MOLECULAR AND CELLULAR BIOLOGY, Apr. 1995, p. 2245–2251 Vol. 15, No. 4
0270-7306/95/$04.00+0

RAD1 and *RAD10*, but Not Other Excision Repair Genes, Are Required for Double-Strand Break-Induced Recombination in *Saccharomyces cerevisiae*

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Received 7 December 1994/Returned for modification 10 January 1995/Accepted 16 January 1995

HO endonuclease-induced double-strand breaks (DSBs) in the yeast *Saccharomyces cerevisiae* can be repaired by the process of gap repair or, alternatively, by single-strand annealing if the site of the break is flanked by directly repeated homologous sequences. We have shown previously (J. Fishman-Lobell and J. E. Haber, Science 258:480–484, 1992) that during the repair of an HO-induced DSB, the excision repair gene *RAD1* is needed to remove regions of nonhomology from the DSB ends. In this report, we present evidence that among nine genes involved in nucleotide excision repair, only *RAD1* and *RAD10* are required for removal of nonhomologous sequences from the DSB ends. *rad1*Δ and *rad10*Δ mutants displayed a 20-fold reduction in the ability to execute both gap repair and single-strand annealing pathways of HO-induced recombination. Mutations in *RAD2*, *RAD3*, and *RAD14* reduced HO-induced recombination by about twofold. We also show that *RAD7* and *RAD16*, which are required to remove UV photodamage from the silent *HML* locus, are not required for *MAT* switching with *HML* or *HMR* as a donor. Our results provide a molecular basis for understanding the role of yeast nucleotide excision repair genes and their human homologs in DSB-induced recombination and repair.

Genes belonging to the epistasis group *RAD3* are required for nucleotide excision repair (NER) of UV-damaged DNA in the yeast *Saccharomyces cerevisiae* (for a recent review, see reference 40). Some of these genes also function in other pathways of DNA metabolism. Genomic deletions of *RAD3* (34) and *SSL2* (*RAD25*) (17, 38) are lethal in haploid cells, indicating their essential role in maintaining cell viability. Experiments using conditional lethal mutations of *RAD3* and *SSL2* have demonstrated that their essential function is most likely their role in RNA polymerase II transcription (18, 19, 41). Substantiating the idea of an intimate linkage between NER and transcription were recent findings that protein products of at least *RAD2*, *RAD3*, *RAD4*, and *SSL2* are components of or are physically associated with RNA polymerase II basal transcription factor b (5, 12).

Experimental data also show that some NER genes have a role in mitotic recombination. Mutations in *RAD1* and *RAD10* reduce intrachromosomal recombination between directly repeated sequences and decrease the efficiency of homologous integration of linear DNA fragments and circular plasmids (30, 45, 47, 48). The *RAD1* function is also required for elevated recombination in *cdc9* and *top3* mutants (2, 47) as well as for mitotic recombination stimulated by RNA polymerase I- or II-dependent transcription (59, 67). The effect of *rad1* and *rad10* mutations on recombination seems to be rather specific in the sense that mitotic, but not meiotic, events are affected in the mutant strains (48). Moreover, only *rad1* and *rad10*, not other NER mutants, showed defects in spontaneous recombination between directly repeated genes (47).

A key to understanding a precise role of *RAD1* in mitotic recombination came from our previous studies on the genetic control of recombination induced by DNA double-strand breaks (DSBs) (14, 15). There are two major mechanisms for DSB repair in yeast cells (reviewed in reference 21): (i) gap repair, a conservative mechanism leading to a gene conversion event (58), and (ii) single-strand annealing (SSA), in which repair of a DSB is accompanied by a deletion of one of the flanking direct repeats along with the loss of intervening sequences (15) (Fig. 1). A *rad1* mutant fails to repair a DSB by both mechanisms when its ends contain a small (ca. 50-bp) region of nonhomology (14). Recombination proficiency of the *rad1* mutant is restored when the ends of the DSB are made homologous to donor sequences. We proposed that *RAD1* is required to remove nonhomologous sequences from the 3′ ends of recombining DNA, a process possibly analogous to the excision of photolesions during repair of UV-damaged DNA (14). Supporting this idea is the fact that the Rad1 protein is a component of the Rad1/Rad10 protein complex possessing DNA endonuclease activity specific for 3′-ended tails (3, 6, 8, 57, 60, 61). Recently, additional, and different, biochemical activities have been assigned to the Rad1 and Rad10 proteins (23, 56). Rad10 was shown to promote the renaturation of complementary DNA strands (56), and Rad1 was reported to catalyze cleavage of a synthetic Holliday junction (23; but see reference 66).

