Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 7131–7136, July 1996
Genetics

Double-strand break repair in the absence of *RAD51* in yeast: A possible role for break-induced DNA replication

(DNA repair/recombination/*Saccharomyces cerevisiae*)

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Communicated by Maurice S. Fox, Massachusetts Institute of Technology, Cambridge, MA, March 21, 1996 (received for review December 28, 1995)

ABSTRACT **In wild-type diploid cells of *Saccharomyces cerevisiae*, an HO endonuclease-induced double-strand break (DSB) at the *MAT* locus can be efficiently repaired by gene conversion using the homologous chromosome sequences. Repair of the broken chromosome was nearly eliminated in *rad52Δ* diploids; 99% lost the broken chromosome. However, in *rad51Δ* diploids, the broken chromosomes were repaired approximately 35% of the time. None of these repair events were simple gene conversions or gene conversions with an associated crossover; instead, they created diploids homozygous for the *MAT* locus and all markers in the 100-kb region distal to the site of the DSB. In *rad51Δ* diploids, the broken chromosome can apparently be inherited for several generations, as many of these repair events are found as sectored colonies, with one part being repaired and the other part having lost the broken chromosome. Similar events occur in about 2% of wild-type cells. We propose that a broken chromosome end can invade a homologous template in the absence of *RAD51* and initiate DNA replication that may extend to the telomere, 100 or more kb away. Such break-induced replication appears to be similar to recombination-initiated replication in bacteria.**

Systems that repair chromosomal DNA double-strand breaks (DSBs) are of great importance to all cells. In the yeast *Saccharomyces cerevisiae*, the major pathway of DSB repair is through gap repair (1, 2), leading to a gene conversion that may be associated with a crossover of flanking markers. However, gene conversion is only one of several homologous and non-homologous recombination pathways that are found in yeast (and mammalian cells) to repair chromosomal DSBs (3). Several of these pathways are relatively rare in yeast and can only be studied effectively when the primary homologous recombination pathway is eliminated. This can be accomplished by using deletions of genes, such as *RAD52* or *RAD51*, that prevent chromosomal DSB repair by gene conversion (4, 5).

Although the *RAD52* and *RAD51* gene products have been shown to interact in yeast in both genetic and biochemical assays (6, 7), they are quite different in their effects on recombination. *RAD52* appears to be required for all homologous recombination events, including both gene conversion and single-strand annealing (3). In contrast, a deletion of *RAD51* has no effect on single-strand annealing and has a much less profound effect on gene conversion events than does a *rad52* deletion (5, 8–10). *RAD51* protein appears to be homologous to the bacterial RecA protein (11–13) and has been shown to catalyze strand-exchange events *in vitro* that are similar but not identical to RecA-mediated events (6, 14, 15). However, our studies of DSB recombination suggest that the major role of *RAD51* may be to promote recombination in regions of chromatin that are otherwise inaccessible rather than catalyzing these strand-exchange reactions *per se* (5). Thus, we found that an HO endonuclease-cleaved *MAT* locus on a plasmid could be repaired without *RAD51* using an HO-insensitive *MATα*-inc donor sequence on a plasmid; however, *RAD51* was still required when the donor locus was situated on a chromosome (ref. 5; N. Sugawara, unpublished).

To explore further the role of *RAD51*, we used diploid cells in which the *MAT***a** locus on one chromosome, cleaved by HO, could be repaired by homologous recombination using *MATα*-inc on the homologous chromosome as a donor. This diploid system has the advantage over previously used haploid strains in that we can identify events such as chromosome loss that would be lethal in a haploid and nonreciprocal events that would be either lethal or undetected in haploids. Using this system we confirmed that both *RAD52* and *RAD51* are indeed required for gene conversion but that there is an alternative homologous recombination repair process that is *RAD51*-independent but still *RAD52*-dependent.

MATERIALS AND METHODS

Plasmids and Strains. Plasmid YIp*ade3*HO was used to insert the *GAL::HO* fusion into the chromosomal *ADE3* locus as described by Sandell and Zakian (16). The following plasmids were used to disrupt *RAD* genes: *RAD51*, pJH683 (10); *RAD52*, pSM20 (17). Plasmid pJH1159 (X. Wu, unpublished data) was used to create *hmrΔ::LEU2* that removes all *HMR***a** sequences and surrounding silencing sites and introduces an *Xho*I/*Sal*I *LEU2* fragment.

