Genome-wide high-resolution mapping of chromosome fragile sites in Saccharomyces cerevisiae

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In mammalian cells, perturbations in DNA replication result in chromosome breaks in regions termed “fragile sites.” Using DNA microarrays, we mapped recombination events and chromosome rearrangements induced by reduced levels of the replicative DNA polymerase-α in the yeast Saccharomyces cerevisiae. We found that the recombination events were nonrandomly associated with a number of structural/sequence motifs that correlate with paused DNA replication forks, including replication-termination sites (TER sites) and binding sites for the helicase Rrm3p. The pattern of gene-conversion events associated with cross-overs suggests that most of the DNA lesions that initiate recombination between homologs are double-stranded DNA breaks induced during S or G2 of the cell cycle, in contrast to spontaneous recombination events that are initiated by double-stranded DNA breaks formed prior to replication. Low levels of DNA polymerase-α also induced very high rates of aneuploidy, as well as chromosome deletions and duplications. Most of the deletions and duplications had Ty retrotransposons at their breakpoints.

In response to perturbed DNA replication, mammalian chromosomes break in regions termed “fragile sites.” Human fragile sites can be classified as either common fragile sites (CFSs, present in essentially all individuals) or rare fragile sites (present in less than 5% of the population) (1). Most CFSs are common fragile sites (CFSs, present in essentially all individuals) or rare fragile sites can be classified as either common fragile sites (CFSs) or rare fragile sites (16). Occurring, increasing the probability of a double-strand break (DSB) within single-stranded DNA located at the replication fork (16).

In most (although not all) of the yeast studies described above, the fragile sites identified do not meet the classic criterion of a CFS, because these sites were not detected under conditions in which DNA replication is perturbed. In previous studies, we examined genome instability in yeast strains in which the transcription of the POL1 gene (encoding the catalytic subunit of DNA polymerase-α) was regulated by a galactose-inducible promoter (11). DNA polymerase-α is required to initiate DNA replication at replication origins and to synthesize Okazaki fragments on the lagging strand of the replication fork (17). We found that the low levels of polymerase-α elevated the frequency of chromosome loss and chromosome rearrangements on chromosome III about 100-fold, and most of the chromosome rearrangements involved an inverted pair of retrotransposons located on the right arm of III (fragile site FS2) (11). Rosen et al. (18) mapped mitotic recombination events between homologous copies of chromosome III in strains with low levels of DNA polymerase-α and showed that DSBs formed at FS2 initiated many of these exchanges. Low levels of DNA polymerase-α also stimulated high levels of mitotic and meiotic recombination in the tandemly repeated ribosomal RNA genes (19).

The yeast studies described above were performed using assays that were specific for specific genes or chromosomes. We have recently developed methods of monitoring mitotic recombination events throughout the genome using DNA microarrays (20). Because mitotic recombination events reflect the repair of DNA lesions by homologous recombination, the recombination breakpoints are located near the DNA lesion. By mapping recombination events in strains with low levels of DNA polymerase-α as described below, we conclude that fragile sites in yeast are often associated with structural motifs implicated in mitotic cross-overs | loss of heterozygosity | break-induced replication

Significance

When mammalian cells are treated with drugs that perturb DNA replication, chromosomes break in specific locations called “fragile sites.” Human fragile sites are hotspots for chromosome rearrangements seen in many solid tumors. In the yeast Saccharomyces cerevisiae, DNA breaks are repaired by homologous recombination. We have developed methods of genome-wide mapping of recombination events in yeast strains that have 10-fold reduced levels of DNA polymerase. We find that these yeast fragile sites are often associated with sequence/structural motifs that pause or stall the DNA replication fork. We also show that low levels of DNA polymerase greatly elevate the frequency of deletions and duplications (reflecting unequal sister-chromatid recombination between repeated genes), and result in changes in chromosome number (aneuploidy).

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pausing or stalling of the replication fork. We also show that low levels of DNA polymerase-α greatly elevate the frequency of chromosome-specific aneuploidy, as well as large deletions and duplications. Importantly, the properties of recombination events observed in strains with low levels of DNA polymerase-α are substantially different from the pattern of spontaneous mitotic exchanges, suggesting that most spontaneous recombination events between homologs are not initiated by DNA lesions formed during the S-period.

Results

Experimental Approach for Mapping Regions of Loss of Heterozygosity Induced by Low Levels of DNA Polymerase-α. In diploid strains that are heterozygous for many SNPs, mitotic recombination events between homologous chromosomes can be mapped by identifying regions that have undergone loss of heterozygosity (LOH). Previously, using SNP-specific microarrays (21), we mapped spontaneous recombination events on the right arm of chromosome IV (22), and UV-induced events throughout the genome (23). In our current study, we map unselected mitotic recombination events induced by low levels of DNA polymerase-α throughout the yeast genome, in addition to mapping reciprocal cross-overs selected on chromosomes V and IV.

The diploid strain WS84 was generated by crossing two haploid strains (W303a and YJM789) that differ by about 55,000 SNPs (details in Tables S1 and S2). In addition, the diploid was homozygous for an allele of POL1 (KANMX-GAL1-POL1) in which the transcription of the POL1 gene (encoding the catalytic subunit of DNA polymerase-α) is regulated by the galactose-inducible GAL1 promoter. We previously showed that cells grown in medium containing 0.005% galactose and 3% (wt/wt) raffinose (low-galactose medium) had about 10% of the level of Pol1p as a wild-type strain, whereas cells grown in 0.05% galactose and 3% raffinose (high-galactose medium) had about three times more Pol1p than that observed in wild-type cells (11). Using chromosome-specific assays, Lemoine et al. (11) showed that low levels of DNA polymerase-α elevated the frequency of mitotic recombination about 20-fold; Rosen et al. (18) observed a 10-fold elevation of cross-overs on chromosome III under similar conditions.

