Sister Chromatids Are Preferred Over Homologs as Substrates for Recombinational Repair in *Saccharomyces cerevisiae*

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ABSTRACT

A diploid *Saccharomyces cerevisiae* strain was constructed in which the products of both homolog recombination and unequal sister chromatid recombination events could be selected. This strain was synchronized in G_1 or in G_2, irradiated with X-rays to induce DNA damage, and monitored for levels of recombination. Cells irradiated in G_1 were found to repair recombinogenic damage primarily by homolog recombination, whereas those irradiated in G_2 repaired such damage preferentially by sister chromatid recombination. We found, as have others, that G_1 diploids were much more sensitive to the lethal effects of X-ray damage than were G_2 diploids, especially at higher doses of irradiation. The following possible explanations for this observation were tested: G_2 cells have more potential templates for repair than G_1 cells; G_2 cells are protected by the *RAD9*-mediated delay in G_2 following DNA damage; sister chromatids may share more homology than homologous chromosomes. All these possibilities were ruled out by appropriate tests. We propose that, due to a special relationship they share, sister chromatids are not only preferred over homologous chromatids as substrates for recombinational repair, but have the capacity to repair more DNA damage than do homologs.

MITOTIC recombination in *Saccharomyces cerevisiae* is primarily a result of DNA repair processes responding to spontaneous or induced damage that occurs during vegetative growth (HAYNES and KUNZ 1981; GAME 1983). In order for yeast cells to repair damage recombinationally, there must be present in the same nucleus a second DNA molecule containing sequence homology with the region to be repaired (RESNICK 1976; GAME 1983). In a diploid cell in G_1, such a molecule is present in the form of the homologous chromosome. However, in the G_2 stage of the cell cycle (following replication), a second homologous molecule is also present: the sister chromatid. If DNA damage is present at this stage, it could theoretically be repaired using the homology found on the sister chromatid or on one of the two homologous chromatids. We have studied the relationship between the time in the cell cycle when a recombinogenic lesion is introduced and which chromatid serves as the template for repair.

The question of when in the mitotic cycle spontaneous recombination can occur has been studied in yeast and other organisms. Genetic analyses showing the existence of half-sectored colonies in yeast (JAMES 1955; JAMES and LEE-WHITING 1955; ROMAN and JACOB 1958) or of "twin-spots" in other organisms (STERN 1936; HOLLIDAY 1961; PONTECORVO and KAFER 1958) are evidence that spontaneous homolog recombination can occur at the G_2 stage of the cell cycle. Unequal reciprocal sister chromatid recombination also has been detected in G_2 between the tandemly repeated rDNA genes (SZOSTAK and WU 1980; ZAMB and PETES 1981; PRAKASH and TAILLON-MILLER 1981) or between duplicated sequences on a single chromosome (JACKSON and FINK 1981; FASULLO and DAVIS 1987), and represents most of the reciprocal recombination events between duplicated sequences (LICHTEN and HABER 1989). By analogy with meiotic recombination, which occurs at the four-strand (post-replicative) stage, it might be thought that mitotic recombination would be limited to the G_2 stage. However, studies of homolog recombination in yeast have shown that both spontaneous and induced homolog recombination events can also occur in the G_1 phase of the cell cycle (ESPOSITO 1978; FABRE 1978; GOLIN and ESPOSITO 1981). In studies where recombination was induced by X-irradiating yeast cells at different stages of the cell cycle, much higher levels of homolog recombination were induced in cells irradiated at the G_1 stage than in cells irradiated at the G_2 stage (ESPOSITO 1968; WILDENBERG 1970; FABRE, BOULET and ROMAN 1984). The low level of homolog recombination induced in G_2 cells cannot be explained as failure of those cells to repair X-ray damage, since G_2 diploids are better able to survive exposure to X-irradiation than are G_1 diploids (BRUNBORG and WILLIAMSON 1978; BRUNBORG, RESNICK and WILLIAMSON 1980) and X-rays can induce recombination in G_2 (ROMAN and FABRE 1983). To explain these results, it was suggested that repair occurring in G_2-irradiated cells involves recombination between sister chromatids (FABRE, BOULET and ROMAN 1984); however,

another possibility is that G_2 repair is nonrecombinational.

