Spontaneous Chromosome Loss in *Saccharomyces cerevisiae* Is Suppressed by DNA Damage Checkpoint Functions

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ABSTRACT

Genomic instability is one of the hallmarks of cancer cells and is often the causative factor in revealing recessive gene mutations that progress cells along the pathway to unregulated growth. Genomic instability can take many forms, including aneuploidy and changes in chromosome structure. Chromosome loss, loss and reduplication, and deletions are the majority events that result in loss of heterozygosity (LOH). Defective DNA replication, repair, and recombination can significantly increase the frequency of spontaneous genomic instability. Recently, DNA damage checkpoint functions that operate during the S-phase checkpoint have been shown to suppress spontaneous chromosome rearrangements in haploid yeast strains. To further study the role of DNA damage checkpoint functions in genomic stability, we have determined chromosome loss in DNA damage checkpoint-deficient yeast strains. We have found that the DNA damage checkpoints are essential for preserving the normal chromosome number and act synergistically with homologous recombination functions to ensure that chromosomes are segregated correctly to daughter cells. Failure of either of these processes increases LOH events. However, loss of the G2/M checkpoint does not result in an increase in chromosome loss, suggesting that it is the various S-phase DNA damage checkpoints that suppress chromosome loss. The *mec1* checkpoint function mutant, defective in the yeast ATR homolog, results in increased recombination through a process that is distinct from that operative in wild-type cells.

GENOMIC instability occurs in cells at a low rate, but is enhanced in cancer cells (Hartwell 1992; Hartwell *et al.* 1994; Hartwell and Kastan 1994; Weinert 1997; Kinzler and Vogelstein 1998; Lengauer *et al.* 1998). Mutations and chromosome rearrangements are found. While both types of genome changes can result in loss of heterozygosity (LOH) of a tumor suppressor gene, the chromosome rearrangement events can reduce multiple linked genes to homozygosity. Spontaneous genomic instability is controlled by DNA repair factors, DNA recombination factors, DNA replication factors, sister chromatid cohesion factors, mitotic apparatus factors, and transcription components among other regulatory pathways. In the yeast *Saccharomyces cerevisiae*, many studies have shown that defects in these processes result in increased recombination or rearrangements (Hartwell and Kastan 1994; Foiani *et al.* 1998; Chen and Kolodner 1999; Datta *et al.* 2000; Frei and Gasser 2000; Haber 2000; Karran 2000).

Recent studies of a class of genomic instability events have been described in haploid yeast strains (Chen and Kolodner 1999). These events include interstitial deletions, chromosome arm deletions with addition of a new telomere, and nonreciprocal translocations. Mutations in replication and double-strand break repair functions (Chen and Kolodner 1999) and S-phase checkpoint functions (Myung *et al.* 2001) suppress the occurrence of these rearrangements. Although the mutant strains can greatly increase the occurrence of rearrangements, the rates remain low. Moreover, the use of haploid strains precludes the ability to detect chromosome loss events. Since somatic cells are diploid, it was of interest to determine what was the predominant event leading to LOH of a marker allele.

Diploid *S. cerevisiae* strains undergo mitotic recombination at a rate of $\sim 1 \times 10^{-5}$ for intragenic recombination at the *LEU2* locus (Petukhova *et al.* 1999). Mitotic recombination occurs as gene conversion and crossing over. In wild-type cells reciprocal exchanges occur (Nickoloff *et al.* 1999; Galgoczy and Toczyski 2001), while in cells defective in *RAD51* and *RAD54*, apparent reciprocal exchanges are really break-induced replication (BIR) events (Malkova *et al.* 1996; Signon *et al.* 2001). Mitotic recombination is under control of the mating-type *MAT* locus. Diploid cells are normally heterozygous *MAT***a**/*MAT*α and it is this genotype that regulates the mitotic recombination rate (Friis and Roman 1968; Heude and Fabre 1993). Strains that are hemizygous or homozygous at the *MAT* locus have reduced recombination rates (Friis and Roman 1968; Heude and Fabre 1993). The heterozygous *MAT* information is thought to have a regulatory function in mitotic re-

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combination, although it is not clear how the regulation operates. Diploid cells are also capable of losing a chromosome, as *S. cerevisiae* is relatively tolerant of 2n−1 monosomy. Chromosome loss does not appear to be under control by the *MAT* locus, suggesting that spontaneous chromosome loss does not have its origin as aborted mitotic recombination events.

The first described DNA damage checkpoint mutants of yeast were notable for an absence of phenotype in undamaged cells, although it was reported that *rad9* mutant diploid strains had a chromosome loss increase of 7- to 21-fold (Weinert and Hartwell 1990). Other studies showed that spontaneous chromosome rearrangements were elevated in the *rad9* mutant (Fasullo *et al.* 1998). Initially, the *mec1* and *rad53* DNA damage checkpoint null allele mutants of *S. cerevisiae* were found to be lethal. However, the lethality of the *mec1* mutant, encoding the *S. cerevisiae* ATR homolog, and *rad53*, encoding the *S. cerevisiae* Chk2 homolog, is suppressed by a deletion in the *SML1* gene (Zhao *et al.* 1998), which does not suppress the DNA damage checkpoint defect. In contrast, deletion of the *ATR* gene in mice results in early embryonic lethality and broad genomic instability (Brown and Baltimore 2000; de Klein *et al.* 2000). These results, combined with recent studies on increased chromosome rearrangements in *S. cerevisiae* DNA damage checkpoint mutants (Myung *et al.* 2001), have prompted us to examine the contribution of DNA damage checkpoint functions to chromosome stability.

