Chromosome Break-Induced DNA Replication Leads to Nonreciprocal Translocations and Telomere Capture

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ABSTRACT

In yeast, broken chromosomes can be repaired by recombination, resulting in nonreciprocal translocations. In haploid cells suffering an HO endonuclease-induced, double-strand break (DSB), nearly 2% of the broken chromosome ends recombined with a sequence near the opposite chromosome end, which shares only 72 bp of homology with the cut sequence. This produced a repaired chromosome with the same 20-kb sequence at each end. Diploid strains were constructed in which the broken chromosome shared homology with the unbroken chromosome only on the centromere-proximal side of the DSB. More than half of these cells repaired the DSB by copying sequences distal to the break from the unbroken template chromosome. All these events were RAD52 dependent. Pedigree analysis established that DSBs occurring in G1 were repaired by a replicative mechanism, producing two identical daughter cells. We discuss the implications of these data in understanding telomerase-independent replication of telomeres, gene amplification, and the evolution of chromosomal ends.

Most linear eukaryotic chromosomes terminate in telomeres. Telomeres are specialized structures that protect the ends of eukaryotic chromosomes from degradation and end-to-end joining (Müller 1938; McClintock 1939, 1941). Telomeres may also participate in meiotic and mitotic chromosome segregation (Cenci et al. 1997; Chua and Roeder 1997; Conrad et al. 1997; Kirk et al. 1997). Telomere loss renders a chromosome unstable and may eventually lead to chromosome loss (Kramer and Haber 1993; Sandell and Zakian 1993). Therefore, maintenance and/or replication of telomeres is crucial for the survival of a cell. Evolution has provided most eukaryotic cells with a ribonucleoprotein enzyme, telomerase, that maintains telomeres by its ability to add telomeric repeats onto the 3′ ends of preexisting telomeres (for review see Greider 1996; Melik and Shippen 1996; Zakian 1997). In Saccharomyces, deletion of telomerase components EST1 (Lundblad and Szostak 1989) or TLC1 (Singer and Gottschling 1994) leads to cell death by slow senescence, in which telomeres progressively become shorter over many cell divisions.

Telomerase can also establish de novo telomeres. In ciliates, this process occurs very efficiently at certain developmental stages (reviewed in Melik and Shippen 1996), while in Saccharomyces, de novo telomere formation at ends lacking telomere sequences occurs at a low frequency (Pluta and Zakian 1989; Lustig 1992; Kramer and Haber 1993; Sandell and Zakian 1993).

Telomeres can also be acquired through recombination. Walsley et al. (1984) advanced the idea that telomere maintenance could occur by recombination-dependent DNA replication. Dunn et al. (1984) first showed that Saccharomyces cerevisiae telomeres could be added to telomere-adjacent Y′ sequences present at the end of a linear molecule that was transformed into cells. In a similar fashion, Vollrath et al. (1988) created fragmented chromosomes by demanding that recombination add the distal segments of chromosomes to a transformed fragment. RAD52-dependent recombinational acquisition of telomeres also plays a decisive role in the repair of yeast lacking key telomerase components such as Est1p (Lundblad and Blackburn 1993). In survivors of telomerase-negative cells, recombination can apparently occur between TG13 telomere sequences themselves or between subtelomeric sequences such as X and Y′. In some cases, est1 survivors have added other still-unknown sequences to their chromosomal ends, but in all cases, they appear to have maintained telomere sequences at the very termini. In another yeast, Kluyveromyces lactis, disruption of the telomerase RNA gene TE1 also results in a similar senescent phenotype, and cells are also rescued by a RAD52-dependent mechanism (McEachern and Blackburn 1996).

The acquisition or maintenance of telomeres by recombination might occur by a recombination-dependent replication mechanism analogous to late DNA replication in bacteriophage (Luder and Mosig 1982; Formosa and Alberts 1986; Mosig 1987) or to origin-independent DNA synthesis in Escherichia coli (reviewed...
in Kogoma 1996) and telomere replication in Streptomyces (Asai et al. 1994; reviewed in Chen 1996). A similar mechanism, termed break-induced replication, has been invoked to explain RAD52-dependent, RAD51-independent repair of broken chromosomes in yeast (Malkova et al. 1996). Butler et al. (1996) and Morrow et al. (1997) have also presented evidence favoring break-copy DNA replication in Saccharomyces. However, in previous experiments involving linearized plasmid DNA, it has not been possible to rule out the view that such repair events arise from a nonreciprocal crossover (Haber and Earn 1985) such that the broken chromosome is repaired by acquiring the end of another chromosome, while the distal end of the broken chromosome and the now-broken donor chromosome remain unrepaired. Mitotic separation of chromosomes could then produce one viable cell with a repaired chromosome containing an apparently nonreciprocal translocation and an inviable sister cell. In this article, we present direct evidence that repair of a broken chromosome occurs by a replicative mechanism. This type of DNA end repair can also explain several features of the evolution of chromosome ends.