To compare the roles of sister chromatids and homologs in recombinational repair, we constructed diploid yeast strains in which both recombination between homologs and that between sister chromatids could be monitored, and we used X-rays to induce DNA damage and recombination at different stages of the cell cycle. X-rays induce single- and double-strand breaks in DNA (FRIEFELDER 1968), and unrepaired double-strand breaks are potentially lethal lesions (RESNICK and MARTIN 1976). Since recombination is one of the primary means of repair of double-strand breaks (RESNICK and MARTIN 1976; SZOSTAK, ORR-WEAVER and STAHL 1983), we also investigated how the type of recombinational repair observed at different times of the cell cycle might relate to differences in survival following X-irradiation.

MATERIALS AND METHODS

Yeast strains and culture conditions: YEPD and dropout media have been described (SHERMAN, FINK and HICKS 1981). YM-1 is described by HARTWELL (1967). -N medium is yeast nitrogen base without amino acids and ammonium sulfate, 1.6 g/liter; 0.09 M succinic acid; adjusted to pH 5.8 with NaOH. For α-pheromone arrests of cells in G_1, log-phase cells ($5–10 \times 10^6$/ml) were collected and resuspended at the same concentration in YM-1 adjusted to pH 4.0 with HCl, and α-pheromone was added to 2×10^{-6} M. After 2 hr at 30°, the cells were >99% unbudded and forming projections, indicating arrest in G_1. To arrest cells in G_1 by nitrogen starvation, cells were grown in YM-1 + glucose (2%) to mid-log phase (about 5×10^6 cells/ml), washed once with -N medium and resuspended at $5–10 \times 10^6$ cells/ml in -N + glucose (2%) plus nutrients required to supplement auxotrophies in the strain (0.002%). Cells were allowed to grow 24–48 hr at 23°, until >95% were unbudded. To arrest cells in G_2, log-phase cells were plated to about 5×10^7 cells per plate on YEPD plates containing the microtubule inhibitor drug methyl benzimidazol-2-yl carbamate (MBC), a gift of Du Pont Corp., at a concentration of 100 μg/ml. After 3 hr at 23°, about 95% of cells appeared to be in G_2, as they were large-budded and contained a single nucleus at the neck of the bud or in one cell, as visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining (DAPI staining is described in SHERMAN, FINK and HICKS 1981). The remainder of the cells appear unbudded. This arrest regime typically resulted in about 10% cell lethality. We also confirmed a G_2 DNA content of MBC arrested cells using flow cytometry.

All strains used are congenic with A364a (Table 1). The *ade3-130* allele (JONES 1977) is a spontaneous deletion encompassing at least the 2 kb from nucleotide 508 to nucleotide 2551 of the 2.8-kb open reading frame of *ADE3* (data not shown). This deletion precludes recombination that might generate *ADE3*+ between the sister chromatid recombination substrate SCR::*URA3* (described below) and the endogenous locus. The *MAT***a**/*MAT***a** diploid strains 8301 and 8302 were constructed using the protoplast fusion strategy described by VAN SOLINGEN and VAN DER PLAAT (1977). Strain 8301 is a fusion of strains 8202SCR and 8203-1; strain 8302 is a fusion of strains 8202SCR and 15-1SCR. Two *MAT***a**/*MAT*α strains (8260 and 8261) heterozygous for the *MAT*-linked recessive cryptopleurine resistance marker *cry1* (SKOGERSON, MCLAUGHLIN and WAKATAMA 1973), were used to construct the *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains 8260-1 and 8261-1. Cells were selected for cryptopleurine resistance on YEPD plates spread with 100 μl of cryptopleurine (250 μg/ml in methanol), then were screened for the ability to mate. Strain 8202A10 was made by selecting spontaneous variants of strain 8202-1 (*MAT***a**) on YEPD plates (adjusted to pH 4.0) spread with α-pheromone to a final concentration of 5×10^{-6} M. Among these resistant colonies were some *MAT***a**/*MAT*α diploids resulting from the mating between a cell that had spontaneously switched mating type and another cell of the identical (save for the *MAT* locus) genotype. The homozygosity of the strain was checked by sporulating it and analyzing 11 tetrads; all markers segregated 4:0, except *MAT*, which segregated 2:2.