Strain JKM111 (*MAT***a** *hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1–100 leu2–3, 112 lys5 ura3–52*) was constructed by J. K. Moore (13). In this strain, the *HML* and *HMR* genes and their surrounding silencers have been deleted and replaced by the *ADE1* gene. Strain EI515 is isogenic to JKM111, but also carries the *HO* endonuclease gene under control of the inducible *GAL* promoter, the whole construction being integrated into chromosomal *ADE3* locus (16). Strain AM133 has genotype (*MATα-inc ade1 met13 ura3 leu2–3, 112 thr4 trp1Δ*). Deletions of *RAD51* and *RAD52* were introduced into strains EI515, JKM111, and AM133 by the one-step gene disruption method (18) using the plasmids listed above. For details of strain constructions, see the corresponding references. The lithium acetate method (19) was used for yeast transformation. All the strain constructions were verified by Southern blot analysis. All diploids used in this work resulted from crossing of EI515 to AM133 or from crosses between their isogenic derivatives. Standard methods were used for the analysis of yeast colonies, crosses, and micromanipulating of cells (20).

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Abbreviations: DSB, double-strand break; BIR, break-induced replication.

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Media and Growth Conditions. Rich medium (YEPD) and synthetic complete medium with bases and amino acids omitted as specified were as described (20). YEPGly and YEPGal consisted of 1% yeast extract/2% Bacto Peptone media supplemented with 3% (vol/vol) glycerin or 2% (wt/vol) galactose, respectively. YEPD medium containing 0.015% (vol/vol) methyl methane sulfonate was used to follow the Rad$^-$ phenotype. Cultures were incubated at 30°C.

Induction of DSBs. Cells were grown overnight in 50 ml of YEPGly to a cell density of 10^7 cells per ml. At time zero, an aliquot of cells was removed, while the rest of the culture was harvested and suspended in the same volume of warmed YEPGal, and incubation was continued for 2 hr. Appropriate dilutions of cells were spread on YEPD plates, grown to colonies, and analyzed.

In the case of HO induction on plates, cells grown overnight in YEPGly to a cell density of 10^7 cells were washed twice with water, and dilutions were plated directly on YEPGal plates.

Analysis of DSB Repair Events. DNA was prepared by the glass bead protocol (21), digested with the appropriate restriction enzyme(s), separated on 0.8% neutral agarose gels (22), 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 (23). ^{32}P-labeled probes for hybridization were prepared by the random-primer protocol (24). For the analysis of the *MAT* locus, DNA was digested with *Hha*I. The 0.65-kb *Xho*I/*Hae*III fragment of the *MAT* distal region (25) was used as a probe. This probe detected a 2.7-kb fragment in the case of *MATα*-inc allele and 1.75-kb fragment when *MATα* was present. For the analysis of the *HML* locus, DNA was digested with *Bam*HI, and a 0.5-kb *Bam*HI/*Xho*I fragment of *HML* (26) was used as a probe. This probe hybridized to a 3-kb fragment in the case of a *hml*Δ::*ADE1* allele and to a 6.6-kb fragment in the case of an intact *HML*. To examine the *HMR* locus, DNA was digested by *Hin*dIII. A 0.6-kb *Eco*RI fragment of *HMR* (25) was used as a probe. This probe detected a 5-kb fragment for the intact *HMR* and 4-kb fragment for the *hmr*Δ::*ADE1*.

To establish if *HMR* was homozygous or hemizygous after DSB repair in *rad51*Δ diploids, we used a gene replacement approach (27). We crossed the α-mating, Ade$^+$ Thr$^-$ *leu2* *rad51*Δ diploids to a *MAT***a** *leu2* Rad$^+$ strain carrying *hmr*Δ::*ADE1*. These triploids were then transformed with a *Hin*dIII DNA fragment of pJH1159 carrying *hmr*Δ::*LEU2* to obtain Leu$^+$ Ade$^+$ derivatives. DNA prepared from these triploids was digested by *Hin*dIII and *Kpn*I, and Southern blots were hybridized with an *HMR*-specific probe. If two copies of *HMR* were initially present in the repaired diploid, then three fragments were expected to appear after hybridization with an *HMR*-adjacent fragment: a 5-kb fragment corresponding to intact *HMR*, a 4-kb band corresponding to *hmr*Δ::*ADE1*, and a 3.2-kb fragment corresponding to *hmr*Δ::*LEU2*. If only one *HMR* copy were present, then the 5-kb fragment would be replaced by a 3.2-kb *hmr*Δ::*LEU2* fragment.

RESULTS

To study the roles of *RAD51* in DSB repair, we examined the repair of a single chromosomal DSB in *MAT***a**/*MATα*-inc diploids (Fig. 1). A site-specific DSB can be induced by HO endonuclease, under the control of the *GAL10* promoter. In this strain, only the *MAT***a** locus is cut, as the *MATα*-inc allele contains a mutation destroying the HO cut site (28). The *HMR* and *HML* alleles on the *MAT***a** chromosome were deleted by insertion of the *ADE1* gene; thus, there were no sequences homologous to *MAT***a** on the same chromosome. Consequently, nearly all of the time, repair occurred by recombining with the intact *MATα*-inc locus to produce a *MATα*-inc/*MATα*-inc diploid (see below). Cells that repair the broken chromosome and retain at least one *ADE1* gene will form white colonies, but cells that lose this chromosome will be Ade$^-$ and form red colonies.

