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Two Different Types of Double-Strand Breaks in *Saccharomyces cerevisiae* Are Repaired by Similar *RAD52*-Independent, Nonhomologous Recombination Events

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In haploid *rad52 Saccharomyces cerevisiae* strains unable to undergo homologous recombination, a chromosomal double-strand break (DSB) can be repaired by imprecise rejoining of the broken chromosome ends. We have used two different strategies to generate broken chromosomes: (i) a site-specific DSB generated at the *MAT* locus by HO endonuclease cutting or (ii) a random DSB generated by mechanical rupture during mitotic segregation of a conditionally dicentric chromosome. Broken chromosomes were repaired by deletions that were highly variable in size, all of which removed more sequences than was required either to prevent subsequent HO cleavage or to eliminate a functional centromere, respectively. The junction of the deletions frequently occurred where complementary strands from the flanking DNA could anneal to form 1 to 5 bp, although 12% (4 of 34) of the events appear to have occurred by blunt-end ligation. These types of deletions are very similar to the junctions observed in the repair of DSBs by mammalian cells (D. B. Roth and J. H. Wilson, Mol. Cell. Biol. 6:4295–4304, 1986). When a high level of HO endonuclease, expressed in all phases of the cell cycle, was used to create DSBs, we also recovered a large class of very small (2- or 3-bp) insertions in the HO cleavage site. These insertions appear to represent still another mechanism of DSB repair, apparently by annealing and filling in the overhanging 3′ ends of the cleavage site. These types of events have also been well documented for vertebrate cells.

Repair of double-strand breaks (DSBs) in *Saccharomyces cerevisiae* by homologous recombination is very efficient, in both meiotic and mitotic cells (16, 41, 46). There appear to be two major mechanisms of DSB repair in mitotic cells (14). Gap repair (63) is a conservative mechanism in which a DSB is expanded into a single-stranded or double-stranded gap. Repair from homologous sequences leads to a gene conversion event. An alternative nonconservative pathway is called single-strand annealing (SSA), in which a DSB between direct repeats of homology leads to a deletion and the loss of intervening sequences between the direct repeats (16). Both of these repair mechanisms require extensive homology for efficient recombination (1, 33, 59).

The *RAD52* gene product is required for most mitotic recombination in *S. cerevisiae*, including mating-type switching (31, 66), integration of transformed linear DNA (37), and repair of both enzyme-induced and nonspecific DSBs (4, 36, 45, 50). *RAD52*-independent repair by SSA between direct repeats of 1 to 2 kb occurs at a much lower efficiency than in Rad$^+$ strains (4, 14, 43); however, DSB repair by SSA in large arrays of tandemly repeated genes such as *rDNA* and *CUP1* is efficient in *rad52* strains (38).

Because repair of DSBs by homologous recombination is very efficient in *S. cerevisiae*, a strong selection scheme is needed to look for nonhomologous repair pathways. In three previous studies, illegitimate recombination has been examined using transformation of linear DNA. In one experiment, plasmid DNA was linearized by a cut within *lacZ* sequences that were not homologous to any chromosomal sequences (29). Most of the rare transformants recovered were head-to-head dimer plasmids. The mechanism of end joining appeared to depend on homologous interactions along the length of two molecules of transformed DNA since only head-to-head and no head-to-tail joinings were recovered. In another series of experiments (53, 55), DNA fragments that had been cut with *Bam*HI preferentially integrated into chromosomal GATC sites by interaction of the *Bam*HI-generated GATC ends of the fragment. Finally, linear transformed fragments having homeologous sequences at either end with 52 or 73% sequence identity were found to recircularize to generate a replicative plasmid. The junction of these recombination events involved short (2 to 21 bp) regions of identity but also required a general alignment of the homologous sequences so that most of the repair events maintained the open reading frame between the two diverged gene copies (33). Despite the very small degree of sequence identity at the junctions, these events require *RAD52* gene function, possibly for the general alignment of homeologous sequences.

In mammalian cells, nonhomologous recombination events are far more common than in *S. cerevisiae*, as illustrated by the fact that in mammalian cells only 1 in 100 transformants involves homologous integration of the transforming DNA (10, 47). End joining appears to be the most common pathway of DSB repair in mammalian cells. A variety of both in vivo and in vitro studies of end-joining events arising by nonhomologous DNA interactions in eucaryotic systems has revealed that in many cases short stretches of DNA identity are involved in end-joining events (25, 40, 47, 64), although broken DNA ends can be joined together by simple ligation as well (49, 65).

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We have studied repair of DSBs by illegitimate recombination in nontransformed yeast cells in which chromosomal DSBs were generated in vivo. We have compared end-joining events in two systems: (i) a site-specific DSB generated at the *MAT* locus HO cut site in a *rad52* haploid strain unable to repair the break by homologous recombination and (ii) undefined breaks created between two centromeres of a conditionally dicentric chromosome III during mitosis in a *rad52* haploid. Although a simple rejoining of broken ends would not eliminate the lethal condition, further chromosomal breakage could be prevented by the formation of deletions. In both cases, the sizes of deletions were larger than was required either to remove the HO cutting site or to eliminate the function of the conditional centromere. Our results suggest that microhomologies of a few base pairs frequently direct end joining of broken chromosomes, although in some cases we find no homology at the deletion junction. The spectrum of repair events is very similar to that observed in mammalian cells (49), indicating that mechanisms of end joining may be similar in all eucaryotes.

MATERIALS AND METHODS

Strains. Strain BW330-26A (*HO MATα swi1 rad52 met13 his5 ade2 mal2*) was constructed as described previously (67). Dicentric strain J178-#7-20 (*MAT***a** *ade1 met14 ura3-52 leu2-3,112 his3 his4*::*GALCEN3*::*URA3 rad52*::*LEU2*) was constructed as described previously (4, 21). Monocentric strain J178-20 (*ho MAT***a** *ade1 met14 ura3-52 leu2-3,112 his3 Δrad52*) was constructed as described previously (4, 21). The *URA3-GAL*::*HO* plasmid pJH132 was transformed into strain J178-20 by the lithium acetate method described by Schiestl and Gietz (54) to create strain J178-20T. Growth media have been previously described by Rudin and Haber (50) and Sherman et al. (58).