One question that arises from these studies is whether *RAD1* is unique in its requirement in DSB-induced recombination or if other NER genes are similarly involved. To answer this question, we tested the abilities of different excision-defective mutants to repair single, HO endonuclease-induced DSB, using a plasmid with two directly repeated *lacZ* genes of *Escherichia coli* as a recombination substrate (14, 15). Our results show that a mutation in the *RAD10* gene displays deficiency in DSB-induced recombination, qualitatively and quantitatively similar to that of *rad1* strains, and that *RAD1* and *RAD10* are the only NER genes required for this process. The relevance of these results is discussed in view of the high degree of homology between yeast NER genes and their human (*XP*) counterparts.

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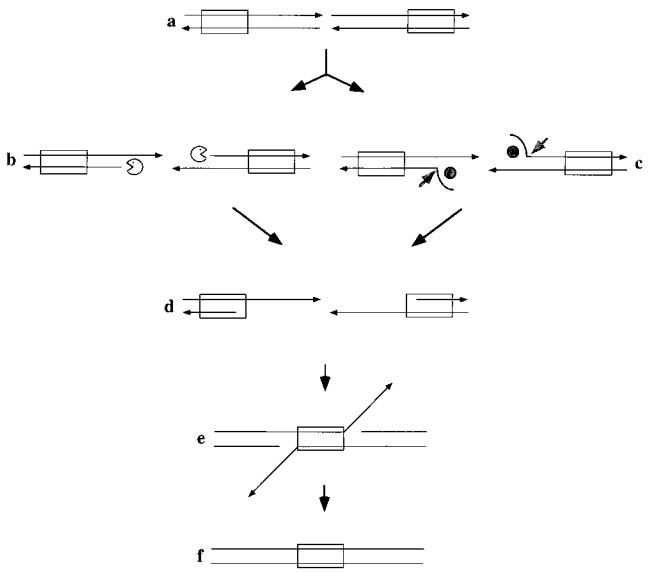

FIG. 1. Two hypothetical mechanisms for the SSA pathway of DSB repair. (a) Repair of a DSB flanked by two directly repeated sequences (shown as boxes) is initiated by 5′-to-3′ degradation of the DSB ends (the 3′ end of DNA strands is indicated by an arrow). According to the first mechanism (b), this degradation is carried out by a 5′-to-3′ exonuclease. According to the second mechanism (c), DSB ends are degraded by the concerted action of a DNA helicase (circles) and an endonuclease specific to single-stranded DNA (arrowheads). One can imagine that both 5′-to-3′ and 3′-to-5′ DNA helicases could be involved; a 5′-to-3′ DNA helicase is arbitrarily presented here. (d) In either case, degradation of the 5′ strands leads to the exposure of complementary strands of repeated sequences followed by their annealing. (e) Nonhomologous 3′ ends form tails that must be removed to restore the integrity of DNA backbone. (f) The whole process results in the deletion of one repeat along with the loss of intervening sequences.

MATERIALS AND METHODS

Plasmids. As a substrate for HO-induced recombination, we used plasmid pJF6 (Fig. 2), which carries two directly repeated copies of the *E. coli lacZ* gene, one of which is interrupted by a 117-bp fragment of the *MAT***a** gene containing the HO endonuclease recognition site (15). This plasmid also contains the yeast selectable markers *LEU2* and *URA3*. Plasmid pFH800 (*GAL1*::*HO ARS1 CEN4 TRP1*) (35) was used for galactose induction experiments. The following plasmids were used to disrupt or replace *RAD* genes: *RAD1*, pL962 (provided by R. L. Keil); *RAD2*, pWS521; *RAD3*, pWS3502-1 (both plasmids provided by W. Siede); *RAD4*, pNF412 (16); *RAD10*, pMTII-RAD10::URA3 (65); *RAD14*, pR14.4 (provided by L. Prakash); *RAD16*, pBLY22 (49); and *RAD25* (*SSL2*), pEP22 (38). Plasmid pNKY1009 was used to disrupt the *TRP1* gene with *hisG-URA3-hisG* sequence (1).

Strains and media. All yeast strains used in this work were derivatives of strain JKM40 (*ho HML*α *MAT*α *HMR***a** *ade1 leu2 trp1 ura3*) provided by J. K. Moore. The following strains were constructed by the one-step gene disruption/replacement method (43): EI457, *rad4*::*URA3*; EI458, *rad10*::*URA3*; EI459, *rad1*::*LEU2*; EI460, *rad14*::*URA3*; EI461, *rad16*::*URA3*, EI462, *rad2*::*TRP1*; and EI463, *rad3-1*::*TRP1*. In the two latter strains, the *TRP1* gene was disrupted with *hisG-URA3-hisG*, giving strains EI511 (*rad2*::*TRP1*::*hisG-URA3-hisG*) and EI513 (*rad3-1*::*TRP1*::*hisG-URA3-hisG*), respectively. Strain EI468 ($rad25_{799am}$) was constructed in two steps. First, pEP22 was linearized with *Sph*I and used to transform recipient cells to Ura^+. Second, Ura^- derivatives were selected on 5-fluoroorotic acid plates (10) and tested for UV sensitivity (38). All strain constructions were verified by Southern blot analysis. Except for EI463, EI468, and EI513, all of the above-mentioned strains carry deletion/disruption alleles of corresponding genes. Strains EI468 and EI513 carry point mutations $rad25_{799am}$ and *rad3-1*, respectively, which confer an extreme UV-sensitive phenotype (34, 38) and most likely to represent true loss-of-function mutations. Strain EI480 (*MAT***a** *ade1 leu2 trp1 ura3 rad7-1*) carries the *rad7-1* mutation which derives from the Yeast Genetic Stock collection and was introduced into the JKM40 background by two backcrosses. For galactose induction experiments, all strains were transformed with plasmids pFH800 and pJF6. The lithium acetate method (26) was used for yeast transformation.