To detect recombination events for the entire yeast genome, we used oligonucleotide-containing microarrays (20, 22). Each individual heterozygous SNP that was assayed was represented by four 25-base oligonucleotides, two that perfectly matched the Watson and Crick strands of the W303a-specific SNP and two that perfectly matched the Watson and Crick strands of the YJM789-specific SNP. DNA samples from individual experimental strains were labeled with Cy3-tagged nucleotides and mixed with DNA isolated from a control heterozygous diploid. The mixture was hybridized to the SNP-microarrays, and we measured the relative hybridization levels to the two different samples (details in SI Text). The whole-genome array (WG-array) examined about 15,000 SNPs located throughout the genome. Because of repetitive sequences in the subtelomeric regions, our analysis of LOH events is limited to about 96% of the genome; the omitted regions are summarized in Dataset S1.

Mapping Unselected LOH Events in WS84. We grew single WS84 cells to colonies on low-galactose medium, resulting in low levels of DNA polymerase-α. We then analyzed 25 colonies by WG-arrays. Subsequent manipulations of the strains derived from this experiment were done using high-galactose medium to reduce the frequency of secondary recombination events.

Classes of unselected LOH events observed in colonies of WS84. Among the 25 colonies, we found an extraordinarily high level of chromosome alterations, including mitotic recombination between homologs, deletions and duplications of genomic regions, and changes in chromosome ploidy. Fig. 1 shows hybridization results for all 16 chromosomes of one isolate (WS84-2-#6). In Fig. 1, the blue and red lines represent the level of hybridization of the DNA sample to YJM789-derived and W303a-derived SNPs, respectively. If the chromosome has no LOH events or changes in chromosome number, both lines have a value of about one from the whole chromosome, as seen for chromosomes IX, XIV, and XVI. Below, we discuss the types of events in which chromosome segments have an altered ratio of hybridization. The Saccharomyces

Fig. 1. Patterns of LOH observed in one unsectored colony of WS84 (WS84-2#6). Genomic DNA was isolated from one colony formed on medium with low levels of galactose. This sample was hybridized to SNP-specific microarrays. The blue and red lines show hybridization to YJM789-specific and W303a-specific SNPs, respectively (normalized to a control strain). Note that the SGD coordinates on the x axis are in base pairs, with the left telomere representing base 1.
Genome Database (SGD) coordinates that define transitions between heterozygous and homozygous regions are listed in Dataset S2 for all LOH events, except those involving deletions and duplications.

One common class is a region of interstitial LOH in which a segment derived from one homolog replaces a segment derived from the other homolog. For example, on chromosome XV, we observed three regions of interstitial LOH (Figs. 1 and 2A). Examining one of these regions (marked with an arrow in Fig. 2A) at higher resolution (Fig. 2B), we determined that the left transition between heterozygous and homozygous markers was between SNPs located at 935 kb and 942 kb, and the right transition was between SNPs at 949 kb and 958 kb. One likely source of such events is a gene conversion unassociated with a cross-over (Fig. 3A). Because the chromosome that has the recombinogenic lesion acts as a recipient in gene-conversion events (24), we infer that the LOH event shown in Fig. 2B was initiated by a DNA lesion on the YJM789-derived homolog. The simple interstitial LOH events are shown schematically in Dataset S3 as classes b1 and b2. In this depiction, heterozygous regions are shown as green lines, and regions homozygous for W303a-derived and YJM789-derived SNPs are shown as red and black lines, respectively. More complex conversion events are shown as classes c1, d1–d3, and e1 (Dataset S3).

In the second common class, we also observed an increase in the signal of SNPs derived from one homolog and a decrease in the signal derived from the other, with the affected segment extending to the telomere. One example in Fig. 1 is on chromosome V, in which the transition between heterozygous and homozygous SNPs occurs near SGD coordinate 384 kb. This pattern of LOH is consistent with a cross-over with or without an associated gene conversion event (Fig. 3B and C). Alternatively, terminal LOH could reflect a break-induced replication (BIR) event (Fig. 3D). Although we cannot distinguish between cross-overs and BIR events, in other experiments described below with sectored colonies we can make such a distinction. The coordinates for these events are given in Dataset S2 and depicted as classes a1–a4 in Dataset S3. Classes a5 and a6 are likely to reflect cross-over/BIR events that were followed by loss of one of the recombined chromosomes. One example of such an event is on chromosome VI (Fig. 1). There are 115 terminal cross-over/BIR events of the sort shown in Fig. 3B–D.
The distribution of these events and interstitial LOH events is shown in Fig. 4.

We also observed deletions and duplications of chromosomal sequences, both interstitial and terminal. The characteristic signature of these events is that the hybridization signal for one homolog is about one, whereas the signal for the other is either reduced (deletion) or increased (duplication). In Fig. 1, chromosome III has an interstitial deletion of W303a-derived sequences between SGD coordinates 150 kb and 170 kb, and an interstitial duplication of W303a-derived sequences between SGD coordinates 200 kb and 293 kb. Most of the interstitial deletions and duplications have directly oriented repetitive genes at the breakpoints. For example, the deletion on chromosome III is located between a pair of Watson-oriented transposable (Ty) elements at 150 K and an inverted pair of Ty elements at coordinate 170 kb (Dataset S4; depictions of events in Dataset S5). The duplication spans the MAT locus near SGD coordinate 200 kb and the silent HMR mating-type information at coordinate 293 kb. Of the 43 deletions/duplications, 42 involved directly oriented repeats that were greater than 1 kb in size.

Several loci were particularly susceptible to duplications and deletions. Of the 43 deletions/duplications, 20 were within the tandem cluster of CUP1 genes, 8 involved the two pairs of Ty elements on chromosome III discussed above, and 3 occurred between inverted pairs of Ty elements located on chromosome IV near SGD coordinates 880 kb and 990 kb. We note that the two pairs of Ty elements on chromosome III were previously shown to be hotspots for chromosome rearrangements (11) and BIR events (18) in strains with low levels of DNA polymerase-α. These observations suggest that large (>1 kb) deletions and duplications in yeast primarily reflect homologous recombination between directly oriented repeats located on sister chromatids. Both deletions and duplications could be produced by unequal crossing over (Fig. 3E), although deletions could also reflect other pathways of homologous recombination (intrachromatid recombination or single-strand annealing).