Generating deletions. (i) BW330-26A derivatives. In cells with *HO* activity but which are unable to complete *MAT* switching in the *rad52* background, a double-strand cut will be lethal unless the cut site becomes deleted or mutated (66). Surviving cells carrying deletions that remove part of the *MATα1* gene are recognized as sterile, while survivors that have parts of both *MATα1* and *MATα2* deleted are **a** mating (67).

(ii) J178-#7-20 derivatives. Deletions that removed all or part of a centromere were recovered by growing cells carrying a galactose-suppressed conditional centromere as well as a normal centromere on chromosome III on glucose medium and analyzing survivors as described elsewhere (4).

(iii) J178-20T derivatives. J178-20 cells were transformed with plasmid pJH132, in which the *HO* gene is carried on a single-copy plasmid under control of the *GAL10* promoter (4, 8, 24, 68). When these cells are grown on glucose, there is little or no HO cutting, while growth on galactose induces HO production, and cutting at *MAT* is very efficient. Strains were grown in nonfermentable yeast extract-peptone (YP)-lactate medium overnight and plated in serial dilutions onto galactose-containing synthetic medium lacking uracil to isolate mutants at the HO cut site and onto glucose-containing synthetic medium lacking uracil to determine survival rates. Colonies surviving galactose growth were picked as deletion derivative candidates. They were classified genetically as *MAT***a** or *mat***a***1* by mating to a *MATα* tester strain. A *MAT***a**/*MATα* diploid has a nonmating phenotype, indicating a functional *MAT***a***1* gene product. *mat***a***1*/*MATα* diploids have an α-mating phenotype because they lack the *MAT***a***1* gene product (for a review, see references 17 and 20). Both types of survivors were further analyzed by Southern mapping and sequence analysis.

PCR and sequencing. Strains were grown overnight in 5 ml of rich yeast extract-peptone-dextrose (YEPD) medium, and DNA was prepared by the sodium dodecyl sulfate (SDS)-potassium acetate method (58). An ~5-ng amount of genomic DNA was used in each PCR amplification reaction (2, 51) with a Techne Programmable Dri-Block PHC-1 temperature cycling machine. The oligonucleotides used were synthesized with a Cyclone V1.05 synthesizer by using phosphoramidite chemistry. Two pairs of primers used for DNA amplifications around the HO-cut site were synthesized on the basis of known sequences at *MAT*. Primers for amplification of the *CEN3* deletions were synthesized on the basis of known sequences flanking the *Bam*HI *GAL1*::*CEN3* fragment.

MAT primers HD (distal to the HO cut site) and HP (proximal to the HO cut site) were as follows: 735 (HD), 5′-ATGTGAACCGCATGGGCAGT-3′; BRL4 (HD), 5′-AATATTAGTGGGTTAATACA-3′; BRL6 (HP), 5′-GG TAAATTACAGCAAATAGA-3′; and #867 (HP), 5′-CTG GTAACTTAGGTAAATTACAGC-3′. *CEN3* primers were as follows: 3050 (left of *GAL1*::*CEN3*), 5′-TCGACTACGC GATCATGGCG-3′; 3051 (right of *GAL1*::*CEN3*), 5′-CAC GATGCGTCCGGCGTAGA-3′.

The parameters of the amplification cycle were as follows: denaturation at 95°C for 1.5 min, annealing at 48°C for 1 min, and polymerization at 72°C for 0.1 min per 100 bp. PCR was run for 30 cycles with a final polymerization step at 72°C for 7 min. The enzyme used for DNA amplification was either Pyrostase (Molecular Genetic Resources, Inc.) or Deep $Vent_R$ Polymerase (New England Biolabs). Primers were removed from the PCR product by treatment with Prep-a-gene (Bio-Rad). The approximate sizes of the deletions were estimated by gel electrophoresis of ethidium-stained PCR product, and an appropriate sequencing primer was used for sequencing with an fmol sequencing kit (Promega). An ~50-ng amount of PCR-amplified DNA was used in each sequencing reaction.

RESULTS

Deletions at the HO cut site. A strong selection process was designed to look for events in which a DSB had healed by illegitimate recombination. Haploid strain BW330-26A has a *HO MATα rad52 swi1-1* genotype. In normal cells, the HO endonuclease makes a specific cut at the *MAT* locus to initiate the mating-type switch (for a review, see reference 17). In BW330-26A, this is a lethal event because *RAD52* is essential for completion of the mating-type switch (31, 66). However, since *HO* expression is low because of the *swi1-1* mutation (18, 35, 66), the DSB occurs in only a small proportion of the cell population, allowing the strain to be propagated. Cells in which an HO cut has occurred have a transient **a**-like mating type because of disruption of the *MATα1* and *MATα2* genes. Thus, the mating type of a population of cells is α > **a**. A few *MATα* cells attempting to switch mating type survived the DSB by deleting sequences around the HO cut site and becoming resistant to subsequent HO cleavage. Nine such subclones were recovered, eight of which had deletions that removed part or all of the *MATα1* sequences in which the HO cut site is embedded. Because cells with the genotype *matα1 MATα2* are sterile, these derivatives had a nonmating phenotype. The remaining subclone with a stable **a**-like phenotype was *matα1 matα2*, having a deletion that removed *MATα1* sequences and part