YEPL medium was 1% yeast extract–2% Bacto Peptone medium supplemented with lactic acid (3.15% [wt/vol]), pH 5.5. YEPD medium, synthetic minimal medium supplemented with adenine (MA) and synthetic complete media lacking leucine (SC-Leu), tryptophan (SC-Trp), or uracil (SC-Ura) were prepared as described previously (50).

Induction of recombination. HO-induced recombination in plasmid pJF6 was carried out as described in detail previously (15). Briefly, cells were pregrown in liquid MA medium, selective for both pFH800 and pJF6, and then in a small volume of YEPL medium. The cultures were then inoculated in 0.5 liter of YEPL and grown overnight to a cell density of 10^7 cells per ml. At the time zero, an aliquot of cells was removed, and galactose was added to a final concentration of 2%. After 30 min, 1/10 volume of 20% glucose was added to repress galactose induction, and incubation was then continued, samples of cells being withdrawn at the time points indicated. Cells were spread on YEPD plates, grown to colonies, and replica plated on SC-Trp and SC-Leu or SC-Ura plates to measure loss of plasmids pFH800 and pJF6, respectively. Colonies were also scored for mating type by using appropriate testers.

Physical monitoring of recombination. DNA from galactose-induced cells was prepared by the glass bead protocol (44), digested with the appropriate restriction enzyme(s), separated on neutral agarose gels, and transferred to Biotrans (+) nylon membranes (ICN) in 0.4 M NaOH. Southern blot hybridization was carried out by the method of Church and Gilbert (11), using a 1.7-kb *Bsu*36I-*Sac*I fragment of the *lacZ* gene (15) as a probe. ^{32}P-labelled hybridization probes were prepared by the random-primer protocol (13). Densitometry of Southern blot filters was carried out by using a Molecular Dynamics Storage PhosphorImager.

RESULTS

***rad1*Δ and *rad10*Δ show similar defects in HO-induced recombination.** In our previous experiments (14, 15), HO-induced recombination in pJF6 plasmid substrate was studied by using a strain with a deleted *MAT* locus, so that an HO-induced DSB could occur only on plasmid DNA. In this work, we made use of derivatives of strain JKM40 with an intact *MAT* gene, so that the efficiency of mating-type switching could be also monitored. In accordance with previous results (14, 15), expression of the HO endonuclease gene leads to the cleavage of the *lacZ* sequence, as evidenced by the appearance of expected restriction fragments (cut fragments in Fig. 3). Both ends of the DSB contain approximately 50 bp of nonhomology to the donor sequence that must be removed in order for recombination to be completed. Southern blots of *Pst*I-digested DNA prepared from wild-type cells showed that as early as 30 to 60 min after the formation of HO-induced DSB, a DNA band that is characteristic of a deletion event resulting from recombination between the two repeated *lacZ* sequences appeared (Fig. 3a). By 6 h, the intensity of this band represented approximately 50% of the total hybridization signal (Table 1). This is the minimal estimate of the efficiency of repair, as deletions represent 80 to 85% of the recombination products in this plasmid system. The other type of recombination product, resulting from a gene conversion event, is not distinguishable from the duplication fragment on Southern blots of *Pst*I-digested DNA but can be visualized after triple *Hin*dIII-*Pst*I-*Sma*I digestion of the same DNA samples (Fig. 4). Comparison of the intensities of deletion and gene conversion bands seen in Fig. 4 allowed us to estimate that deletion and gene conversion events represented 83 and 17% of the recombination products, respectively (Table 1), in agreement with previous results (15).

As expected from the efficient repair of HO-induced DSBs seen on Southern blots, the wild-type strain demonstrated a high level of retention of plasmid pJF6 (Table 1). It is also worth noting that repair of a DSB on plasmid DNA is accompanied by the efficient repair of the DSB at the chromosomal *MAT* locus; indeed, almost 80% of wild-type cells carrying the plasmid containing the HO gene switched from *MAT*α to *MAT***a** (Table 1).

