome III and location of primers used for PCR. A 34 bp deletion of the *his3* gene was constructed and inserted at *MAT*. This deletion spans the *Msc*I site, the site of insertion of the artificial intron in plasmid *his3* sequences. (B) PCR analysis indicating that recombination between the *HIS3* cDNA and chromosomal *his3-ΔMscI* target sequences results from a simple gene conversion event, *i.e.*, no additional sequences are present. The primers used for PCR are indicated above each lane. Primers c + b amplify target sequence present at *MAT* and serve as a positive control for the PCR reaction. Primers a + b were used to demonstrate that the His$^+$ prototroph is linked to *MAT* and that no additional sequences are present downstream. Primers d + e were used to demonstrate that no additional sequences are present upstream. Samples are from strains: *rad51* (lanes 1, 4, 7); *rad55* (lanes 2, 5, 8); *rad57* (lanes 3, 6, 9).

rad51, *rad55* and *rad57* strains gave the expected size PCR products, indicative of a simple RNA-mediated gene conversion event.

The role of the excision repair gene *RAD1*: *RAD1* is required for the incision step of excision repair (Reynolds and Friedberg 1981; Wilcox and Prakash 1981; Tomkinson *et al.* 1993) and has been shown to also have a role in mitotic recombination (Schiestl and Prakash 1988). As shown in Table 2, mutation of *RAD1* reduces RNA-mediated recombination relative to the wild type. Although a greater proportion of the His$^+$ prototrophs are plasmid-borne in *rad1* strains relative to members of the *RAD52* epistasis group, it appears that the rate of both plasmid and chromosomal events is affected by mutation of *RAD1*. Recovery and analysis of several of the plasmid-borne His$^+$ prototrophs revealed no gross rearrangements, but appeared to represent simple RNA-mediated gene conversion of plasmid *his3-AI* sequences (data not shown). Like *RAD52*, *RAD1* is

Figure 1.—RNA-mediated recombination. (A) The *GAL1-his3-AI* plasmid acts as the donor of *HIS3* cDNA. The yeast *his3* gene is interrupted by an artificial intron inserted into a unique *Msc*I site in the unspliceable orientation relative to the *HIS3* promoter, but in the spliceable orientation relative to the *GAL1* promoter. The *HIS3* sequences on the *GAL1-his3-AI* plasmid extend from nucleotide −172 → +703 (Struhl 1985). Transcripts are shown as wavy lines and the presence of the intron is indicated by thicker lines. SD, splice donor; SA, splice acceptor. The formation of *HIS3* cDNA requires reverse transcription of the spliced, antisense *his3* transcript. This cDNA is then involved in a subsequent recombination event. (B) RNA-mediated gene conversion of plasmid *his3-AI* sequences, resulting in intron loss. (C) Insertion into a chromosome in the absence of *HIS3* homology. J3, 3′ junction and J5, 5′ junction created upon insertion of *HIS3* cDNA. Physical analysis has revealed that in all cases the junctions are Ty1 sequences; insertion can be mediated by Ty1 integrase or the cellular recombination machinery. (D) RNA-mediated gene conversion of chromosomal *his3-ΔMscI* sequences. Checkered sequences are chromosomal sequences; stippling indicates a deletion mutation. The chromosomal *his3-ΔMscI* sequences extend from nucleotide −72 → +703 with a 34bp deletion extending from nucleotide +219 → +252 (Struhl 1985), spanning the *Msc*I site. (A′) The *GAL1-his3-ΔATG* plasmid acts as the donor of *HIS3* cDNA. An expansion of the deleted region (cross-hatching) in *GAL1-his3-ΔATG* is shown, a 199bp deletion, nucleotides −172 → +27, removes the *HIS3* promoter (Pr), Gcn4p binding site (*GCN4*), and initiation codon (ATG). The *HIS3* sequences present on this plasmid extend from nucleotide +28 → +703 (Struhl 1985). His$^+$ prototroph formation requires recombination with chromosomal *his3-ΔMscI* sequences (D).

required for recombination between the *HIS3* cDNA and chromosomal *his3-ΔMscI* allele (Table 2). From 100 *rad1* cultures, only two His^+ prototrophs were detected. Both were linked to *MAT*, as determined by Southern hybridization analysis (data not shown).