We also observed terminal deletions and duplications; a terminal deletion is shown on chromosome XV in Fig. 1. As for the interstitial deletions and duplications, the breakpoints of these events were usually in repetitive elements (Dataset S4). Although we did not characterize these deletions and duplications in detail, previously, we found that terminal deletions and duplications were generated by the mechanism shown in Fig. 3F in which a break in or near a repetitive element on one chromosome was repaired by a BIR event using a repetitive element on a nonhomolog (11). Such events would be expected to result in strains with paired deletions and duplications in the same cell. However, most of our observed terminal deletion and duplications were in subtelomeric regions that were poorly represented in the SNP microarrays, making it difficult to detect this coupling. In summary, among the 25 strains examined, we observed 77 deletion/duplication events, 43 interstitial and 34 terminal.

Finally, we detected 79 alterations in ploidy (Dataset S6), 55 monosomy (for example, chromosome I in Fig. 1), and 12 trisomy events. In addition, we observed 12 events of uniparental disomy. In such strains, there is a deletion of one homolog and duplication of the other. Although such events are often explained as a consequence of loss of one homolog followed by duplication of the remaining homolog in a subsequent cell division, we recently showed that some cell divisions in yeast undergo a meiosis I-like segregation in which one daughter cell receives two copies of the W303a-derived homolog and the other daughter cell receives two copies of the YJM789-derived homolog (25); this process was termed “reciprocal uniparental disomy.” We cannot determine whether the UPD events observed in WS84 are a consequence of chromosome loss followed by reduplication or reciprocal uniparental disomy events.

The summary of all alterations in unsectored colonies of WS84 is presented in Dataset S7. There were a total of 357 unselected events observed in 25 isolates generated in a single cycle of growth from a cell to a colony (about 25 cell divisions).

Association of unselected LOH events in WS84 strains with genomic features. The transitions between heterozygous and homozygous markers should represent the approximate position of the DNA lesion that initiates mitotic recombination. For an interstitial LOH region, the initiating lesion could be located anywhere between the two transitions that define the LOH transition.
Thus, to define the “window” containing the putative recombinogenic lesion for interstitial LOH events, we used the SGD coordinates of the heterozygous sites of the rightmost and leftmost transitions (Dataset S2). For terminal LOH events representing either cross-overs or BIR events, we used a 20-kb “window” generated by using SGD coordinates that were 10 kb to each side of the first homozygous SNP (Dataset S2). For all of the windows in Dataset S2, we then determined whether certain chromosomal elements were significantly over- or underrepresented within the windows (SI Text).

We chose to examine the association of genomic elements to LOH events that have been proposed or demonstrated by others (references in Dataset S8) to have a connection to chromosome fragility including tRNA genes, ARS elements, transposable elements, and solo LTRs, centromeres, palindromic sequences, G4 (quadruplex) motifs, highly transcribed genes, Rrm3p binding regions, regions with high levels of γ-H2AX, and replication-termination regions. By a χ² analysis (SI Text), we found five types of elements that were overrepresented at recombination breakpoints (P value corrected for multiple comparisons in parentheses): noncoding RNA genes (0.006), solo LTRs (0.006), G4 sequences (0.001), binding sites for Rrm3p (<0.0001), and replication-termination (Ter) sequences (<0.0001). The basic finding of this analysis (details discussed later) is that motifs associated with slow-moving replication forks in cells grown under normal laboratory conditions are hotspots for mitotic recombination between homologs in strains with low levels of DNA polymerase-α.

Mapping Reciprocal Cross-Overs Induced by Low Levels of DNA Polymerase-α. The experiments described above did not allow us to distinguish cross-overs and BIR events, nor to examine the types of gene conversions associated with cross-overs. For this analysis, we used diploid strains in which cross-overs could be detected by a sectoring assay (22, 23, 26). In these strains, one homolog has the ochre-suppressor SUP4-o located near the telomere and the hygromycin-resistant HYG gene located at a similar position on the other homolog; in addition, the diploid is homozygous for ade2-1, an ochre mutation that, when unsuppressed, results in red colonies. Cells with one, two, or no copies of the SUP4-o suppressor result in pink, white, or red colonies, respectively. Thus, cross-overs within single cells result in red/white sectored colonies (Fig. S4). Most cross-overs selected with this method are associated with an adjacent gene-conversion event (27). A conversion event associated with repair of a single broken chromatid (3:1 conversion) is shown in Fig. 5B. This type of conversion results in red and white sectors in which the position of the transition between heterozygous and homozygous markers is different in the two sectors, with the difference representing the extent of the conversion tract. In the boxed region of Fig. 5B, there are three chromosomes with sequences derived from the YJM789-related homolog and only one with sequences derived from the W303a-related homolog.

In our previous studies of spontaneous (22) and UV-induced mitotic recombination events (23), more than half of the cross-over–associated gene-conversion events were 4:0 conversions (Fig. S5C) or 3:1/4:0 hybrid events (Fig. S5D), reflecting the repair of two sister chromatids that were broken at approximately the same position. We suggested that the simplest explanation of such events was that many cross-overs between homologs are initiated by a DSB in an unreplicated chromosome, followed by replication of the broken chromosome to yield two broken sister chromatids. It might be expected that low levels of DNA polymerase-α would result in DSBs in the S-period, resulting in primarily 3:1 conversion events. To confirm this expectation, we isolated red/white sectored colonies and examined patterns of LOH in both the red and white sectors. Finally, we note that a BIR event, unlike a cross-over, does not generate red/white sectored colonies (Fig. 5E). Among unselected events in the red/white sectored colonies, cross-overs and BIR events were equally frequent (SI Text).