MATERIALS AND METHODS

Strains: All strains are on the W303 *RAD5* background and carry the *leu2-3*, *112 his3-11*, and *15 trp1-1 ura3-1* markers. For each diploid strain one parent was also *hom3-10 ade2-1 can1-100* and the other parent was *HOM3 ADE2 CAN1*. All strains are isogenic with the exception of the indicated mutant gene and were constructed through crosses to the wild-type strains. All of the checkpoint gene mutations and recombination repair gene mutations are null alleles, with the exception of the *sml1-1* mutation in the *rad53*Δ strains. The *sml1* allele in the *mec1*Δ strains is a null allele. All strains were grown at 30° with the exception of the *pds1* strains, which were grown at 25°. Strains used were the following: for wild type, HKY947-14D and HKY953-3A; for *rad9*Δ, HKY965-12B and 964-6D; for *rad17*Δ, HKY960-5A and HKY961-9D; for *mec3*Δ, HKY949-2D and 967-2D; for *rad24*Δ, HKY947-1B and HKY966-4A; for *mec1*Δ *sml1*Δ, HKY978-9D and HKY986-10D; for *rad53*Δ *sml1-1*, HKY987-6C and HKY983-2A; for *sml1*Δ, HKY1164-1C and HKY1165-7C; for *dun1*Δ, HKY1026-4D and 1025-9B; for *chk1*Δ, HKY1031-5C and HKY1032-5C; for *chk1*Δ *dun1*Δ, HKY1087-4A and HKY1088-23C; for *rad53*Δ *sml1-1 chk1*Δ, HKY1119-24A and HKY1121-41B; for *pds1*Δ, HKY1013-5C and HKY1014-7B; for *bub1*Δ, HKY1104-1D and HKY1103-2C; for *mad3*Δ, HKY1125-2C and HKY1126-4C; for *rad54*Δ, HKY947-22C and HKY950-15B; for *rad51*Δ, HKY1039-1A and HKY1038-6C; for *rad51*Δ *rad24*Δ, HKY973-3C and HKY974-13B; for *rad51*Δ *mec3*Δ, HKY971-12D and HKY972-14B; for *yku70*Δ, HKY1066-22A and HKY1067-2B; for *yku70*Δ *rad51*Δ, HKY1083-2A and HKY1084-4B; and for *rad50*Δ, HKY1047-1A and HKY1049-10D. The *MAT*Δ mutation was made by transforming a *MAT*α strain with a *MAT***a**::*hisG-URA3-hisG* fragment derived from pFP19, a gift from F. Paques and J. Haber. *MAT* disruption transformants were confirmed by a change in the mating capability of the transformed strain. Selection on 5-fluoroorotic acid medium resulted in loss of the *URA3* marker of the disruption, to give a *MAT* null allele of the genotype *MAT::hisG*. Strains used for the *MAT*α by *MAT*Δ crosses were the following: for wild type, HKY1025-47D and HKY1045-5C; for *mec1*Δ *sml1*Δ, HKY1045-5A and HKY986-10D; and for *rad54*Δ, HKY950-9C and HKY975-2A.

Determination of chromosome loss and mitotic recombination rates: Diploid zygotes were isolated from freshly mated strains by micromanipulation on solid YEPD medium. For each cross 50 zygotes were isolated. After growth for 3–4 days at 30° (or 25°), the plates were photographed and then nine zygotic colonies were picked for each fluctuation test. Appropriate dilutions were plated onto complete medium, to determine viable cell number, and complete medium containing 60 μg/ml canavanine. After growth at 30° for 2 days (or 25° for 3 days), the number of cells was counted and then the canavanine-containing plates were replica plated to complete plates lacking threonine. After 2 more days of growth at 30° (or 3 days at 25°), the fractions of Can^r colonies that were Thr^+ and Thr^- were determined. The data from the total number of viable cells and cells growing on plates containing canavanine were analyzed by the median method (Lea and Coulson 1948) to determine the rate of Can^r formation. The fraction of Can^r that was also Thr^- was taken as the rate of chromosome loss. The remaining fraction that was Thr^+ reflects mitotic recombination events and spontaneous mutagenesis. Spontaneous mutagenesis rates were determined in haploid strains that were mating or nonmating due to the presence of a *sir4* mutation to reflect the nonmating status of the diploid strains. These mutagenesis rates were subtracted from the Can^r Thr^+ rates to give the mitotic recombination rates. Fluctuation tests were repeated two to three times for each genotype.

Cell viability: Two methods were used to determine cell viability. Cells from new zygotic colonies were resuspended in 1 ml water and appropriate dilutions were made. The total number of cells was determined by duplicate cell counts with a hemacytometer. Colony-forming units were determined by plating 100 μl, in duplicate, from the appropriate dilution to YEPD plates. Second, cells from new zygotic colonies grown for 3 days were streaked onto fresh YEPD plates. One hundred unbudded colonies per zygotic colony were micromanipulated to the YEPD medium and then incubated at 30° for 3 days (or 25° for 4 days). Three zygotic colonies were used for each genotype.

