Cell, Vol. 120, 587–598, March 11, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2004.12.039

Chromosomal Translocations in Yeast Induced by Low Levels of DNA Polymerase: A Model for Chromosome Fragile Sites

Francene J. Lemoine,[1,3,4] Natasha P. Degtyareva,[1,3,5] Kirill Lobachev,[2] and Thomas D. Petes[1,4,*]

[1]Department of Biology and
Curriculum in Genetics and Molecular Biology
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599

[2]School of Biology
Institute for Bioengineering and Bioscience
Georgia Institute of Technology
Atlanta, Georgia 30322

Summary

In the yeast *Saccharomyces cerevisiae*, reduced levels of the replicative α DNA polymerase result in greatly elevated frequencies of chromosome translocations and chromosome loss. We selected translocations in a small region of chromosome III and found that they involve homologous recombination events between yeast retrotransposons (Ty elements) on chromosome III and retrotransposons located on other chromosomes. One of the two preferred sites of these translocations on chromosome III involve two Ty elements arrayed head-to-head; disruption of this site substantially reduces the rate of translocations. We demonstrate that this pair of Ty elements constitutes a preferred site for double-strand DNA breaks when DNA replication is compromised, analogous to the fragile sites observed in mammalian chromosomes.

Introduction

In wild-type organisms, the rates of mutation and chromosomal rearrangements (deletions, duplications, and translocations) are quite low, usually less 10^{-6}/cell division. Mutations that elevate the rate of genome instability often lead to increased cell death and in higher organisms an increased incidence of cancer (Lengauer et al., 1998). Common fragile sites are regions of the mammalian chromosome that break when cells are grown under conditions of thymidylate or folate stress or when incubated in drugs (such as aphidicolin) that inhibit DNA replication (Glover et al., 1984). These sites are also "hotspots" for the integration of viruses. In addition, chromosome translocations sometimes have breakpoints at common fragile sites (Glover and Stein, 1988; Wang et al., 1997).

*Correspondence: tompetes@duke.edu

[3]These authors contributed equally to this work.

[4]Present address: Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710.

[5]Present address: Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322.

What makes common fragile sites prone to breakage is not clear. Common fragile sites are relatively large (several hundred kb) chromosomal regions of unremarkable sequence that are replicated late in S phase (LeBeau et al., 1998; Arlt et al., 2003). Although the mechanism responsible for fragile sites is not yet understood, mammalian cells with an ATR mutation have high levels of breaks at fragile sites (Casper et al., 2002). A mutation in the orthologous yeast gene, *MEC1*, results in high levels of breaks in regions of the chromosome that replicate slowly (Cha and Kleckner, 2002). These findings suggest that common fragile sites are regions of DNA that are difficult to replicate and any condition that inhibits or delays DNA replication may induce chromosome breakage at these positions.

Formation of a chromosome aberration requires at least two steps: breakage of the chromosome(s) and rejoining of the broken ends in a novel arrangement by either homologous recombination (presumably involving repetitive DNA sequences) or nonhomologous end joining. In one experiment, in a hamster-human hybrid cell with a single human chromosome, the breakpoints in the fragile site of the human chromosome were mapped to two regions (Wang et al., 1997). In one of these regions, several of the sequenced breakpoints were within members of the THE-1 family of transposable elements.

Although the evidence for the involvement of transposable elements in fragile site-related translocations is not yet conclusive, there is clear evidence that recombination between transposable elements in yeast is a source of genomic rearrangements. Ty1 elements are the most common retrotransposon, with 32 full-length element insertions identified in the sequenced genome (Kim et al., 1998). The 5.9 kb Ty1 elements are flanked by 330 bp long terminal repeat (LTR) sequences (delta elements) in direct orientation. In several studies (for example Roeder and Fink, [1980] and Dunham et al. [2002]), recombination events between Ty elements or between solo delta elements were shown to generate chromosome translocations, deletions, and inversions. From these studies, it is not clear whether Ty elements are particularly susceptible to DNA breakage compared to other genomic sequences.

In the present study, we show that reduced expression of Pol1p (the catalytic subunit of DNA polymerase α) results in high frequencies of chromosome translocations and chromosome loss. We employed a genetic method (an illegitimate mating assay) that allowed us to select haploid strains with chromosome translocation and chromosome loss events, even when these events are haploid lethal. Using DNA microarrays and Southern analysis, we showed that the breakpoints of the translocations are within Ty elements. A preferred site for these events is associated with a head-to-head pair of Ty elements, and we suggest that this region may share some of the properties of mammalian fragile sites.

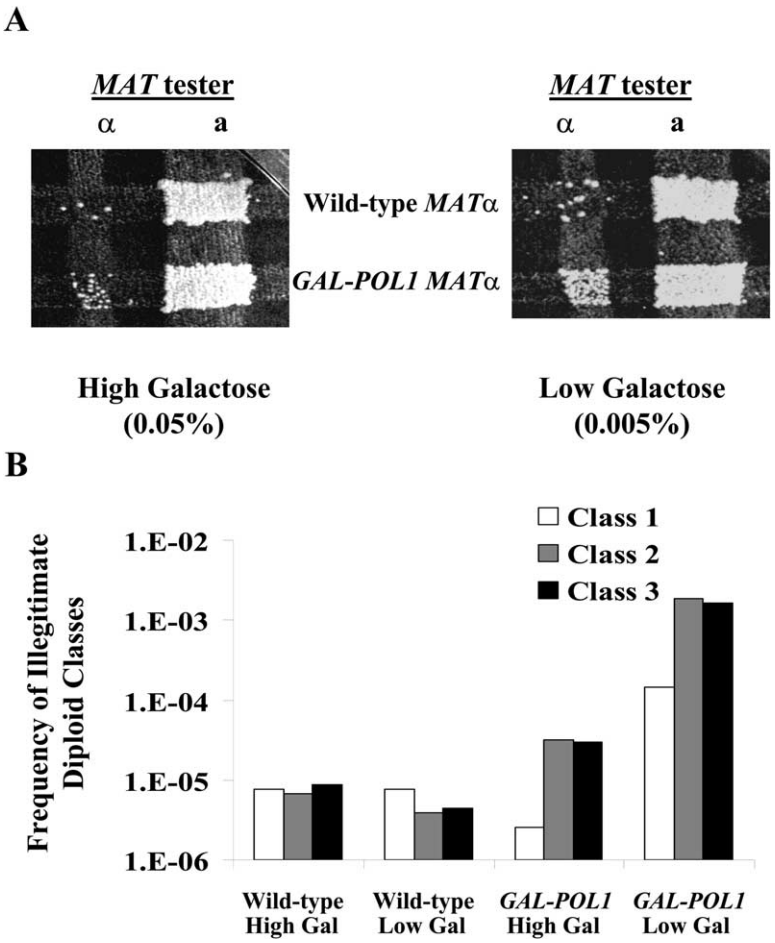

Figure 1. Elevated Frequencies of Illegitimate Mating in Strains with Low Levels of DNA Polymerase α and Frequencies of Different Classes of Diploids Resulting from Illegitimate Matings

(A) The *MAT*α wild-type and *GAL-POL1* strains were streaked onto rich growth medium supplemented with low or high concentrations of galactose. After the strains had grown, they were mated (by replica plating) to *MAT*α or *MAT***a** tester strains, and the resulting diploids were selected on appropriate omission medium.

(B) *MAT*α wild-type (MS71) and *GAL-POL1* (NPD1) haploid strains grown in the presence of high (0.05%) or low (0.005%) levels of galactose were mated to a *MAT*α tester strain (1225), and the resulting illegitimate diploids were analyzed. We examined the ratios of each class (Class 1, His^+ Thr^+; Class 2, His^- Thr^- ; Class 3, His^+ Thr^-) for about 100 diploids for each strain under each condition. The frequencies shown in this figure were determined by multiplying these ratios by the total frequency of illegitimate mating (Table S1).