Analysis of cross-overs on chromosome V in sectored colonies derived from WS84. In WS84, the SUP4-o and HYG markers are located at allelic positions on the two homologs near the CAN1 locus at the left end of chromosome V. To stimulate mitotic cross-overs, we grew cells in high-galactose medium [3% (wt/wt) raffinose, 0.05% galactose] overnight, and then resuspended the cells in medium lacking galactose [2% (wt/wt) glucose] for 6 h to deplete DNA polymerase-α. The cells were then plated on solid high-galactose medium. This protocol elevates the frequency of mitotic cross-overs about sevenfold relative to cells that are not
incubated in the absence of galactose (18). Eleven red/white sectored colonies were examined for LOH events by microarrays, and the coordinates for the detected LOH events are given in Dataset S9 (depictions in Dataset S10).

Nine of the 11 sectored colonies examined had associated conversions, seven were unambiguously 3:1 conversion events (classes B1 and B2 in Dataset S10), and two were complex events involving two different donor chromosomes. Although the number of conversion events that we examined was limited, these results suggest that most of the cross-overs induced by low levels of DNA polymerase-α are initiated by a break of single chromatin in S or G2, rather than by a break in G1. This conclusion was supported by our examination of cross-overs in MS47.

**Analysis of cross-overs on chromosome IV in sectored colonies derived from MG47.** Previously, we analyzed more than 100 spontaneous cross-overs on the right arm of chromosome IV (a 1.1-Mb region representing about 10% of the yeast genome). In the present study, we examined 44 sectored colonies induced by low levels of DNA polymerase-α derived from MG47, in which the SUP4-o and HYG markers are located at allelic positions near the right end of chromosome IV. Thirty-seven of these colonies appeared to reflect a single cross-over (depictions in Dataset S10, coordinates in Dataset S11), whereas seven appeared to be a consequence of multiple initiation events (Dataset S12). Because the interpretation of the events in Dataset S12 is not straightforward, our conclusions are based primarily on the events depicted in Dataset S10. The following types of events were observed (class and number of events in parentheses): no conversion (class A2, 10 events), 3:1 conversion (classes B3 and B4, 22 events), simple 4:0 or 3:1/4:0 hybrid tracts (0 events), complex tracts without a segment of 4:0 (C2–C5, 4 events), and a complex tract with a segment of 4:0 (C6, 1 event). In summary, if we include only the simple 3:1, 4:0, and hybrid 3:1/4:0 conversion tracts of both WS84 and MG47, we observed 29 3:1 tracts and none of the other two classes. In addition, only one of the complex tracts observed in the two strains had a 4:0 region. In contrast, for spontaneous cross-overs on chromosome IV, we previously observed 29 3:1 events, 7 4:0 events, and 45 hybrid tracts (22). By the Fisher’s exact test, the types of conversion events associated with spontaneous cross-overs and those induced by low levels of DNA polymerase-α are very significantly different ($P < 0.0001$). These results are consistent with the expectation that low levels of DNA polymerase generate high levels of S/G2-associated DNA lesions.

We also examine the distributions of low-polymerase-induced recombination events on the right arm of chromosome IV compared with spontaneous events previously examined (Fig. S1). The events induced by low polymerase-α have an elevated frequency of events in the region between coordinates 845 and 1,045 kb (SI Text and Fig. S1A). The hotspots HS3 and HS4, previously described for spontaneous events (Fig. S1B), do not appear to be induced in strains with low levels of α DNA polymerase, although this difference is not statistically significant.

We also calculated lengths of conversion tracts associated with cross-overs in WS84 and MG47 (Datasets S9 and S11). The median tract length was 6.3 kb (95% confidence limits of 4.9–10.3 kb). This length is similar to those observed for spontaneous conversions [10.6 kb (8.2–13.6); 22 events] and UV-induced cross-over–associated conversions (7.6 kb [6.4–9.6]; 23 events).

**Discussion**

Our analysis demonstrates that a single cycle of growth from a cell to a colony in conditions in which the level of DNA polymerase-α is depleted (about 10% the wild-type level) results in high frequencies of genomic alterations, about 11 chromosome alterations (LOH events, deletions/duplications) and three ploidy alterations per cell. In an analysis of 13 wild-type cells, we observed 0.08 LOH events per cells and no ploidy alterations (20). Most of the recombination events in the cells with low polymerase-α have the pattern of conversion (3:1) indicative of DNA damage induced in S/G2. Below, we discuss the likely mechanisms associated with these events.

**Recombination Events in Unsectored Colonies of WS84. Fragile sites in yeast.** The term “fragile site” was originally defined in mammalian cells as the position of breaks or gaps in metaphase chromosomes following exposure to conditions that perturb DNA replication (1). In studies done in *Saccharomyces cerevisiae*, the term is used more loosely to refer to genomic regions that accumulate proteins associated with DNA damage/repair or delayed/stalled replication forks. Some of these studies were done in wild-type cells grown without replication stress (12), whereas others were performed in cells with repair- or checkpoint-defective cells mutations or in the presence of drugs affecting DNA precursor pools (16).

In several studies, DSBs were mapped for single sites. For example, the triplet repeats (CTG)N are broken in yeast cells treated with hydroxyurea (28), and a variety of other microsatellites are associated with stalled replication forks and recombogenic DNA lesions (29, 30). DSB formation and elevated frequencies of recombination are also associated with inverted Ty elements or other inverted repeats, particularly in strains with perturbed DNA replication (11, 31). Ravendranathan et al. (32) found DSBs that were associated with ARS elements that failed to get activated in mec1 and rad53 strains (compromised early origins), and Feng et al. (16) showed that the location of DSBs in mec1 strains in HU-treated strains are often near “checked” replication origins that delay firing in the presence of a wild-type DNA damage checkpoint.

In our analysis, we identified fragile sites as breakpoints in recombination events between homologs that occur in strains with low levels of DNA polymerase-α. Unlike many studies that are restricted to a single gene or single chromosome arm, we investigated events throughout the genome. By examining many independent recombination breakpoints, we established that there were significant correlations between breakpoints and chromosome elements associated with slow or paused replication forks including: solo LTRs, binding sites for Rrm3p, and replication-termination sequences. These associations suggest that regions of the genome with slow-progressing replication forks are particularly susceptible to recombogenic lesions in cells with low levels of DNA polymerase-α. It is likely that these stalled forks have large single-stranded regions that are susceptible to nucleaseases or topoisomerases (8, 33). It is also possible that DSBs can be generated by cytokinesis during an attempt to segregate incompletely replicated chromosomes (34).