We examined the fate of an HO-induced DSB in three isogenic strains: wild type, *rad52*Δ/*rad52*Δ, and *rad51*Δ/*rad51*Δ. HO endonuclease was induced in liquid galactose medium for 2 hr, and then cells were spread on dextrose-containing YEPD plates to turn off HO expression (see *Materials and Methods*). In wild-type cells, the cell titer after induction was 110% of the number of cells plated before induction, indicating that some wild-type cells had started to divide by the end of the second hour in the galactose medium. A similar value was found in diploids not carrying the *GAL*::*HO* gene. There was also very little reduction in viability for the *rad52*Δ diploid, where 90% of the cells were recovered after induction. The viability of the *rad51*Δ diploid was 60%, compared with 100% for an isogenic *rad51*Δ strain without *GAL*::*HO*.

We then analyzed the colonies derived from single cells. With wild-type cells, 90% of the colonies (1207 of 1341) changed from nonmating (*MAT***a**/*MATα*-inc) to α-mating (*MATα*-inc/*MATα*-inc). We presume that nonmating cells had not experienced an HO cleavage or had used *HMR***a** as a donor to repair the DSB. Southern blot analysis performed on DNA from 40 α-mating colonies indicated that in 38 cases (95%), the *MATα*-inc located on the homologous chromosome served as a donor. Only in 5% of the cases were the DSBs at *MAT* repaired by recombination with the *HMLα* cassette located on the *MATα*-inc chromosome. Thus, the vast majority of DSBs induced in our experimental system were repaired by allelic interchromosomal recombination. Nearly all of the α-mating diploids were Ade$^+$, but 2.8% (34 of 1207) (Fig. 1, class 5) were red (Ade$^-$) and were also Thr$^-$. This result suggests that wild-type repair of the DSB is not 100% efficient; a small but significant fraction of cells lost the entire broken chromosome. As shown in Fig. 1, about 81% of the colonies (978 of 1207) were Ade$^+$ Thr$^+$, as expected from a gene conversion event that replaced *MAT***a** by *MATα*-inc without an associated crossover (class 1). Another 13% of colonies (157 of 1207) were Ade$^+$, but sectored for Thr$^+$/Thr$^-$ (Fig. 1, class 2). As described below, further analysis of such colonies confirmed that most of these represented gene conversions of *MAT*, accompanied by reciprocal exchange of markers more distal to *MAT*. If we assume that all crossovers occur or are resolved in the G2 stage of the cell cycle, then for every detected crossover, there should be an undetected one, because of the way the recombined chromosomes segregate. Thus, about 25% of the events are likely to have been crossover-associated.

***rad52* Diploids Cannot Efficiently Repair a Chromosomal DSB.** The induction of HO breaks in *rad52*Δ diploids resulted in the loss of the broken chromosome in 99% (395 of 399) of the cases, leading to the formation of α Ade$^-$ Thr$^-$ colonies. The remaining four colonies were α Ade$^+$ Thr$^-$. Southern blot analysis of these α Ade$^+$ Thr$^-$ derivatives showed that they were either homozygous or hemizygous for *hml*Δ::*ADE1* and not heterozygous *hml*Δ::*ADE1*/*HMLα*. This suggests they are likely to be 2n-1 diploids carrying a single, recombined chromosome III as illustrated in Fig. 1 (class 6). These chromosomes appear to have arisen by a nonreciprocal crossover event, producing one recombined chromosome III with the concomitant loss of the reciprocal chromosome segments, reminiscent of the events described previously for spontaneous recombination in *rad52* diploids by Haber and Hearn (29).

***rad51* Diploids Can Repair an HO-Induced Chromosomal Break.** Results obtained after induction of DSBs in *rad51*Δ diploids were different from both the wild type and *rad52*Δ. Although a significant fraction of colonies derived from induced cells (43.4%) apparently had experienced complete chromosome loss, most of them (56.6%) had successfully repaired the DSB, as they were α-mating but retained one or both copies of *ADE1*. All of them were α-mating and Thr$^-$; approximately 1/4 of these were Ade$^+$ while 3/4 were sectored