Confirming our previous observations (14), formation of the deletion product was dramatically reduced in a *rad1*Δ strain (Fig. 3b). Densitometric analysis of bands seen on the Southern blots at the 6-h time point showed an approximately 20-fold decrease in the intensity of deletion products (Table 1).

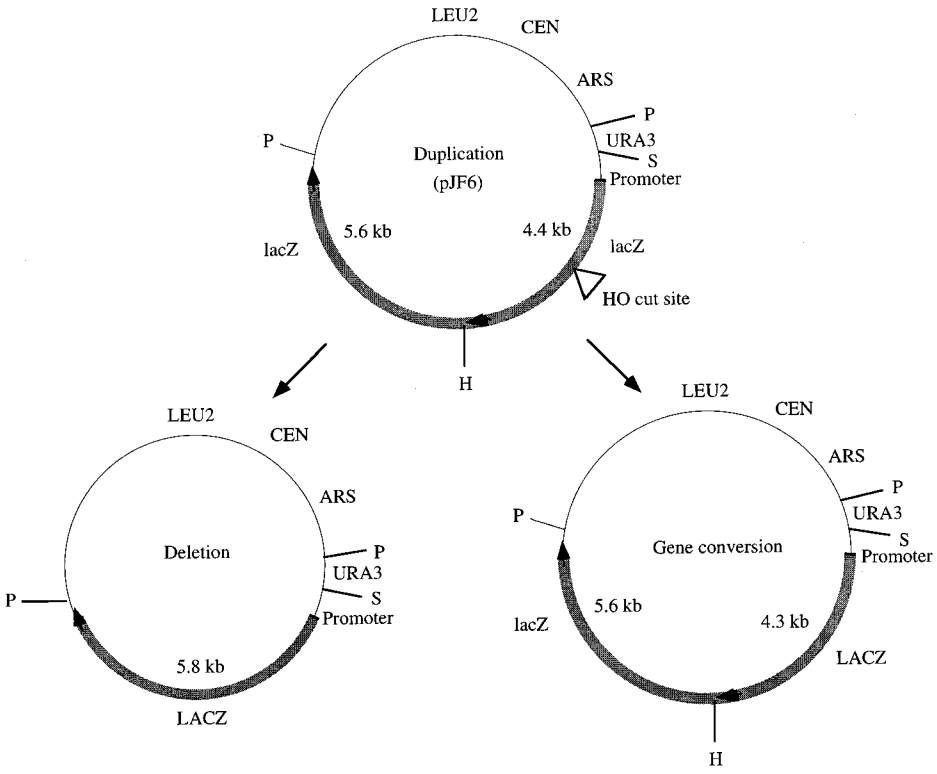

FIG. 2. Structure of plasmid pJF6 and its recombination derivatives. The positions of relevant *Hin*dIII (H), *Pst*I (P), and *Sma*I (S) sites are shown; for more detail, see reference 14. pJF6 contains two inactive copies of *lacZ*; one is promoterless, while the other is interrupted with an HO cut site. The galactose-induced HO cut cleaves an 11.1-kb *Pst*I-fragment containing the *lacZ* duplication into 8.7- and 2.4-kb cut fragments. Repair of the resulting DSB by SSA leads to the appearance of a 7.0-kb *Pst*I fragment containing the deletion product. Repair of the DSB by gene conversion results in the transformation of a 4.4-kb *Hin*dIII-*Sma*I fragment into a 4.3-kb *Hin*dIII-*Sma*I fragment. Use of a triple *Hin*dIII-*Pst*I-*Sma*I restriction digest allows one to monitor the formation of both recombination products simultaneously. In the latter case, the deletion product is represented by a 5.8-kb *Pst*I-*Sma*I fragment.

There was an apparently equivalent reduction in the abundance of gene conversion products (Fig. 4). In good agreement with these results, the *rad1*Δ mutant displayed substantial loss of plasmid pJF6 (Table 1); however, no HO-induced lethality of *rad1*Δ cells occurred, as seen by the wild-type level of retention of the *GAL*::*HO*-containing plasmid pFH800. HO-induced mating-type switching in *rad1*Δ cells was almost as efficient as in wild-type cells (Table 1). These data suggest that the inability of *rad1*Δ cells to repair an HO-induced DSB is specific to the substrates for which nonhomologous DNA ends must be removed before recombination can be completed.

Genetic and biochemical evidence predict similar defects of *rad1*Δ and *rad10*Δ mutants in mitotic recombination, although some biochemical differences between the two proteins have been reported (see the introduction). Thus, we expected that a *rad10*Δ mutant would show the same defect in HO-induced recombination as displayed by *rad1*Δ mutant strains. Confirming this expectation, a *rad10*Δ strain showed a pronounced reduction in the deletion formation (Fig. 3c). The magnitude of this reduction was exactly the same as for an isogenic *rad1*Δ strain (Table 1). Triple *Hin*dIII-*Pst*I-*Sma*I digestion of the same DNA samples was used to show that essentially no gene conversion product of recombination could be detected in the *rad10*Δ strain (Fig. 4). Importantly, all of the other tested parameters (i.e., loss of plasmid pJF6, lack of HO-induced cell lethality, and extent of mating-type switching) were affected qualitatively and quantitatively similarly in *rad10*Δ and *rad1*Δ cells (Table 1).