Results

Reduced Levels of Pol1p Increase Sensitivity to DNA Damaging Agents

To examine the phenotypes associated with low levels of DNA polymerase α, we constructed a strain (NPD1) in which the galactose-regulatable *GAL1/10* promoter was fused to *POL1* (the gene encoding the catalytic subunit of DNA polymerase α). When this strain is grown in 0.005% (low) galactose, the expression of DNA polymerase α is approximately 10% of the wild-type level; since the *GAL1/10* promoter is active throughout the cell cycle, whereas the *POL1* promoter is S phase-specific, it is likely that the level of α DNA polymerase in S phase is less than 10-fold decreased in the *GAL-POL1* strain. When the strain is grown in 0.05% (high) galactose, α DNA polymerase is expressed at a level approximately 3-fold higher than that seen in wild-type cells (see Figure S1 in the Supplemental Data available with this article online). Additionally, the *GAL-POL1* strain grew slowly in low-galactose-containing medium (details in the Supplemental Data) and progressed slowly through S phase (Figure S2). These effects are not a consequence of a low amount of a carbon source in the medium since for both low- and high-galactose medium 3% raffinose was included but are likely a result of slowed DNA replication in response to reduced levels of DNA polymerase α. We also examined the effects of reduced expression of *POL1* on the ability of cells to repair or tolerate DNA damage (Figure S3). When grown in low-galactose medium, the *GAL-POL1* strain was more sensitive than the wild-type strain to ultraviolet light (UV), methyl-methane sulfonate (MMS), and hydroxyurea (HU). Since sensitivity to DNA-damaging agents is a phenotype often associated with cells that are genetically unstable, we used a different assay to determine the effect of low DNA polymerase on genome stability. As described below, this assay revealed a very high rate of chromosome rearrangements and chromosome loss in strains with low levels of DNA polymerase α.

Reduced Levels of Pol1p Increase the Frequency of Illegitimate Mating

The mating of a haploid yeast cell is regulated by the mating type, or *MAT*, locus. The genetic information present at this locus, which is located on the right arm of chromosome III, determines whether a haploid cell is of the α or **a** mating type. Wild-type haploid cells mate efficiently only to cells of the opposite mating type to form a *MAT***a**/α diploid cell. As shown in Figure 1A, the wild-type *MAT*α haploid (MS71) mated effi-

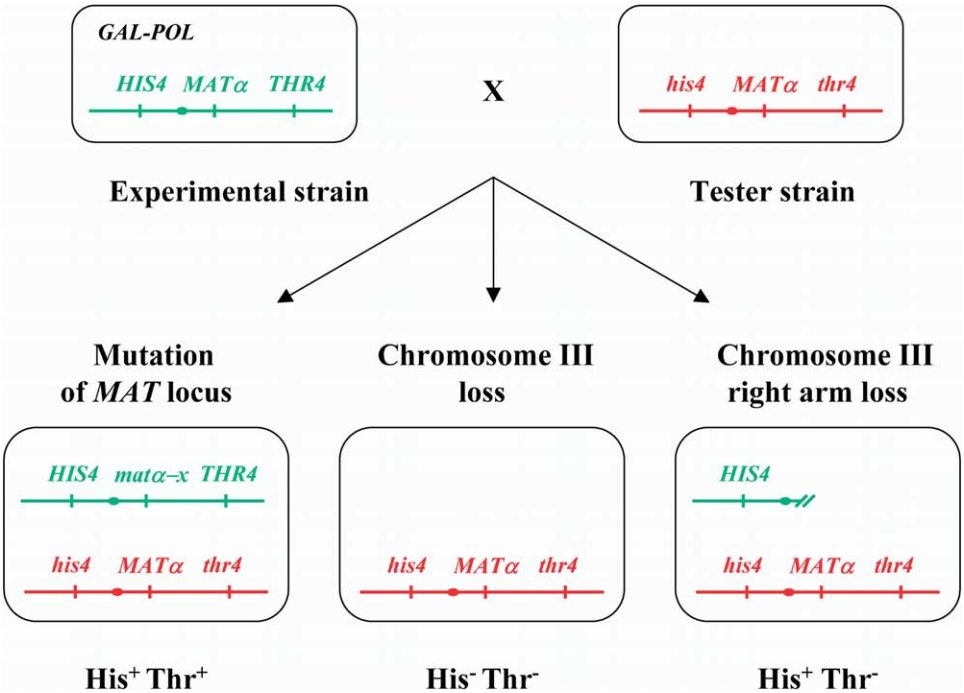

Figure 2. Genetic Events Leading to Illegitimate Mating of a *MAT*α Strain

The *GAL-POL1* (NPD1) strain is wild-type at the *HIS4* and *THR4* loci, markers on the left and right arms of chromosome III, respectively. The tester strain (1225) has mutant alleles of these genes. Point mutations in the *MAT*α locus (indicated by *matα-x*), loss of chromosome III, and loss of part of the right arm of chromosome III would lead to His$^+$ Thr$^+$, His$^-$ Thr$^-$, and His$^+$ Thr$^-$ diploids, respectively.

ciently to a tester strain of the opposite mating type (**a**) but very inefficiently to a strain of the same mating type (α). Similarly, the *MAT*α *GAL-POL1* mutant haploid grown in high-galactose-containing medium mated efficiently to a tester strain of the opposite mating type (**a**) but inefficiently to a strain of the same mating type (α). However, when this mutant strain was grown in low-galactose-containing medium, while legitimate (α × **a**) mating was unaffected, the efficiency of illegitimate (α × α) mating was dramatically increased. Under the conditions in which these experiments were performed (5-fold excess of the tester maters), all viable cells of both wild-type and *GAL-POL1* strains mated legitimately with an efficiency of approximately 1 in both high- and low-galactose-containing medium (data not shown). In contrast, there was a 210-fold increase in the illegitimate mating efficiency of the *GAL-POL1* strain, relative to the wild-type strain, when grown in the presence of low levels of galactose (Table S1). When the *GAL-POL1* strain was grown in medium with high levels of galactose, the frequency of illegitimate mating was elevated about 3-fold.

There are two types of events that can lead to the illegitimate mating of an α haploid to another α haploid: (1) a mating type switch which converts a *MAT*α haploid to a *MAT***a** haploid or (2) a mutation (either a point mutation or a deletion) that inactivates the *MAT*α locus information resulting in a haploid with an "Alf" or *a-l*ike *f*aker phenotype (Strathern et al., 1981). If illegitimate mating is a result of a mating type switch event, the resulting diploid will be *MAT***a**/α and should be capable of sporulation but incapable of further mating. However, if illegitimate mating is a result of a *MAT* locus mutation, the resulting diploid will be *MAT*–/α and should be incapable of sporulation but capable of further mating to a *MAT***a** haploid. None of the 289 diploids generated by the illegitimate mating of the *GAL-POL1* strain pregrown on low-galactose medium were capable of sporulation. Additionally, all of the illegitimate diploids were capable of further mating to a *MAT***a** tester strain. These findings suggest that the illegitimate matings that generated these diploids were not caused by a mating type switch event, but rather by a mutation or deletion of the *MAT* locus.

Because deletion of the *MAT* locus leads to the mating of haploid cells of the same mating type, illegitimate mating can be used to detect chromosome deletions and chromosome loss involving yeast chromosome III (McCusker and Haber, 1981; Gordenin et al., 1991). To determine whether the Alf phenotype of the *GAL-POL1*-derived illegitimate diploids described above was a result of a point mutation of the *MAT*α locus or a more extensive deletion of chromosome III, we mated the α haploids of the two genotypes (wild-type and *GAL-POL1*), pregrown on low-galactose medium, to a *MAT*α tester strain (1225) that had recessive mutations located on the left (*his4*) and right (*thr4*) arms of chromosome III. As shown in Figure 2, a point mutation or a small deletion within the *MAT*α locus would result in His$^+$ Thr$^+$ illegitimate diploids (Class 1); this class could also be the result of a direct fusion, rather than mating, of the haploid cells without any mutation of the *MAT* locus. Loss of chromosome III would result in His$^-$ Thr$^-$ illegitimate diploids (Class 2) and a large deletion that