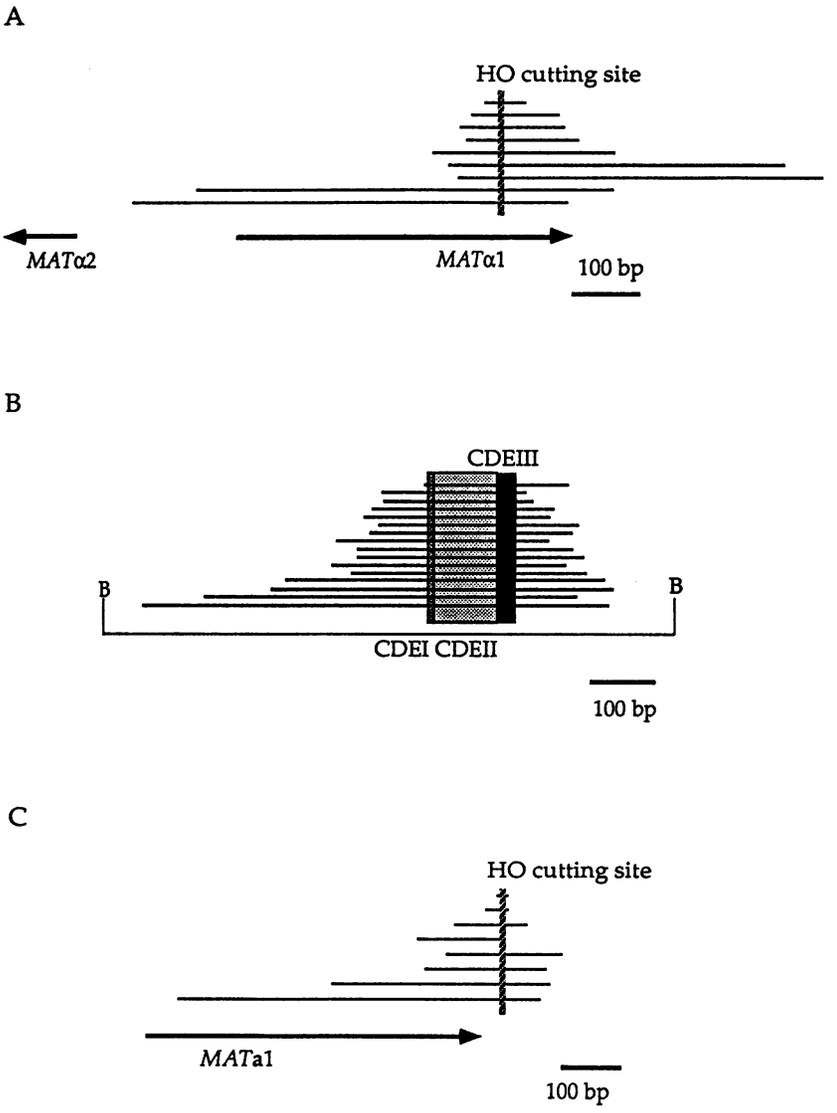

FIG. 1. Sizes and positions of nonhomologous deletions. Deletion events from each strain are shown in their chromosomal contexts. Each horizontal line represents sequences deleted relative to the relevant chromosomal sequences shown at the bottom of each deletion group. (A) Deletions recovered from *HO swi1-1 rad52* strain BW330-26A. Sequences deleted are shown relative to the HO cut site on chromosome III. *MATα1* and *MATα2* open reading frames are indicated by arrows. (B) Deletions recovered from *GAL1::CEN3 rad52* dicentric strain J178-#7-20 after transfer to glucose medium. Sequences deleted are shown relative to *CEN* elements CDEI to CDEIII, indicated as shaded bars. (C) Deletions recovered from *MATa GAL1::HO rad52* monocentric strain J178-20T. Sequences deleted are shown relative to the HO cut site and the *MATa1* transcript. The deletions shown do not include a large class of very small (1- to 3-bp) insertions and deletions at the HO cutting site (see text).

of the promoter shared by *MATα1* and *MATα2* (67) (Fig. 1A). Deletions around the HO cut site occurred at a frequency of 1% among *HO rad52 swi1-1 MATα* cells (67). The actual frequency of deletions is likely to be higher, since the *HO* gene is expressed only in a small proportion of cells with this genotype.

Deletion junctions were sequenced by amplifying the relevant DNA sequences by using PCR and directly sequencing the PCR product. The sizes of the deletions varied between 62 and 698 bp, with a mean size of 282 bp, in good agreement with a previously published Southern analysis of these events (67). The locations of the deletions relative to the HO cut site are shown in Fig. 1A. We note that the deletions are much larger than necessary to relieve lethality, given that a single nucleotide deletion at the HO cut site eliminates cutting by the HO endonuclease (67; see below).

To understand how these deletions arose, we looked for the presence of nucleotide homologies at the junction site that could effect joining of flanking DNA by annealing of complementary single strands. For example, in deletion strain BW-D2, there is a five-nucleotide junctional homology of GAAGA; Fig. 2A illustrates how strand annealing might have played a role in this particular deletion event. The convention that we have used to represent sequences at the deletion junctions is shown in Fig. 2B. When diagrammed in this way, junctional homologies (underlined) occupy the same position in all three lines. The sequences of the nine BW330-26A deletion derivatives are shown in Fig. 3A. Eight