Deficiency in HO-induced recombination is not a common property of excision repair mutants. We then wanted to know whether the deficiency in HO-induced recombination seen in *rad1*Δ and *rad10*Δ strains is specific for mutations in these genes or if some other mutants belonging to the *RAD3* epistasis group have the same property. We first tested mutations in genes which, in addition to *RAD1* and *RAD10*, are absolutely required for NER: *RAD2*, *RAD3*, *RAD4*, *RAD14*, and *RAD25* (*SSL2*) (for details, see reference 40). As shown in Fig. 3 and 4 and in Table 1, mutations in *RAD4* and *RAD25* genes did not affect HO-induced recombination of directly repeated *lacZ* sequences. *rad2*Δ, *rad3-1*, and *rad14*Δ mutants also displayed general proficiency in HO-induced recombination, as evidenced by the formation of both deletion (Fig. 3) and gene conversion (Fig. 4) products. However, in these strains, the production of both deletions and gene convertants was two- to threefold lower than in the wild-type strain (Table 1). One factor that might be responsible for the less efficient recombination is less efficient cutting of the substrate. We tested this possibility by comparing intensities of cut fragment bands on the Southern blots at the 0.5-h time point, when only parental and cut fragment bands can be detected (Fig. 3). In the wild-type strain, cut fragment bands represent 28 to 32% of the total DNA. The same amount of the cut DNA was produced in the *rad2*Δ and *rad14*Δ mutant strains, 28 and 29%, respectively. However, in the *rad3-1* mutant strain, the amount of the cut fragment represented only 16% of the total DNA content. Thus, at least part of the less efficient deletion formation seen in the *rad3-1* mutant is due to less efficient cutting of plasmid DNA, leading also to a slightly higher than wild-type level of its