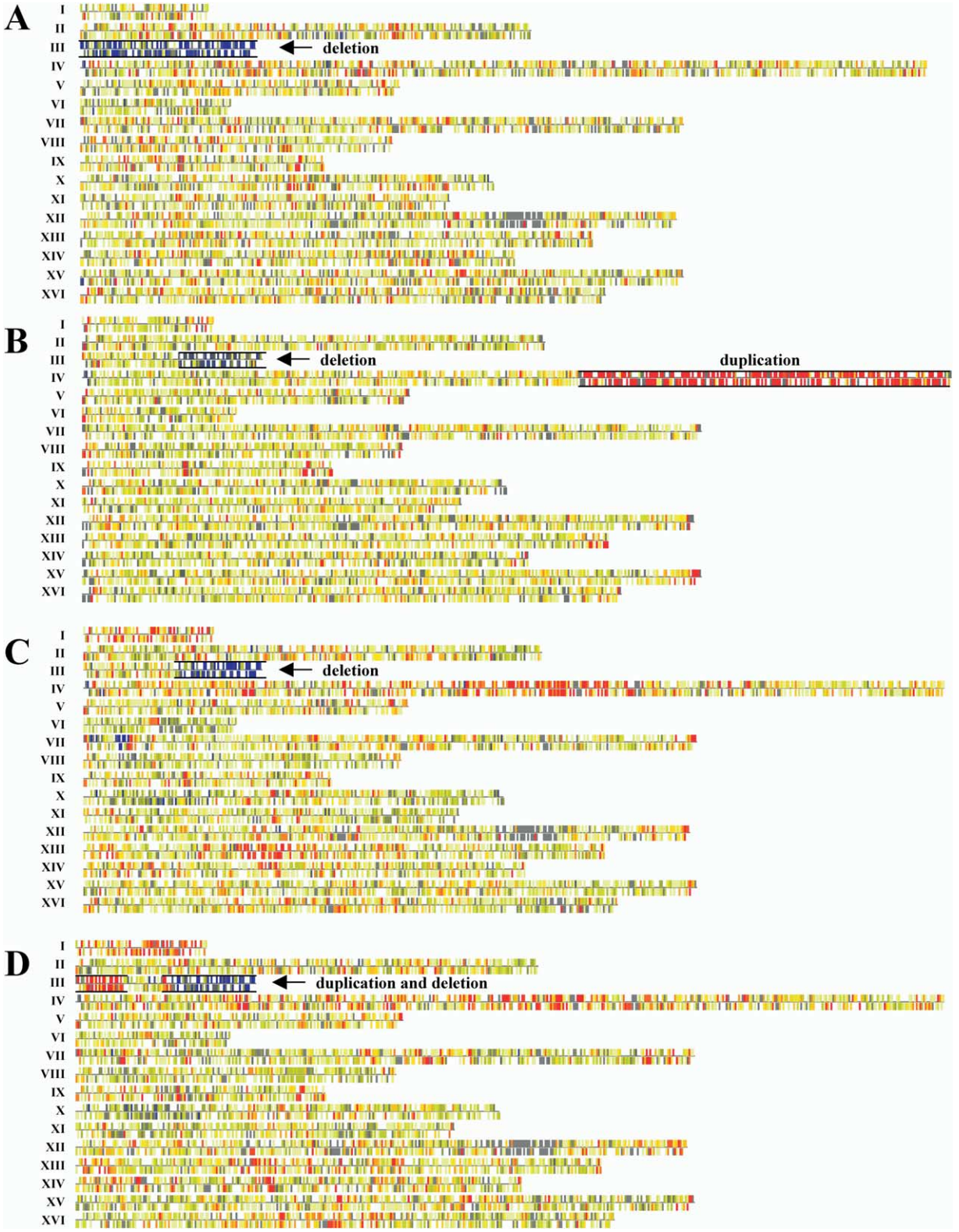

Figure 3. Genomic Microarray Analysis of Diploids Resulting from Illegitimate Mating

Genomic DNA was isolated from diploids generated by the illegitimate mating of the *GAL-POL1* (grown in low levels of galactose, [A] and [B]) or wild-type haploids (C and D) to the 1225 tester strain. This DNA was labeled with a Cy5-labeled fluorescent nucleotide (details in the Supplemental Experimental Procedures), and DNA from a reference strain (MS71) was labeled with a Cy3 nucleotide. The two samples were mixed and hybridized to a DNA microarray containing all of yeast ORFs. In this depiction, each vertical bar represents an ORF. Yellow indicates approximately equal amounts of DNA from the experimental and control strains; blue signifies approximately 2-fold less DNA in the

removed both the *MAT*α and *THR4* loci would result in His^+ Thr^- illegitimate diploids (Class 3). Events generating the illegitimate diploids of the latter two classes would result in the loss of multiple essential genes located on chromosome III. This loss would prevent the growth of the haploid cells in which these events occurred. However, these growth-inhibited cells can be rescued by mating to the tester haploid, generating an illegitimate diploid containing at least one copy of the essential genes. Therefore, this system allows for the specific identification of lethal chromosomal aberrations that are rescued by illegitimate mating.

The distributions of these three classes for the wild-type and *GAL-POL1* strains grown in low- and high-galactose-containing media are shown in Figure 1B. Wild-type cells grown in either type of media had similar frequencies of the three classes. In the *GAL-POL1* strain, grown in either type of media, Classes 2 and 3 were equally frequent and both were about 10-fold more frequent than Class 1. These data, coupled with the above-described results on the rates of illegitimate mating, indicate that low levels of α DNA polymerase lead to very high rates of chromosome loss and large deletions. This conclusion was confirmed by DNA microarray analysis, as described below. This technique has been used previously to characterize yeast chromosome alterations associated with the continuous growth of cells in limiting glucose (Dunham et al., 2002). Finally, it should be mentioned that our estimates of the frequencies of chromosome loss and rearrangements are likely to be underestimates, since some cells with these events might be incapable of being rescued by mating.

The data shown in Figure 1B and Table S1 also indicate that overexpression of DNA polymerase (*GAL-POL1* strain grown in high galactose) results in elevation in chromosome loss and chromosome rearrangements, although this effect is relatively subtle compared to that observed in cells with low polymerase levels. It is possible that the overexpression of one subunit of α polymerase reduces the number of properly assembled polymerase-primase complexes.

Reduced Levels of Pol1p Induce Chromosome Loss and Chromosome Rearrangements

DNA was isolated from two Class 1 (one wild-type-derived and one *GAL-POL1*-derived), one Class 2 (*GAL-POL1*-derived), and twenty-four Class 3 (sixteen wild-type-derived and eight *GAL-POL1*-derived) illegitimate diploids for microarray analysis; all diploids were the result of illegitimate matings of cells pregrown in low-galactose-containing medium. The DNA samples derived from individual illegitimate diploids were labeled with a Cy5 fluorescent nucleotide (details in the Supplemental Experimental Procedures). Control DNA from the wild-type (MS71) strain was labeled with a Cy3 fluorescent nucleotide. The experimental and control samples were mixed and hybridized to a DNA microarray containing all of the yeast ORFs. The relative amount of hybridization at each ORF was measured. Examples of this analysis are shown in Figure 3. As described in the legend, each ORF is represented by a vertical bar. Equal, decreased, and increased gene dosages of the experimental DNA relative to the control are shown by yellow, blue, and red colors, respectively.

In the two Class 1 diploids, no alterations in gene dosage were observed (data not shown), consistent with the possibility of a point mutation inactivating the *MAT*α locus or a fusion between the two *MAT*α strains. In the Class 2 diploid (Figure 3A), all of the ORFs on chromosome III were 2-fold underrepresented, consistent with a single copy of chromosome III in the diploid. There are two plausible mechanisms for this chromosome loss. First, low levels of DNA polymerase may result in failure to complete chromosome replication prior to chromosome segregation, resulting in loss of the chromosome from one cell and, presumably, duplication of the chromosome in the daughter cell. Alternatively, low levels of DNA polymerase may lead to an increased frequency of double-strand DNA breaks (DSBs). If the broken chromosomes are degraded rather than repaired, chromosome loss would occur.

There were three different types of Class 3 illegitimate diploids that were found for both the wild-type and *GAL-POL1* strains. Class 3A diploids had both a deletion of sequences from the right arm of chromosome III and a duplication of sequences from a different chromosome. The segment duplicated varied among independent Class 3A diploids. For example, in the diploid shown in Figure 3B, a segment from the right arm of chromosome IV is duplicated. Class 3B diploids had a deletion of sequences from the right arm of chromosome III but no duplication (Figure 3C). Class 3C diploids had no obvious deletion or duplication (data not shown). The numbers of Class 3A:3B:3C diploids (as determined by microarray analysis) were 3:1:7 for the wild-type strain and 3:1:4 for the *GAL-POL1* mutant strain.

Several types of Class 3 events were unique to the wild-type strain including (1) a deletion resulting from recombination between the *MAT*α and *HMR* loci (identical to the "Hawthorne" deletion [Hawthorne, 1963]), (2) a deletion of sequences on the right arm of III and a duplication of sequences on the left and right arms (Figure 3D), (3) loss of one copy of chromosome III, with the remaining copy composed of the left half derived from MS71 and the right half from 1225, (4) duplication

experimental strain relative to the control, and red indicates approximately 1.5-fold more DNA in the experimental strain. The ORFs indicated in gray on chromosome XII are not present on the microarray.