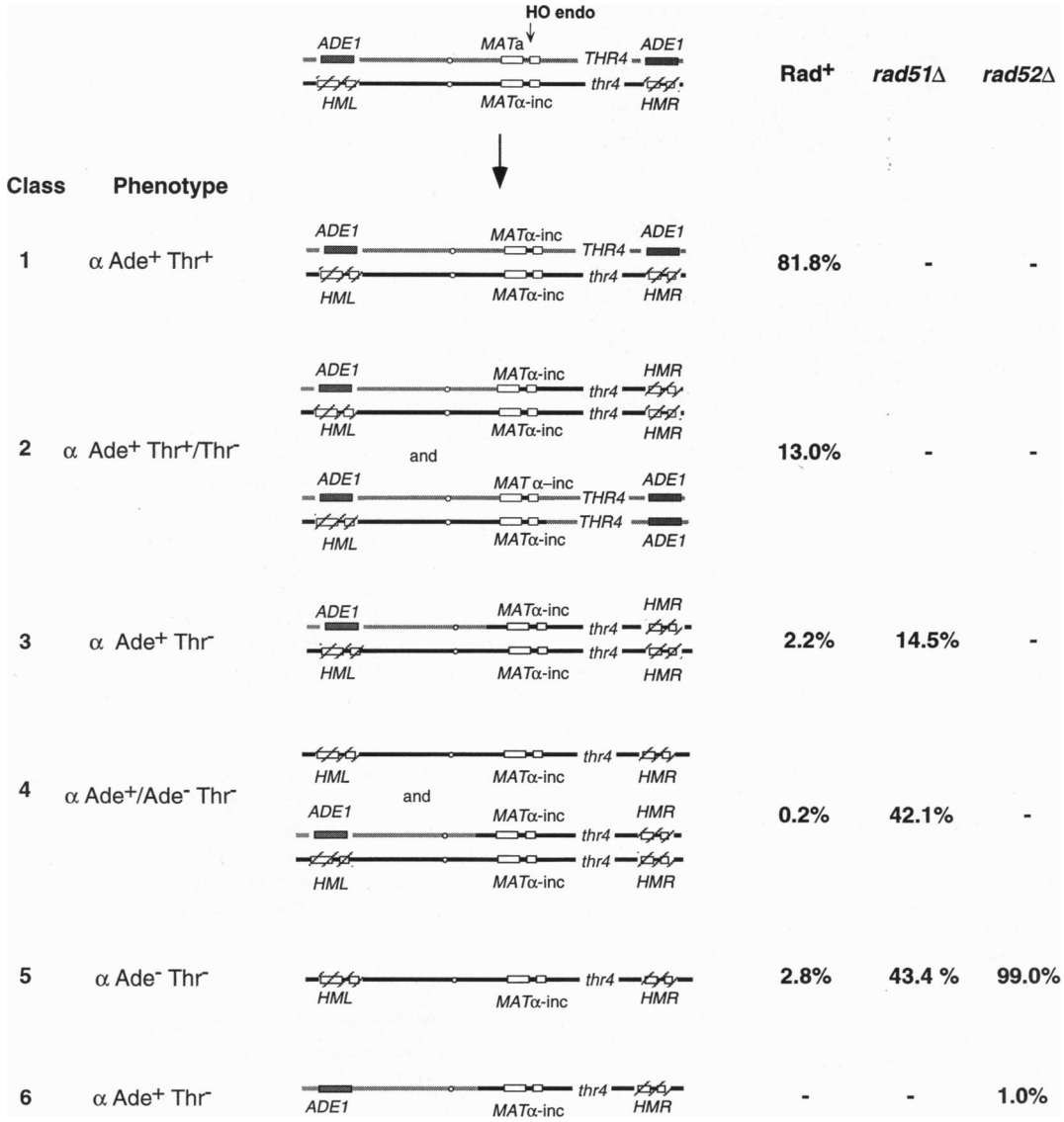

FIG. 1. Repair of HO DSBs in diploids. A site-specific DSB was introduced by HO endonuclease at the *MAT***a** locus of three isogenic diploid strains: wild type, *rad51*Δ/*rad51*Δ, and *rad52*Δ/*rad52*Δ. In the top chromosome, both *HML*α and *HMR***a** and their adjacent silencing sequences have been deleted and replaced by the *ADE1* gene. The lower chromosome contains *MAT*α-inc, which cannot be cleaved by HO, and the two unexpressed silent mating-type donors, *HML*α and *HMR***a**, shown with hatched lines. A DSB at *MAT***a** was repaired 95% of the time in wild-type cells by recombination with *MAT*α-inc (see text). α-Mating colonies that arose after induction were analyzed as described in the text. The total numbers of α-mating colonies analyzed were: 1207 for wild type, 399 for *rad52*Δ, and 587 for *rad51*Δ. The presumed structures of chromosome III corresponding to each phenotypic class are presented.

for Ade^+/Ade^- (Fig. 1). Taking into account the overall survival of induced cells (60%), we conclude that approximately 35% of plated cells (or their descendants) were able to repair the DSB in the absence of *RAD51*. It is important to stress that there were no α-mating Ade^+ Thr^+ colonies that would arise by a simple gene conversion of *MAT***a** to *MAT*α-inc.