His^+ prototroph formation in *rad1rad52* background: Because *RAD52* and *RAD1* define distinct epistasis groups (reviewed in Game 1983), I analyzed the effect of the double mutant on RNA-mediated recombination. As shown in Table 2, the rate of His^+ prototroph formation is higher, and the proportion of chromosomal events appears to be increased in the *rad1rad52* double mutant relative to the *rad1* single mutant. Furthermore, it is clear that RNA-mediated gene conversion of plasmid *his3-AI* sequences can occur in the absence of both *RAD52* and *RAD1*. The plasmid events that were analyzed showed no gross rearrangements, but again seemed to reflect a simple RNA-mediated gene conversion event (data not shown). Not unexpectedly, no His^+ prototrophs were detected when the *GAL1-ΔATG* plasmid was used as the source of cDNA (Table 2).

DISCUSSION

In this paper, I investigate the involvement of cellular genes, known to have a role in recombination, in the novel pathway of RNA-mediated recombination. Previously, we have shown that the reverse transcription of a cellular mRNA (*his3-AI*) requires expression of the yeast retrotransposon Ty1 to provide a source of reverse transcriptase. Physical analysis of the chromosomal His^+ prototrophs, inserted in the absence of *HIS3* homology, revealed *HIS3* sequences embedded in Ty1 sequences, suggesting additional roles for Ty1 in priming and/or insertion of the *HIS3* sequences into the chromosome. The presence of Ty1 sequences flanking *HIS3* sequences suggested two potential mechanisms for insertion of the cellular *HIS3* sequences into the chromosome. Insertion might be mediated by the host recombination machinery, using the homology provided by Ty1, for homologous recombination with endogenous Ty1 elements or solo LTR sequences. Alternatively, insertion might be mediated by Ty1 integrase acting on the LTR sequences that flanked the cellular *HIS3* sequences (Derr *et al.* 1991). The *RAD52* gene of *S. cerevisiae* is involved in most recombination in yeast. Strains bearing a mutation in *RAD52* are defective in mitotic recombination, specifically gene conversion (Malone *et al.* 1988). Results showed that mutation of *RAD52* does not reduce the overall rate of RNA-mediated recombination, and in fact chromosomal events increase significantly. However, *RAD52* is specifically required for RNA-mediated gene conversion of homologous, chromosomal sequences (Derr and Strathern 1993). It has been reported that a *rad52* mutation increases Ty1 transposition (Curcio and Garfinkel 1994). Therefore, it is likely that the chromosomal events observed in a *rad52*-mutant background are mediated by Ty1 integrase. That is, in the absence of *RAD52*, RNA-mediated gene conversion of chromosomal sequences is eliminated, and chromosomal insertion is mediated by Ty1 integrase.

The mitotic RecA homologs *RAD51*, *RAD55*, and *RAD57* are required for most mitotic recombination in yeast and are not functionally redundant (Petes *et al.* 1991; Shinohara *et al.* 1992; Lovett 1994). *RAD51* has been shown to possess DNA-dependent ATPase and strand exchange activities (Sung 1994). It is thought that Rad55p and Rad57p function as a heterodimer to promote strand exchange by Rad51p (Sung 1997). *RAD51*, *RAD55*, and *RAD57* are required for wild-type levels of mitotic gene conversion (Rattray and Symington 1995). Mutation of these genes increases recombination between direct repeats, including Ty LTR sequences (McDonald and Rothstein 1994; Liefshitz *et al.* 1995) by a mechanism thought to involve single-strand annealing (Ivanov *et al.* 1996). *RAD51* is also required for cDNA-mediated conversion of Ty elements, but *RAD57* is not (Nevo-Caspi and Kupiec 1994). I show that RNA-mediated recombination is increased in *rad51*, *rad55*, and *rad57* strains relative to the wild type and that a greater percentage of the events are chromosomal (Table 2). Interestingly, *RAD51*, *RAD55*, and *RAD57* are not required for RNA-mediated gene conversion of chromosomal *his3-ΔMscI* sequences.