Other methods: Whole chromosomes were prepared from candidate chromosome loss colonies and separated on contour-clamped homogeneous electric field (CHEF) gels according to standard procedures. Chromosome identity and ploidy was determined by Southern blot analysis using *HOM3* DNA as a probe. The chromosome *V* pair contained a size polymorphism, which enabled rapid identification of the monosomic strains through Southern analysis. In every case, chromosome loss candidates (Can^r Thr^- colonies) were monosomic for chromosome *V*.

RESULTS

Chromosome loss rates in checkpoint mutants: Chromosome stability was measured by a simple genetic assay for loss of one chromosome of a pair of chromosome *V* homologs (Figure 1). After correcting for background

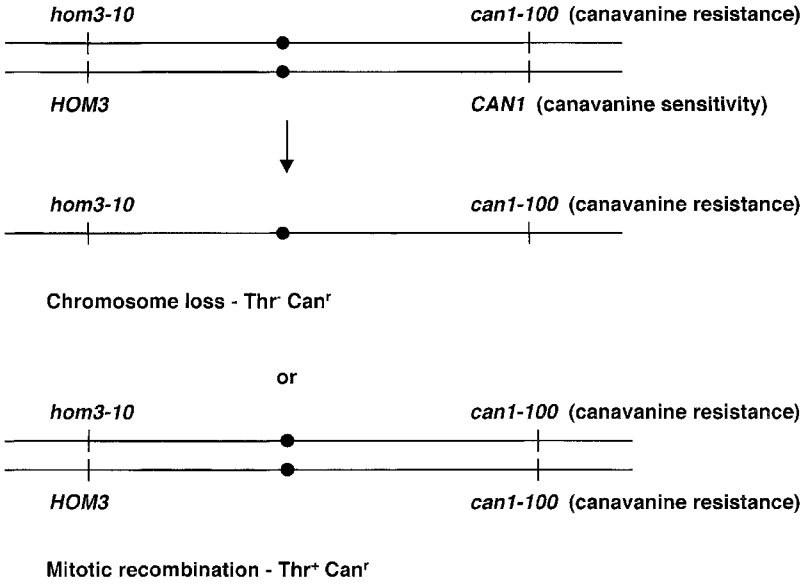

FIGURE 1.—A schematic of the chromosome *V* homologs used to monitor mitotic recombination and chromosome loss. *hom3-10* mutants require homocysteine and threonine and do not grow on complete medium lacking threonine. The *can1-100* mutation makes cells resistant to the arginine analog canavanine. Cells are first plated to complete medium containing canavanine to select for cells that have lost the *CAN1* allele. The canavanine-resistant colonies are then replica plated to complete medium lacking threonine to determine the fraction of canavanine-resistant colonies that also require threonine. Chromosome loss events are reflected in colonies that have lost markers from both chromosome arms, which are separated by the large black dot depicting the centromere. Mitotic recombination events are those that lose only the *CAN1* marker. In Tables 2 and 4 these numbers are corrected for background mutation events that inactivate the *CAN1* allele.

mutational events, the results were classified into chromosome loss events and mitotic recombination events, which include gene conversion of the *CAN1* allele to *can1-100*, mitotic crossing over in the *CENV* to *CAN1* interval, a genetic distance of ~50 cM, and BIR occurring within the same interval (PAQUES and HABER 1999). Whole chromosome CHEF gel analysis and Southern blotting confirmed chromosome loss events in samples. No chromosome rearrangements, including gross deletions and translocations, were found. This is not to imply that such events do not occur. Rather, from our selection scheme the major event that results in simultaneous loss of the *CAN1* and *HOM3* alleles is chromosome loss.

Mutation rates of the *CAN1* gene were determined in mating and nonmating wild type, *rad51*Δ, *rad54*Δ, *rad24*Δ, and *mec1*Δ *sml1*Δ haploid strains, constructed as described in MATERIALS AND METHODS. The rate in wild type was 9.8×10^{-8}; in *rad51*Δ, 1.2×10^{-6}; in *rad54*Δ, 1.5×10^{-6}; in *rad24*Δ, 1.7×10^{-7}; and in *mec1*Δ *sml1*Δ, 2.1×10^{-7}. These rates were unchanged in nonmating haploid strains.