(A) Class 2 (His^- Thr^-) diploid (DNPD103, loss of chromosome III).

(B) Class 3A diploid (DNPD104; loss of sequences from right arm of chromosome III and duplication of sequences from chromosome IV).

(C) Class 3B diploid (DMS71-27); loss of sequences from right arm of chromosome III unassociated with a duplication of sequences from another chromosome).

(D) Illegitimate diploid (DMS71-23) with deletion of chromosome III sequences centromere-distal to FS2, duplication of sequences between FS1 and FS2, and duplication of sequences on the left arm.

of all chromosome III sequences, and (5) duplication of sequences from the left arm of III (between a Ty2 element and the telomere) and a duplication of sequences on the right arm.

We performed a detailed analysis of four Class 3A illegitimate diploids (one wild-type-derived and three *GAL-POL1*-derived). From the microarray analysis, it was clear that the breakpoints on chromosome III marking the deleted sequences in these strains occurred at one of two sites that were centromere-proximal to the *MAT* locus, one immediately centromere-distal to *SRD1* (termed "FS1" for *f*ragile *s*ite 1) and one immediately centromere-distal to *RHB1* (FS2). The boundaries of the duplicated sequences on the other chromosomes were adjacent to a Ty element. Although there were no Ty elements at FS1 or FS2 indicated in the Stanford Genome Database, by Southern and PCR analysis (details in the Supplemental Data), we showed that in MS71 and its derivatives, FS1 had a 3.2 kb deletion (bases 148610–151846) and an insertion of two Watson-oriented Ty1 elements in a tandem head-to-tail array, sharing a single central delta element (Figure S4); this structure, which was also present in the tester strain 1225, is exactly as reported for the similar strain AB972 by Wicksteed et al. (1994).

By additional Southern analysis, we found that in MS71 and its derivatives, the FS2 region also had two closely-linked Ty1 elements, but these are arranged head-to-head with 283 bp between the delta elements. This arrangement was also described previously in the strain AB972 by Wicksteed et al. (1994). However, we found that the tester strain 1225 had a single Ty1 element in the FS2 region instead of the inverted pair (Supplemental Data; Figure S5). It should be noted that, although the Stanford Genome Database (based on sequencing an isogenic derivative of the strain S288c) has no indication of Ty elements at the positions of FS1 and FS2, restriction analysis of our lab isolate of S288c indicates the presence of Ty elements at FS1 and FS2 in the same positions as observed for MS71; we have no evidence for additional Ty1 elements on chromosome III (although we did not examine the whole chromosome), but we confirmed the presence of a Ty2 element (*YCLWTy2-1*) on the left arm of III (data not shown).

The presence of Ty elements in FS1 and FS2 suggests that the patterns observed in the microarray analysis of Class 3A strains likely reflect the presence of chromosomal translocations generated by homologous recombination between the Ty elements located in FS1 or FS2 with Ty elements on nonhomologous chromosomes. This conclusion was confirmed by a detailed Southern analysis. The particular Ty elements involved in the translocations from the four strains that were analyzed were as follows: (1) the centromere-proximal Ty1 element at FS1 and the *YBRWTy1-2* element of chromosome II (strain DNPD101; *GAL-POL1*-derived), (2) the centromere-proximal Ty1 element at FS2 and the *YDRCTy1-2* element of chromosome IV (strain DNPD104; *GAL-POL1*-derived), (3) the 3′ delta element of the centromere-proximal Ty1 element at FS2 and the 5′ delta element of the *YDRCTy1-2* element of chromosome IV (strain DNPD106; *GAL-POL1*-derived), and (4) the centromere-proximal Ty1 element at FS1 and the *YPRWTy1-3* element of chromosome XVI (strain DMS71-12; wild-type-derived). In summary, the Class 3A diploids contain a translocation between a centromere-proximal Ty1 element of FS1 or FS2 and a Ty element of a nonhomologous chromosome. The orientation of the involved Ty elements is such that monocentric rather than dicentric chromosomes were generated. The presence of the predicted translocation chromosomes was verified by analysis of intact chromosomes (CHEF gels) (data not shown).

The generation of Class 3A diploids can be explained by the model shown in Figure 4. We propose that cells with low levels of DNA polymerase have elevated frequencies of DSBs on chromosome III. These DSBs occur within either FS1 and FS2 or at some point centromere-distal to these sites, and the ends then are degraded until they are within the FS1 and FS2 regions. Regardless of the site of formation, the DSB results in a broken chromosome III molecule that has lost the *MAT*α locus. The loss of the *MAT*α locus renders the haploid cell capable of mating illegitimately with the *MAT*α tester strain. This mating rescues the haploid from its lethal chromosome loss. Following the formation of the *MAT*–/α illegitimate diploid, the broken chromosome III molecule is repaired by a break-induced replication (BIR) event (Paques and Haber, 1999). The BIR event involves the invasion of the chromosome III end with the Ty sequences into a Ty element on a different chromosome, followed by duplication of all of the sequences centromere-distal to the site of invasion.

Although the BIR event described above could occur in the haploid cell prior to mating rather than in the diploid cell (as shown), several arguments suggest that the recombination event in the *GAL-POL1* strain occurs in the diploid. First, since this strain has low levels of DNA polymerase, a BIR event involving the replication of hundreds of kb of DNA by a single replication fork is likely to be difficult. In the diploid generated by illegitimate mating, DNA polymerase levels would be elevated because transcription of the *POL1* gene in the haploid tester strain 1225 is regulated by the endogenous promoter. Second, for two of the illegitimate diploids (DNPD104 and DNPD106), we determined that the translocations were generated in the diploid following mating. Both of these illegitimate diploids had a translocation between chromosomes III and IV. We identified a sequence polymorphism located near the telomere of chromosome IV, and we showed that the 1225-derived allele was present in two copies in the strains containing the translocation, indicating that the BIR event occurred in the diploid (details of the analysis in the Supplemental Data). Finally, as will be discussed below, Class 3C diploids are very likely to represent BIR events that could occur only in the diploid.

We identified two Class 3B illegitimate diploids that had a deletion of sequences on chromosome III but no duplication of other sequences (Figure 3C); one was derived from the illegitimate mating of the wild-type (MS71) strain and the other was derived from the illegitimate mating of the *GAL-POL1* mutant strain. By microarray analysis, the deletion had a breakpoint near FS2 (for the wild-type-derived diploid) or FS1 (for the *GAL-*