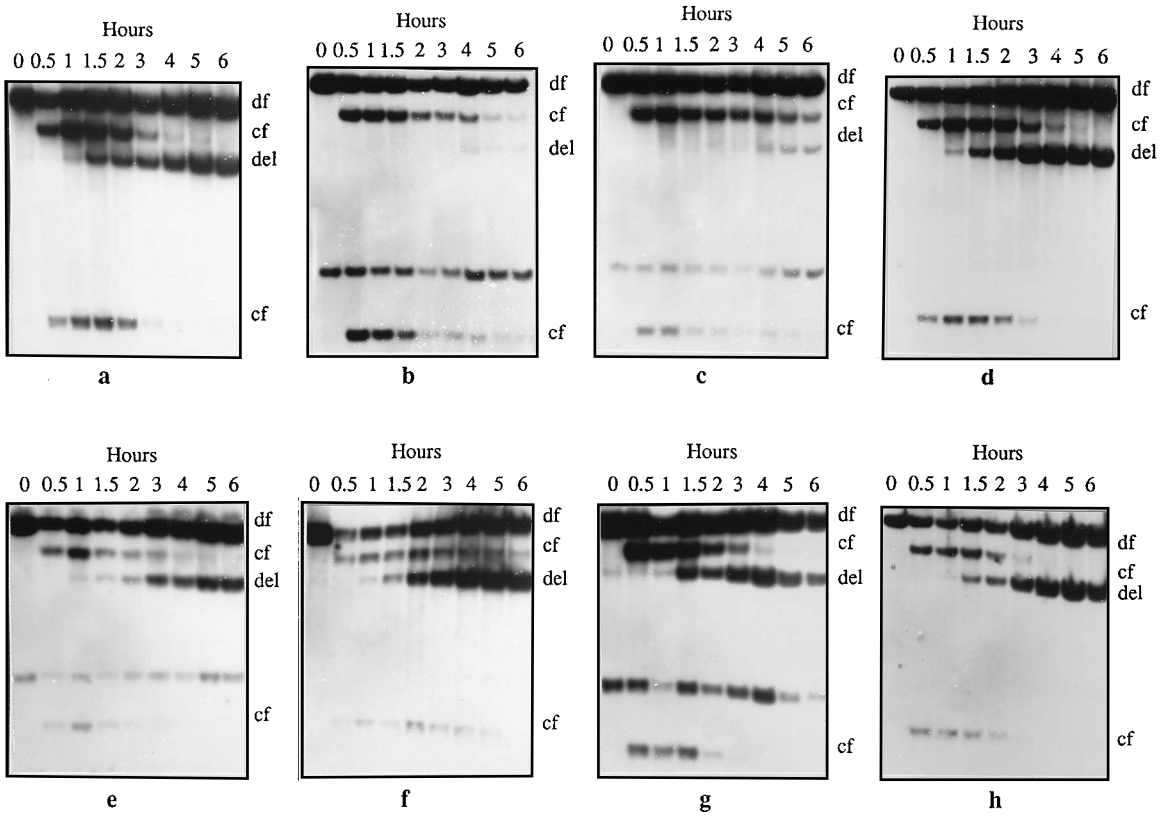

FIG. 3. Southern blot analysis of the kinetics of deletion formation in wild-type and mutant strains. Expression of the HO endonuclease was initiated by the addition of galactose at time zero. At the time points indicated, samples of cells were taken, and DNA was prepared and digested with *Pst*I. The DNA fragments were separated on neutral 0.8% agarose gels and probed with a *lacZ*-specific probe as described in Materials and Methods. Positions of the 11.1-kb duplication fragment (df), 8.7- and 2.4-kb cut fragments (cf), and the 7.0-kb deletion product (del) are indicated. The other product of recombination, a gene conversion event, cannot be seen on these Southern blots. A DNA band at the position of ~3.4 kb seen on some of the blots is probably due to nonspecific hybridization. (a) Wild type; (b) *rad1*Δ; (c) *rad10*Δ; (d) *rad2*Δ; (e) *rad3-1*; (f) *rad4*Δ; (g) *rad14*Δ; (h) $rad25_{799am}$.

retention (Table 1). It is possible that HO expression is lowered in this strain, as we noticed that *MAT* switching is also slightly less efficient in the *rad3-1* strain (Table 1). Thus, the *rad2*Δ, *rad3-1*, and *rad14*Δ mutations slightly reduce deletion and gene conversion induced by a DSB flanked by nonhomologous ends, but recombination in these mutants is still 7 to 10 times more frequent than in *rad1*Δ and *rad10*Δ strains.

We also tested strains bearing mutations in *RAD7* and

TABLE 1. HO-induced recombination, plasmid loss, and mating-type switching in wild-type and mutant strains

Strain	Cells containing plasmids after HO induction (%)		*MAT*α→*MAT***a**[a] (%)	Relative efficiency of deletion formation[b]	Fraction of gene conversion[c] (%)
	pFH800/total cells[d]	pJF6/Trp$^+$ cells[e]			
Wild type	83	71	76	1	17
*rad1*Δ	78	13	60	0.05	NA
*rad2*Δ	82	54	69	0.53	14
rad3-1	76	81	57	0.35	17
*rad4*Δ	84	61	81	1.28	16
rad7-1	74	67	65	0.91	18
*rad10*Δ	79	12	70	0.06	NA
*rad14*Δ	76	59	71	0.56	25
*rad16*Δ	75	64	55	0.92	15
$rad25_{799am}$	81	63	82	0.87	14

[a] Fraction of cells that switched mating type among Trp$^+$ cells.
[b] Densitometric analysis of Southern blot data shown in Fig. 3. Intensities of all *lacZ*-hybridizing bands seen at the 6-h time point were integrated. The intensity of the deletion product band was normalized to the resulting sum and expressed as a fraction of overall DNA content, the value being 45.0% ± 9.7% for the wild-type strain. The fractions of the deletion product in mutant strains were related to that of the wild-type strain. Presented are data averaged from two to five measurements for each strain.
[c] Densitometric analysis of Southern blot data shown in Fig. 4. Intensities of *lacZ*-hybridizing bands corresponding to the recombination product (deletions plus gene conversions) were integrated, and the proportion of the gene conversion bands was calculated. NA, not applicable.
[d] Fraction of Trp$^+$ colonies among all the colonies grown on YEPD plates after HO induction; 150 to 250 colonies were routinely analyzed for each strain.
[e] Fraction of colonies retaining plasmid pJF6 among Trp$^+$ colonies. These were either Trp$^+$ Leu$^+$ or Trp$^+$ Ura$^+$.

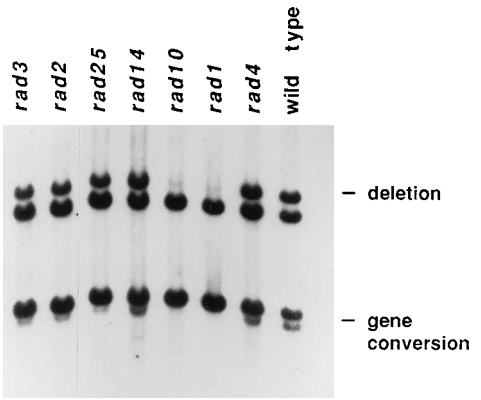

FIG. 4. Southern blot analysis of the formation of deletion and gene conversion products of HO-induced recombination in wild-type and mutant strains. Samples of cells were taken 6 h after expression of HO; DNA was prepared and digested with *Hin*dIII-*Pst*I-*Sma*I. The DNA fragments were separated on neutral 0.5% agarose gels and probed with a *lacZ*-specific probe. Positions of the 5.8-kb deletion product and the 4.3-kb gene conversion product are indicated. The two other fragments represent parental bands. The fragments created by HO cleavage of the 4.4-kb *Hin*dIII-*Sma*I fragment cannot be seen on this blot.

