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-ΔM sc 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-ΔM sc 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 intertact. 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-
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-DΔsc 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 (MATα his3-200 ura3-52 trpl-289 lys) obtained from G. R. Fink. Strains were constructed by gene disruption (Rothstein et al. 1983; Alani et al. 1987). Strain YLD125 was transformed with a 6.6 kb Bgl II Iau2::hisG-Ura3::hisG fragment from plasmid pNYK85, resulting in the Iau2::hisG-Ura3::hisG disruption strain YLD615. Excision of the URA3 gene from the Iau2::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 HindIII rad1::LEU2 disruption fragment from plasmid pL962 (R. Keil), a XbaI-PstI rad51::LEU2 disruption fragment from plasmid pAM28 (M. Aker), a HindIII rad55::LEU2 disruption fragment from plasmid pSTL11 (S. Lovett), or a Sad rad57::LEU2 disruption fragment from plasmid pSM51 (D. Schildd). 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 60Co. The UV-sensitivity of strains was scored by replica plating test strains to YEPD medium and irradiating with 160 J/m² 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, rad57, or rad57 or 100 (GAL1-his3-AI; rad1, and rad1rad52; GAL1-his3-ΔATG; 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°C. 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°C.

**Chromosomal versus plasmid events:** One His+ prototroph was picked randomly from each culture and patched to SC-his 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 electrotransportation 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 PstI. Standard molecular techniques were performed as described in Maniatis et al. (1982).

**PCR analysis:** Linkage between HIS3 and MAT was determined by primer extension reaction (PCR). Primer alpha (5'-CAT GCT CTG GCC AAAGCAT TCC (nucleotides 222-242; St ruhler 1985) is complementary to sequences spanning the Msd site,
deleted in his3-ΔM scl and interrupted in plasmid his3-AI sequences. Primer b: 5'-CTGGGTAGAGTCTATGTGGCA (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'-CCCCCGCGACATCAGACAGAAGCCCTAGTA (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°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, for 25 cycles, followed by a 7 min extension at 72°C. Primer c: 5'-GGAATGCTTGGCCA-9 and primer d: 5'-GGAATGCTTGGCCA-9 are complementary to sequences spanning the MAT sequences. If no additional sequences are present, then a 1 kb PCR product is expected. Primer b is complementary to sequences upstream of the MAT target. Primer d: 5'-GGAATGCTTGGCCA-9 and primer e: 5'-GGTGTCGGTTTG CTGCAAAGT (nucleotides 196869–196899; Oliver et al. 1992) is complementary to MAT sequences upstream of the inserted his3-ΔM scl target. Primer e: 5'-GGTGTCGGTTTG CTGCAAAGT (nucleotides 196869–196899; Oliver et al. 1992) is complementary to MAT sequences spanning the M scl 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°C.

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-ΔM scl allele, i.e., plasmid events and chromosomal events not linked to MAT are eliminated (Figure 1; Derr and Stathern 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-ΔM scl 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>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>YLD125</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys</td>
</tr>
<tr>
<td>YLD157</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys rad52::hisG</td>
</tr>
<tr>
<td>YLD615</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG-URA3-hisG</td>
</tr>
<tr>
<td>YLD855</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad51::LEU2</td>
</tr>
<tr>
<td>YLD658</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad55::LEU2</td>
</tr>
<tr>
<td>YLD659</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad57::LEU2</td>
</tr>
<tr>
<td>YLD655</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad1::LEU2</td>
</tr>
<tr>
<td>YLD746</td>
<td>MATα::his3-ΔM scl his3-Δ200 ura3-52 trp1-289 lys leu2::hisG rad1::LEU2 rad52::hisG</td>
</tr>
</tbody>
</table>
TABLE 2  
Rate of His\textsuperscript{+} prototroph formation in various rad strains

<table>
<thead>
<tr>
<th>Strain (rad mutation)</th>
<th>Rate (× 10\textsuperscript{-3})\textsuperscript{a} GAL1-his3-AI (% chromosomal)</th>
<th>Rate (× 10\textsuperscript{-3})\textsuperscript{b} GAL1-his3-ΔATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>YLD125 (RAD)</td>
<td>6.5 (33)</td>
<td>0.2</td>
</tr>
<tr>
<td>YLD157 (rad52)</td>
<td>6.7 (87)</td>
<td>0 (0/ 3.4 × 10\textsuperscript{4})</td>
</tr>
<tr>
<td>YLD855 (rad51)</td>
<td>31.6 (79)</td>
<td>1.2</td>
</tr>
<tr>
<td>YLD658 (rad55)</td>
<td>17.9 (66)</td>
<td>1.1</td>
</tr>
<tr>
<td>YLD659 (rad57)</td>
<td>19.0 (68)</td>
<td>0.9</td>
</tr>
<tr>
<td>YLD655 (rad1)</td>
<td>1.1 (33)</td>
<td>0.01 (2/1.3 × 10\textsuperscript{9})</td>
</tr>
<tr>
<td>YLD746 (rad1rad52)</td>
<td>6.1\textsuperscript{c} (63)</td>
<td>0 (0/ 3.7 × 10\textsuperscript{9})</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Rates were computed by the method of the median Lea and Coulson (1949).
\textsuperscript{b} Rates were computed by the Poisson distribution as the fraction of the tubes with no mutants.

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\textsuperscript{+} 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\textsuperscript{+} 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 MscI site in the spliceable 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 inteinase or the cellular recombination machinery. (D) RNA-mediated gene conversion of chromosomal his3-ΔM spr sequences. Checkered sequences are chromosomal sequences; stippling indicates a deletion mutation. The chromosomal his3-ΔM spr sequences extend from nucleotide −72 → +703 with a 34bp deletion extending from nucleotide +219 → +252 (Struhl 1985), spanning the MscI 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\textsuperscript{+} prototroph formation requires recombination with chromosomal his3-ΔM spr sequences (D).
required for recombination between the HIS3 cDNA and chromosomal his3-ΔM sc allele (Table 2). From 100 rad1 cultures, only two His<sup>+</sup> prototrophs were detected. Both were linked to MAT, as determined by Southern hybridization analysis (data not shown).

**His<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> prototrophs were detected when the GAL1-AATG 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<sup>+</sup> 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 Stathern 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). Mutations 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-ΔM sc 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-ΔM sc 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 (Schietzel 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-ΔM slc 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 (Der 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-ΔM slc 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 (Der 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-ΔM slc 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 (Fischel 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 his3-3M 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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LITERATURE CITED


Rattray, A. J., and L. S. Symington, 1994 Use of a chromosomal inverted repeat to demonstrate that the RAD51 and RAD52 genes of Saccharomyces cerevisiae have different roles in mitotic recombination. Genetics 138: 587–595.


Schiestl, R. H., M. Dominici and T. D. Petes, 1993 Transformation of Saccharomyces cerevisiae with nonhomologous DNA: Illegiti-


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