We also found a statistically significant association between G-quadruplex DNA sequences (motif 1 in Dataset S8) and recombination breakpoints. G-quadruplex sequences result in slowing of the replication fork in yeast (35, 36). Although we observed a significant association with these sequences using the motif proposed by Capra et al. (37), with a more restrictive definition of G-quadruplex–forming sequences (motifs 2 and 3 in Dataset S8), we found no significant association. Motifs 2 and 3 are included in the motif proposed by Capra et al.; when these motifs are removed, the association remains significant ($P = 0.0007$).

No very strong mitotic recombination hotspots were observed in our studies; the events were distributed widely over all of the chromosomes (Fig. 4). Although certain motifs were enriched at the breakpoints, no single motif was observed at all of the breakpoints. Because a number of different types of sequences are capable of affecting DNA fork movement, this result is not surprising. We found a strong correlation between chromosome size and the number of LOH events ($r = 0.78$, $P < 0.001$), as expected if the motifs associated with recombogenic lesions are broadly distributed. There is a region near the middle of chro-
mosome XII that has many cross-over events but no conversion events. This observation reflects a property of the microarray rather than a biologically interesting finding. The apparent hot-spot for cross-overs is the location of the array of 150 9-kb ribosomal RNA genes. The microarray contains SNPs flanking the cluster but not SNPs within the cluster. Thus, we can detect cross-overs within the array but not gene-conversion events. Because conversion events are usually shorter than 20 kb, they would likely be undetectable even if the microarray contained SNPs that distinguished the two types of RNA genes.

Deletions/duplications and changes in chromosome number. Our observation that most (>90%) of deletions and duplications involve repeated sequences is consistent with many other studies demonstrating that most chromosome rearrangements (deletions, duplications, and translocations) in S. cerevisiae reflect homologous recombination between ectopic repeats rather than non-homologous end-joining. One region of the genome that is particularly susceptible to interstitial deletions and duplications is the tandemly repeated CUP1, which was altered in more than half of the isolates examined.

We also observed frequent chromosome loss and duplication events with losses (monosomies) outnumbering duplications (trisomies) 55 to 11. One possible mechanism to explain this high frequency of aneuploidy is that partially replicated chromosomes are sometimes segregated into daughter cells, leading to trisomy in one daughter and monosomy in the other. Although this mechanism would be expected to produce equal frequencies of trisomies and monosomies, it is possible that the recovery of aneuploid cells is affected by the relative growth rates of monosomic and trisomic cells. However, tell mel1 diploids have a substantial excess of trisomic chromosomes relative to monosomic and trisomic cells. In our previous mapping of spontaneous recombination events on chromosome IV (22), we showed that 64% of the cross-overs associated with a gene-conversion event involved the repair of two sister chromatids broken at the same position (4:0 or 3:1/4:0 hybrid event) (Fig. 5 C and D). Our interpretation of this result is that most spontaneous recombination events between homologs reflect the repair of a DSB in an unreplicated chromosome that is then duplicated to yield two sister chromatids broken at the same position. This interpretation is supported by the observation that diploid yeast cells irradiated in G1 of the cell cycle produce primarily 4:0 and 3:1/4:0 hybrid conversion tracts, whereas cells irradiated in G2 produce primary 3:1 conversions (47). In addition, DSBs can be physically detected in G1/G0 cells at the very strong mitotic recombination hotspot caused by GAA/TTC repeats, and such tracts result in primarily 4:0 and 3:1/4:0 tracts (48, 49).

Our previous conclusion that most spontaneous recombination events between homologs were initiated in unreplicated chromosomes is surprising because Rad52p repair foci, presumably marking DSB formation, are much more common in S and G2 than in G1 (50). We previously suggested the model shown in Fig. 6A (22) in which DSBs are more common in S/G2 than in G1, but are usually repaired by sister-chromatid recombination. A small fraction of S-generated DSBs uses the homolog rather than the sister, resulting in 3:1 conversion events. Because replication of a G1-associated DSB results in two sister chromatids broken at the same position, sister-chromatid recombination cannot occur and the DSBs are repaired by recombination with the homolog. Our observation that low levels of DNA polymerase-α greatly elevate the fraction of 3:1 gene-conversion events relative to the fraction of 4:0 and 3:1/4:0 hybrid conversions is consistent with this model.
likely associated with sister-strand recombination. In contrast, with 3:1 conversion events), and deletion/duplication events stimulate both recombination between homologs (associated with 3:1 conversion events), and deletion/duplication events were used in our experiments (genotypes and strain construction details in SI Text) were homozygous for the KANMX-GAL1-POL1 gene. The primary difference between WS84 and MG47 is the location of the gene (SUP4-α) that allows detection of crossovers. This gene is located near the left end of chromosome V in WS84 and near the right end of chromosome IV in MG47.

Construction of Yeast Strains. The genotypes of all strains in this study are given in Table S1; primers used in strain constructions are listed in Table S2. Two isogenic diploids WS84 and MG47 were used in our experiments (genotypes and strain construction details in SI Text) were homozygous for the KANMX-GAL1-POL1 gene. The primary difference between WS84 and MG47 is the location of the gene (SUP4-α) that allows detection of crossovers. This gene is located near the left end of chromosome V in WS84 and near the right end of chromosome IV in MG47.

Identification and Mapping of Reciprocal Cross-overs in WS84 and MG47. As discussed in the text, in strains WS84 and MG47, cross-overs can be identified as red/white sectored colonies. To induce crossovers, we grew these strains in YPR-HG, followed by harvesting the cells and incubating them in liquid YPD medium for 6 h at 30 °C. The cells were then plated on SR-HG-Arg medium for 4 d at room temperature. The plates with the colonies were then incubated at 4 °C overnight, and the red HygR/white HygS sectored colonies were identified.