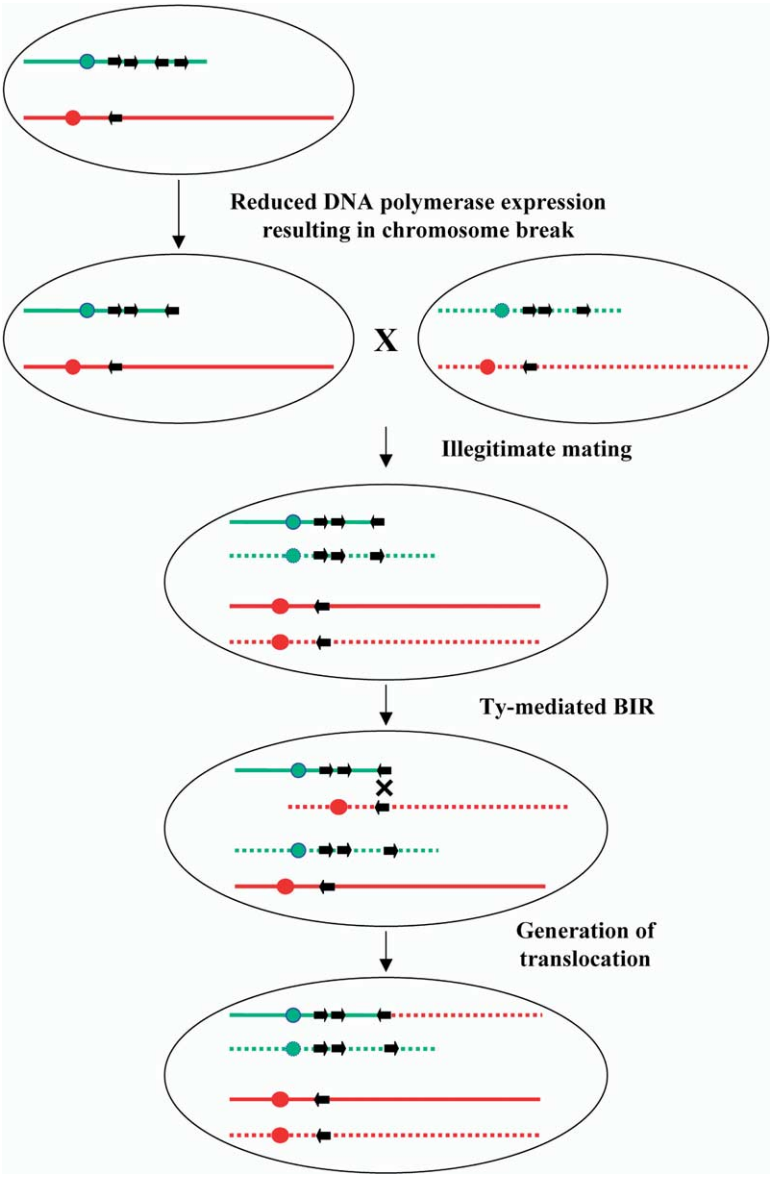

Figure 4. Chromosome Translocations Induced by Double-Strand Breaks at FS2

We suggest that the low levels of DNA polymerase in the *GAL-POL1* strain results in elevated levels of DSBs at FS2. Chromosome III is shown in green, a nonhomologous chromosome in red, and Ty elements are indicated by arrows; continuous and dotted lines represent chromosomes from the experimental and tester strains, respectively. Loss of the chromosome III sequences distal to the break allows the illegitimate mating of these strains to the 1225 tester strain. In the resulting diploid, the broken chromosome with a Ty element at the break engages in a BIR event, resulting in a translocation (Class 3A diploid). If the BIR event involves an allelic invasion of chromosome III (not depicted in this figure), one would obtain a Class 3C diploid (no net loss or duplication of genomic sequences).

POL1-derived diploid). These isolates may represent broken chromosomes that were "capped" by telomeric sequences, although it is also possible that they are also a result of a translocation involving a Ty element located very near a telomere (resulting in a duplication that was undetectable by microarray analysis). CHEF analysis of intact chromosomes confirmed a truncated form of chromosome III in both of these Class 3B diploids (data not shown).

In Class 3C illegitimate diploids, no obvious deletions or duplications were observed. Since the diploids mate as though they were *MAT*α strains and are threonine auxotrophs, they lack two linked genes derived from the *GAL-POL1* parent. The microarray analysis, however, indicates that these strains are homozygous rather than hemizygous for the *MAT* and *THR4* loci. One simple explanation of Class 3C diploids is that a broken chromosome III is repaired by a BIR event in the diploid. However, unlike the BIR events that lead to the generation of Class 3A illegitimate diploids, these events utilize allelic sequences from chromosome III of the tester 1225 strain as a template.

As described previously, we found several additional types of Class 3 events only in diploids derived from the wild-type strain. One of these rearrangements (in strain DMS71-23) was extensively characterized by Southern analysis and CHEF gels (details in the Supplemental Data). In this strain, one copy of chromosome III had a deletion of the sequences distal to FS2, a duplication of sequences between FS1 and FS2, and a duplication of sequences centromere-distal to a Ty2 element on the left arm of III (Figure 3D).

We also examined the effects of low α DNA polymerase on the rates of mitotic recombination and chromo-

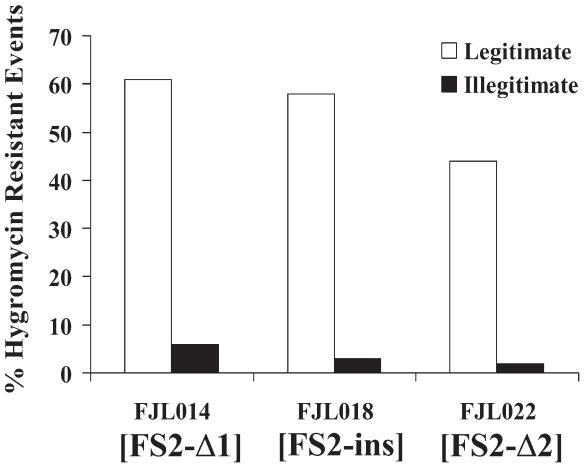

Figure 5. Efficiency of Illegitimate Mating for Strains with Alterations of the FS2 Region

The *GAL1-POL1* strain (NPD1 [FS2]) with the intact FS2 (two inverted Ty elements separated by about 200 bp) was allowed to form colonies on solid medium with high galactose. Individual colonies were mixed with individual colonies of other strains (FJL014 [FS2-Δ1], FJL018 [FS2-ins], and FJL022 [FS2-Δ2]) grown on medium with high levels of galactose. All three of these strains had the *GAL-POL1* gene but also had alterations of the structure of FS2. FJL014 [FS2-Δ1] lacked the centromere-proximal Ty element, FJL022 [FS2-Δ2] lacked the centromere-distal Ty1, and FJL018 [FS2-ins] had an insertion that increased the distance between the FS2 Ty elements about 10-fold; all three strains were hygromycin-resistant, unlike NPD1. The mixed *MAT*α cells were grown on medium with low levels of galactose then mated illegitimately to the *MAT*α strain 1225 and legitimately to the *MAT***a** strain A364a (*ade1 ura1 gal1 ade2 tyr1 his7 lys2*; provided by L. Hartwell). We tested one diploid from each mating to determine whether it was derived from NPD1 (resulting in hygromycin sensitivity) or one of the strains with the altered FS2 structure (resulting in hygromycin resistance). About 100 independent competitive matings were examined for each pair of strains.

some loss for chromosome V in diploid strains (details in the Supplemental Data). The rates of chromosome loss were elevated only modestly (4-fold), but the mitotic recombination rates were elevated 22-fold by low levels of α DNA polymerase.

Genetic Evidence that the Ty Elements at FS2 Are Preferred Sites for DNA Damage

Although it is clear that FS1 and FS2 are preferred sites for the chromosome rearrangements, it is not clear whether these are preferred sites for the DNA lesions (presumably DSBs) that initiate the rearrangements. To address this issue, we constructed derivatives of the *GAL-POL1* strain with alterations to FS2 and determined the effects of these alterations on the frequency of illegitimate mating. These derivatives had (1) a replacement of the centromere-proximal Ty1 of FS2 with the *HYG*^{R} gene (FJL014 [FS2-Δ1]), (2) a replacement of the centromere-distal Ty1 of FS2 with the *HYG*^{R} gene (FJL022 [FS2-Δ2]), or (3) an insertion of the *HYG*^{R} gene between the two Ty elements of FS2 (FJL018 [FS2-ins]). Independent colonies of NPD1 and these derivative strains were mixed (either NPD1 [FS2] and FJL014 [FS2-Δ1], NPD1 [FS2] and FJL022 [FS2-Δ2], or NPD1 [FS2] and FJL018 [FS2-ins]), and the mixed cultures were mated separately to a strain of the same mating type (α) or to a strain of the opposite mating type (**a**). A single diploid was selected from each mated mixture and examined to determine whether it was hygromycin-resistant (derived from the derivative with the disrupted FS2 region) or hygromycin-sensitive (derived from the haploid with the intact FS2 region). At least 100 independent matings were analyzed for each pair of strains.

As shown in Figure 5, the alteration of FS2 did not affect legitimate mating but substantially (average of 24-fold) reduced the frequency of illegitimate mating in strains with low α polymerase. Since the frequency of illegimate mating reflects genome rearrangements on chromosome III, these results demonstrate that much of the genetic instability on chromosome III is FS2 related. The ratio of Classes 1:2:3 in the strains with the alterations of FS2 were 1:0:5 (FJL014 [FS2-Δ1]), 1:0:2 (FJL018 [FS2-ins]), and 1:0:1 (FJL022 [FS2-Δ2]). From these data and the data in Figure 1B, we calculate that disruption of the structure of FS2 reduces the rates of translocations more than 15-fold and chromosome III loss more than 20-fold in the *GAL-POL1* strain. The simplest interpretation of these observations is that the closely linked, inverted Ty1 elements are a preferred site for the DNA damage that initiates the chromosome loss and translocation events. This interpretation is supported by the physical analysis described below.