MATERIALS AND METHODS

Plasmid constructs: Plasmid pGB3 is derived from pGEM3 (Promega, Madison, WI) that has the BamH I site destroyed by Klenow fill-in. Plasmid pCW9-1 contains a BamHI fragment from HML with internal Xho I sequence replaced by a yeast LEU2 Xho I-Sall fragment. Plasmid pGB1 was constructed by first purifying the BamH I-hml::LEU2 fragment from pCW9-1, digesting it into two fragments with Xho I, and ligating both fragments to a Sall-linearized pGB3. pGB6 was constructed by deletion of a 1.94-kb Sphi to EcoRI fragment of the LEU2 gene from pGB1 and blunt-end recircularization. A 2.4-kb HindIII-Clal fragment from pKK154.HO2 contains a URA3 gene, 13 repeats of the Tetrahymena telomere DNA (T. G. 13), and a 117-bp HO endonuclease cut site placed 300 bp from (T2G4)13. pGB7 was constructed by inserting a blunt-ended, 2.4-kb HindIII-Clal fragment from pKK154.HO2 into a Smal site of pGB6. Orientation of fragments in pGB1 and pGB7 were confirmed by DNA sequencing of the cloned junctions.

Yeast strains: Strain YGB8 (ho trp1 leu2 ade1 lys5 ura3 thr4 cys1 rta12 HMLa MATa inc HMRa inc) was made by an integration and excision of a TRP1 deletion using a URA3-marked plasmid pH449 in strain TNR238-6C. YGB30 was constructed by transplacing BamH I-linearized pGB1 into YGB8, thereby deleting HMLa. YGB30 was used to construct YGB34 by integrating HindIII, BstXI-linearized pGB7. Integrations were confirmed by Southern blot analysis of stable Ura+ transformants. YGB61 was made RAD52 deficient by transplacement with a THR4-marked disruption of RAD52 (BamH I fragment from pNSU165) in YGB34. Thr+ transformants were confirmed to be rad52 by methyl methanesulfonate sensitivity (0.015% methyl methanesulfonate; Aldrich Chemical Co., Milwaukee) and failure to complement known rad52 cells. All strains were determined to be Gal+ by their ability to grow on galactose under anaerobic conditions. YGB65 was made MATα inc by integrating the MATα inc-containing plasmid pH32 at the MATα inc locus of YGB30, by selecting Ura+ nonrevertor clones and by subsequent selection of α-mate-resistant 5-fluoroorotic acid (5-FOA)-resistant isolates (Boeke et al. 1984). YGB80 was constructed by integrating HindIII, BstXI-linearized pGB7. Diploid strain YGB85 was constructed by mating YGB80 to a MATα inc survivor (YGB116) that had healed broken chromosome III by nonreciprocal translocation of HMRα inc onto the left arm of chromosome III. Diploid strain YGB87 was constructed by mating YGB80 to a MATα inc survivor (YGB157) that had healed a broken chromosome III by a de novo telomere addition 18 bp proximal to the HO cleavage site (HOcs).

Induction of double-strand breaks in vivo: The plasmid pFH800 (Nickoloff et al. 1986) is a centromeric, TRP1-marked shuttle vector that carries a galactose-inducible H0 endonuclease gene. Cells were grown to ~1010 cells/ml in 5 ml of synthetic complete medium lacking tryptophan (SC-TRP) and typically diluted to 1-5 × 108 cells/ml in 5 ml of YEP-glycerol medium, as described previously (Rudin and Haber 1988). YEP-glycerol cultures were allowed to grow from 5 to 20 hr. Cultures were serially diluted in sterile water and plated onto dextrose or galactose SC-TRP plates. Because only cells containing the TRP1-marked GAL::H0 plasmid are selected, we assume that all colonies from galactose SC-TRP plates have induced a double-strand break (DSB). All plates and cultures were incubated at 30°C.

Molecular analysis of DSB repair events: Genomic DNA from all yeast was prepared by glass bead breakage and phenol extraction (Rudin and Haber 1988). All electrophoreses of genomic DNA were performed in 0.8% agarose 1× TAE gels. Enzymes used in all experiments were purchased from New England Biolabs (Beverly, MA). Southern hybridizations were done in 6× SSC, 5× Denhardt’s, 0.1% SDS, and 0.1 ng/ml sheared carrier DNA. Probes were made by random primer extension and hybridized at 65°C. pGEM3 DNA or its equivalent was used for probes in determining HindIII and BamHI restriction fragments from healed chromosomes. Healed break junctions were cloned in E. coli by plasmid rescue of genomic DNA: 5-50 μg of genomic DNA was digested with HindIII (~20–80 units) in 1× NBE (New England Biolabs) buffer #2 (200 ml final volume) overnight at 37°C. Digested DNA was blunted ended by adding 1 unit of T4 DNA polymerase, 1 unit of Klenow, and dNTPs (0.1 mm each final) directly to the 200-ml digestion mix and incubated overnight at 16°C. The ligation mix was transformed into MC1061 bacterial cells by electroporation, and transformants were selected on LB ampicillin plates. To discount cloning artifacts, two individual bacterial clones for each of the YGB34 and YGB61 healed events were analyzed by DNA sequencing. Sequencing was performed on double-strand plasmid DNA using the fmol DNA sequencing system (Promega) according to the manufacturer’s instructions.

Analysis of broken chromosomes in G1 cells and one generation pedigree: YGB85 cells were grown to ~109 cells/ml in 5 ml of SC-TRP liquid medium to maintain plasmid pFH800. Cells were diluted to ~109 cells/ml in 500 ml of YEP-glycerol (3% glycerol, w/v) and grown overnight at 30°C with vigorous agitation. Galactose was added to a final concentration of 2% (w/v), and cells were allowed to incubate at 30°C for 1 hr. An aliquot was removed and streaked on a YEPD plate. Unbudded G1 cells were picked by micromanipulation and placed at known coordinates. G1 cells were monitored over time, and sister cells were separated by micromanipulation after the first cell division was completed. Sister colonies were allowed to grow on YEPD (with no selection) and were scored phenotypically by replica-plating onto standard leucine and uracil drop-out plates. These colonies were also tested for mating type.