A sample of mutants in DNA damage sensor genes, signal transduction genes, and effector genes in the DNA damage checkpoint pathways are shown in Tables 1 and 2. Wild-type spontaneous chromosome loss rate is 7-fold less than the spontaneous recombination rate. The increased recombination rate is due to heterozygosity at the *MAT* locus as the wild-type *MAT* α/Δ strain, hemizygous at *MAT*, has a 5-fold lower rate of mitotic recombination. Mutants in the DNA damage sensor genes *rad9*, *rad17*, *mec3*, and *rad24* have a 6.5- to 14-fold increase in chromosome loss. With the exception of the *rad17* mutant, mitotic recombination is only modestly increased (see Table 2). Mutants in the signal transduction kinases *MEC1* and *RAD53* have a more substantial increase in chromosome loss (Table 1) while mitotic recombination is modestly increased, and in the case of the *mec1* mutant, this is not under *MAT* regulation (Table 2). The *mec1* effect on mitotic recombination is in contrast to a measurement of spontaneous intragenic recombination at the *LEU2* locus, where a 5-fold reduction in the recombination rate was observed in the *mec1* mutant (BASHKIROV *et al.* 2000). Our recombination measurements include gene conversion, reciprocal crossing over, and BIR and other rearrangements, and the increase in the checkpoint mutants suggests that some event other than gene conversion is augmented in these mutants. Mutants in the downstream kinases encoded by *DUN1* and *CHK1* have only a 2-fold increase in chromosome loss and no increase in mitotic recombination (Tables 1 and 2). The Chk1 and Rad53 kinases have been proposed to function in distinct pathways for the control of the DNA damage checkpoint (SANCHEZ *et al.* 1999). However, no synergistic or additive effect on chromosome loss or mitotic recombination was observed in the double mutant. The *dun1* mutant is reported to have an increase of 200-fold in chromosome rearrangements (MYUNG *et al.* 2001), but our assay did not show a significant increase in chromosome loss or mitotic recombination, in either the *dun1* single mutant or the *dun1 chk1* double mutant (Tables 1 and 2). This implies that strains completely defective for the G2/M checkpoint have no increase in chromosome loss. Finally, mutants defective in M phase checkpoint genes *PDS1*, *BUB1*, and *MAD3* showed a significant increase in chromosome loss, particularly the *pds1* mutant, as previously reported (YAMAMOTO *et al.* 1996). This may reflect the suggested role of *PDS1* in a DNA damage checkpoint (COHEN-FIX and KOSHLAND 1997; CLARKE *et al.* 1999). Curiously, the *bub1* and *mad3* mutants had a significant increase in recombination rates. We do not know yet what type of recombination event is increased in these mutants. The *mad3* mutant had no reported

TABLE 1

Chromosome *V* loss rates in DNA damage checkpoint mutants

Strain	Rate	Fold increase
Wild type	2.0×10^{-6} ($1.3–2.9 \times 10^{-6}$)	
Wild-type *MAT*α/Δ	3.1×10^{-6} ($1.6–4.1 \times 10^{-6}$)	1.6
rad9	2.0×10^{-5} ($1.6–2.3 \times 10^{-5}$)	10
rad17	1.3×10^{-5} ($1.2–1.4 \times 10^{-5}$)	6.5
mec3	2.7×10^{-5} ($1.2–3.5 \times 10^{-5}$)	14
rad24	2.6×10^{-5} ($1.8–3.3 \times 10^{-5}$)	13
mec1 sml1	4.7×10^{-5} ($3.4–6.0 \times 10^{-5}$)	24
*mec1 sml1 MAT*α/Δ	7.0×10^{-5} ($6.2–7.7 \times 10^{-5}$)	35
rad53 sml1-1	3.6×10^{-5} ($2.4–4.9 \times 10^{-5}$)	18
sml1	6.2×10^{-6} ($4.8–7.7 \times 10^{-6}$)	3.1
dun1	4.4×10^{-6} ($1.5–7.1 \times 10^{-6}$)	2.2
chk1	4.5×10^{-6} ($1.4–7.6 \times 10^{-6}$)	2.2
chk1 dun1	4.8×10^{-6} ($3.4–6.3 \times 10^{-6}$)	2.4
rad53 sml1-1 chk1	1.4×10^{-5} ($1.1–1.7 \times 10^{-5}$)	7.0
pds1	1.1×10^{-3} ($0.7–1.6 \times 10^{-3}$)	550
bub1	6.1×10^{-5} ($3.4–8.8 \times 10^{-4}$)	30
mad3	1.2×10^{-4} ($0.9–1.6 \times 10^{-4}$)	60

All diploid strains were derived from isogenic W303 parental haploid strains. One copy of chromosome *V* is marked with the *HOM3* and *CAN1* alleles while the other chromosome *V* copy is marked with the *hom3-10* and *can1-100* alleles. Fluctuation tests were performed on freshly formed diploid colonies as described in MATERIALS AND METHODS. For each genotype, rates were determined two to three times using freshly formed zygote colonies. The average of these rates is presented with the range of values shown in parentheses.

effect on gross chromosomal rearrangements (MYUNG *et al.* 2001).

Chromosome loss rates in recombination and repair mutants: Next, the effect of mutations in different DNA repair pathways was examined (Tables 3 and 4). *RAD51* encodes a DNA strand exchange protein and functions

TABLE 2

Chromosome *V* recombination rates in DNA damage checkpoint mutants

Strain	Rate	Fold increase
Wild type	1.4×10^{-5} ($1.3–1.6 \times 10^{-6}$)	
Wild-type *MAT*α/Δ	3.0×10^{-6} ($2.0–4.0 \times 10^{-6}$)	0.21
rad9	3.8×10^{-5} ($3.2–4.3 \times 10^{-5}$)	2.7
rad17	1.2×10^{-4} ($0.9–1.4 \times 10^{-4}$)	8.6
mec3	5.4×10^{-5} ($4.8–6.5 \times 10^{-5}$)	3.9
rad24	4.9×10^{-5} ($4.7–5.1 \times 10^{-5}$)	3.5
mec1 sml1	9.6×10^{-5} ($8.3–11.0 \times 10^{-5}$)	6.8
*mec1 sml1 MAT*α/Δ	3.4×10^{-5} ($2.3–4.5 \times 10^{-5}$)	2.4
rad53 sml1-1	1.4×10^{-5} ($1.0–1.9 \times 10^{-5}$)	1.0
sml1	2.3×10^{-5} ($1.9–2.7 \times 10^{-5}$)	1.6
dun1	2.0×10^{-5} ($1.7–2.2 \times 10^{-5}$)	1.4
chk1	2.3×10^{-5} ($2.2–2.4 \times 10^{-5}$)	1.6
chk1 dun1	1.3×10^{-5} ($1.1–1.5 \times 10^{-5}$)	0.9
rad53 sml1-1 chk1	2.7×10^{-5} ($2.6–2.8 \times 10^{-5}$)	1.9
pds1	3.0×10^{-5} ($2.8–3.3 \times 10^{-5}$)	2.1
bub1	1.4×10^{-4} ($1.2–1.7 \times 10^{-4}$)	10
mad3	1.9×10^{-4} ($1.2–2.6 \times 10^{-4}$)	14