RAD16, which are important but not absolutely required for NER, as corresponding mutants show only moderate sensitivity to UV light (40). The *RAD7* and *RAD16* genes were recently shown to be essential for pyrimidine dimer removal from the silent loci *HML* and *HMR* but not from the transcribed *MAT* locus (4, 64). HO-induced recombination and pJF6 plasmid loss were shown to be at wild-type levels in a strain with a deletion mutation in *RAD16* and in a strain with a presumed point mutation *rad7-1* (Table 1; Southern blots not shown). The *rad7-1* allele confers the same level of UV sensitivity as that conferred by a disruption of *RAD7* (39); thus, it is unlikely that the absence of the effect of the *rad7-1* mutation on HO-induced recombination is due to its leakiness. We also found that the *RAD7* and *RAD16* genes are not required for *MAT* switching with *HML* or *HMR* as a donor, although some reduction in the amount of *MAT* switching was seen for the *rad16*Δ strain (Table 1). The kinetics of *MAT* switching in the *rad16*Δ strain are not delayed compared with a wild-type strain (27).

DISCUSSION

RAD1 and *RAD10*, but not other excision repair genes, are required to repair an HO-induced DSB via both the SSA and gene conversion mechanisms. The Rad1 and Rad10 proteins from a stable complex that was recently shown to constitute a single-stranded DNA-specific endonuclease (3, 6, 8, 57, 60, 61). Our finding that mutations in these genes lead to identical defects in *lacZ* direct-repeat recombination suggests that these proteins work in concert during repair of HO-induced DSBs. The identity of phenotypes of *rad1*Δ and *rad10*Δ mutants with respect to HO-induced recombination was an expected but not trivial result. Protein products of *RAD51* and *RAD52* genes have also been shown to interact physically (33, 51), but *rad51*Δ and *rad52*Δ mutants behave quite differently with respect to spontaneous (31, 32, 42) as well as HO-induced (54) recombination.

A current model of the SSA process (Fig. 1) implies that 5′-to-3′ degradation of the DSB ends followed by the annealing of exposed complementary sequences leaves behind 3′-ended regions of nonhomology that must be removed to complete the formation of deletion product. Previously (14), we suggested that *RAD1* was required to remove these nonhomologous DNA tails, in a way that is probably analogous to its function in excision repair. In excellent agreement with this suggestion, it was recently shown that Rad1/Rad10 protein complex is an endonuclease that cleaves at the junction between duplex and single-stranded DNA containing 3′ tails (7), a substrate which is identical to that predicted by the SSA model (Fig. 1e). Rad10 was previously shown to promote the renaturation of complementary DNA strands (56). Thus, the possibility that Rad10 also functions at an earlier step of the SSA process cannot be formally excluded.

*rad1*Δ and *rad10*Δ inhibit not only SSA but also the gene conversion pathway of repair of HO-induced DSB when the 3′ ends of the DNA are not homologous to the donor sequences (reference 14 and this work). This finding provides a general basis for understanding the nature of inhibitory effects of *rad1* and *rad10* mutations on mitotic recombination. We expect that mitotic recombination would require *RAD1* and *RAD10* whenever the initiation of recombination occurs in a region lacking homology with donor sequences. For example, in the case of directly repeated genes separated by an intervening sequence (30, 47, 48), recombination is thought to be initiated by a DSB (or any other lesion whose processing leads to a DSB) in the intervening sequence and to proceed by the SSA mechanism essentially as outlined in Fig. 1. *RAD1* and *RAD10* would then step in to remove nonhomologous 3′-ended tails. In the case of recombination between homeologous sequences, *RAD1* and *RAD10* would be needed to ensure a gene conversion event if the initiation of recombination occurred in a region of nonhomology. At present, we do not know the lower limit of the amount of nonhomology that cannot be removed without *RAD1* and *RAD10*. In the experiments reported here, gene conversion is prevented when there are approximately 50 bp of nonhomologous DNA at both sides of the DSB.

Recently, it was shown that the Rad1 protein binds specifically to a synthetic Holliday junction and catalyzes its endonucleolytic cleavage (23). The relevance of this finding to the in vivo recombination process remains, however, unclear (66). If the *RAD1* function were really required for the resolution of recombination intermediates, then one would expect more general effects of *rad1* mutation on homologous recombination. In contrast, available genetic data suggest that *RAD1* is needed only when recombining substrates contain regions of nonhomology.