RESULTS

Repair of a broken chromosome in haploid yeast by intrachromosomal telomere capture: An H0 endonuclease-induced DSB was created on the left arm of chromo-
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Figure 1.—Healing of broken chromosome III in YGB34 by telomere capture. (A) The structure of the relevant regions of chromosome III is illustrated. HML has been completely replaced by an 8-kb HindIII construct that contains LEU2, a HOcs, and URA3 DNA. The HOcs is a 117-bp fragment derived from MATa. Both MATa-inc and HMRa-inc contain a 1-bp mutation of their HO recognition sites that prevents cleavage by HO endonuclease. The mutant HOcs at the HMRa-inc locus is ~20 kb from the right telomere. The white circle represents the centromere. (B) HO-induced chromosome breakage produces a 72-bp centromere-proximal end that shares perfect homology with the HOcs at MATa-inc and at HMRa-inc. (C) This 72-bp homologous end is able to initiate homologous recombination with HMRa-inc and capture the ~20 kb from HMRa-inc to the right telomere. (D) The resulting recombinant chromosome contains a nonreciprocal translocation of the right arm end onto the left arm. The recombinant chromosome creates a novel 5-kb HindIII fragment.

some III in strain YGB34. In this strain, the region containing HML was deleted and replaced by a LEU2 gene, an HO endonuclease recognition site, and the URA3 gene (Figure 1). There are no essential genes distal to this insertion. HO cleavage produced a broken chromosome with an ~12-kb, telomere-containing fragment containing LEU2 and with the fragment containing the rest of the chromosome, terminating in a broken end 300 bp distal to URA3 (Figure 1). Cells that fail to repair the DSB will die, as essential genes centromere proximal to the URA3 gene are degraded and lost. Those that survive must have restored a telomere to the end, before the first essential gene. This can happen in several ways. The broken ends could rejoin in a fashion that destroys the HO endonuclease recognition site (Kramer et al. 1994; Moore and Haber 1996), or a new telomere could be added to the end of the broken chromosome (Kramer and Haber 1993; Sandell and Zakian 1993). Alternatively, a nonreciprocal translocation could produce a new chromosome end.

Plating strain YGB34 on galactose-containing medium resulted in 95% cell death compared to plating on glucose (Table 1). There is no difference in viability when cells lacking the GAL::HO gene are plated on these two media. Among the survivors, 60% of them (3% of total cells) were still Leu−Ura+ (Table 2). These could have rejoined the broken ends either by nonhomologous end joining or by repairing the 117-bp HOcs by recombination (gene conversion) with a homologous MATa-inc or HMRa-inc sequence. Both of these a-inc sequences contain a single base pair mutation that cannot be cleaved by HO (Weiffenbach and Haber 1981). As shown below, nearly all of these Leu−Ura+ events depend on RAD52 and, hence, are most likely the result of homologous recombination. Another 35% of the viable colonies (1.7% of total cells plated) were Leu−Ura2 and either could have acquired a new telomere distal to the URA3 gene or could have been repaired by some other means. The remaining classes of Leu−Ura− and Leu+Ura− cells were not analyzed.

Thirty-eight Leu−Ura+ colonies were characterized by Southern blot analysis. Thirty-five (~92%) were

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colony-forming units (cfu)</th>
<th>Dextrose</th>
<th>Galactose</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>YGB34 (Rad+)</td>
<td>4 exp.</td>
<td>7.1 × 10⁴</td>
<td>3.6 × 10⁴</td>
<td>5</td>
</tr>
<tr>
<td>YGB61 (rad52)</td>
<td>4 exp.</td>
<td>41.9 × 10⁴</td>
<td>4.6 × 10⁴</td>
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<tr>
<td>YGB85 (Rad+)</td>
<td>3 exp.</td>
<td>5.7 × 10²</td>
<td>5.6 × 10²</td>
<td>98</td>
</tr>
<tr>
<td>UGB87 (Rad+)</td>
<td>3 exp.</td>
<td>8.9 × 10²</td>
<td>5.8 × 10²</td>
<td>65</td>
</tr>
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</table>

Cells were diluted in sterile H2O and plated on SC-TRP dextrose and SC-TRP galactose. Growth on galactose induces expression of HO endonuclease (see materials and methods) and causes a DSB (see Figure 1). Cells that survive must have repaired the broken chromosome because loss of a chromosome in haploid cells is lethal. Three or four experiments (exp.) were done for each strain. Survival was calculated by the following formula: % survival = [cfu on galactose/ cfu on dextrose] × 100.
found to have identical terminal restriction fragments when DNA was digested with either HindIII or BamHI (Figure 2). Three of these were cloned by plasmid rescue (see materials and methods) and propagated in E. coli. Sequence analysis revealed that all three appeared to have engaged in homologous recombination between the 72 bp of the HO recognition site centromere proximal to the DSB and the nearly identical sequences in the opposite chromosomal orientation at the silent-mating-type donor locus HMRα-inc on the right arm of chromosome III (Figure 1). HMRα-inc contains a 1-bp substitution that prevents HO cleavage (Weiffenbach and Haber 1981), so the resulting chromosomal rearrangement is also resistant to HO cutting. Thus, it appears that most of these chromosome breaks had been repaired by homologous recombination that yielded a partial duplication of the sequences at the right end of the chromosome, now at the left end.