All diploid strains were derived from isogenic W303 parental haploid strains. One copy of chromosome *V* is marked with the *HOM3* and *CAN1* alleles while the other chromosome *V* copy is marked with the *hom3-10* and *can1-100* alleles. Fluctuation tests were performed on freshly formed diploid colonies as described in the MATERIALS AND METHODS. The distinction between chromosome loss and mitotic recombination was made as described in MATERIALS AND METHODS and as outlined in Figure 1. For each genotype, rates were determined two to three times using freshly formed zygote colonies. The average of these rates is presented with the range of values shown in parentheses.

TABLE 3

Chromosome *V* loss rates in DNA recombination and repair mutants

Strain	Rate	Fold increase
Wild type	2.0×10^{-6} ($1.3–2.9 \times 10^{-6}$)	
Wild-type *MAT*α/Δ	3.1×10^{-6} ($1.6–4.1 \times 10^{-6}$)	1.6
rad54	5.8×10^{-5} ($5.5–6.2 \times 10^{-5}$)	29
*rad54 MAT*α/Δ	7.6×10^{-5} ($6.3–8.9 \times 10^{-5}$)	38
rad51	5.6×10^{-5} ($4.7–6.4 \times 10^{-5}$)	28
rad51 rad24	2.5×10^{-4} ($2.3–2.7 \times 10^{-4}$)	125
rad51 mec3	2.3×10^{-4} ($2.1–2.5 \times 10^{-4}$)	115
yku70	8.3×10^{-6} ($7.4–9.2 \times 10^{-6}$)	4.2
yku70 rad51	3.3×10^{-5} ($2.8–3.8 \times 10^{-5}$)	16
rad50	2.6×10^{-5} ($1.4–3.7 \times 10^{-5}$)	13

in several homologous recombination pathways, but not in the BIR pathway (Malkova *et al.* 1996; Signon *et al.* 2001). *RAD54* encodes a member of the SNF/SWI superfamily and functions in the formation of heteroduplex DNA promoted by Rad51 protein (Petukhova *et al.* 1998, 1999). Similar to *RAD51*, *RAD54* is required for most homologous recombination, but not BIR (Signon *et al.* 2001). *YKU70* encodes the *S. cerevisiae* Ku70 homolog. *YKU70* is required for nonhomologous end joining (Boulton and Jackson 1996). *RAD50* is also involved in homologous recombination, but in mitosis the mutant has a hyperrecombination phenotype (Malone *et al.* 1990). If components of the homologous recombination repair pathways functioned to repair spontaneous double-strand breaks, one would expect a *rad51* or *rad54* mutant to have an increase in chromosome loss. This was observed for the *rad51* and *rad54* mutants (Table 3). The lack of decrease in spontaneous recombination probably reflects *RAD51*- and *RAD54*-independent recombination pathways such as BIR (Table 4). The *yku70* mutant had no effect on chromosome loss or mitotic recombination and the *rad50* mutant had a modest effect on chromosome loss and the anticipated increase in mitotic recombination. The observation that the *rad50*, *rad51*, and *rad54* mutations had different effects on chromosome loss and mitotic recombination underscores the fact that these genes act in different mitotic recombination repair pathways (Malone *et al.* 1990; Paques and Haber 1999). The *yku70* mutation had no effect on the *rad51* mutant increase in chromosome loss and the demonstrated wild-type level of mitotic recombination. This is in contrast to the essential role of Ku80 in mammalian cells in maintaining genomic stability (Karanjawala *et al.* 1999; Difilippantonio *et al.* 2000; Ferguson *et al.* 2000).

If the DNA damage checkpoint mutants were causing increased chromosome loss simply from a failure to arrest damaged cells and permit recombinational repair of the damage, then one would expect a strain defective in both a DNA damage checkpoint and a recombination repair function to have the same rate of chromosome loss as a strain defective only in recombination repair. However, *rad51 rad24* and *rad51 mec3* double mutants showed a 4.5-fold and 4.1-fold increase, respectively, over the *rad51* chromosome loss rate (Table 3). The mitotic recombination rate of these double mutants also was increased over the *rad51* mutant recombination rate, although the single mutant increases are low (Table 4). These data are consistent with the observed combined effect of *rad9* and *rad52* mutations on sponta-