Plasmids: Plasmid pLK22 contains *LEU1* on a 5.1-kb *Bam*HI fragment (HSU and SCHIMMEL 1984), *MAT*α on a 4.1-kb *Bam*HI fragment (NASMYTH and TATCHELL 1980), *ARS1* on a 0.8-kb *Eco*RI/*Hin*dIII fragment (STRUHL *et al.* 1979), and *CEN3* on a 1.7-kb *Hin*dIII/*Bam*HI fragment (FITZGERALD-HAYES, CLARK and CARBON 1982) in pHSS6 (SEIFERT *et al.* 1986). The construction of plasmid pLK19, containing the SCR::*URA3* substrate, is described below.

Construction of the sister chromatid recombination substrate: The *ADE3* gene product has two domains required for adenine biosynthesis (MCKENZIE and JONES 1977; STABEN and RABINOWITZ 1986). The amino terminal domain is required for histidine biosynthesis as well. We constructed 5′ and 3′ deletions of the *ADE3* gene which fail to complement either the adenine or histidine auxotrophies of a strain carrying a deletion of the native *ADE3* locus. Starting with pDK206 (KOSHLAND, KENT and HARTWELL 1985), a plasmid containing a 5.5-kb *Bam*HI/*Sal*I fragment including the 2.8-kb open reading frame of the *ADE3* gene, we constructed a 5′ deletion of the gene by subcloning the 4.5-kb *Bgl*II-*Sal*I fragment of *ADE3* (Figure 1A) into a modified version of pHSS6 (SEIFERT *et al.* 1986) containing a *Sal*I site in place of the *Xba*I site in the polylinker (pHSS6 (S)). The resulting 5′ deletion of *ADE3* removed the first 507 bp of coding sequence and 510 bp of upstream sequence. To construct a 3′ deletion, the *Bam*HI site at the 5′ end of the *ADE3*-containing fragment in pDK206 was modified to a *Sal*I site, and the*Cla*I site at position 2554 in the *ADE3* open reading frame was modified to a *Bam*HI site, according to standard methods (SAMBROOK, FRITSCH and MANIATIS 1989). The resulting modified version of pDK206 was digested with *Sal*I and *Bam*HI to release a 3.0-kb fragment missing 284 bp from the 3′ end of the *ADE3* open reading frame, and this fragment was cloned into *Bam*HI and *Sal*I-cut pHSS6 (S) to create pLK9. When transformed into yeast strain 8202-1, this deletion fragment still complemented the histidine auxotrophy of the *ade3-130* allele, so more of the 3′ end of the gene was removed in the following way: plasmid pLK9 was restriction digested with *Eco*RI and reclosed to generate pLK11, containing a 3′ deletion of 1505 bp of the *ADE3* open reading frame. This deletion also complemented the histidine auxotrophy of the *ade3-130* allele, so pLK11 was digested unidirectionally to various extents with exonuclease III into the 3′ end of the *ADE3* gene, according to HENIKOFF (1987). A his$^-$ 3′ deletion constructed by this method was identified that failed to complement the histidine auxotrophy of strain 8202-1, and the deletion endpoint was determined by sequence analysis to be at nucleotide 812 of the coding sequence. There are 305 bp of sequence in common between the 5′ and 3′ deletions of the gene (Figure 1A). A 1.2-kb *URA3* fragment with *Sal*I linkers cloned onto the ends was cloned between