The high proportion of sectored colonies could arise because the *MAT***a** loci in the already replicated chromosomes of a G2 cell were each cleaved by HO and produced two daughter cells, one of which then lost the broken chromosome, while the other repaired the DSB. Alternatively, such sectored colonies might mean that a DSB was produced in a G1 cell, but the broken chromosome was replicated and inherited without repair for one or more cell divisions (16), after which some of the broken chromosomes were lost and others were repaired. To investigate this point, we plated cells on YEPD medium directly after HO induction (Fig. 2). Here, too, it is evident that many cells could repair the DSB in the absence of *RAD51*, as the majority of colonies were white (7%) or contained white sectors (61%). If repair had occurred independently in G2, we would have expected all sectors to be half red and half white. However, the proportion of the colony that was white was frequently less than half, suggesting again that repair of the DSB may have occurred after the broken chromosome had been replicated and segregated to daughter cells for several generations. The inheritance of a broken chromosome through several cell divisions has previously been shown by Sandell and Zakian (16) in *rad52* diploids. Moreover, in many cases, it appears that several independent repair events had occurred, as there was more than one distinct white sector in the colony. The irregular shape of most of these colonies also suggests that there was significant lethality associated with the inheritance of a broken chromosome, consistent with our observation, presented above, that only 60% of HO-induced cells were viable.

We also followed the fate of *rad51*Δ cells that were induced for 2 hr and then transferred to liquid YEPD medium (where HO expression is repressed). Microscopic examination of cells taken at intervals during YEPD incubation showed that for at

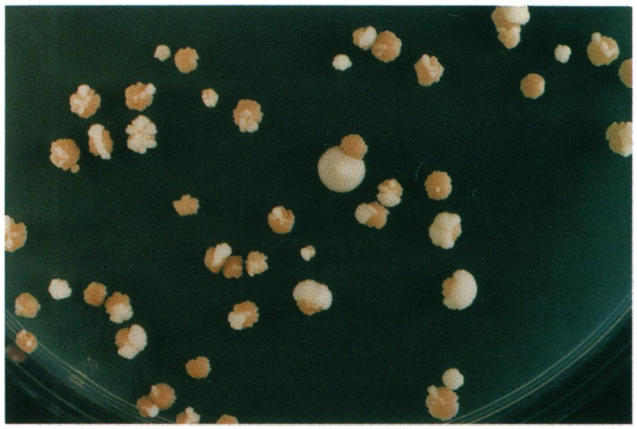

FIG. 2. Repair of an HO-induced DSB in a diploid homozygous for *rad51*Δ. Cells were induced in liquid YEPGal medium and plated on YEPD plates. When the broken chromosome, carrying *ADE1*, is lost, the colony or the sectored part of the colony becomes *ade1* and turns red. Cells that retain at *hml*Δ::*ADE1* and or *hmr*Δ::*ADE1* remain white. Subsequent testing of the colonies showed that all cells had been induced except for the large white colony in the center. Some of the colonies show several white sectors, suggesting that they arose by more than one independent repair event.

least 3 hr, *rad51*Δ cells did not divide and appeared to be arrested at the G2 stage of the cell cycle, as large budded cells. (In contrast, wild-type cells had started to divide by the time of transfer to YEPD.) *rad51*Δ cells finished their first division after approximately 6 hr of incubation in YEPD medium. Even after 18 hr of incubation in YEPD, when the *rad51*Δ culture completed three to four cell divisions, 10% (17 of 160) of the cells plated gave rise to Ade^+/Ade^- sectored colonies. This again suggests that a broken chromosome could be inherited for several generations before being repaired.

Characterization of *RAD51*-Independent Repair Events. By Southern blot analysis, we characterized α Ade^+ Thr^- colonies obtained after the DSB induction in *rad51*Δ diploids. We found that 12 of 18 of the white α Ade^+ Thr^- colonies and 13 of 15 of the white sectors of Ade^+/Ade^- Thr^- colonies had repaired the DSB in the same way (Fig. 1, class 4).§ Hybridization with an *HML*-specific probe showed that these cells were heterozygous for *HML* and *hml*Δ::*ADE1*. In contrast, these colonies were homozygous (or hemizygous) for the *HMR* marker located on the right end of *MAT*α-inc chromosome, as an *HMR*-specific probe illuminated only one restriction fragment.