An important result from our work is that the *RAD2* gene has only a small effect on *lacZ* direct-repeat recombination compared with the *RAD1* and *RAD10* genes. Previously, it was demonstrated that the product of *RAD2* is also a single-stranded DNA-specific endonuclease that is believed to function in concert with the Rad1/Rad10 endonuclease at the incision step of NER (22). From studies of a *RAD2* homolog (*YKL510*) and of an internally deleted derivative of *RAD2*, it appears that Rad2 cleaves at the junction between duplex and single-stranded DNA containing 5′ tails but not their 3′ counterparts (25). Thus, the requirement for *RAD1* and *RAD10*, but not *RAD2*, genes in mitotic recombination might simply be a reflection of the fact that during SSA, the degradation of the DSB ends is always 5′ to 3′ (Fig. 1b and c) and that during gene conversion, it is always a 3′ strand that invades, so that regions of nonhomology (if present) always form branched structures with 3′-ended single-stranded tails (Fig. 1e).

We cannot exclude the possibility that *RAD2* plays some minor role in HO-induced recombination, as a *rad2*Δ mutant showed slightly less efficient deletion formation. One could imagine that the Rad2 and Rad1/Rad10 endonucleases are involved in a multiprotein repair complex so that the absence

of Rad2 would affect to some extent the activity of Rad1/Rad10. It is also possible that this presumed repair complex involves proteins targeting both endonucleases to their respective substrates. In this respect, we note that the formation of deletions is somewhat lowered in a *rad14*Δ strain. Rad14 protein was shown to be a damage recognition protein which binds specifically to UV-irradiated DNA (20). Although one would not expect a high affinity of Rad14 to 3′- or 5′-ended branched molecules, we speculate that proteins targeting Rad1/Rad10 and Rad2 endonucleases to their substrates, recognize distortions of the DNA helix rather than particular damage.

Repair of a DSB via the SSA mechanism (Fig. 1) absolutely depends on the 5′-to-3′ degradation of DSB ends (15, 53). However, the precise mechanism of this degradation is still obscure. Hypothetically, two mechanisms are possible. According to the first one (Fig. 1b), this degradation is accomplished by a 5′-to-3′ exonuclease. According to the second mechanism (Fig. 1c), DSB ends are degraded by a concerted action of a DNA helicase and a single-strand-specific endonuclease. If the latter mechanism were operating, then we could expect that some DNA helicase mutant(s) would be deficient in deletion formation. In this respect, we note that the *RAD3* and *RAD25* genes code for DNA helicases (19, 55), and the predicted amino acid sequence of the *RAD16* protein contains several helicase-specific motifs (49). However, mutations in all three genes show general proficiency in deletion formation, although we cannot entirely exclude some minor effects on this process in the *rad3-1* mutant. We also tested a mutation in the *RAD5* gene coding for a protein containing all seven conserved domains associated with DNA helicases (29). Again, the *rad5*Δ mutant displayed wild-type level of deletion formation (27). We also note that if the 5′-to-3′ degradation of DSB ends were initiated by the action of a DNA helicase, then the resulting 5′-ended branched structures (Fig. 1c) would constitute an expected substrate for the Rad2 endonuclease (25). However, as discussed earlier, the *RAD2* gene does not play a major role in the deletion formation. Therefore, currently available data do not support a helicase/endonuclease mechanism of 5′-to-3′ degradation of DSB ends. We note that the exonuclease(s) responsible for this degradation also has not been identified, though deletion alleles of *RAD50* and *XRS2* markedly retard the process of degradation (28).

Our results have some important implications given the high degree of homology between yeast NER genes and their human counterparts. The human homolog of *RAD10* is the *ERCC1* gene (62), whose product has been recently shown to form a complex with the *XPF* or *ERCC4* gene product. The latter two genes may be equivalent to one another and homologous to *RAD1* (9, 63). The yeast *RAD2* gene is homologous to another human gene responsible for the hereditary disease xeroderma pigmentosum, *XPG* (46). The *XPG* gene has recently been shown to code for a structure-specific endonuclease with a mode and polarity of cleavage identical to those of Rad2 endonuclease (24, 36). Our results predict that *ERCC1*, *XPF*, and/or *ERCC4* genes, in addition to their role in NER, are involved in recombination between repeated sequences, which are abundant in the human genome and are considered as an important source of genome rearrangements (52). It is also worth noting that neither Rad1/Rad10 endonuclease (57, 61) nor the ERCC1/XPF (ERCC4) protein complex (37) display specificity to altered DNA, so other factors targeting these complexes to their respective substrates in DNA repair and recombination must exist. Thus, additional yeast and human genes playing a role in NER and/or mitotic recombination are expected to be identified.

ACKNOWLEDGMENTS

We thank E. Alani, R. L. Keil, J. K. Moore, L. Prakash, and W. Siede for providing strains and plasmids. We are grateful to A. Malkova, S. Lovett, and N. Sugawara for helpful comments on the manuscript.

This work was supported by NIH grant GM20056.

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