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype
8202-1	*MATa ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 trp1 sap3*
8202SCR	*MATa SCR::URA3 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 trp1 sap3*
8203-1	*MATa ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3*
15-1SCR	*MATa SCR::URA3 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 tyr1*
8301	*MATa SCR::URA3 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 trp1 sap3* *MATa ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 TRP1 sap3*
8302	*MATa SCR::URA3 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 trp1 sap3 TYR1* *MATa SCR::URA3 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 TRP1 sap3 tyr1*
8260	*MATa cry1 ade2 ADE3 CYH2 URA3 CAN1 his7 TRP1 SAP3* *MATα CRY1 ade2 ade3-130 cyh2 ura3-52 can1 HIS7 trp1 sap3*
8260-1	*MATa cry1 ade2 ADE3 CYH2 URA3 CAN1 his7 TRP1 SAP3* *MATa cry1 ade2 ade3-130 cyh2 ura3-52 can1 HIS7 trp1 sap3*
8261	*MATa cry1 ade2 ADE3 LEU1 CYH2 URA3 CAN1 hom3 his7 SAP3* *MATα CRY1 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 HOM3 HIS7 sap3*
8261-1	*MATα cry1 ade2 ADE3 LEU1 CYH2 URA3 CAN1 hom3 his7 SAP3* *MATα cry1 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 HOM3 HIS7 sap3*
8294	*MATa ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 TYR1* *MATa SCR::URA3 ade2 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 tyr1*
8297	*MATa SCR::URA3 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 HIS7 sap3 trp1* *MATa ADE2 ADE3 leu1-1 CYH2 URA3 CAN1 his7 SAP3 TRP1*
8285	*MATa ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1 TYR1* *MATα SCR::URA3 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 TRP1 tyr1*
8202A10	*MATa ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1* *MATα ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1*
8298	*MATa SCR::URA3 CRY1 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 HOM3 sap3 trp1 HIS7* *MATa CRY1 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 HOM3 sap3 TRP1 HIS7* *MATa cry1 ade2 ADE3 leu1-1 CYH2 URA3 CAN1 hom3 SAP3 TRP1 his7* *MATa cry1 ADE2 ADE3 LEU1 CYH2 URA3 CAN1 HOM3 SAP3 TRP1 his7*
8247	*MATa ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1 rad9::TRP1 LYS2* *MATa SCR::URA3 ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 trp1 rad9::TRP1 lys2*
8291	*MATa ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 TRP1 cdc13-1* *MATα SCR::URA3 ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1 cdc13-1*
8316	*MATa ade2 ade3-130 LEU1 cyh2 ura3-52 can1 sap3 trp1* *MATα ADE2 ade3-130 leu1-1 cyh2 URA3 can1 SAP3 TRP1*
8317	*MATa SCR::URA3 (tel) ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1* *MATα ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 TRP1*
8318	*MATa SCR::URA3 (tel) ade2 ade3-130 leu1-1 cyh2 ura3-52 can1 sap3 trp1* *MATα SCR::URA3 (tel) ade2 ade3-130 leu1-12 cyh2 ura3-52 can1 sap3 TRP1*

All of the strains described above were generated during the course of this study and are congenic with the A364a strain background.

the 4.5-kb *Bgl*II-*Sal*I 5′ deletion fragment and the 1.3-kb *Sal*I-*Bam*HI 3′ deletion fragment (Figure 1B). The resulting 7.0-kb fragment was isolated from the vector by a *Bam*HI/partial *Bgl*II digestion. This fragment was ligated into the *Bam*HI site of a 2.5-kb fragment from the left arm of chromosome *III* (from λ clone L4, NEWLON *et al.* 1991) cloned into pHSS6. The resulting plasmid, pLK19, was digested with *Not*I to generate a 9.5-kb fragment containing SCR::*URA3* in chromosome *III* DNA, and this fragment was transformed into strain 8202 using a standard procedure (ITO *et al.* 1983). A transformant, 8202SCR, was isolated that had integrated the construct by recombination into the left arm of chromosome *III*, 1.7 kb distal to *LEU2* (Figure 1B). Southern blot analysis was used to verify the structure and function of the sister chromatid recombination substrate; genomic DNA was isolated from strains 8202, 8202SCR, and from an *ADE3*$^+$ recombinant isolated from 8202SCR. This DNA was cut with *Bgl*II, *Eco*RI and *Hin*dIII, electrophoresed, blotted, and probed with two different probes (Yip5, containing homology to *URA3*, and pLK6, containing the 2.5-kb *Eco*RI fragment of chromosome *III*

into which the sister chromatid recombination substrate was cloned). All structures were as expected.