TABLE 4

Chromosome *V* recombination rates in DNA recombination and repair mutants

Strain	Rate	Fold increase
Wild type	1.4×10^{-5} ($1.3–1.6 \times 10^{-5}$)	
Wild-type *MAT*α/Δ	3.0×10^{-6} ($2.0–4.0 \times 10^{-6}$)	0.21
rad54	6.5×10^{-5} ($4.1–8.9 \times 10^{-5}$)	4.6
*rad54 MAT*α/Δ	4.1×10^{-6} ($3.3–4.9 \times 10^{-6}$)	0.3
rad51	1.2×10^{-5} ($1.2–1.3 \times 10^{-5}$)	0.8
rad51 rad24	1.4×10^{-4} ($0.8–2.0 \times 10^{-4}$)	10
rad51 mec3	9.4×10^{-5} ($6.7–12 \times 10^{-5}$)	6.7
yku70	2.8×10^{-5} ($2.8–2.8 \times 10^{-5}$)	2.0
yku70 rad51	1.0×10^{-5} ($0.9–1.1 \times 10^{-5}$)	0.6
rad50	3.4×10^{-4} ($2.2–4.6 \times 10^{-4}$)	24

TABLE 5

Cell survival from diploid colonies

Strain	Survival of unbudded cells (%)	Total plating efficiency (%)
Wild type	97.3 ± 2.5	88.7 ± 6.7
Wild-type *MAT*α/Δ	94.7 ± 8.4	82.3 ± 11.0
rad9	90.3 ± 7.1	84.0 ± 4.6
rad17	91.7 ± 7.5	72.7 ± 20.7
mec3	89.3 ± 4.9	75.3 ± 8.1
rad24	92.7 ± 4.7	82.3 ± 6.8
mec1 sml1	84.0 ± 7.2	87.7 ± 7.5
*mec1 sml1 MAT*α/Δ	85.0 ± 6.1	89.3 ± 7.2
rad53 sml1-1	66.0 ± 5.3	44.7 ± 16.8
sml1	92.3 ± 3.5	67.3 ± 6.5
dun1	94.3 ± 3.2	93.0 ± 5.6
chk1	92.7 ± 5.8	90.0 ± 9.2
chk1 dun1	80.7 ± 2.5	59.3 ± 8.1
rad53 sml1-1 chk1	76.7 ± 4.7	77.0 ± 22.5
pds1	56.3 ± 7.1	48.3 ± 9.8
bub1	96.7 ± 2.1	92.7 ± 3.2
mad3	90.0 ± 12.1	62.0 ± 6.6
rad50	90.0 ± 6.2	35.3 ± 8.1
rad54	79.3 ± 15.3	64.7 ± 2.5
*rad54 MAT*α/Δ	79.7 ± 14.6	68.0 ± 3.6
rad51	90.3 ± 7.4	60.2 ± 17.1
rad51 rad24	77.0 ± 4.6[a]	60.3 ± 7.2[a]
rad51 mec3	77.3 ± 10.7[a]	65.3 ± 5.0[a]
yku70	90.3 ± 2.1	82.0 ± 13.5
yku70 rad51	73.7 ± 6.7	51.7 ± 8.5

Cell survival was determined from the colony-forming ability of 100 unbudded cells micromanipulated to fresh YEPD medium from each of three newly formed diploid colonies of each genotype and from plating efficiencies of three newly formed diploid colonies of each genotype. The mean and standard deviation of the percentage of survival is shown.

[a] Over half the colonies were microcolonies, visible only after 3 days of growth. An example of this is seen in Figure 2.

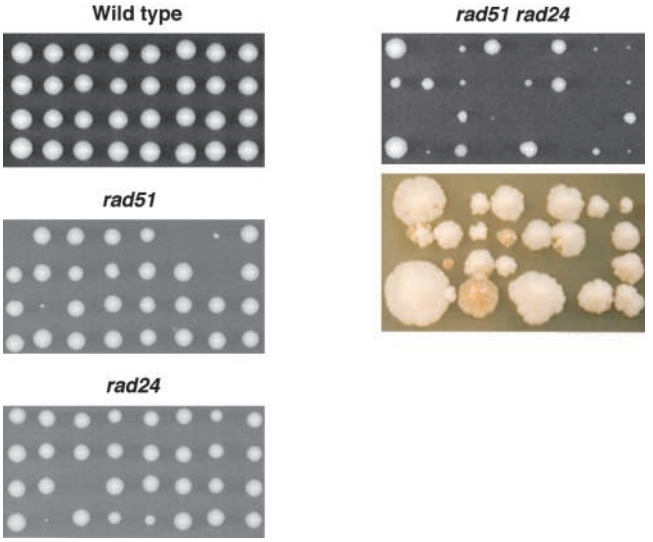

FIGURE 2.—Poor growth potential of unbudded cells from *rad51 rad24* zygotes. Unbudded cells from newly formed zygotes of the indicated genotypes were micromanipulated to fresh YPD medium and grown at 30°. Each black and white photograph shows growth of 32 cells after 3 days incubation at 30°. It can be seen that the *rad51 rad24* diploid cells show poor and variable growth after 3 days. The color photograph shows the same 32 *rad51 rad24* cells after 8 days of growth at 30°. The colonies are irregularly shaped. The diploids are heterozygous at the *ADE2* locus. Loss of chromosome *XV* where the *ADE2* locus is situated and recombination events result in red sectors. The wild-type, *rad51*, and *rad24* diploids did not give visible red sectors nor were the colonies irregularly shaped.

neous chromosome loss and chromosome loss after elimination of a telomere (SANDELL and ZAKIAN 1993).