Of the 38 Leu⁻ Ura⁺ survivors that were tested, 2 gave HindIII restriction patterns consistent with the addition of a new telomere between the HOcs and the URA3 gene (Figure 2, lanes 4 and 15). The remaining single survivor of the 38 tested exhibited a HindIII fragment that was close in size to the uninduced parent and was not analyzed further (Figure 2, lane 3).

We confirmed that Leu⁻ Ura⁺ rearrangements containing an isochromosomal end depended on the RAD52 gene, which is required for homologous recombination. In the rad52-derivative strain YGB61, both the Leu⁺ Ura⁺ and Leu⁻ Ura⁻ classes of events were essentially eliminated (Table 2). Southern analysis of rare rad52 Leu⁻ Ura⁺ survivors shows that none of 40 tested had healed by recombination with HMRα-inc or MATα-inc; instead, their URA3-homologous restriction fragments had the diffuse migration behavior characteristic of telomere-containing ends (data not shown). Healed chromosome junctions from two independent rad52 survivors were cloned by plasmid rescue in E. coli (see materials and methods). DNA sequence analysis confirmed that these rad52 chromosomes healed by de novo telomere addition in the 300-bp region between the HOcs and the URA3 gene (data not shown). Therefore, the majority of Leu⁻ Ura⁺ events in wild-type YGB34 cells were repaired through RAD52-dependent homologous recombination between a 72-bp sequence from the HOcs introduced on the left arm of chromosome III and the mutant α-inc cut site at the silent HMRα-inc locus, yielding a nonreciprocal translocation that duplicated ~20 kb from the right arm of the same chromosome (Figure 1C). This process is at least 100-fold more efficient than de novo telomere formation (Table 2).

**Broken chromosomes can recombine efficiently with interchromosomal targets to acquire new telomeres:** In haploid cells, we showed that a very small (72-bp) region of homology was sufficient to recombine with an intrachromosomal target, although this could also have involved a sister chromatid in G2 cells. To determine if interchromosomal targets were used efficiently, we constructed a Rad⁺ diploid strain YGB85 (Figure 3A) in which significant homology between the broken chromosome and the donor chromosome is present only on the centromere-proximal side of the ensuing DSB. This was accomplished by mating a MATα-inc strain carrying the LEU2-HOcs-URA3 construct inserted in place of HML with a MATα-inc Leu⁻ Ura⁺ derivative of YGB34 that had a nonreciprocal translocation of sequences from the right arm of chromosome III at the left arm. Hence, there are only 45 bp distal to the HOs that are homologous with the second chromosome III (Figure 3B). In this diploid, chromosomal breaks leading to Leu⁻ Ura⁺ nonmater survivor colonies can arise in three ways. First, the broken chromosome can initiate recombination with its homolog, thereby making all sequences distal to the break homozygous (Figure 3C). Second, the broken chromosome could recombine with the HMRα-inc locus at the other end of the same chromosome. Third, the broken chromosome could be healed by the addition of a new telomere at any point proximal to the break site, making all sequences distal to the healing site hemizygous (Figure 3D).

HO endonuclease was induced by plating cells on galactose-containing plates. In this diploid strain, 98% of all cells survived the induction of a DSB, 68% of which were Leu⁻ Ura⁺ nonmating (MATα-inc/MATα-inc) diploids (Table 2). The nonmating phenotype confirms that both MATα-inc and MATα-inc are present, and it indicates that both homologs of chromosome III are present. Therefore, the majority of Leu⁻ Ura⁺ survivors have not lost the broken chromosome. Most of the remaining colonies were Leu⁺ Ura⁺ and most likely resulted from homologous gene conversion of the cleavage site to the α-inc sequence, either from HMRα-inc or from the opposite chromosome.
In this diploid, the two chromosomes differ in the size of HindIII and BamH1 restriction fragments containing the URA3 gene (Figure 3A). The difference in size of the BamH1 fragment is caused by the presence of a BamH1 polymorphism 212 bp proximal to the chromosomal break site. The difference in size of the HindIII fragment reflects a polymorphism 2.5 kb distal to the break site. The BamH1 RFLP allows for physical detection of the two polymorphic chromosomes before and after the break is healed and, therefore, is diagnostic for the presence of sequences immediately proximal to the break. To determine whether or not the broken chromosome from Leu\(^{-}\) Ura\(^{+}\) nonmater survivors had retained the BamH1 URA3-containing fragment, 20 survivors were tested by Southern blot analysis. Six of 20 Leu\(^{-}\) Ura\(^{+}\) nonmater derivatives had not lost this marker (Figure 4A), thus confirming that they had healed the broken chromosome at some position distal to the BamH1 site. These 6 survivors could have healed the broken chromosome by addition of a new telomere distal to the BamH1 site (Figure 3D) or by break-induced replication using its homolog as a template (Figure 3C). If survivors had healed the broken chromosome by de novo telomere addition, then HindIII fragments should also be different, and one of them should differ from the parent chromosome that originally carried the HOcs. If, on the other hand, they have repaired the broken chromosome by recombination and nonreciprocal translocation, then the HindIII fragments of both chromosomes should be identical. All six diploids still heterozygous for the BamH1 site were homozygous for the HindIII site, demonstrating that they had repaired by recombination, not by new telomere addition (Figure 4B).