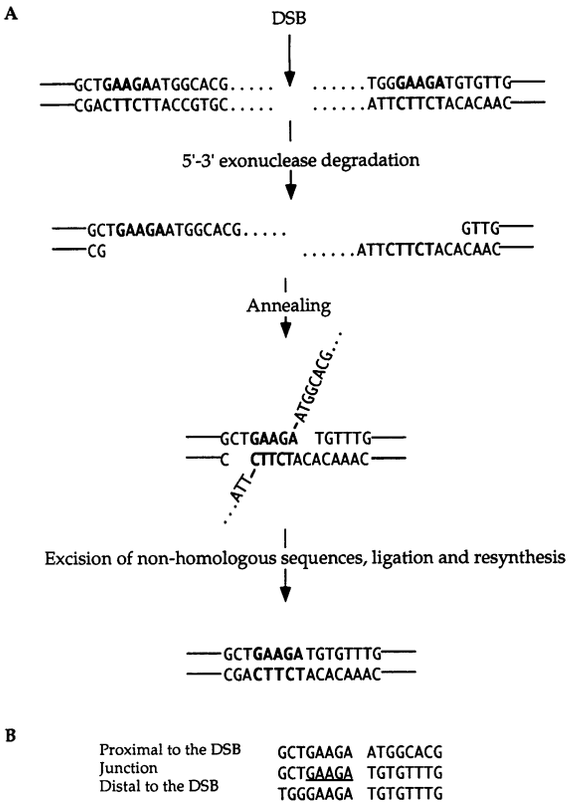

FIG. 2. A model for strand annealing to create deletions between short homologies. (A) 5′ to 3′ exonucleolytic degradation of the ends of a DSB exposes single-stranded regions of homology (GAAGA). After annealing of the partner strands, nonhomologous sequences are excised and gaps are filled in by DNA repair synthesis and ligation. (B) The convention used in Fig. 3 for representing deletion junctions is shown. Only the top strand (5′ to 3′) is shown; junctional nucleotide homologies are underlined.

of the nine deletion junctions contain one or more nucleotides of homology, although only two have 3 or more overlapping bp.

Deletions on a conditional dicentric chromosome. Nonspecific DSBs were generated in a conditional dicentric *rad52* haploid, J178-#7-20, as described elsewhere (4). A dicentric chromosome III was constructed by inserting *GAL1*::*CEN3* at the *HIS4* locus 45 kb to the left of the resident *CEN3* (Fig. 4). This *CEN* is under the control of an inducible *GAL1* promoter, so that transcription through the inserted *CEN3* during growth in galactose medium inactivates it, allowing normal chromosome III segregation during mitosis (4, 21). In glucose medium, transcription from the *GAL1* promoter is repressed, resulting in a functionally dicentric chromosome. As has been previously observed, dicentric chromosomes in *S. cerevisiae* are mitotically unstable and prone to DNA rearrangements, often resulting in the loss of one of the centromeres (4, 19, 21, 22, 62). Hence, the mitotic lethality could be rescued by deleting one of the centromere sequences. A total of 0.04% of cells survived growth on glucose, most of which contained centromere deletions that ranged from about 200 bp to more than 4 kb (4). Deletions of either the conditional centromere or the normal *CEN3* were recovered (Fig. 4), with the majority of events removing the conditional centromere. Approximately 13% of all deletions were contained within the 900-bp *GAL1*::*CEN3* *Bam*HI

A

deletion strain	deletion junction sequence	deletion size	deletion strain	deletion junction sequence	deletion size
	CTGCTCGC TGAAGAAT			TACTTCTT TTAACCTT	
BW-B13	CTGCTCGC TGGTCGTG	173 bp	BW-A3	TACTTCTT CCTATTTG	653 bp
	ATAGAGTG TGGTCGTG			ATAGCTAT CCTATTTG	
BW-B17	ATGTTTCA AAACATTA		BW-D2	GCTGAAGA ATGGCACG	
	ATGTTTCA TATAGAGT	689 bp		GCTGAAGA TGTGTTTG	138 bp
	TTTGGCCT TATAGAGT			TGGGAAGA TGTGTTTG	
BW-F32	GAGCATAT TACTCACA		BW-G30	GTCTTGTC TTCTCTGC	
	GAGCATAT CCTATTTG	282 bp		GTCTTGTC TGTACATT	162 bp
	ATAGCTAT CCTATTTG			TATGTGTT TGTACATT	
BW-D11	CGGTGTAA AACAAAAT		BW-B28	TCTCTGCT CGCTGAAG	
	CGGTGTAA CGGGACGG	485 bp		TCTCTGCT ACCTTCGG	575 bp
	TGGCTATA CGGGACGG			TGCTAGTT ACCTTCGG	
BW-A5	AATGCAGC ACGGAATA				
	AATGCAGC AGAGTGGT	61 bp			
	GGTTTTGT AGAGTGGT				

B

deletion strain	deletion junction sequence	deletion size	deletion strain	deletion junction sequence	deletion size
7-03	TATACTTT AACGTCAA		4-2	CCAAACAA TATGGAAA	
	TATACTTT AATGGTAT	234 bp		CCAAACAA TATTATTC	242 bp
	TTTCATTG AATGGTAT			TCATATAT TATTATTC	
7-04	GTACAAAT AAGTCACA		10	TAAAAGTA TCAACAAA	
	GTACAAAT CAACTTAA	233 bp		TAAAACTA TCATATAT	305 bp
	TGGTAAAG CAACTTAA			AGTTTTGC TCATATAT	
7-06	TCTTATTC AAATGTAA		2-6	ACCTCTAT ACTTTAAC	
	TCTTATTC AAAGAAAA	349 bp		ACCTCTAT ACTTTAACA	325 bp
	GAAGTAAT AAAGAAAA			GTAAAGCA ACTTAACA	
7-19	TTCTTATT CAAATGTA		3-10	ACTTTAAC TAATACTT	
	TTCTTATT CAACTTAA	369 bp		ACTTTAAC TACTGGTG	343 bp
	TGGTAAAG CAACTTAA			GAAACGTT TACTGGTG	
7-28	CATAACCA CTTTAACT		1-3	TATTTCTG GGGTAATT	
	CATAACCA CAATAGAA	383 bp		TATTTCTG AGGTAATG	502 bp
	TTATTATT CAATAGAA			CAGTAAAA AGGTAATG	
7-40	CAAATTAA CAACCATA		3-8	TGAAACGC AGATGTGC	
	CAAATTAA TTGAAAAA	556 bp		TGAAACGC AGTTTTGC	602 bp
	GGTAATGA TTGAAAAA			CTGGTGGA AGTTTTGC	
7-54	ACAAAAAA TTGTTAAT		2-5	GAACTTTC AGTAATAC	
7-59	ACAAAAAA TTGGTAAA	332 bp		GAACTTTC AGTAATAA	760 bp
	AGAAAAAG TTGGTAAA			TCAATAGA AGTAATAA	
7-64	TTTCTATT ACTTCTTA		7-61	AATATACC TCTATACT	
	TTTCTATT ACAGTAAA	295 bp		AATATACC TCAATAGA	386 bp
	GCAACTTA ACAGTAAA			ATTATTAT TCAATAGA	