It was possible that the Ade^+ Thr^- colonies were hemizygous for markers distal to *MAT*, if the Ade^+ chromosome had been healed by the formation of a new telomere to produce a terminally deleted chromosome (30). Therefore, we demonstrated that these diploids do indeed have two copies of *HMR*, by using the gene replacement method described in *Materials and Methods*. In essence, we crossed the α-mating, Ade^+ Thr^--*leu2 rad51*Δ diploids to a *MAT***a** *leu2* Rad^+ strain carrying *hmr*Δ::*ADE1* to create a Leu^- Ade^+ triploid. We then targeted a linear fragment of DNA carrying *hmr*Δ::*LEU2* to obtain Leu^+ Ade^+ transformants in which one *HMR***a** locus was replaced by *hmr*Δ::*LEU2*. In six out of six cases, Southern blots showed that there were three bands, corresponding to *HMR***a**, *hmr*Δ::*LEU2*, and *hmr*Δ::*ADE1* (data not shown). Thus, the repaired *rad51*Δ diploid strains must have had two copies of *HMR*. We conclude a chromosomal DSB repair can be repaired in *rad51*Δ mutants, resulting in diploids heterozygous for the left arm of chromosome III, but homozygous for *MAT*α-inc and for *thr4* and *HMR* distal to the break site.

Comparison of Wild-Type and *RAD51*-Independent DSB Repair. Homozygosis of markers distal to *MAT* can be explained by two different mechanisms: (*i*) a gene conversion at *MAT* with an associated reciprocal crossover in the *MAT*–*THR4* interval or (*ii*) a nonreciprocal acquisition of information distal to the break site, copied from the *MAT*α-inc chromosome (Fig. 3). In the former case, colonies should appear as Thr^+/Thr^- sectors if there was no cell separation in liquid medium between the time of DSB induction and the time of plating. Therefore, we induced DSBs by *GAL*::*HO* in a liquid culture and then micromanipulated single unbudded cells on YEPD plates to examine repair events emanating from a single G1 cell.

When *rad51*Δ mutant cells were plated in this way, 35% of colonies (9 of 26) were α Thr^- Ade^+/Ade^- and apparently repaired the broken chromosome. Again, the fact that an unbudded cell gave rise to a sectored colony is evidence of a repair event occurring in G2 or in later cell divisions. The rest of the colonies we obtained were Ade^- Thr^-, resulting from loss of the broken chromosome. There were no Thr^+/Thr^- sectors, again indicating that the *RAD51*-independent events arose by a nonreciprocal pathway. We also did not find any Thr^+/Thr^- sectors when we analyzed another 161 events that arose from random cells plated directly on YEP-Gal plates (data not shown).

For the Rad^+ diploid, 80% of the α-mating cells (72 of 90) were Ade^+ Thr^+, in which the DSB was repaired by gene conversion. The remaining 20% (18 of 90) were α-mating Ade^+ but sectored for Thr^+/Thr^-. These sectors have apparently arisen from a G1 cell in which the DSB was induced. By Southern blot analysis, we found that 13 of 17 analyzed Thr^+ parts of such sectors were homozygous for *hmr*Δ::*ADE1* (and

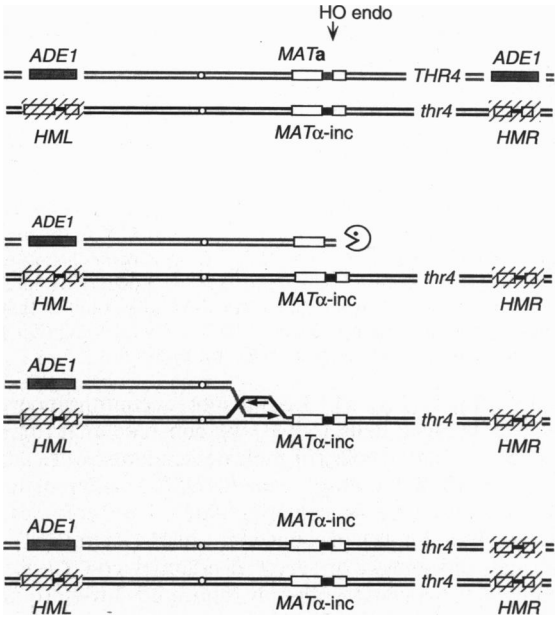

FIG. 3. A break-induced replication model of *RAD51*-independent DSB repair. After induction of a DSB at *MAT***a** (*A*), the ends of the DNA are acted upon by a 5′ to 3′ exonuclease (*B*) and strand invasion can occur, with the removal of at least 700 bases of the nonhomologous **Ya** DNA (*C*). The D loop formed by strand invasion is either converted into a unidirectional replication fork that can copy both strands by semiconservative replication (*C*) or the leading strand is displaced from a migrating D loop and the lagging strand is copied from the newly synthesized template (*C*). This leads to restoration of the broken chromosome by the apparent gene conversion or homozygosis of all markers distal to the DSB (*D*).