Physical Evidence that the Ty Elements at FS2 Are Preferred Sites for DNA Damage

To look for physical evidence for fragile sites associated with illegitimate mating, we separated intact yeast chromosomal DNA using CHEF gels and hybridized the separated chromosomes to chromosome III-specific probes, as previously done by Cha and Kleckner (2002) to map DSBs that occur in strains with a *mec1-ts* mutation. When DNA was isolated from *GAL-POL1* cells grown in low-galactose-containing medium, we detected DNA fragments that were smaller than the intact chromosome III of no obvious preferred size class (data not shown). However, when we shifted the *GAL-POL1* cells from medium with high levels of galactose to medium containing raffinose but lacking galactose, after 6 hr, we found a discrete DNA fragment of approximately the size expected as a consequence of an FS2-associated break (about 200 kb; Figure 6, lane 2). This fragment was not observed in the wild-type strain grown under the same conditions (Figure 6, lane 1) nor in FJL014 [FS2-Δ1], the derivative of the *GAL-POL1* strain that lacks the centromere-proximal Ty element of FS2 (Figure 6, lane 4). In addition, no such fragment was observed in RJK341, a strain with a fusion between the *GAL* promoter and *POL3* (Kokoska et al., 2000), encoding DNA polymerase δ. Furthermore, this fragment was not detected in any of these strains when they were grown in medium containing high levels of galactose (data not shown). These results support the conclusion that the inverted Ty configuration at FS2 is a hotspot for DNA breaks under conditions of low α DNA polymerase.

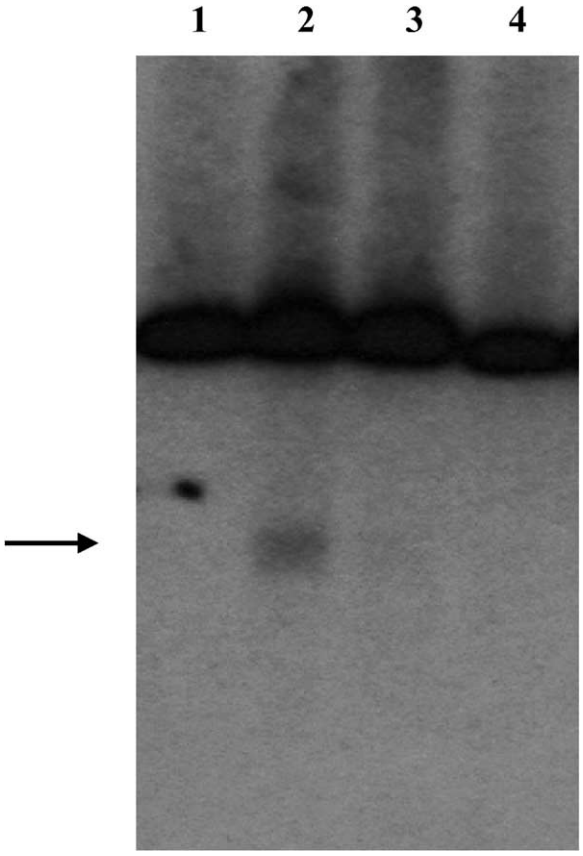

Figure 6. Identification of an FS2-Dependent DSB on Chromosome III

Strains of various genotypes were grown on medium containing 0.05% galactose, 3% raffinose to midlog phase. The cells were then washed and resuspended in medium with 3% raffinose, but no galactose, and incubated at 30°C for 6 hr. DNA samples were collected, and intact yeast chromosomal DNA molecules were separated by pulsed field gel electrophoresis. The separated molecules were transferred to a membrane and hybridized to a probe (*HIS4*) from the left arm of chromosome III. The strains in each lane are: MS71 (wild-type, lane 1), NPD1 (*GAL-POL1*, intact FS2, lane 2), RJK341 (*GAL-POL3* [described in Kokoska et al. (2000)], lane 3), and FJL014 [FS2-Δ1] (*GAL-POL1*, deletion of centromere-proximal Ty of FS2, lane 4).

Discussion

Although genomic regions with high levels of meiotic recombination (hotspots) are well documented in yeast (reviewed by Petes [2001]), there is relatively little information about hotspots for mitotic recombination. There appear to be two somewhat general stimulatory factors. The first is transcription. In a variety of different assays (most involving ectopic recombination), transcription stimulates mitotic exchange (reviewed by Aguilera [2002]). A second likely factor is DNA replication. Cha and Kleckner (2002) showed that a *mec1-ts* mutant, when incubated at the restrictive temperature, had DSBs in regions of the genome that had slowly moving DNA replication forks. Since DSBs, caused by X-rays or site-specific nucleases, efficiently stimulate mitotic recombination (Paques and Haber, 1999), it is likely that these replication slow zones (RSZs) would be hotspots for mitotic exchange, at least in cells with low levels of Mec1p.

There are several types of nonyeast sequences (closely spaced inverted repeats or palindromes) that function as sites for DSB formation and/or hotspots for mitotic recombination in yeast. Gordenin et al. (1993) showed that the bacterial transposon Tn5 (two inverted repeats of 1.5 kb separated by a spacer of 2.7 kb), when inserted in the yeast genome, resulted in increased rates of mitotic recombination. These rates were further elevated in strains with mutations in either DNA polymerase α or δ (Gordenin et al., 1993). Ruskin and Fink (1993) found that short (30 and 80 bp) inverted repeats were excised at very high rates in yeast strains with mutations in DNA polymerase α. Freudenreich et al. (1998) showed that tandem repeats of $(CTG)_{130–250}$, which can form "hairpins" in which every third base is mismatched, are capable of stimulating mitotic recombination and that DSBs occur within these repetitive tracts. A head-to-head duplication of the human *Alu* repeat also strongly stimulates mitotic recombination and is a site for DSB formation in vegetative cells (Lobachev et al., 2002). The mechanism proposed for these effects is that the palindromic sequences are sometimes extruded to form a cruciform that is then processed to form the recombinogenic lesion (Lobachev et al., 2002).

The study presented here demonstrates that naturally occurring yeast sequences are capable of producing effects similar to those described above. These previously reported observations suggest several nonexclusive explanations for our results. One explanation is that low levels of DNA polymerase result in a delay in the synthesis of Okazaki fragments on the lagging strand, leading to an increase in the size of the single-stranded region of the lagging strand. This large single-stranded region increases the probability of forming a hairpin structure involving the Ty elements of FS2 (left side of Figure 7). Processing of this hairpin, perhaps within the single-stranded region separating the Ty element, followed by dissociation of the hairpin stem results in the recombinogenic DSB near the centromere-proximal Ty element. This mechanism is similar to that proposed to explain the instability of inverted repeats in *E. coli* (Leach, 1994) and yeast (Lobachev et al., 1998). The translocations that involve FS1 could reflect extensive processing of a DNA end resulting from an FS2 break, or, alternatively, it may also be a preferred site for chromosome breaks through a different mechanism.

A second explanation is that the FS2 inverted repeat becomes extruded as a cruciform that is then processed by DNA resolvase (right side of Figure 7). The resulting structure could have two possible fates. Endonucleolytic removal of the single-stranded loop at the end would produce an intermediate very similar to that resulting from processing of the hairpin. Alternatively, replication of the structure, without removal of the single-stranded loop, could produce a palindromic chromosome (Figure 7). Cruciform extrusion, followed by processing of the structure, has been observed in yeast previously (Lobachev et al., 2002), although the