C

deletion strain	deletion junction sequence	deletion size	deletion strain	deletion junction sequence	deletion size
603	GTATGAGA TCTAAATA		620	AATTTGTA GTTCATAA	
	GTATGAGA CGAATAAT	117 bp		AATTTGTA TGTACATT	189 bp
	GTGGTTGA CGAATAAT			GATGTGTT TGTACATT	
604	TTGGGTAT GTAATATG		636	TATCCTAT ACTAACAA	
	TTGGGTAT AATTTTAT	146 bp		TATCCTAT ATGTGTTT	
	AACAGTAT AATTTTAT			TTGGGAAG ATGTGTTT	195 bp
647	TCAGCTTT CCGCAACA		642	TCAATATC ACCCCAAG	
	TCAGCTTT ATAAACC	18 bp		TCAATATC ACATTTGG	
	ATAATTTT ATAAACC			GTGTTTGT ACATTTGG	360 bp
614	TTGTAGTA TGGCGAAA		629	TCGGGTTT TTCTTTTA	
	TTGTAGTA CGTGGTGA	596 bp		TCGGGTTT ATAAACCC	37 bp
	CTGAAGTA CGTGGTGA			ATAATTTT ATAAACCC	

FIG. 3. Sequences of deletion junctions. For each deletion junction presented, only top-strand sequences are shown. The top line represents the original sequence at the left side of the junction, the middle line represents the deletion junction itself, and the bottom line represents the original sequence at the right side of the junction (see Fig. 2). Nucleotide homologies present at the junction occupy the same position in all three lines and are underlined in the middle line. The site of the joint is represented by a space, although in cases in which homologies are present the nucleotides in question could have come from either side of the junction. Sequences of deletion derivatives from *MAT*α *ho swi1 rad52* strain BW330-26A (A), from *MAT***a** *GAL1*::*CEN3 rad52* strain J178-#7-20 (B), and from *MAT***a** *rad52 GAL*::*HO* strain J178-20T (C) are shown.

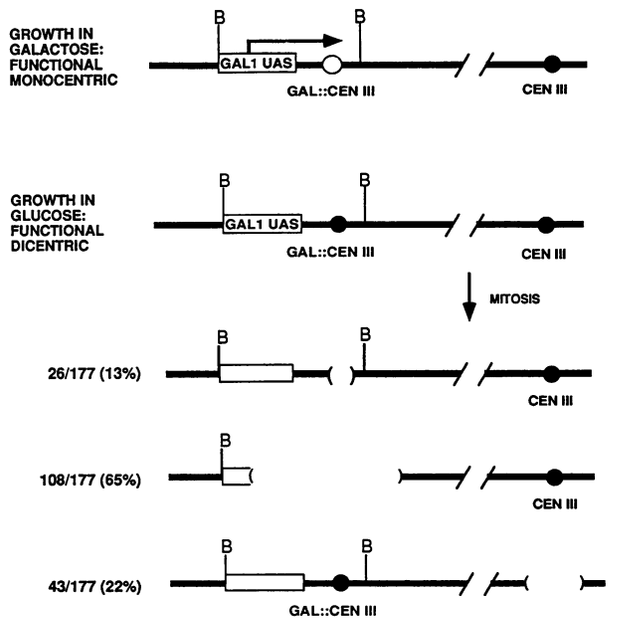

FIG. 4. Deletions of the conditional dicentric chromosome III in strain J178-#7-20. Chromosome III carries a second *CEN3* under control of a *GAL1* promoter inserted 45 kb from the resident copy of *CEN3* (4). During mitotic growth in galactose-containing medium, the inserted *CEN3* is inactivated as a result of transcription from the *GAL* promoter, yielding a functionally monocentric chromosome. During growth in glucose, transcription from the *GAL* promoter is repressed, resulting in a functionally dicentric chromosome. Glucose medium is largely lethal to this strain, which lacks *RAD52* function because DSBs generated during mitotic growth cannot be repaired by homologous recombination. Cells can survive by deleting one of the *CEN3* sequences. The frequencies of deletions that removed one or the other *CEN3* are indicated. We have analyzed strains in which deletions of the inserted *CEN3* are confined within the *Bam*HI (B) fragment.

fragment (Fig. 4), of which we have examined 17. As before, the region of interest was PCR amplified and sequenced. The sizes of these deletions ranged from 233 to 760 bp, with a mean size of 343 bp (Fig. 1B). We note that these deletions represent a selected subset that retain the proximal *Bam*HI site and that many other deletions were substantially larger.

During mitotic segregation of the dicentric chromosome, the DSB presumably occurred between the two *CEN3* sequences (Fig. 4) and therefore to the right of the CDEIII element of the inserted *CEN3*. Although a deletion of only a few nucleotides of CDEIII would be sufficient to eliminate centromere function (5, 15, 39, 57), all of these deletions removed all three of the CDE elements constituting a yeast centromere (Fig. 1B). Thus, as with the HO-induced deletions, far more sequences are removed than are required to overcome lethality by eliminating centromere function. Under the assumption that the DSB occurred in the 160 bp between the *CEN3* CDEIII element and the right-hand *Bam*HI site, it appears that in most cases, more sequences were deleted to the left of the break site than to the right (Fig. 1B). However, by restricting our analysis to deletions contained within the *Bam*HI *GAL1*::*CEN3* fragment, we may have selected events in which the deletion was asymmetric around the break site.