DNA samples derived from sectors with the appropriate phenotypes were analyzed by SNP-specific microarrays as described previously (20). The locations and sequences for the oligonucleotides used in this analysis have been published previously (20, 22).

Analysis of Unsectored Colonies Derived from WS84 Strains with Low Levels of α-DNA Polymerase. For our analysis of the effects of low α-DNA polymerase on genome stability, we used two independently constructed isolates of WS84 (WS84-2 and WS84-5) that had previously been shown by microarray analysis to be euploid and devoid of chromosome rearrangements. Each isolate was grown on YPR-HG for 2 d. Cells from the resulting patches were then streaked on solid medium containing YPR-LG to obtain isolates derived from single cells. After a 4-d incubation at 30 °C, 25 individual colonies were selected from the YPR-LG plates (19 from WS84-2 and 6 from WS84-5), restreaked on plates containing high levels of galactose (YPY-HG), and incubated at 30 °C for 2 d. Single colonies derived from each of these restreaked 25 colonies were used for our subsequent analysis. These derivatives were grown in YPR-HG to prepare DNA. Samples were examined using whole-genome SNP-specific microarrays.

Statistical Analysis. Most of the statistical tests were performed using VassarStat (http://vassarstats.net) or the statistical functions in Excel. Corrections of P values for multiple comparisons were performed as described by Hochberg and Benjamini (52). The 95% limits on median values were calculated according to table B11 of Altman (53). Details of the statistical analyses are in SI Text.

ACKNOWLEDGMENTS. We thank Y. Yin, J. Kelly, and J. St. Charles for their help in data analysis; M. Gawel for help with the construction and microarray analysis of WS84; A. Nicolas for providing information about G4 motifs in the yeast genome; all members of the T.D.P. and Jinks-Robertson laboratories for helpful advice; and Sue Jinks-Robertson and members of the T.D.P. laboratory for comments on the manuscript. The research was supported by National Institutes of Health Grants GM24110 and GM52319 (to T.D.P.) and National Institutes of Health Training Grant T32-AI52080 (to W.S.).

We expect a defect in DNA replication to elevate the frequency of S-associated DSBs without altering the frequency of G1-associ-
ated DSBs, resulting in the elevation of 3:1 events (Fig. 6). If low levels of DNA polymerase-α increase the frequency of S-associated DSBs, this model also predicts an increase in the frequency of unequal sister-chromatid recombination events; this increase was observed. Rosen et al. (18) showed that low levels of DNA polymerase-α also result primarily in 3:1 conversion events (90%) relative to 4:0 events (10%) on chromosome III.

In summary, our analyses demonstrate that DNA lesions generated under conditions of perturbed DNA replication stimulate both recombination between homologs (associated with 3:1 conversion events), and deletion/duplication events likely associated with sister-strand recombination. In contrast, most spontaneous crossovers are associated with 4:0 conversion events and are not initiated by chromosome breaks associated with DNA replication.

Materials and Methods

Genetic Analysis and Media. Standard yeast procedures were used for mating, sporulation, and tetrad dissection (51). Rich growth medium YPD (yeast extract, peptone, dextrose) and omission media [synthetic dextrose (SD) medium lacking single amino acids or bases] were made following standard protocols (27, 51). As described previously (11), the growth media YPR-LG and YPR-HG were made with yeast extract, peptone, and raffinose [3% (wt/vol)] with either 0.005% galactose (LG) or 0.05% galactose (HG). For the detection of red/white sectored colonies, we used plates containing the omission medium SR-HG-arginine (equivalent to SD plates lacking arginine, containing 10 μg/mL adenine, 3% (wt/vol) raffinose, and 0.05% galactose). To confirm cross-overs (as described below), we also used SR-HG medium lacking adenine and SR-HG medium containing hygromycin (300 μg/mL).

**Fig. 6.** Repair of DNA breaks induced in G1 or in S/G2 in wild-type strains and in strains with low levels of DNA polymerase-α. The widths of the arrows reflect the frequency of use of various pathways. (A) Spontaneous LOH events in wild-type cells. We suggest that most DSBs are formed in S or G2 (Right side of panel). The majority of these breaks are repaired by sister-chromatid recombination with the remainder being repaired using the homolog as a template. All of the G1-induced events involve exchange between the homologs. (B) LOH events induced by low levels of DNA polymerase-α. In these strains, the number of DSBs occurring in S is greatly elevated without an elevation in G1-initiated breaks. Consequently, the frequency of 3:1 events relative to 4:0 events is greatly elevated.


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Construction of Yeast Strains. The genotypes of all strains in this study are given in Table S1 and oligonucleotides used in the constructions of these strains are in Table S2. Each haploid in the study was isogenic (except for changes introduced by transformation), with the haploid strains W503a (1) and YJM789 (2). The three diploids, JSC25, WS84, and MG47, were constructed by crossing haploids isogenic with W503a and YJM789. These diploids were heterozygous for about 55,000 SNPs. WS84 and MG47 are homoyzgous for the KANMX-GAL1-POL1 gene (3), and JSC25 is the wild-type control. All diploids are isogenic except for the changes introduced by transformation. JSC25 and MG47 are heterozygous for an insertion of the SUP4-o gene near the end of chromosome IV, and WS84 is heterozygous for the insertion of this gene near the end of chromosome V. As discussed in the text, this gene allows the detection of cross-overs.

The haploid strain WS68-3a was obtained in several steps. First, the YJM789-related strain YJMS50 was transformed to geneticin-resistant by using a PCR fragment containing the KANMX-GAL1-POL1 cassette; this transformant was WS50. This cassette contains the KANMX gene located upstream of the GAL110 promoter that is fused to the POL1, encoding the catalytic subunit of DNA polymerase-α (3), and was obtained by amplifying the plasmid pFA6a-PGA3-3HA (4) with the primers GALPOL1F and GALPOL1R. Because we found that WS50 was dicyomic for chromosome XIV (the location of POL1), we constructed a monosomic strain with the KANMX-GAL1-POL1 cassette by mating WS50 to an isogenic MATα derivative (YJM8494), and sporulating the resulting diploid (WS68). One of the resulting spores was WS68-3a.