The remaining 13 Leu\(^{-}\) Ura\(^{+}\) nonmater survivors have lost both the BamH1 and HindIII RFLPs (Figure 4, A and B). This can be explained in two ways. The broken chromosome could have been healed by the addition of a new telomere proximal to the HindIII site, thereby deleting the URA3 gene, or it could also result from nonreciprocal recombination. As described below, further analysis of similar diploids suggests that they all have arisen by nonreciprocal recombination.

**Repair of a broken chromosome by a replicative mechanism:** The acquisition of a new chromosome end by homologous recombination resulting in a nonreciprocal translocation can be imagined to occur in two ways (Figure 5). First, a centromere-proximal end of the broken chromosome could invade a homologous template and initiate DNA replication to the end of the template chromosome (Figure 5A; Walmsley et al. 1984; Malkova et al. 1996; Morrow et al. 1997). This could happen in either the G1 or G2 stage of the cell cycle. Alternatively, the repair might involve a nonreciprocal exchange of sequences, such that the recipient chromosome acquired an intact end, while the broken telomere-containing end was segregated with the unrepaired donor chromosome (Figure 5B). In G1 cells, this might be expected to yield a diploid monosomic for a recombined chromosome III. Such events have been documented in RAD52-independent spontaneous recombination on the left arm of chromosome III (Haber and Hearn 1985) and in RAD52-dependent, RAD51-independent repair of broken chromosomes (Malkova et al. 1996). In G2 cells, a similar event could yield a diploid with two identical intact ends after segregation of chromatids.

To distinguish between these two possible mechanisms, HO endonuclease was induced in a liquid culture of diploid YGB85, cells were plated on YEPD, where HO was no longer induced, and then unbudded (G1) cells were selected. The cells were monitored for viability, and after they had divided once, the mother and daughter cells were separated and moved apart so that each

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**Table 2**

<table>
<thead>
<tr>
<th>Haploid strain</th>
<th>Cells tested</th>
<th>% survival</th>
<th>% Leu(^{-}) Ura(^{+})</th>
<th>% Leu(^{-}) Ura(^{+})</th>
<th>% Leu(^{-}) Ura(^{-})</th>
<th>% Leu(^{-}) Ura(^{-})</th>
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<tr>
<td>YGB34</td>
<td>1710</td>
<td>5</td>
<td>1.7 ± 0.15</td>
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<td>YGB61</td>
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<td>0.032 ± 0.003</td>
<td>0.076 ± 0.006</td>
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<tr>
<th>Diploid strain</th>
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<th>% survival</th>
<th>% Leu(^{-}) Ura(^{+})</th>
<th>% Leu(^{-}) Ura(^{+})</th>
<th>% Leu(^{-}) Ura(^{-})</th>
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<td>YGB85</td>
<td>400</td>
<td>98</td>
<td>68 ± 3</td>
<td>19 ± 2</td>
<td>10 ± 1</td>
<td>1.0 ± 0.3</td>
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<td>YGB87</td>
<td>400</td>
<td>65</td>
<td>49 ± 3</td>
<td>7.0 ± 0.9</td>
<td>8.0 ± 0.7</td>
<td>0.5 ± 0.06</td>
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Distribution of healed events in haploid and diploid cells. YGB61 is a rad52 derivative of YGB34, which is illustrated in Figure 1. The structure of the relevant regions of chromosome III of YGB85 (Rad\(^{+}\)) is shown in Figure 3. The arrangement of sequences in YGB87 is similar to that in YGB85, except that the donor chromosome contains a new telomere sequence added 18 bp centromere-proximal to the HO-cut site on the top chromosome. "Cells tested" represents the actual number of survivors that were tested for leucine and uracil auxotrophy and mating type. Survival was calculated as described in Table 1.
could grow into a colony (Figure 6A). Of 15 G1 cells that were analyzed, all gave rise to viable pairs of sister cells that grew into colonies. This is consistent with the high level of survival obtained in the previous experiment (Table 1). All 15 pairs of sister colonies were Leu- Ura+ and nonmating, confirming that they had not undergone chromosome loss. We expected that ~30% (~5 of the 15) would not fall into this phenotypic class because the distribution of events for an asynchronous population only gave 68% Leu- Ura+ nonmater cells (Table 2). It is possible that this bias may be caused by the fact that we have specifically selected G1 cells in which a DNA repair may differ in S, G2, and M phase cells. Thirteen pairs were checked by Southern analysis for loss of the HindIII RFLP distal to the break site (cf. Figure 4B). In 12 of 13 pairs, both mother- and daughter-derived colonies had become homozygous for the HindIII fragment present on the unbroken homologue (Figure 6B). The one remaining pair of cells had not repaired the G1 break identically (Figure 6B). One sister had repaired by recombination, while the other sister seems to have undergone de novo telomere addition because it is identical in size to a previously characterized, newly formed telomeres. Thus, in 12 of 13 cases, the repair event produced two identical progeny in which high level of survival obtained in the previous experiment (Table 1). All 15 pairs of sister colonies were Leu- Ura+ and nonmating, confirming that they had not undergone chromosome loss. We expected that ~30% (~5 of the 15) would not fall into this phenotypic class because the distribution of events for an asynchronous population only gave 68% Leu- Ura+ nonmater cells (Table 2). It is possible that this bias may be caused by the fact that we have specifically selected G1 cells in which a DNA repair may differ in S, G2, and M phase cells. Thirteen pairs were checked by Southern analysis for loss of the HindIII RFLP distal to the break site (cf. Figure 4B). In 12 of 13 pairs, both mother- and daughter-derived colonies had become homozygous for the HindIII fragment present on the unbroken homologue (Figure 6B). The one remaining pair of cells had not repaired the G1 break identically (Figure 6B). One sister had repaired by recombination, while the other sister seems to have undergone de novo telomere addition because it is identical in size to the previously characterized, newly formed telomeres. Thus, in 12 of 13 cases, the repair event produced two identical progeny in which high level of survival obtained in the previous experiment (Table 1). All 15 pairs of sister colonies were Leu- Ura+ and nonmating, confirming that they had not undergone chromosome loss. We expected that ~30% (~5 of the 15) would not fall into this phenotypic class because the distribution of events for an asynchronous population only gave 68% Leu- Ura+ nonmater cells (Table 2). It is possible that this bias may be caused by the fact that we have specifically selected G1 cells in which a DNA repair may differ in S, G2, and M phase cells. Thirteen pairs were checked by Southern analysis for loss of the HindIII RFLP distal to the break site (cf. Figure 4B). In 12 of 13 pairs, both mother- and daughter-derived colonies had become homozygous for the HindIII fragment present on the unbroken homologue (Figure 6B). The one remaining pair of cells had not repaired the G1 break identically (Figure 6B). One sister had repaired by recombination, while the other sister
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Figure 5.—Two alternative models for nonreciprocal translocations. (A) A broken chromosome invades a homologous target only with its centromere-proximal end and initiates DNA synthesis (dashed arrow). Leading strand repair synthesis is converted into semiconservative DNA replication where both leading and lagging strands are synthesized. Replication proceeds to the end of the chromosome, thereby copying all sequences from the invasion point to the telomere itself without any damage to the template chromosome. (B) Alternatively, nonreciprocal translocations can also arise by half crossover events (Haber and Hearn 1985). This nonreciprocal exchange proceeds such that the recipient chromosome acquires an intact end from its donor, while the donor chromosome assumes the break and is eventually degraded.