The results of PCR amplification and DNA sequencing of the *CEN3* deletion junctions are presented in Fig. 3B, with nucleotide homologies underlined as described above. Of 17

Wild type *MAT*a

GCTTTCCGCAACAGTATAATTT

		number of examples
Deletions		
	GCTTTCCGC$_{A}$ACAGTATAATTT	1
Insertions		
	GCTTTCCGCAACA^{CA}GTATAATTT	6
	GCTTTCCGCAACA^{ACA}GTATAATTT	2

FIG. 5. Small duplicative insertions and a single-base pair deletion recovered in the *GAL1*::*HO MAT*a *rad52* strain J178-20T. The 4-bp 3′ overlapping ends of the HO cleavage site are underlined in the wild-type sequence. The deletion of a single base pair is indicated by the boldface subscripted base. The duplications of 2 or 3 bp at the HO cutting site are indicated by boldface superscripted letters.

deletion events, all but 2 had junctional homologies ranging from 1 to at least 7 nucleotides. The other two (7-04 and 1-3) had, respectively, 3 and 6 nucleotides of homology offset from the junctions by 1 bp.

Deletions in *GAL*::*HO* strains. The deletions created by HO endonuclease were generated in a strain background different from that used to analyze the survivors of dicentric chromosome breakage. Therefore, we undertook another experiment to compare the two types of deletion events in the same genetic background. We used a monocentric *rad52* haploid J178-20T strain isogenic to dicentric J178-#7-20 but carrying a plasmid-borne *HO* gene under the control of a *GAL* promoter (8, 24). Growth on galactose medium in the monocentric strain is almost always lethal, since the HO cut at *MAT* cannot be repaired by homologous recombination in a *rad52* background. Deletions around the HO cut site were isolated by plating cells on galactose-containing medium and by analyzing the survivors. In several experiments, the frequency with which survivors appeared was 1×10^{-4} to 4×10^{-4}. Deletions around the HO cutting site will leave strains either *MAT*a or *mat*a*1*, depending on the size of the deletion. These two types can be distinguished by mating them to *MAT*α tester strains, as described in Materials and Methods. A combination of Southern analysis and DNA sequence analysis (see below) of 50 galactose-insensitive survivors has revealed that they can be grouped into four classes. The distribution of deletion sizes in J178-20T *MAT*a *rad52 GAL*::*HO* derivatives is as follows (numbers are the numbers that were analyzed): for 1- to 3-bp deletions and small insertions, 36 (72%); 20- to 100-bp deletions, 9 (18%); 100-bp to 1-kb deletions, 4 (8%); >1-kb deletions, 1 (2%). Deletions of approximately 20 bp or larger could be resolved on Southern blots of *Sty*I-digested genomic DNA probed with a *MAT* distal-specific probe (61).

We have determined the deletion end points of eight randomly selected deletions that were detectable by Southern blot analysis (greater than approximately 20 bp) (Fig. 1C). They have an average size of 207 bp and have the same degree of shared homology at their junctions as did the centromere deletions made in the same strain background or the HO-induced deletions in a different background (Fig. 3C).

The majority of survivors (72%) make up a new class of small deletion or insertion events not observed in the *HO swi1 rad52* strain. We have PCR amplified and sequenced nine of the junctions of these events. As shown in Fig. 5, all of these deletions and insertions involved the 4 bp that are the site of the staggered 3′ overhanging cuts on the two

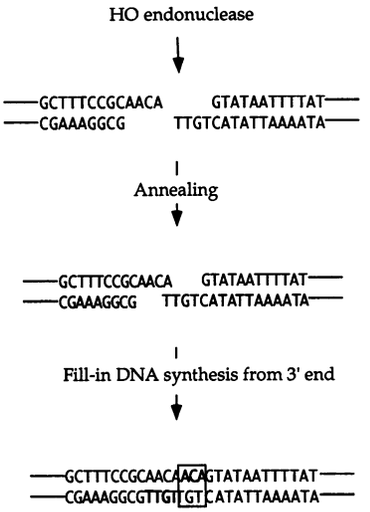

FIG. 6. Possible mechanism to create small duplications of the *MAT*a cutting site. The terminal bases at the 3′ ends of the HO cleavage site can anneal and permit primer extension from the 3′ ends to fill in the bases missing in the gap and create a 3-bp (ACA) insertion. If the terminal 3′ T of the lower strand is removed, the annealing of the adjacent T followed by primer extension will create a 2-bp insertion (CA). Deletion of several nucleotides from both ends and annealing can also give rise to the 1-bp deletion (606) seen in Fig. 5.

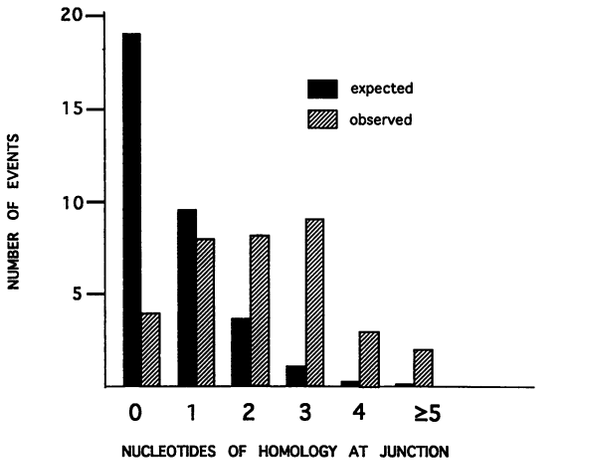

FIG. 7. Expected versus observed junctional homologies. The probability of finding X nucleotides of homology at the junction of a deletion was calculated by using the formula $P(X) = (X + 1)(1/4)^X(3/4)^2$ assuming unbiased base composition (48). The expected numbers are represented by black bars. The observed numbers (based on the 34 examples in Fig. 3) are represented by striped bars.

strands of the HO recognition site. One was a deletion of a single base pair; the others were insertions of 2 or 3 bp. These types of duplication events can best be explained by an annealing of complementary bases near the 3′ overhanging single-stranded ends, after which the 3′ end may be used as a primer to fill in the missing bases (Fig. 6). Such events have been well documented in vertebrate cells and in in vitro studies (25, 42, 49, 65).