Cell viability: Although all of the genotypes listed in Tables 1–4 were viable and gave apparently normal growth as haploid and fresh diploid colonies, this is not an accurate measure of cell viability. To better assess cell growth potential, two approaches were taken. First, single unbudded cells from freshly formed diploid colonies were individually micromanipulated to fresh growth medium and the potential of 100 such cells of each genotype to form a colony was determined. Unbudded cells were chosen as these were thought to reflect cells that had gone through the cell cycle and were not arrested in the G2/M phase of the cell cycle. Some of the mutants have an excess of large doublet cells, cells with a large bud, and these cells have very poor growth potential. The results, shown in Table 5, indicate that most genotypes had good continued viability, although viability dropped in some of the checkpoint function mutants. Plating efficiency of cells following cell number determination was also used to assess cell viability. Those data, shown in Table 5, are for the most part similar to the first method of cell viability assessment, with some notable exceptions such as the *rad53*, *sml1*, *mad3*, *rad50*, *rad54*, *rad51*, and *yku70 rad51* mutants.

Loss of the homologous recombination repair functions that utilize *RAD51* reduced cell viability, suggesting that *RAD51*-independent repair pathways may not be optimum in some damage contexts. The *rad51 rad24* and *rad51 mec3* diploids showed reduced viability of single unbudded cells and over half of those colonies that grew formed microcolonies only after several days of growth (Figure 2). A similar variable poor growth phenotype was observed in the plating efficiency experiments. This most likely reflects the increased chromosome loss observed in this mutant combination. The rates reported here are for one chromosome of a pair of chromosomes, and *S. cerevisiae* has 32 chromosomes. Although chromosome loss rates are not uniform (HIRAOKA *et al.* 2000), the total chromosome loss rate should be at least 10-fold higher than the rate determined for a single chromosome.

DISCUSSION

We have shown that loss of function of genes in the DNA damage sensor and transducer components of the DNA damage checkpoint pathways results in increased

chromosome loss. Inactivation of some of the homologous recombination repair pathways gives a similar increase in chromosome loss. Loss of both DNA damage checkpoint functions and homologous recombination repair functions results in a higher increase in chromosome loss. The spontaneous loss rates reported here are sufficiently high as to be a significant factor in LOH events. Diploid *S. cerevisiae* strains have 32 chromosomes. We have measured loss of only one chromosome. Thus overall chromosome loss for a cell is expected to be at least 10-fold higher. Intact homologous recombination repair pathways provide some protection against spontaneous LOH events when the DNA damage checkpoints are compromised, underscoring the multiple pathways that suppress genomic instability and preserve the cell karyotype.

Effect of mating-type heterozygosity and recombination pathways: We have measured chromosome loss and mitotic recombination in three strains that were hemizygous at the *MAT* locus: wild type, *rad54*Δ as representative of the homologous recombination repair pathway, and *mec1*Δ *sml1*Δ as representative of the DNA damage checkpoint pathways. In no case was chromosome loss affected by the mating-type genotype, although the *rad54*Δ and *mec1*Δ *sml1*Δ genotypes increased chromosome loss. However, mitotic recombination was sensitive to the mating-type genotype. The recombination rate was decreased 4.6-fold in the wild-type strain when mating-type heterozygosity was lost. The recombination rate was reduced 15.8-fold in the *rad54 MAT*α*/*Δ strain compared to the *rad54 MAT*α*/MAT***a** strain. In contrast, loss of mating-type heterozygosity in the *mec1 sml1* mutant reduced recombination only 2.8-fold. The wild-type result reproduces previous reports on the effect of mating-type heterozygosity on mitotic recombination (Friis and Roman 1968; Heude and Fabre 1993). The *rad54*Δ mutant did not show any decrease in mitotic recombination compared to wild type when mating type was heterozygous. This suggests either that spontaneous mitotic recombination is *RAD54* independent, possibly occurring through a BIR type of event (Signon *et al.* 2001), or that in the absence of the *RAD54* pathway, spontaneous lesions are channeled into a novel *RAD54*-independent pathway of recombination. It is known that *RAD54* and *RAD51*, *RAD52*, *RAD55*, and *RAD57* are required for mitotic gene conversion (Rattray and Symington 1994, 1995; Paques and Haber 1999; Petukhova *et al.* 1999). The wild-type rate of gene conversion at the *LEU2* locus, measured as intragenic recombination, is $\sim 1 \times 10^{-5}$. If the gene conversion rate at *CAN1* is similar, then most of the recombination observed at the *CAN1* locus in wild type would be gene conversion. Thus it is not surprising that a reduction in spontaneous recombination occurs in the wild-type *MAT* hemizygous strain. However, the *rad54*Δ strain is not decreased in mitotic recombination when *MAT* is heterozygous, and this recombination level is dependent on *MAT* heterozygosity. This suggests that spontaneous *RAD54*-independent events, whether they occur through BIR or another mechanism, are sensitive to the mating-type genotype. A recent study using haploid yeast strains disomic for chromosome *VII* has found that spontaneous BIR events occur in haploid *rad51* mutant cells (Galgoczy and Toczyski 2001).