The diploid strain WS84 was constructed by crossing the haploid strains MG40 and MG44. The W503a-related haploid MG40 was constructed by transforming PSL2 (5) using a PCR fragment obtained by amplifying W68-3a genomic DNA with primers XIV F 429839 and XIVR 430329. The resulting transformant (MG40) contained the KANMX-GAL1-POL1 cassette and was genomic resistant. We confirmed the correct insertion of this cassette using PCR with the primer pairs Pol1F 430028 with pFA6a-PGA3-3HA R, and Pol1F with Pol1R. MG41 was constructed by transforming PSL5 (5) using a PCR fragment obtained by amplifying WS68-3a genomic DNA with primers XIV F 429839 and XIVR 430329. The location of the KANMX-GAL1-POL1 cassette was confirmed using the same primers that were used for MG40. Because the diploid WS84 is homozygous for the ade2-1 ochre mutation and is heterozygous for the insertion of SUP4-o near the left telomere of chromosome V, cross-overs on the left arm of chromosome V result in a red/white-sector colony, as described in the main text.

The diploid MG47 was generated by crossing the haploid strains MG44 and MG46, which are isogenic with strains YJM789 and W503a, respectively, except for alterations introduced by transformation. MG44 was constructed by transforming the YJM789-related haploid JSC21-1 (6), with a PCR fragment obtained by amplifying WS68-3a genomic DNA with primers XIV F 429839 and XIVR 430329. MG44 has a deletion of the CAN1 locus on the left arm of chromosome V, and an insertion of the SUP4-o gene near the right telomere of chromosome IV (IV1510386::SUP4-o) in addition to the KANMX-GAL1-POL1 cassette. The W503a-related haploid MG46 was constructed in several steps. First, we replaced the KANMX gene associated with the can1-100 allele in JSC12-1 (6) with the HYG gene. This construction involved transformation of JSC12-1 with a HYG-containing PCR fragment generated by amplification of the plasmid pAG32 (7), with the primers IV1510386::can1-100 F and IVHygKANMX R. The replacement was confirmed using the primer pairs AMC012 with EXT IV1510386 F, EXT IV1510386 F with EXT IV1510386 R, and EXT IV1510386 F with KANMX INT R. The strain MG46 was obtained by transformation of MG45 with the KANMX-GAL1-POL1 fragment previously described. In the diploid strain MG47, resulting from mating of MG44 and MG46, the SUP4-o marker allowing detection of cross-overs on the right arm of chromosome IV was inserted near the right telomere (IV1510386::SUP4-o) allelic to the HYG marker (IV1510386::HYG-can1-100).

Analysis of Loss of Heterozygosity by SNP Microarrays. As in previous experiments (6, 8, 9), we monitored loss of heterozygosity (LOH) using oligonucleotide-containing microarrays (Agilent) in which 25 base oligonucleotides were designed to distinguish between SNPs. The sequences of the oligonucleotides used for the preparation of the arrays are given in Table S1 and oligonucleotides used in the genotyping of the three diploids were heterozygous for about 55,000 SNPs. The sequences of the oligonucleotides used for the preparation of the arrays are given in Table S1 and oligonucleotides used in the genotyping of the three diploids, JSC25, WS84, and MG47, were obtained in several steps. First, the YJM789-related strain YJMS50 was transformed to geneticin-resistant by using a PCR fragment containing the KANMX-GAL1-POL1 cassette; this transformant was WS50. This cassette contains the KANMX gene located upstream of the GAL110 promoter that is fused to the POL1, encoding the catalytic subunit of DNA polymerase-α (3), and was obtained by amplifying the plasmid pFA6a-PGA3-3HA (4) with the primers GALPOL1F and GALPOL1R. Because we found that WS50 was dicyomic for chromosome XIV (the location of POL1), we constructed a monosomic strain with the KANMX-GAL1-POL1 cassette by mating WS50 to an isogenic MATα derivative (YJM8494), and sporulating the resulting diploid (WS68). One of the resulting spores was WS68-3a.

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Analysis of Over- or Underrepresentation of Various Genomic Elements with LOH Breakpoints. From our analysis of unsectored colonies of WS84, we identified 201 cross-over/ break-induced replication (BIR) and conversion events. For each event, we determined a “window” likely to contain the initiating recombination event, as described in the main text. As we have done previously (6, 9), we examined the sequences within these windows to determine whether various chromosome elements were over- or underrepresented. Any element that was overrepresented at recombination breakpoints is implicated as a potential hotspot for recombination DNA lesions. Based on our previous studies and those of others, most of the motifs examined have been previously

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associated with accumulation of DNA-damage–associated proteins, elevated rates of chromosome breakage, or elevated recombination. Our analysis incorporated data derived from 25 unsectored colonies of WS84. The approach that we used was based on calculating the number of bases of genomic DNA that was included within the 201 events observed in these colonies (3,804,944 bp), and the number of bases that were not included (286,194,331 bp). The second number was calculated by subtracting the total number of bases in the LOH regions from the total number of bases examined (289,999,275 bp). The total number of bases examined was the product of the genomic region represented by SNPs in the microarrays (11,599,975 bp) (Dataset S1) multiplied by 25 (the number of colonies examined). From these numbers, we calculate that about 1.3% of genomic DNA is included within the LOH windows and about 98.7% is located outside of the LOH windows. Based on the number of chromosome elements within the genomic sequences represented in the microarrays, we can then calculate the expected numbers of these elements within the LOH and non-LOH fractions of the genome. For example, as shown in Dataset S8, there are 71 replication-termination (TER) sequences in the genome (10). In examining 25 genomes, we expect about 23.3 TER sequences within the LOH regions, and 1,751.7 TER sequences outside of the LOH regions. We observed 49 TER sequences within the LOH regions and 1,726 outside of the LOH regions. We compared these numbers by \( \chi^2 \) analysis, and found a very significant (\( P < 0.0001 \)) over-representation of the TER sequences at the recombination breakpoints. The \( \chi^2 \) analysis was done using both the VassarStats Web site (http://vassarstats.net), which has a correction for continuity, and the \( \chi^2 \) function in Excel, which does not correct for continuity. Both values were in good agreement qualitatively, and both are presented in Dataset S8.