DISCUSSION

We have examined the process of illegitimate recombination in *S. cerevisiae* resulting from a DSB generated in vivo by studying deletion events in recombination-deficient strains. On the basis of sequence analysis of the deletion junctions, we conclude that nucleotide homologies play a significant role in the end joining of the partner DNA molecules; 39 of 43 events had 1 to at least 7 bp of homology at the junctions. The presence of very short regions of DNA sequence identity at the end points of the deletions is statistically significant; this can be seen by calculating the random probability that any deletion event would contain X nucleotides of homology at a given junction, with unbiased base composition, based on the formula $P(X) = (X + 1)(1/4)^X(3/4)^2$ (48). This formula applies only to nucleotides exactly at the junction; therefore, for the purposes of this calculation, events such as the dicentric derivatives 7-04 and 1-3 are considered to have no junctional nucleotide homologies, even though homologies around the junction may have had a role in the end-joining event, as discussed below. The comparison between the number of expected and observed homologies, shown in Fig. 7, revealed that there are significantly more events with 2 or more nucleotides of homology than would be expected by random occurrence. This suggests that these homologies play a significant role in the joining of the DNA ends, possibly by an annealing event which stabilizes the partner molecules, allowing ligation. This small but significant number of homologous nucleotides at the junctions of deletions has also been found in mammalian cells (48).

We point out that in some cases, notably in the dicentric derivatives 7-54 and 7-59 (Fig. 3B), the homology at the deletion junction is discontinuous. Thus, we have assigned only 3 nucleotides of homology to those junctions, even though there is a total of 11 potential base pairings with two single-nucleotide interruptions. Three other events (7-04 and 1-3 [Fig. 3B] and 620 [Fig. 3C]) had 3 and 6 nucleotides of homology offset by 1 bp at the junction which may have participated in the end-joining event. Schiestl et al. (53) have reported similar observations in the insertion of nonhomologous DNA into *S. cerevisiae* and have suggested that such cases may have necessitated mismatch repair of the heteroduplex DNA which is an efficient process in *S. cerevisiae* (41, 44). The idea that longer, discontinuous regions of homology may help align sequences that are to be joined is also suggested from the homologous recombination studies described by Mézard et al. (33).

How, then, does *RAD52*-independent deletion repair of a DSB occur? Our previous studies have shown that the creation of a DSB by HO endonuclease is followed by extensive 5′ to 3′ exonuclease DNA degradation (14, 59, 61, 68) resulting in the formation of long 3′-ended single strands. In Rad^+ cells, such DSB can be repaired very efficiently by SSA between flanking homologous regions that can be as short as 30 to 60 bp (59, 60). In *rad52* cells, SSA is also possible, but only at much lower efficiency or when the flanking homologous regions are very long, as in rDNA repeats (14, 38, 43). In our present work, we find that at efficiencies of between 0.05 and 1%, single strands may also anneal in the absence of *RAD52* function. Here, there are but a few bases of complementary sequence that might act to stabilize the partner DNA molecules long enough to permit any necessary excision of nonhomologous sequences and complete ligation (Fig. 2). It is unclear whether the similarities between *RAD52*-dependent annealing of longer homologous regions and these *RAD52*-independent junctions of as

few as 1 bp of homology are a reflection of some common processes or whether they represent truly independent pathways. Recently, we have shown that a UV repair gene, *RAD1*, plays a crucial role in the removal of nonhomologous DNA from the 3′ ends of HO-cut molecules undergoing Rad$^+$ SSA and deletion formation (13). It will be interesting to determine whether the *RAD52*-independent deletion events that we have documented here also require *RAD1*. *RAD1* is not needed for integration of linear transformed DNA by illegitimate recombination (56).

An inspection of the current data also argues that there are several different pathways for nonhomologous recombination in *S. cerevisiae*. All of the systems used to study illegitimate recombination in *S. cerevisiae* have shown that most events were mediated through base pairing of at least a few nucleotides. However, both the integration of linear transformed nonhomologous DNA (53, 55, 56) and recombination between highly diverged genes of common function (33) have proven to be *RAD52* dependent, even though the junctions of recombination involve only a few base pairs. It is possible that the HO-induced deletion-repair events that we have studied also occur more frequently in *RAD52* cells; however, this is unlikely. The most compelling evidence that this type of deletion repair does not occur much more frequently in Rad$^+$ cells comes from studies of HO-induced attempts to switch the mating type in cells from which the two homologous donor sequences, *HML* and *HMR*, are deleted (26). In these studies, cells that suffer a DSB at *MAT* must either repair the break by nonhomologous means or die. Essentially, all cells died. Survivors, which arose at no more than a few percent, had generated base substitutions or small deletions at the HO cut site such as those reported here. This is similar to our results with *rad52* cells unable to complete recombination (66) and in Rad$^+$ strains that lacked mating-type donors (69).

We have found at least 1, and perhaps 4, of 43 deletions that appear to have resulted from blunt-end ligation when there was no homology at the junction. Such events have also been seen when nonhomologous transforming DNA integrates at sites that are likely to have been cleaved by topoisomerase I (53). It is unclear whether these events arise by still another pathway of repair. Ligation involving no homology can occur by two blunt-ended molecules coming together or by a blunt end and a 5′ protruding end coming together followed by 5′-3′ repair synthesis filling in of the 5′ overhang and ligation, as described elsewhere for mammalian cells (49).