A strain containing the SCR::*URA3* substrate near the telomere of chromosome *III* was made by BARBARA GARVIK. This was done by cloning the 7.0-kb *Bam*HI/*Bgl*II partial digestion fragment containing SCR::*URA3* into a 4.6-kb *Eco*RI fragment (from lambda clone P78, NEWLON *et al.* 1991) located 1.5 kb distal to *HML*. The entire 11.6-kb fragment was then transplaced into chromosome *III*.

Induction of sister chromatid recombination and homolog recombination by X-irradiation: Overnight cultures of diploid strains 8301, 8302 or 8285 were started from stationary liquid cultures or from single colonies on a plate. In all experiments duplicate cultures were used. Cells were grown overnight in YM-1 + glucose (2%) to mid-log phase ($0.5–1 \times 10^7$/ml). The cells were then collected by centrifugation and half were arrested in G_1, the other half in G_2, as described above. Following arrest in either G_1 or G_2, cells were irradiated on agar plates with varying doses of X-rays delivered by a Machlett OEG 60 X-ray tube operated at 50 kV and 20 mA and delivering a dose rate of 106 rad/sec. The cells were then washed from the plates with -N medium, collected by centrifugation, resuspended in a small volume of -N, diluted, and plated on complete medium plates to monitor viable cells per ml, and on selective plates to monitor recombination (-leu plates for homolog recombination, and -his plates for sister chromatid recombination). These plates were incubated for 3 days at 30°, then colonies were counted to calculate recombination frequency per viable cell. The viability of cells following X-irradiation was measured in one of two ways. Absolute viability was measured by plating 5×10^3 to 1×10^4 cells on half of a complete synthetic medium plate and then microscopically monitoring microcolony formation following 24–48 hr of incubation at either 30° or 23°. Very small microcolonies (less than 100 cells) were scored as dead, whereas colonies consisting of at least 100 cells were considered viable. In most cases the dead cells formed fewer than 10-cell colonies, and the live cells had many more than 100 cells per colony, although at some doses there was a fraction of the population which was of an intermediate size which were counted as dead due to their extremely slow growth. In this method, viability was the fraction of live cells over the total counted. A second way that viability was determined was to dilute unirradiated cells onto rich medium plates, irradiate them and then count colony formation after 3 days at 30°. Viability at a given dose was calculated as the viable cell concentration at that dose divided by the viable cell concentration of unirradiated cells.