§Subsequent Southern blot analysis showed that the remaining eight white colonies or sectors had a variety of different genotypes. One was a 2n-1 diploid with a recombined chromosome, apparently identical to the rare *rad52*Δ repair events; the others were more complicated cases that could be explained by double repair events. These were not characterized further.

presumably *THR4*). This result establishes that in the wild-type cell, most of the Thr^+/Thr^- sectors arose by a reciprocal crossover accompanying gene conversion at *MAT*, producing *THR4/THR4* and *thr4/thr4* progeny (see Fig. 1, class 2). Given that we plated unbudded (G1) cells, these sectored colonies probably arose by recombination that began in G1 but were only resolved postreplicationally in G2, possibly by the mechanism proposed by Esposito (31). The remaining 4 out of 17 cases contained a Thr^+ half-sector that was heterozygous *HMR/hmrΔ::ADE1*. These four appear to have replicated the broken chromosome and then repaired it in two different ways in G2: one yielding the Thr^+ sector with no exchange of flanking markers (by gene conversion) (see Fig. 1, class 1) and the other producing α Thr^- cell by a mechanism that appears to be similar to the nonreciprocal exchange events we observed in *rad51Δ* cells (e.g. Fig. 1, class 3).

DISCUSSION

We have found that a single chromosomal DSB can be repaired in *rad51Δ* diploids by a mechanism that appears to be quite different from the classical DSB gap repair mechanism of Szostak and coworkers (2). *RAD51*-independent repair results in the homozygosis of the 100-kb chromosomal region distal to the break point by a nonreciprocal repair event. The type of repair events in *rad51Δ* cells is also distinctly different from the rare 2n-1 aneuploid α Ade^+ Thr^- colonies observed in *rad52Δ* diploids.

The efficiency of DSB repair in *rad51Δ* mutants is 30-fold higher than that in *rad52* diploids. In about 14% of the colonies, all of the progeny of a cell containing a broken chromosome were α Ade^+ Thr^-. Another 40% yielded α Thr^- colonies that were sectored for Ade^+/Ade^-. We have shown that most of the Ade^+ Thr^- cells from both unsectored and sectored colonies are heterozygous for a marker on the left arm of the chromosome and homozygous for *MATα*-inc and for a marker at the right end of the chromosome. Cases in which the entire colony is white (Ade^+) either represent instances in which the repair occurred in G1 or in which both of the chromatids were repaired in G2. We note that some sectors appear to have repaired the DSB only after several cell divisions, producing white sectors that are much less than half the size of the colony. This result suggests that a broken chromosome can be inherited for several generations before it is repaired. Evidence that a broken chromosome in a *rad52* diploid can be replicated and segregated, without repair, was previously presented by Sandell and Zakian (16).

We emphasize that *RAD51*-independent repair is a moderately efficient homologous repair process in diploids. In wild-type cells, events apparently similar to what we observed in *rad51* diploids were indeed found (Fig. 1), but they were 50-fold less efficient than gene conversions that preserve markers on both sides of the DSB. However, when gene conversion is absent, this *RAD51*-independent mechanism can rescue approximately 35% of the cells experiencing a DSB, although in some cases this does not occur for several generations. This mechanism is two orders of magnitude more efficient than the nonhomologous end-joining repair events that have been observed in the wild-type and both *rad52* and *rad51* strains when homologous recombination is not possible (13, 32).

Break-Induced Replication: A Mechanism to Account for *RAD51*-Independent DSB Repair. To explain at a molecular level how these nonreciprocal, *RAD51*-independent events might occur, we invoke a mechanism analogous to the model of recombination-dependent initiation of late replication in T4 phage proposed by Mosig (33) and substantiated by the biochemical studies of Formosa and Alberts (34). In the phage system, as we imagine in yeast, a DNA end invades an intact DNA molecule to initiate replication. This process involves strand invasion of a DNA end to prime new DNA synthesis and requires the UvsX protein, the phage homologue of *Escherichia coli* RecA. A similar mechanism was proposed by Asai and coworkers (35) to explain the recA-dependent, homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in *E. coli*. We term the similar process in yeast as break-induced replication (BIR). Despite the requirement for RecA or RecA homologues in the bacterial systems, BIR in yeast does not require *RAD51*.

We envision several steps in BIR (Fig. 3): nucleolytic degradation of broken ends to produce a 3′-ended single strand; strand invasion of the broken chromosome into the homologue occurring somewhere proximal to *MAT*; initiation and elongation of DNA synthesis using the homologous chromosome as a template; and, as a result, restoration of the broken chromosome by homozygosis of all markers distal to the break point. This mechanism has not previously been described in detail in eukaryotes, but several groups have observed a nonreciprocal inheritance of markers distal to a selected site of gene conversion in (Rad^+) yeast (31, 36–38), all of which could be explained by a similar mechanism. In addition, Dunn and Szostak (39) and Vollrath and coworkers (40) provided evidence that a transformed linear plasmid in which one telomere had been cut off could repair itself by recombination with homologous chromosomal regions. This repair could also occur by BIR rather than by a nonreciprocal crossover.