In contrast, the mitotic recombination rate in the *mec1*Δ *sml1*Δ mutant is not greatly affected by mating-type hemizygosity. We do not know if the same spectrum of recombination events occurs in the *mec1 sml1* mutant as in wild type and whether all types of recombination events are equally increased in the *MAT* heterozygous *mec1 sml1* mutant. The fact that mitotic recombination in this mutant is not greatly decreased in the *MAT* hemizygous background suggests that a specific type of mitotic recombination is increased in the *MAT* heterozygous strain that is immune to mating-type heterozygosity. Interestingly, the original report of a *mec1* mutant noted that diploids were not altered in mitotic intragenic or gene conversion recombination, but were greatly increased in intergenic recombination, which could be crossing-over or BIR events (Kato and Ogawa 1994), although another study found gene conversion to be decreased in the *mec1* mutant (Bashkirov *et al.* 2000). Loss of the *MEC1* checkpoint control could result in aberrant mitotic recombination, a channeling of sister chromatid recombination to interchromosome recombination, for example. Sister chromatid recombination, the preferred mode of recombinational repair in mitotic yeast cells, is not regulated by mating-type heterozygosity (Kadyk and Hartwell 1992). However, checkpoint functions can influence repair of damage by sister chromatid recombination. In mitosis *RAD9* and *RAD17* are required for suppression of UV-induced sister chromatid exchange (Paulovich *et al.* 1998), although it is not known if *MEC1* is also involved in this suppression. In contrast, loss of these same checkpoint gene functions had no effect on spontaneous sister chromatid exchange (Paulovich *et al.* 1998). In meiosis, partner choice for recombination is controlled by the checkpoint genes *MEC1*, *RAD17*, *RAD24*, and *MEC3* (Grushcow *et al.* 1999; Thompson and Stahl 1999). The elevated ectopic meiotic recombination observed in *mec1* mutants (Grushcow *et al.* 1999) might be similar to the elevated mitotic interchromosome recombination that was observed by Kato and Ogawa (1994) and the increased mitotic recombination that we find in the *mec1*Δ *sml1*Δ mutant. It will be of interest to compare the types of recombination events observed in wild type and *mec1*Δ *sml1*Δ in mating and nonmating diploids.

Effects of mutations in DNA damage checkpoint functions and homologous recombination: The spontaneous lesions that result in increased recombination and chromosome loss have not been identified. The spontaneous lesions most likely occur during S phase. In wild type, recombination is higher than chromosome loss. This may mean that most lesions are efficiently repaired through recombination or other repair pathways. In

the checkpoint mutants recombination is only slightly increased over wild type. We do not expect that new lesions are formed in the checkpoint mutants, although aberrant processing may change a lesion destined for a strictly repair pathway into a recombinogenic lesion. However, most spontaneous lesions that are present in wild type are also present in the checkpoint mutants, but are not correctly repaired in a timely manner.

Why is chromosome loss increased in the *rad51*Δ and *rad54*Δ mutants whereas mitotic recombination is not decreased? The mitotic recombination must reflect *RAD51*- and *RAD54*-independent recombination such as crossing over or BIR (Rattray and Symington 1995; Signon *et al.* 2001). The fact that these events are not greatly increased over wild type suggests that this pathway is inefficient or that lesions such as a double-strand break (DSB) that occur in the context of a replication fork are generally targeted for repair via a pathway that uses the Rad51 and Rad54 proteins. When such spontaneous damage is not repaired or is repaired incorrectly, the result is loss of a chromosome. Spontaneous chromosome loss in a *rad51* mutant requires the adaptation function of *CDC5*, which adapts cells arrested at a DNA damage checkpoint. BIR is especially sensitive to adaptation and is reduced in adaptation-defective strains (Galgoczy and Toczyski 2001). Spontaneous damage that is not repaired in recombination-deficient strains induces a checkpoint response. Loss of the DNA damage checkpoint functions may increase chromosome loss by not arresting the cell cycle in S phase to allow recombinational repair, resulting in an extra increase in loss when both checkpoint and recombination repair functions are missing. If the DNA damage checkpoint functions have an additional role in regulating repair through sister chromatid recombination, especially in the context of a DSB at the replication fork, then loss of both recombinational repair functions and checkpoint functions will have an increased effect, even though both types of gene functions operate on the same substrate. The observation that the *rad51*Δ *rad24*Δ and *rad51*Δ *mec3*Δ double mutants have a reduced ability to form robust colonies most likely reflects the increased chromosome loss.

Our results from studies on the *dun1 chk1* double mutants, which should be completely defective in the G2/M checkpoint, suggest that this checkpoint is not a major factor in suppressing chromosome loss from spontaneous damage. If the damage occurs during S phase, it is likely that S-phase checkpoints, those related to arrested or collapsed replication forks and perhaps checkpoint pathways not yet described for blocked or aberrant recombination intermediates, stop or slow down replication fork firing and progression to allow repair. Such checkpoint functions may also be part of the adaptation and recovery aspects of the cellular response to DNA damage.

Finally, we note that the *bub1* and *mad3* spindle checkpoint mutants have an increased mitotic recombination rate. Until we have determined the type of recombination event that is increased in these mutants, it is difficult to speculate as to the origin of this curious effect on recombination, but it is possible that recombination repair events that normally occur between sister chromatids may now occur between nonsister strains, possibly in the G_1 phase of the cell cycle.

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