Figure 4.—Southern analysis of YGB85 Leu− Ura+ nonmater survivors. (A) DNA from uninduced YGB85 (lane 1) and 20 independent healing events digested with BamHI and probed with pGEM3 DNA. Six of 20 healed chromosomes have retained the BamHI 4.8-kb parental fragment (lanes 2, 3, 5, 9, 10, and 12), confirming that these cells indeed have at least two copies of the left arm of chromosome III. Three of 20 healed events (lanes 6, 7, and 17) acquired novel 4.9-kb BamHI fragments, and the remaining (11 of 20) events have eliminated the parental BamHI 4.8-kb fragment without acquiring a novel fragment. The pFH800 plasmid gives rise to a 7-kb BamHI fragment. (B) DNA from uninduced YGB85 (lane 1) and the same 20 independent healing events described in A were digested with HindIII and probed with pGEM3 DNA. The same six survivors that contained healed chromosomes that retained the BamHI 4.8-kb parental fragment (A) have lost the 8-kb HindIII parental fragment from the broken chromosome. They also exhibit a 5-kb HindIII fragment from the unbroken chromosome (lanes 2, 3, 5, 9, 10, and 12). This strongly suggests that the broken chromosome healed by telomere capture events where sequences to the left of the break are homozygous for those of the unbroken homolog (cf. Figure 3C). Lanes 6, 7, and 17 give 4.1-kb HindIII fragments that are presumed to reflect new telomere addition. The remaining 11 of 20 events have eliminated the parental HindIII 8-kb fragment without acquiring a novel fragment. The pFH800 plasmid gives rise to a 3.1-kb HindIII fragment.

repair had occurred by nonreciprocal recombination. Each gave rise to four viable spores, all of which were Ura+ (data not shown). Therefore, the loss of the HindIII RFLP is indicative of chromosome healing by recombination-dependent replication.

Telomere capture events tolerate 3'-nonhomology at the break site: Spontaneous chromosomal breaks may not always have perfect homology between the very end of the break and the homologous target. Therefore, we tested whether nonhomology at the chromosomal break site would inhibit interchromosomal recombination-dependent replication. Diploid strain YGB87 (Rad+) contains one copy of chromosome III derived from YGB81, which contains a de novo telomere added 18 bp proximal to the HOs, and the second chromosome III contains an HOs and is identical in structure to that of YGB85. Thus, there is no homology distal to the HOs. The first 18 bp at the centromere-proximal end of the DSB are also not homologous to the unbroken chromosome and must be removed before DNA synthesis can initiate. YGB87 is also heterozygous for a URA3-containing HindIII RFLP. In YGB87, 49% of all cells with broken chromosomes had healed as Leu− Ura+ nonmaters, compared to 68% in YGB85 (Table 2). The major difference in survival between YGB85 and YGB87 appears to be a reduction in gene conversion events that replace the HOs with a-inc sequences because YGB87 lacks such a donor sequence on the homologous chromosome. There was no significant difference in the proportion of Leu− Ura+ nonmater cells. Southern analysis of 20 Leu− Ura+ nonmaters showed that all were homozygous for the HindIII RFLP of the unbroken chromosome (data not shown). Thus, the presence of an 18-bp heterology at the end of the broken chromosome had no significant effect on the repair of the chromosome by break-induced replication (BIR).