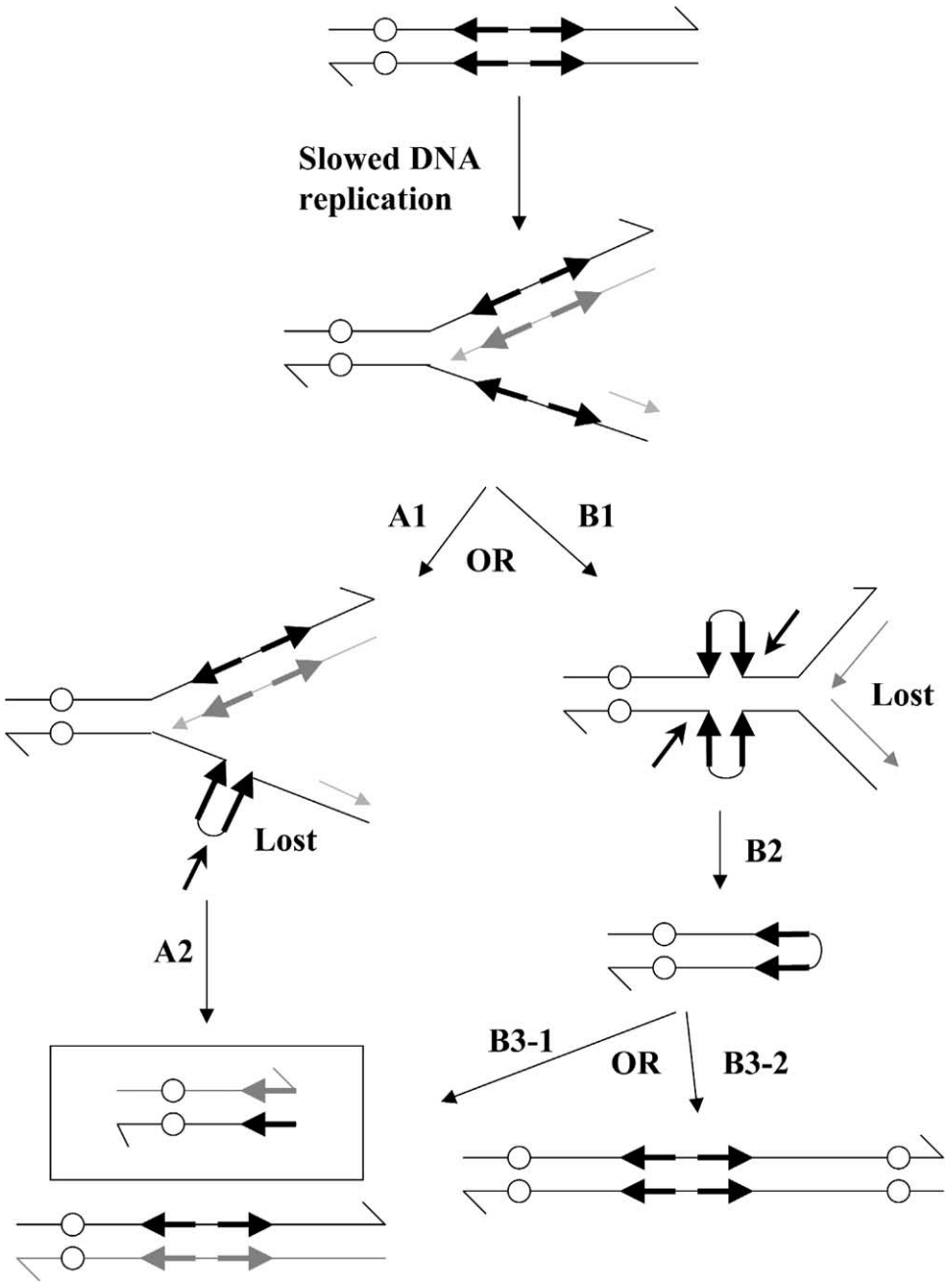

Figure 7. Generation of DSBs during the Replication of FS2

When DNA replication is slowed by reduced levels of DNA polymerase, we suggest that there are large single-stranded regions on the lagging strand. In this diagram, thick arrows indicate the Ty elements of FS2 and the circles indicate centromeric DNA. DSBs can form in two different ways:

(1) As shown in A1, the palindromic Ty elements of FS2 (shown by thick arrows) form a large hairpin. Endonucleolytic cleavage of the single-stranded DNA at the tip of the hairpin (A2), followed by continued DNA replication, results in an intact chromosome and a chromosome with a break located at the Ty element (shown in a box).

(2) Alternatively, the primer leading strand could be degraded, allowing reannealing of the template strands and cruciform formation (B1). Processing of the cruciform by resolvase would result in two DNA molecules with hairpins (B2), one lacking centromeric DNA (not shown) and one with centromeric DNA. The single-stranded region at the tip of the hairpin could be degraded, resulting in a chromosome with a DSB at the Ty element (B3-1) or replicated, resulting in a palindromic dicentric chromosome (B3-2).

inverted repeats in this study were much smaller than those in our study (320 bp versus 6 kb) and only 12 bp separated the repeats.

Despite these differences, at least one of the chromosome rearrangements, that observed in DMS71-23 (derived from an illegitimate mating of the wild-type haploid), has the structure expected as a consequence of resolvase-mediated resolution of a cruciform. From

the microarray and Southern analysis (described in detail in the Supplementa; Data), we found that the sequences located between FS1 and FS2 were duplicated in inverted orientation, forming a large palindrome. In addition, the sequences between a Ty2 element on the left arm of chromosome III were duplicated. As depicted in Figure S6, this chromosome structure can be explained by resolution of a cruciform structure at FS2, followed by replication of the resulting giant hairpin structure, followed by a secondary recombination event between the Ty2 element on the left arm and a Ty1 element of FS1.

Our studies indicate that the frequency of DNA breaks at FS2 is much higher in strains with low levels of α DNA polymerase. Since we find, however, similar classes of chromosome rearrangements in diploids resulting from the illegitimate mating of wild-type strains, FS2 is likely to be an important source of genomic instability even when the levels of α DNA polymerase are normal. In support of this conclusion, it should be noted that FS2 has also been identified as a region (Malkova et al., 2001) that is required for the Rad51p-independent BIR events needed to repair an HO-catalyzed DSB at the *MAT* locus (A. Malkova, J. Haber, J. Theis, and C. Newlon, personal communication).

The presence of yeast fragile sites has been suggested previously by Cha and Kleckner (2002). They demonstrated that certain regions of chromosome III in which replication was slowed were susceptible to DSB formation in a *mec1-ts* strain grown at the restrictive temperature. DSB formation was not shown to occur at specific sites but rather occurred within regions of approximately 10 kb. These regions were not mapped with respect to chromosome III sequences or associated with specific chromosome rearrangements. It is also not possible to compare the positions of the DSBs observed by Cha and Kleckner with the positions of FS1 or FS2, since the locations of Ty elements in their strain have not yet been determined.

Our analysis suggests that yeast fragile sites may require two properties, slow-moving DNA replication forks or other perturbations in DNA replication and inverted repeated sequences. Certain features of the mammalian and yeast fragile sites are conserved. Fragile sites in mammalian cells replicate late, and mutations in ATR (the mammalian homolog of *MEC1*) result in DSBs at fragile sites (Casper et al., 2002). In addition, several mammalian fragile sites have DNA sequences that are capable of forming hairpin-like secondary structures (Zlotorynski et al., 2003). There are also differences between the mammalian and yeast fragile sites. The mammalian fragile sites are much larger (extending over regions greater than 100 kb) and contain no single type of conserved repeat (Arlt et al., 2003).

Of the four translocations that we examined in detail, two involved an interaction between FS2 and an inverted pair of Ty elements (one Ty1 and one Ty2) on chromosome IV. Since there are only five such pairs in the genome (Kim et al., 1998), including FS2, it is likely that the pair on chromosome IV is also susceptible to breakage and recombination. There is a strikingly similar situation in human cells. The most common non-Robertsonian translocation in humans involves a fusion between chromosomes 22 and 11. The breakpoints in both chromosomes occur in the middle of palindromic AT-rich repeats (Kurahashi and Emanuel, 2001; Gotter et al., 2004).

It is likely that the Ty retrotransposons are important in generating chromosomal rearrangements in two different ways. First, they represent a common dispersed repetitive sequence, and ectopic recombination between dispersed repeats can produce translocations, inversions, deletions, and duplications (summarized in Umezu et al. [2002]). Second, if a Ty element transposes very near a second Ty element, resulting in an inverted repeat, the resulting structure would be expected to generate a very high rate of chromosome rearrangements by the mechanisms discussed above. Both of these properties are probably relevant to the observation that karyotypic differences between different yeast species are consistent with ectopic exchanges between Ty elements (Fischer et al., 2000). In addition, chromosomal rearrangements that accumulate in experimental evolution studies performed in a chemostat are usually a consequence of recombination between Ty and LTR elements (Dunham et al., 2002).

Experimental Procedures

Genetic Analysis and Media

Most genetic procedures (transformation, tetrad analysis, etc.) and most media were standard (Guthrie and Fink, 1991). High- and low-galactose media had 0.05% and 0.005% galactose, respectively, in addition to 3% raffinose (as well as the standard supplements for rich growth media [excluding glucose]). Strains were grown at 30°C. Detailed procedures for measurement of the rates of legitimate and illegitimate mating, measurements of rates of chromosome loss and mitotic recombination in diploids, and measurements of rates of mutation at the *CAN1* locus are given in the Supplemental Experimental Procedures.