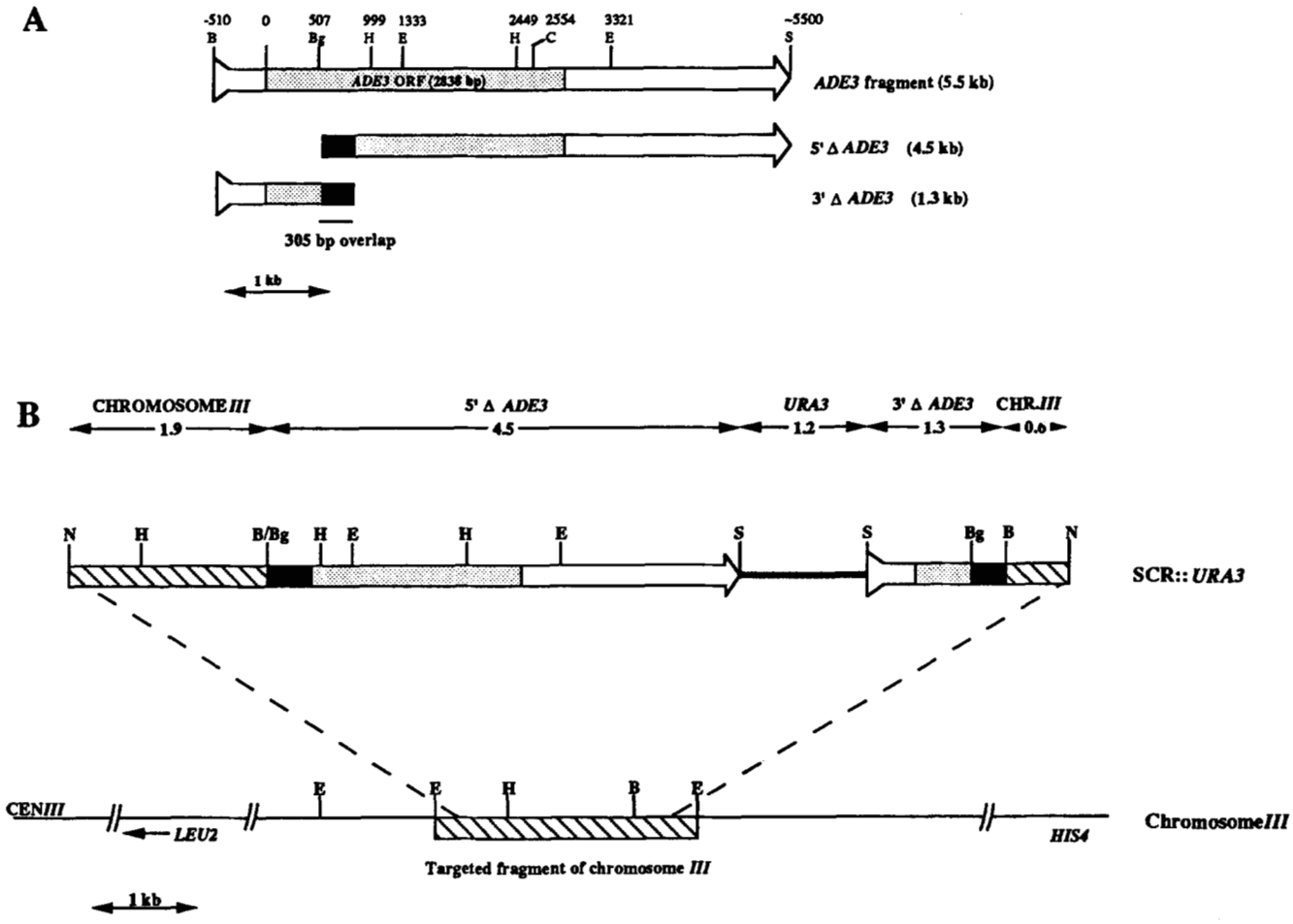
Effect of X-irradiation on mutation rates to *ADE3* and *LEU1*: One concern in these experiments is whether the events being measured were truly recombinants or radiation-induced reversions or suppressors. The possibility of intragenic reversions was eliminated for the sister chromatid recombination substrate since the mutations are caused by large deletions from either end of the locus. The possibility that second site suppressors were induced that bypassed the requirement for the *ADE3* gene to grow on -his plates was eliminated by the following experiment. Irradiation of diploid 8316, homozygous for *ade3-130* but lacking the sister chromatid recombination substrate, resulted in revertants at a rate of 2.4×10^{-7} per viable cell, approximately 100 times lower than the rate of 3.7×10^{-5} per viable cell observed in strains containing the sister chromatid recombination substrate.

The nature of the mutations in *leu1-1* and *leu1-12* is not known, but they may be revertable both intragenically and by second site mutations. Diploid strains 8297 and 8294, homozygous for *leu1-1* or *leu1-12*, respectively, were irradiated with either 4 or 8 krad while growing asynchronously and plated on -leu plates to measure the frequencies of Leu+ revertants. At 4 and 8 krad the mutations induced by X-irradiation accounted for no more than 0.8% and 1.2%, respectively, of the recombination rate, and therefore these events were not a significant factor in our calculations.