The majority of the events that we have analyzed can be accounted for by the kind of process illustrated in Fig. 2. Studies of higher eucaryotic as well as in bacterial systems have reported deletions flanked by small direct repeats (7, 25, 28, 40, 47, 64). It has been estimated that roughly 60% of mammalian end-joining events involve short regions of nucleotide homology (49), including junctions of spontaneous deletions as well as those resulting from end joining of transformed or transfected DNA molecules (47). These types of events also occur in vitro in the presence of *Xenopus* egg cell extracts (65) and in human cell extracts (64). Taken together, these data suggest that there is a common nonhomologous deletion repair process that is similar in a wide variety of organisms ranging from bacteria to yeasts to mammals. Moreover, we have shown that the same kinds of events occur after DSBs are created in two quite different ways, one by a site-specific enzymatic cleavage and the other by mechanical rupture of DNA.

An unusual feature of the deletions of *CEN3*. The deletions described here are generally much larger than those that would be required to relieve lethality. In strains in which the deletion resulted from an HO cut, removal of a single base pair could have overcome the lethal condition. This is also the case in the monocentric derivatives of the dicentric strain. Because the events that we analyzed preserved a *Bam*HI site 160 bp to the right of *CEN3*, the initial rupture of the dicentric chromosome presumably occurred in that interval (Fig. 4). *CEN3* itself consists of a 125-bp sequence which can be subdivided into three elements: CDEI, CDEII, and CDEIII (5, 9, 39). Removal of CDEI plus parts of CDEII diminishes the quality of chromosome transmission significantly but does not abolish it. Removal of CDEIII totally abolishes CEN activity (39). Additionally, CDEIII is the only element in which single-base pair mutations have been shown to abolish CEN function (23, 32). In fact, a dicentric plasmid containing one normal CEN3 and another with a mutation in CDEIII does not exhibit dicentric behavior (27). In our experiments, *CEN3* is oriented so that the DSB should arise between CDEIII and the right-hand *Bam*HI site (Fig. 4). Surprisingly, although removal of only CDEIII should suffice, every deletion removed the entire centromere region (Fig. 1B). This suggests that the entire *CEN3* must be removed before an end-joining event can occur. *CEN* sequences are known to interact with protein components in a nuclease-resistant complex (3, 52). Perhaps the DNA ends within the *CEN* sequences are unavailable to participate in end joining because of their interactions with proteins, and only when sequences outside *CEN3* are uncovered can the deletion event occur. An alternative interpretation is that the breakage of dicentric chromosomes does not occur by simple mechanical rupture of DNA but is mediated by nucleases that cleave the stretched chromatin fiber, perhaps on both sides of the centromere.

Another type of DSB repair: creation of small duplications at the HO cleavage site. When *MAT* deletions were generated in a strain carrying a *GAL::HO* plasmid, the majority of galactose survivors belonged to a new class of insertion events (Fig. 5). These events were not seen when deletions of *MAT* were selected in the *HO swi1-1 rad52* strain. There are two possible explanations for this difference, both related to the expression of HO endonuclease from a galactose-regulated promoter. The first is that the galactose-induced HO gene is expressed in all phases of the cell cycle and in both mother and daughter cells, while the normal HO gene is tightly regulated and confined to the end of the G_1 phase of the cell cycle in mother cells (8, 24, 34). Possibly, the repair of HO-induced DSBs occurs by different pathways at different points in the cell cycle. Alternatively, the level of HO endonuclease may influence the repair process. Compared with normal HO gene expression, *GAL::HO* levels appear to be 100-fold higher. However, this is somewhat misleading, in that the gene is expressed in both mothers and daughters and throughout the cell cycle, instead of being confined to perhaps 10 to 20% of the cell cycle in mother cells. Thus, the level of HO endonuclease induced by galactose may be only 5 to 10 times what is normally present. The relative level of HO expression may be even lower in *HO swi1-1* cells; however, it has not been established whether the *swi1-1* mutation permits a low level of expression in most cells or a normal level of HO transcription in a few cells. It should be noted that even in galactose-induced cells, 28% of the survivors contain deletions that are virtually identical to those obtained in the *HO swi1-1* cells.

We believe that the mechanism for forming small duplications of nucleotides in the HO cleavage site is best explained

by the annealing and filling-in mechanism diagrammed in Fig. 6. This type of mechanism has been studied extensively in vertebrate cells by Pfeiffer and her colleagues (42, 65). Possibly, when HO endonuclease is present at higher levels, it helps hold the two ends of the cleaved DNA in proximity so that the offset base pairing can occur. However, it is difficult to see how HO endonuclease could remain bound while the small gaps in the annealed sequence were being filled in by a repair DNA polymerase. These considerations lead us to suspect that these novel duplication insertions arise by repair at a time in the cell cycle when HO is not normally expressed.

Deletions between very short homologous regions are rare in *S. cerevisiae*. In *S. cerevisiae*, the process of homologous recombination is so efficient that until recently it has been difficult to analyze illegitimate recombination. One might expect to see such events among spontaneous mutations, especially after ionizing radiation treatments, but in fact spontaneous deletions in *S. cerevisiae* are rare (11, 12). There is one interesting exception to the lack of deletions among spontaneous events. The telomere-associated Y′ element of *S. cerevisiae* exist in two forms (Y′ long and Y′ short) that differ from each other in about 1% of their nucleotide sequences (30). The size difference between the two forms arises from 11 small deletions, each of which appears to have arisen by recombination between flanking regions of 4 to 10 bp. Y′ elements are also unusual in that they are not found in many other members of the genus *Saccharomyces* and have some resemblance to viral elements (30). This may suggest that the deletions arose as a consequence of some unusual feature of the replication of these elements.

In summary, we have shown that two very different types of DSBs—one by a site-specific endonuclease cleavage and the other by mechanical breakage of DNA—are repaired by a similar mechanism in the absence of the recombination gene *RAD52*. The types of deletions that we described are reminiscent of analogous events described in many organisms, ranging from bacterial cells to higher eucaryotes (6, 25, 47, 65). The ability to analyze the genetic control of these events in yeasts, as we have done for HO-induced homologous recombination events (14, 59), should make it possible to determine what gene products are required for illegitimate recombination.

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