Strains

The strains used in this study (with the exception of mating type testers) were isogenic (except for changes introduced by transformation) with MS71, a *LEU2* derivative of AMY125 (α *ade5-1 leu2-3 trp1-289 ura3-52 his7-2*; Kokoska et al., 2000). Most of our studies were done with NPD1, which had the galactose-regulated *POL1* gene. Complete genotypes for all haploid and diploid strains used in our study are in Tables S2 and S3, respectively, and details of the strain constructions are in the Supplemental Experimental Procedures.

Analysis of Chromosome Rearrangements Using Microarrays and Southern Analysis

The initial characterization of the chromosome alterations was done by microarray analysis. Details of this analysis are described in the Supplemental Experimental Procedures. Following the microarray analysis, we performed Southern analysis to map the translocation breakpoints with a higher degree of resolution. We also generated restriction maps of the FS1 and FS2 regions in the parental haploid experimental and tester strains. The description of the restriction enzymes and hybridization probes used in these studies are given in the Supplemental Experimental Procedures, and the resulting maps of the FS1 and FS2 regions are shown as Figures S4 and S5.

Supplemental Data

Supplemental Data include six figures, three tables, Supplemental Discussion, and Supplemental Experimental Procedures and can

be found with this article online at http://www.cell.com/cgi/content/full/120/5/587/DC1/.

Acknowledgments

We thank M. Vernon, P. Mieczkowski, and J. Lieb for help with the microarray analysis, C. Newlon, J. Haber, and A. Malkova for information concerning the arrangement of Ty elements on chromosome III, and R. Farber, J.L. Argueso, T. Glover, and D. Gordenin for useful discussions. The research was supported by NIH grant GM52319 (T.P.), an institutional NCI training grant CA09156 (F.J.L.), and NSF grant 0417088 (K.L.).

Received: July 22, 2004
Revised: November 11, 2004
Accepted: December 22, 2004
Published: March 10, 2005

References

Aguilera, A. (2002). The connection between transcription and genomic instability. EMBO J. *21*, 195–201.

Arlt, M.F., Casper, A.M., and Glover, T.W. (2003). Common fragile sites. Cytogenet. Genome Res. *100*, 92–100.

Casper, A.M., Nghiem, P., Arlt, M.F., and Glover, T.W. (2002). ATR regulates fragile site stability. Cell *111*, 779–789.

Cha, R.S., and Kleckner, N. (2002). ATR homolog Mec1 promotes fork progression, thus averting breaks in replication slow zones. Science *297*, 602–606.

Dunham, M.J., Badrane, H., Ferea, T., Adams, J., Brown, P.O., Rosenzweig, F., and Botstein, D. (2002). Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *99*, 16144–16149.

Fischer, G., James, S.A., Roberts, I.N., Oliver, S.G., and Louis, E.J. (2000). Chromosomal evolution in Saccharomyces. Nature *405*, 451–454.

Freudenreich, C.H., Kantrow, S.M., and Zakian, V.A. (1998). Expansion and length-dependent fragility of CTG repeats in yeast. Science *279*, 853–856.

Glover, T.W., and Stein, C.K. (1988). Chromosome breakage and recombination at fragile sites. Am. J. Hum. Genet. *43*, 265–273.

Glover, T.W., Berger, C., Coyle, J., and Echo, B. (1984). DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum. Genet. *64*, 136–142.

Gordenin, D.A., Proscyavichus, Y.Y., Malkova, A.L., Trofimova, M.V., and Peterzen, A. (1991). Yeast mutants with increased transposon Tn5 excision. Yeast *7*, 37–50.

Gordenin, D.A., Lobachev, K.S., Degtyareva, N.P., Malkova, A.L., Perkins, E., and Resnick, M.A. (1993). Inverted DNA repeats: a source of eukaryotic genomic instability. Mol. Cell. Biol. *13*, 5315–5322.

Gotter, A.L., Shaikh, T.H., Budarf, M.L., Rhodes, C.H., and Emanuel, B.S. (2004). A palindrome-mediated mechanism distinguishes translocations involving LCR-B of chromosome 22q11.2. Hum. Mol. Genet. *13*, 103–115.

Guthrie, C., and Fink, G.R. (1991). Guide to Yeast Genetics and Molecular Biology (San Diego: Academic Press).

Hawthorne, D.C. (1963). A deletion in yeast and its bearing on the structure of the mating type locus. Genetics *48*, 1727–1729.

Kim, J.M., Vanguri, S., Boeke, J.D., Gabriel, A., and Voytas, D.F. (1998). Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete Saccharomyces cerevisiae genome sequence. Genome Res. *8*, 464–478.

Kokoska, R.J., Stefanovic, L., DeMai, J., and Petes, T.D. (2000). Increased rates of genomic deletions generated by mutations in the yeast gene encoding DNA polymerase delta or by decreases in the cellular levels of DNA polymerase delta. Mol. Cell. Biol. *20*, 7490–7504.

Kurahashi, H., and Emanuel, B.S. (2001). Long AT-rich palindromes and the constitutional t(11;22) breakpoint. Hum. Mol. Genet. *10*, 2605–2617.

Leach, D.R. (1994). Long DNA palindromes, cruciform structures, genetic instability, and secondary structure repair. Bioessays *16*, 893–900.

LeBeau, M.M., Rassool, F.V., Neilly, M., Espinosa, R., Glover, T.W., Smith, D.I., and McKeithan, T.W. (1998). Replication of a common fragile site, FRA3B, occurs late in S phase and is delayed further upon induction: implications for the mechanism of fragile site induction. Hum. Mol. Genet. *7*, 755–761.

Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1998). Genetic instabilities in cancer. Nature *386*, 623–627.

Lobachev, K.S., Shor, B.M., Tran, H.T., Taylor, W., Keen, J.D., Resnick, M.A., and Gordenin, D.A. (1998). Factors affecting inverted repeat stimulation of recombination and deletion in Saccharomyces cerevisiae. Genetics *148*, 1507–1524.

Lobachev, K.S., Gordenin, D.A., and Resnick, M.A. (2002). The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell *108*, 183–193.

Malkova, A., Signon, L., Schaefer, C.B., Naylor, M.L., Theis, J.F., Newlon, C.S., and Haber, J.E. (2001). *RAD51*-independent break-induced replication to repair a broken chromosome depends on a distant enhancer site. Genes Dev. *15*, 1055–1060.

McCusker, J.H., and Haber, J.E. (1981). Evidence of chromosomal breaks near the mating-type locus of Saccharomyces cerevisiae that accompany *MAT*α × *MAT*α matings. Genetics *99*, 383–403.

Paques, F., and Haber, J.E. (1999). Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. *63*, 349–404.

Petes, T.D. (2001). Meiotic recombination hot spots and cold spots. Nat. Rev. Genet. *2*, 360–369.

Roeder, G.S., and Fink, G.R. (1980). DNA rearrangements associated with a transposable element in yeast. Cell *21*, 239–249.

Ruskin, B., and Fink, G.R. (1993). Mutations in *POL1* increase the mitotic instability of tandem inverted repeats in Saccharomyces cerevisiae. Genetics *133*, 43–56.

Strathern, J., Hicks, J., and Herskowitz, I. (1981). Control of cell type in yeast by the mating type. The alpha1-alpha2 hypothesis. J. Mol. Biol. *147*, 239–244.

Umezu, K., Hiraoka, M., Mori, M., and Maki, H. (2002). Structural analysis of aberrant chromosomes that occur spontaneously in diploid Saccharomyces cerevisiae: retrotransposon Ty1 plays a crucial role in chromosomal rearrangements. Genetics *160*, 97–110.

Wang, L., Paradee, W., Mullins, C., Shridhar, R., Rosati, R., Wilke, C., Glover, T., and Smith, D.I. (1997). Aphidicolin-induced FRA3B breakpoints cluster in two distinct regions. Genomics *41*, 485–488.

Wicksteed, B.L., Collins, I., Dershowitz, A., Stateva, L.I., Green, R.P., Oliver, S.G., Brown, A.J., and Newlon, C.S. (1994). A physical comparison of chromosome III in six strains of Saccharomyces cerevisiae. Yeast *10*, 39–57.

Zlotorynski, E., Rahat, A., Skaug, J., Ben-Porat, N., Ozeri, E., Hershberg, R., Levi, A., Scherer, S., Margalit, H., and Kerem, B. (2003). Molecular basis for expression of common and rare fragile sites. Mol. Cell. Biol. *23*, 7143–7151.