A similar analysis was done for multiple other chromosome elements including: tandem repeats (repeats between 2 and 1,998 bp with a minimum repeat tract of 24 bp), tRNA genes, ARS elements, triplet repeats, long terminal repeats (primarily \( \delta \) elements), peaks of \( \gamma \)-H2AX, Rrm3p pause sites, small nuclear/nucleolar RNA genes, peaks with elevated GC content, and noncoding RNA genes. The references for the location of these elements are in Dataset S8; the map locations of most elements were obtained from the Saccharomyces Genome Database (SGD) Web site (http://www.yeastgenome.org/cgi-bin/seqTools). We also looked for correlations with highly transcribed genes. For this analysis, we determined the number of highly transcribed genes in the breakpoints, defining a highly transcribed gene as a gene ranking in the top 5%, using the database of Nagalakshmi et al. (11). A similar analysis was done for weakly transcribed genes using the 5% of genes that were most weakly transcribed. There are several other features of some of the comparisons that should be discussed. First, for G-quadruplex DNA, we did three different analyses. G-quadruplex sequences (G4 DNA) have been associated with slow-moving replication forks and genetic instability (12, 13). We found a significant correlation between the G4 motif predicted by Capra et al. (14) (four tracts of 3 G’s separated by spacers < 25 bp) and nonrandomness in the distribution of recombination events on the right arm of chromosome IV independently of the location of Ty elements. These regions are not significantly different from a random distribution in Fig. S1A). The \( \chi^2 \) test was done using the VassarStats Web site. The intervals used were: interval 1 (445–645 kb), interval 2 (645–845 kb), interval 3 (845–1,045 kb), interval 4 (1,045–1,245 kb), interval 5 (1,245–1,445 kb), and interval 6 (1,445–1,520 kb). For this analysis, each recombination event was represented as a single SGD coordinate representing the middle of the “window.” This procedure resulted in each event being located nonrandomly within the intervals 1–6, rather than any elements being counted in two intervals.

We found that the bin between SGD coordinates 1,045 and 1,245 kb had significantly more events than expected from a random distribution (Fig. S1A). The peak region contains three closely-linked nearly identical HXT genes. In the wild-type strain, there are two hotspots for spontaneous cross-overs (marked HS3 and HS4 in Fig. S1B). Both HS3 and HS4 contain inverted pairs of Ty elements. These regions are not significantly “hot” in the strain with low levels of DNA polymerase-\( \alpha \). Because the H3 and H4 hotspots are both G1-specific (6), it is not surprising that these hotspots do not appear to be active in strains with low levels of DNA polymerase because the recombination events observed in these strains are G2-specific.

Analysis of Unselected Recombination Events in Sectored Colonies Derived from WS84 and MG47. We examined eleven sectored colonies derived from WS84 and five derived from MG47 using whole-genome arrays to detect unselected LOH events. Of the 20 unselected events in WS84, we observed the following categories (numbers shown in parentheses): 3:1 conversions (10 events), 4:0 conversions (2 events), reciprocal cross-overs (3 events; two of which were apparent double cross-overs), potential BIR events (4 events), and one heterozygous deletion. In MG47, we found: 3:1 conversions (3 events), one reciprocal cross-over, and two potential BIR events. We classify a sectored colony as having a potential BIR event if one sector has a terminal LOH event and...
the other sector is heterozygous for the same region. Because we performed single-colony purification from each sector before analyzing the event by microarray, it is possible that a secondary cross-over occurring during the purification could mimic a BIR event. Nonetheless, based on our data and our previous analysis (9), it is likely that the long terminal LOH regions in our analysis of unsectored WS84 colonies reflect both cross-overs and BIR events.


Fig. S1. Distribution of recombination events induced by low levels of DNA polymerase-α on the right arm of chromosome IV compared with the distribution of spontaneous events. The x axis shows SGD coordinates on the right arm of chromosome IV extending from the centromere (449 kb) to the telomere (1,532 kb). The y axis shows the number of times SNPs on the array were included in an LOH region. (A) Mapping of LOH regions induced by low levels of DNA polymerase-α in MG47. The highest peak of LOH activity is in a region (marked by an arrow) containing three tandem closely-related HXT genes. (B) LOH regions in sectored colonies derived from the wild-type strain JSC25 (6). The two peaks marked HS3 and HS4 have inverted pairs of Ty elements.
Table S1. Strain genotypes

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<th>Strain</th>
<th>Genotype</th>
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<tr>
<td>W303a</td>
<td>MATa ade2-1 his3-11,15 ura3-1 leu2-3,112 tryp1-1 rad5-535 can-100</td>
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<td>JYM789</td>
<td>MATa ho::hisG lys2 gal2</td>
</tr>
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<td>JYM850</td>
<td>MATa ho::hisG lys5 Gal+ (YJM789-related)</td>
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<td>WS50</td>
<td>MATa ho::hisG lys5 KANMX-GAL1-POL1 Gal+ Disomic for chromosome XIV (YJM789-related)</td>
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<tr>
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<td>MATa ho::hisG lys5 Gal+ (YJM789-related)</td>
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<td>MATa ade2-1 his3-11,15 ura3-1 leu2-3,112 tryp1-1 can1-100Δ::NAT RADS IV1510386::KANMX-can1-100 (W303a-related)</td>
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Table S2. Primers used in strain constructions

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<td>GALPOL1R</td>
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<td>Po1F 430028</td>
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<td>pFA6aPGAL3HA R</td>
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<tr>
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Other Supporting Information Files