DISCUSSION

A DSB in yeast cells can be repaired in several different ways. If the DSB occurs in a region that shares homology on both sides, most events are repaired by gene conversion (Szostak et al. 1983). In a haploid, this condition is fulfilled in G2 cells and in special cases, such as yeast mating-type gene switching, where the HO-cut locus has two distant homologous donor sequences. If the DSB is flanked by homologous sequences, repair
can occur by single-strand annealing (Fishman-Lobell and Haber 1992). In the absence of any homologous sequences, or when homologous recombination is prevented by mutations such as rad52, repair can also occur, albeit rarely, either by nonhomologous end joining or in telomere addition (Pluta and Zakian 1989; Lustig 1992; Kramer and Haber 1993; Sandell and Zakian 1993; Kramer et al. 1994; Moore and Haber 1996).

If, however, there is homology only on one side of the DSB, alternative forms of repair become possible. This type of repair has been described previously and called break-induced replication (Malkova et al. 1996). With as little as 72 bp of homology centromere-proximal to the DSB, nonreciprocal translocations arise at frequencies ≈100 times higher than de novo telomere formation. These events are RAD52 dependent. Our results are similar to previous studies of the acquisition of new chromosome ends after transformation of linearized DNA into yeast (Dunn et al. 1984; Vollrath et al. 1988; Morrow et al. 1997). Following an earlier suggestion of Walmsley et al. (1984), it has been suggested that the addition of the distal end of a chromosome to these fragments occurred by a break-copy mechanism of DNA replication (Morrow et al. 1997).

Previous experiments studying telomere acquisition by recombination could not actually distinguish between a recombination-dependent replication mechanism and a reciprocal exchange of chromosome ends in the G2 stage of the cell cycle that would have produced one viable cell and one inviable chromosome. By pedigree analysis, we have shown for the first time that a cell with a DSB in G1 produced two surviving cells, both of which had the same rearranged chromosome end. This is a direct demonstration that the mechanism of repair occurs by a replicative mechanism that is quite efficient in wild-type cells when there is extensive homology proximal to the break. Moreover, 18 bp of nonhomology at the break site does not significantly impair this type of repair.

The results obtained in wild-type cells are also consistent with a previous analysis of broken chromosome repair in the absence of the RAD51 gene (Malkova et al. 1996). While RAD52 is required for this process, RAD51 is important but not essential. About 10% of cells at each division were estimated to be able to repair chromosomes by BIR, apparently by replicating >100 kb of DNA from the site of the DSB to the chromosome end (Malkova et al. 1996). In that case, too, repair must occur using homologous sequences that were >650 bp proximal to the DSB. It is not yet known how the length of the DNA to be replicated affects the efficiency of these events. Whether similar events occur in wild-type cells was not established.

It is interesting to note that while many survivors were obtained that had recombined using the 72 bp of homology between the unexpressed HMR a-inc locus, with its heterochromatic chromatin structure, none of the survivors had recombined with identical sequences at the more accessible MAT a-inc locus. As noted above, Malkova et al. (1996) found that BIR events could emanate from MAT and extend all the way to the end of the chromosome, at least in rad51 cells. It is possible that the >100 kb that must be copied from MAT a-inc is much more difficult to copy than the terminal 20 kb in events that initiate at HMR a-inc.

**Rearrangements at chromosome ends may be explained by BIR:** The recombinant chromosomal structure that results from telomere capture is very similar in structure to naturally occurring chromosomes in yeast and human cryptic translocation chromosomes. A very striking example in yeast is the structure of chromosome I, where the left end is nearly identical to the inversely oriented sequences at the opposite end of the.

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**Figure 6.** Pedigree analysis of G1-induced chromosome breaks in YGB85. (A) Describes the following: (1) Cells were induced for HO expression for 1 hr by incubation with galactose. (2) Induced cells were spread on YEP-dextrose plates, and un budded G1 cells were micromanipulated onto fixed positions (row a). (3) G1 cells were allowed to divide for one generation. (4) Sister cells were separated vertically and allowed to grow into full colonies (a and b designate pairs of sister cells derived from a single G1 cell). (B) HindIII-digested DNA from uninduced parental YGB85 (lanes P) and 13 pairs (a and b) of sister cells derived from G1-induced YGB85 cells. The parental chromosome III with the HO cassettes gives an 8-kb HindIII fragment, and the unbroken parental chromosome III gives a 5-kb HindIII fragment (cf. Figure 3A). All 13 pairs of induced cells have lost the parental 8-kb fragment. With the exception of one pair (#5) of sister cells, none have acquired a novel HindIII fragment. This indicates that the break has been repaired to give either a hemizygous left arm of chromosome III (cf. Figure 3C), or it has been repaired to give a hemizygous left arm of chromosome III (cf. Figure 3D).
same chromosome (Bussey et al. 1995). Another naturally occurring example concerns the resistance to toxic molasses (RTM) gene family in industrial strains of S. cerevisiae (Ness and Aigle 1995). These genes are located between the X and Y' subtelomeric elements of various chromosomes. Strains resistant to toxic levels of molasses have amplified these RTM genes by recombination among subtelomeric regions of different chromosomes. A similar proliferation appears to have occurred in the origin of SUC genes (Carlson et al. 1985).