The observed increase in RNA-mediated recombination in *rad51*, *rad55* and *rad57* strains may reflect, at least in part, increased Ty1 transposition (*rad51* and *rad57* strains have been shown to increase Ty1 transposition; A. Rattray, personal communication). We previously reported that expression of a plasmid-borne *GAL1*-Ty1 element, known to increase Ty1 transposition (Boeke *et al.* 1985), increased RNA-mediated recombination (Derr *et al.* 1991). Thus, by increasing Ty1 transposition, the level of cDNA substrate for RNA-mediated recombination may be increased. Additionally, because RNA-mediated gene conversion is not compromised in *rad51*, *rad55*, and *rad57* strains, insertion of the cDNA can be mediated by homologous, RNA-mediated gene conversion and by Ty1 integrase.

I show that recombination between the *HIS3* cDNA and chromosomal *his3-ΔMscI* target sequences results from a simple gene conversion event, *i.e.*, there is no evidence of additional sequence or rearrangement at the site of insertion (for example, Ty1 sequence; Figure 2). If one considers the homologous integration of transformed DNA, in some respects comparable to RNA-mediated recombination in terms of the donor of genetic information being diffusible, *rad51* and *rad57* strains exhibit a 10-fold reduction relative to the wild type (Schiestl *et al.* 1994). Further, cDNA-mediated conversion of Ty elements requires *RAD51*, but not *RAD57* (Nevo-Caspi and Kupiec 1994). In contrast, conversion of a chromosomal allele by *HIS3* cDNA requires neither *RAD51* nor *RAD57*. Perhaps *RAD51*, *RAD55*, and *RAD57* are not required

for RNA-mediated gene conversion, because the recombinational intermediate is not double-stranded DNA, but rather a single-strand cDNA or an RNA/DNA duplex. For example, cDNA-mediated conversion of Ty elements may utilize a double-stranded DNA intermediate, because Ty has a mechanism for priming second strand synthesis of its own genome. Thus, the requirement for different cellular genes may reflect the utilization of a discrete pathway that is active on single-strand cDNA or an RNA/DNA duplex. It is also of interest that double-strand break induced single-strand annealing does not require *RAD51*, *RAD55*, or *RAD57* (Ivanov *et al.* 1996).

RAD1 has been reported to have a role in mitotic recombination (Schiestl and Prakash 1988). Rad1p together with Rad10p function as an endonuclease, required for incision of UV-damaged DNA (Bailly *et al.* 1992; Sung *et al.* 1993; Tomkinson *et al.* 1993) and for removing nonhomologous sequences from the 3′ ends of recombining DNA (Fishman-Lobell and Haber 1992). I show that RNA-mediated recombination is reduced in *rad1* strains relative to the wild type (Table 2), and that both plasmid and chromosomal rates appear to be affected. This reduction is alleviated somewhat in the *rad1rad52* double mutant, in part due to increased Ty1 transposition (see above). Surprisingly, *RAD1* is specifically required for RNA-mediated gene conversion of chromosomal sequences (Table 2). By contrast, *RAD1* is not required for cDNA-mediated conversion of Ty elements (Nevo-Caspi and Kupiec 1996), or for the homologous integration of transformed DNA (Schiestl *et al.* 1994). The observation that *RAD1* is required for RNA-mediated gene conversion most likely reflects its role in removing nonhomologies from the 3′ ends of recombining DNA, and thus, lends insight into the nature of the recombination intermediate. That is, the cDNA intermediate possesses a region of nonhomology at its 3′ end, relative to the *his3-ΔMscI* target sequence. Consistent with this proposal, prior physical analysis of the chromosomal His^+ prototrophs revealed that the 3′ end of the *HIS3* sequence was polyadenylated and flanked by Ty1 sequences (Derr *et al.* 1991). Therefore, the intermediate utilized for RNA-mediated gene conversion possesses a region of nonhomology at the 3′ end provided by the poly(A) tail and/or flanking Ty1 sequences that must be removed by *RAD1* during recombination with chromosomal *his3-ΔMscI* target sequences.