RESULTS

Construction and function of the sister chromatid recombination substrate: To select unequal sister chromatid recombination events, we constructed a recombination substrate similar to the design of FASULLO and DAVIS (1987, 1988), as described in detail in MATERIALS AND METHODS. Briefly, two nonfunctional deletions of the yeast *ADE3* gene were constructed from either end of the coding sequence such that an overlapping region of 305 bp of homology remained, within which a recombination event could occur to regenerate the intact gene (Figure 1A). In the sister chromatid recombination substrate, denoted as SCR::*URA3*, the deletions flank a wild-type *URA3* gene; this substrate was integrated into chromosome *III* between *LEU2* and *HIS4* of strain 8202-1 to create strain 8202SCR (Figure 1B). This strain and others derived from it also contain the *ade3-130* mutation, a deletion including all of the chromosomal region containing homology with the 305 bp overlap between 5′Δ*ade3* and 3′Δ*ade3* in SCR::*URA3*. Therefore, only a recombination event within the sister chromatid recombination substrate can regenerate the *ADE3* gene. The *ade3* deletions used in the substrate as well as that in the genome (*ade3-130*) create auxotrophy for both adenine and histidine; since the strains are also mutant for the *ade2* gene, which is required for adenine biosynthesis, *ADE3* recombinants were selected as His+ colonies. We have observed microscopically that cells containing the *ade3-130* deletion and the sister chromatid recombination substrate arrest growth within one cell cycle after being plated on plates lacking histidine; therefore, all the recombinants studied occurred in the same cell cycle that received irradiation.

Three types of recombination events can generate a functional *ADE3* gene from the SCR::*URA3* construct (Figure 2). The first type is an intramolecular reciprocal recombination ("popout") within a single chromosome in G_1 or a single chromatid in G_2. This event generates an extrachromosomal circle carrying the *ADE3* and *URA3* genes (Figure 2A) and will not be detected because the resulting episome lacks an origin of replication and is not stably maintained unless it reintegrates into the genome. Reintegration of a popout at the same location would simply restore the structure of the SCR::*URA3* construct and be *ade3*. Thus, only popouts that reintegrated at *URA3* or *ADE3* would be selectable as *ADE3*, and can be distin-



INSERTION OF SCR::*URA3* CONSTRUCT INTO CHROMOSOME *III*

FIGURE 1.—(A) Restriction map of the yeast *ADE3*-containing fragment and creation of deletion fragments. The full-length clone containing *ADE3* is shown, with nucleotide positions relative to the start of the open reading frame indicated above the restriction sites. The shaded box indicates the *ADE3* open reading frame (ORF). Below the full length fragment are shown the 5′ and 3′ deletions used to make the sister chromatid recombination construct. The 5′ deletion (5′Δ*ADE3*) removes 507 bp and the 3′ deletion (3′Δ*ADE3*) removes 2026 bp of the 2838 bp open reading frame. The region of overlap between the two deletions is shaded black and is 305 bp. The arrowhead indicates the 3′ end and the tail indicates the 5′ end of the *ADE3*-containing fragment. (B) Structure and integration of the sister chromatid recombination substrate. The sister chromatid recombination substrate (denoted as SCR::*URA3*) consists of the 5′ and 3′ deletions separated as shown by the selectable marker *URA3*, which is indicated as a thick line. The SCR::*URA3* fragment was cloned into a 2.5 kb fragment of chromosome *III* from a region between *LEU2* and *HIS4*, indicated by stripes; the entire linear fragment was then integrated into the chromosome, as shown. A description of the construction and integration of SCR::*URA3* is in MATERIALS AND METHODS. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; N, *Not*I; S, *Sal*I.

guished from sister chromatid recombination events by Southern analysis. We have examined 20 recombinants induced by 4 krad of X-irradiation, 10 following G_1-irradiation and 10 following G_2-irradiation. All had the structure expected for recombination between sister chromatids. We expect popout events followed by reintegration would be rare, since they occur with a frequency equal to the product of the frequency of popping out times the frequency of reintegration of a nonreplicating episome.