These observations raise the possibility that BIR leading to telomere capture occurs in nature as a result of spontaneous breaks at some distance from telomeres, using short dispersed regions of homology such as duplicated genes or retrotransposon elements as sites to initiate replication. It is also possible that telomeres themselves are occasionally resected (Makarov et al. 1997; Welling er et al. 1997; Wright et al. 1997) either naturally or as a consequence of mutations that affect telomere maintenance, thereby creating recombinogenic, single-strand regions that may lead to the capture of other telomeres. This mechanism can account for the "healing" of the est1 mutation by the proliferation to many chromosome ends of telomeric and subtelomeric sequences (Lundblad and Blackburn 1993).

**Alternative DSB repair in regions with dispersed repeated sequences:** BIR may also explain how subtelomeric DNA, once established on many chromosomal ends, have been maintained as extraordinarily homogenous sequences within a given species. In yeast, there is an apparent gradient of end homogenization: The sequences closest to the ends share the highest degree of homology, and this homology decreases as the distance from the telomere increases (for review see Louis 1995). For example, Y' elements, the most distal of subtelomeric repeats, share the highest degree of homology, yet they are not essential for cell viability, while the more proximal X' subtelomeric elements share less homology, and sequences still more proximal than X elements are even less conserved. The mechanism that maintains this end homogenization almost certainly involves recombination among heterologous chromosomal ends (for review see Louis 1995; Pryde et al. 1997). Repair of DSBs by gene conversion would not necessarily account for such a gradient, unless spontaneous DSBs were much more prevalent close to chromosome ends.

BIR leading to telomere capture does not require any predetermined gradients to establish and maintain a gradient of sequence homology at chromosomal ends. Breaks may occur with equal frequency at any chromosomal position. Those far from the telomere will most likely lie in unique sequences and be repaired by gene conversion from a homologous chromosome. In subtelomeric or telomeric regions, some events can also be repaired by gene conversion using one of many homologous targets dispersed among many chromosomal ends. In some of these cases, however, the two ends of a DSB may invade different templates (Hastings et al. 1993; Paques et al. 1998), neither of which may be at the allelic chromosome end. The centromere-distal end can initiate DNA synthesis to the end of the chromosome (Figure 7). Invasion of only the centromere-distal end may be nonproductive, as it must copy the entire chromosome, and progression through the centromere may be greatly discouraged (Morrow et al. 1997). Moreover, DSBs near telomeres will have little homology distal to the break and be more likely to engage in BIR. Every BIR event will copy sequences all the way to the chromosome end. Sequences close to chromosome ends will more frequently be copied than those at a greater distance, thus establishing a gradient of homogenous sequence.

**Telomerase-independent chromosome maintenance:** A telomerase-independent pathway for healing broken chromosomes and acquiring telomeres has also been revealed in human cells. Terminal deletions (also known

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**Figure 7.—Two models for DSB repair for ends of chromosomes.** (A) Most DSB are thought to be repaired by gap repair (Szostak et al. 1983). (1) One of the two ends invades a homologous target. Strand invasion initiates DNA synthesis (dashed arrow). (2) The non-template strand of the target DNA is displaced by leading strand synthesis and creates a single-stranded substrate for the distal end of the break. The 3' distal end also initiates DNA repair synthesis and proceeds to fill the gap that was created by the DSB. (3) This intermediate structure is then resolved with or without a cross-over (Szostak et al. 1983). (B) Telomere capture can initiate as in gap repair. (1) A broken chromosome invades a homologous target with its centromere-proximal end. Invasion by the distal end may be less efficient because it may be very short in length relative to the proximal end. Furthermore, because most chromosomal ends contain repeated elements, many recombination targets are available for either end to invade. Invasion of a homologous target is not necessarily coordinated for the two ends of a DSB and, therefore, each end is free to invade independent targets. In the case of repeated sequences, this may be a common occurrence. Strand invasion initiates DNA synthesis (dashed arrow), just as in gap repair. (2) Unlike gap repair, leading strand repair synthesis is converted into semiconservative DNA replication where both leading and lagging strands are synthesized. The distal end of the DSB is then resolved with or without a cross-over (Szostak et al. 1983). (B) Telomere capture can initiate as in gap repair. (1) A broken chromosome invades a homologous target with its centromere-proximal end. Invasion by the distal end may be less efficient because it may be very short in length relative to the proximal end. 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as cryptic translocations) of human chromosome 6 in melanoma cell lines or normal cell lines with radiation-induced 6q- derivative chromosomes can heal by duplicating subtelomeric and telomeric sequences from heterologous chromosomes (Meltzer et al. 1993). Examples of Wolf-Hirschhorn syndrome (Altherr et al. 1991), tumor cells from hereditary nonpolyposis colon carcinoma patients (Jenkin et al. 1987), and Miller-Dieker syndrome (Kuwano et al. 1991) also have been found to have nonreciprocal translocations. Bryan et al. (1995) have reported that some immortalized human cell lines lack any detectable telomerase activity, and they show that these cells have suffered rearranged and unusually elongated telomeres. In some immortalized human cells, expression of human telomerase is not necessary for cell proliferation (Strahl and Blackburn 1996; Bryan et al. 1997; Stoppler et al. 1997). Telomere length and stability in some immortal human cells could be regulated by a mechanism independent of telomerase, in a manner analogous to the est1 cells of S. cerevisiae or that of some insects (Lundblad and Blackburn 1993; Roth et al. 1997). This may also explain how mice lacking telomerase are viable for many generations before dying (Blasco et al. 1997; Lee et al. 1998).

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