BIR appears to involve the same initial steps of homologous recombination as does gap repair (i.e., a search for homology and strand invasion), but clearly gap repair does not occur in *rad51* diploids. Gap repair of *MAT***a** by *MATα*-inc depends on both *RAD52* and *RAD51*, because a deletion of either gene eliminates gene conversions both without and with an accompanying reciprocal exchange of flanking markers (class 1 and class 2 events in Fig. 1). BIR is essentially a "one-ended" event. These nonreciprocal events apparently depend on *RAD52*.

The Role of *RAD51* in Homologous Recombination. The role of *RAD51* protein has been the subject of several recent inquiries. A variety of experiments have shown that deleting *RAD51* is much less deleterious to several forms of recombination than is the deletion of *RAD52* (5, 9, 10). Specifically, in the case of HO-induced DSB repair, we showed that *RAD51* protein is not required for gene conversion when the DNA substrates have a chromatin structure that is sufficiently accessible to the strand invasion machinery (5). Why then can BIR, which also appears to involve strand invasion, occur without *RAD51* when gene conversion of the same chromosomal locus cannot? One explanation could be that strand invasion is much less efficient without Rad51p, so that the probability of each end of a DSB independently invading its homologue is very low. This seems unlikely, given that the rate of one-end invasion is at least 15% per generation, so that about 2% of the repair events should have been gene conversions. An attractive alternative reason is that there are only a few sites along chromosome III that have a sufficiently "open" chromatin structure so that strand invasion can occur in the absence of *RAD51*. This might also explain why there were no events in which the right arm distal to the DSB was used as the primer to copy all the chromosome through the centromere and out to the left telomere. This reverse reaction might also have failed, because the distance to be copied is twice as long and must also go through the centromere. Replication itself might provide a more open chromatin structure that would permit BIR to proceed in the absence of *RAD51*. In any case, we imagine that there is at least one site proximal to *MAT* at which strand invasion can occur without *RAD51* protein. By marking the region proximal to *MAT*, it should be possible to establish if there are such preferential repair sites.

Another explanation for the existence of *RAD51*-independent BIR when gene conversion does not occur could be that the invading DNA strand becomes associated with different DNA polymerases for the two processes. Possibly, Rad51p is part of a complex of proteins that is associated with a "repair" DNA

polymerase that is incapable of duplicating the 100-kb of double-stranded DNA that is required in the experimental situation we have devised. The assimilation of the invading strand into the regions undergoing DNA replication could be *RAD51*-independent (though still *RAD52*-dependent).

One question that emerges from this study is why is BIR 50-fold less efficient relative to gap repair in a Rad^+ diploid, even though a significant fraction of cells can use this pathway when gap repair is prevented? We believe that this reflects the fact that different homologous repair pathways are in competition with each other, so that the more efficient pathway will prevail when it is present, but the alternate pathway will take over when the principal pathway is eliminated (8, 41). For example, in a yeast centromeric plasmid containing two inverted repeats of an homologous sequence, one of which has been cut by HO, gene conversion, with and without crossover, occurs as efficiently in a *rad51* strain as in Rad^+. When the two homologous segments are in direct orientation, however, single-strand annealing (80%) predominates over gene conversion (20%) in the wild-type cell, but in *rad51* cells, 99.5% of the events were by single-strand annealing. We conclude that gene conversions are not prevented in the absence of *RAD51*, but they are less efficient than in wild-type cells, and this is revealed when there is competition between alternative pathways.

It is also possible that *RAD51* is actively involved in preventing BIR in wild-type cells. This might reflect the fact that the Rad51 protein apparently catalyzes strand exchange in a 5′ to 3′ direction, opposite to that carried out by RecA, so that the strand invasion intermediate for BIR might be unstable, while intermediates for gap repair would be stable.

A second question is why were 40% of HO-induced *rad51* diploids inviable, while there was no such lethality in wild-type or *rad52* diploids? It is possible that strand invasion in the absence of *RAD51* may sometimes lead to the formation of an interchromosomal recombination intermediate that cannot be resolved and thus traps both the broken and intact homologues in an inviable state. Alternatively, BIR might interfere with normal replication of the intact chromosome and cause cell death.

We thank Susan Lovett, Michael Lichten, Bernard Lopez, and members of the Haber laboratory for their insightful comments and suggestions. This work was supported by National Institutes of Health Grant GM 20056 and Department of Energy Grant DE-FG02-91ER61235.

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