In all of the mutant strains assayed, RNA-mediated gene conversion of plasmid-borne *his3-AI* sequences is detected (Tables 2). Further, although both *RAD1* and *RAD52* are required for homologous, RNA-mediated gene conversion of chromosomal sequences, neither are strictly required for homologous, RNA-mediated gene conversion of plasmid sequences. These observations strongly support the utilization of distinct pathways for plasmid and chromosomal RNA-mediated gene conversion.

One possible explanation for the observed differences in plasmid and chromosomal RNA-mediated gene conversion is that the plasmid-borne His^+ prototrophs do not represent simple gene conversion events. We previously reported that in wild-type strains, plasmid-borne His^+ prototrophs resulted from precise splicing of the intron, no gross rearrangements were observed (Derr *et al.* 1991). Plasmid-borne multimeric insertions were observed when Ty1 integrase-mediated insertion was blocked (Sharon *et al.* 1994). Accordingly, plasmid-borne His^+ prototrophs were recovered from *rad1*, *rad52*, *rad1rad52*, and *rad51* strains. No gross rearrangements were observed. Rather, all of the plasmid-borne His^+ prototrophs resulted from precise splicing of the intron and a simple RNA-mediated gene conversion event between the *HIS3* cDNA and plasmid *his3-AI* sequences.

Differences in plasmid and chromosomal recombination have been attributed to chromatin structure. It has been suggested that *RAD51*, *RAD55*, and *RAD57* are required to promote recombination in the context of chromatin (Sugawara *et al.* 1995). Additionally, transcription might distinguish these two recombination events. Nevo-Caspi and Kupiec (1994) have reported that high levels of Ty transcription induce its participation as a recipient of information in conversion events, and that this induction is distinct from the induction observed upon increased production of Ty cDNA. They suggest that *RAD52* and *RAD1* are required for transcription-induced Ty conversion, but not for cDNA-mediated Ty conversion (Nevo-Caspi and Kupiec 1996). However, we find that neither *RAD52* nor *RAD1* are required for conversion of the actively transcribed plasmid *his3-AI* sequences, but both are required for conversion of chromosomal *his3-ΔMscI* sequences by the *HIS3* cDNA.

It has been reported that *rad52*-mutant strains are relatively proficient for plasmid recombination (Dornfeld and Livingston 1992). A recently identified *RAD52* homolog, *RAD59*, has been suggested to have a role in plasmid recombination (Bai and Symington 1996). Perhaps an analogy can be drawn between plasmid recombination in yeast and *E. coli*. Plasmid recombination in *E. coli* is primarily mediated by *RecF* (Fishel *et al.* 1981; James *et al.* 1982; Cohen and Laban 1983). In this regard, it will be of interest to determine the effect of a *rad59* strain on RNA-mediated recombination, specifically RNA-mediated gene conversion of plasmid *his3-AI* sequences.

In summary, we have determined the involvement of several yeast genes, known to have a role in mitotic recombination, in RNA-mediated recombination. We have found that mutation of *RAD51*, *RAD55*, and *RAD57* increased RNA-mediated recombination. Although these genes are required for wild-type levels of gene conversion, occurring between two DNA substrates, no decrease is observed in RNA-mediated gene conversion upon

mutation of these genes. Importantly, we have found that *RAD1* is required for RNA-mediated gene conversion of chromosomal sequences, lending insight into the nature of the cDNA recombination intermediate. We propose that the *HIS3* cDNA possesses a region of nonhomology at the 3′ end provided by the poly(A) tail and perhaps Ty1 sequences (acquired during priming of reverse transcription by Ty1) that must be removed by *RAD1* during RNA-mediated gene conversion of chromosomal *his3-ΔMscI* sequences. Additionally, we show that homologous RNA-mediated gene conversion of plasmid and chromosomal sequences utilize a distinct subset of cellular genes.

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