The other two types of recombination events that can generate a stable Ade3$^+$ colony are unequal reciprocal recombination or gene conversion between sister chromatids in G_2 (Figure 2, B and C). Whether sister chromatid recombination events occurred by reciprocal exchange or gene conversion can be determined using a red/white sectored colony assay based on changes in pigmentation that result from mutations in the adenine biosynthetic genes (ROMAN 1956). The *ade2 ade3* strains we use are white; unequal sister chromatid recombination in the SCR::*URA3* construct results in a red *ADE3 ade2* colony. To distinguish reciprocal recombination and gene conversion events in the SCR::*URA3* construct, cells are plated nonselectively to recover both products of any recombination events that occur in the first division after plating. Such events will generate red/white half-sectored colonies, representing the two products of the recombination event. White, His- half-sectors generated by reciprocal exchange events are Ura$^-$, while those re-

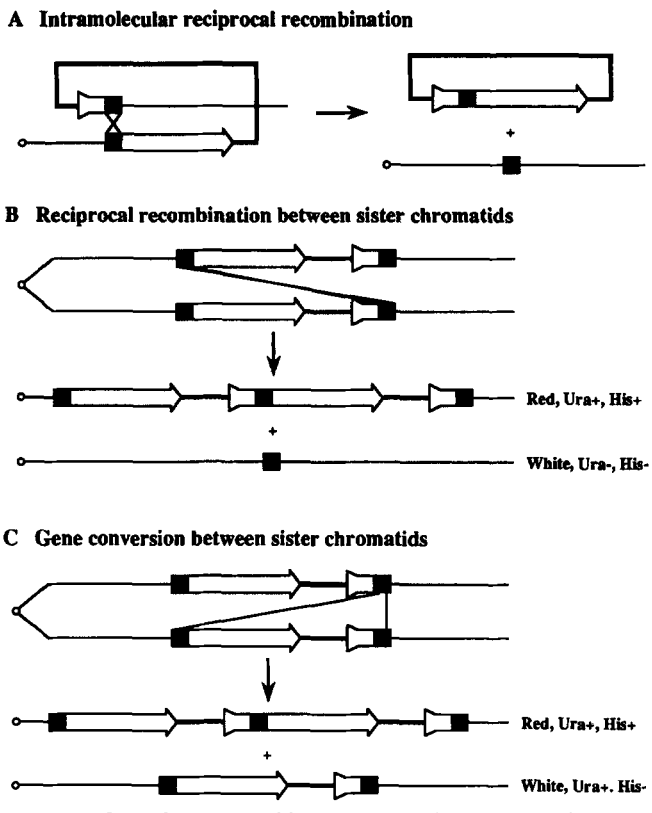


FIGURE 2.—Three recombination events in SCR::*URA3* which generate *ADE3*. SCR::*URA3* is diagrammed as in Figure 1B. (A) An intramolecular event ("popout") can occur before or after DNA replication. This type of recombination generates an extrachromosomal circle containing *ADE3* and *URA3* but lacking an origin of replication; these episomes are unstable and are not detected as viable colonies. (B) Following replication, an unequal reciprocal recombination event can occur between sister chromatids to generate an *ADE3, URA3* chromatid and an *ade3, ura3* chromatid. (C) A gene conversion event may occur via double-strand break gap-repair, generating an *ADE3 URA3* chromatid and an *ade3 URA3* chromatid. The events described in panels B and C can be distinguished when they occur on a nonselective plate in a strain mutant for *ade2*: red/white half-sectored colonies are formed since the progeny arising from one half of the recombination event are *ade2 ade3* and white, whereas the progeny arising from the other half are *ade2 ADE3* and red. By scoring the Ura phenotype of the white half of the sector, reciprocal exchange and gene conversion events can be distinguished, as indicated in panels (B) and (C).

sulting from gene conversion events are Ura$^+$ (Figure 2, B and C).

We used the sectored colony assay described above to examine the unselected half of 12 sectored colonies resulting from X-ray induced sister chromatid recombination in diploid strain 8301, heterozygous for the SCR::*URA3* substrate. We found that approximately 2/3 (8) of X-ray-induced sister chromatid recombination events are gene conversions and 1/3 (4) are reciprocal exchanges. This ratio is similar to that observed for spontaneous recombination events between duplicated *his4* genes (JACKSON and FINK 1981). In that study, gene conversion events, but not reciprocal recombination events, are dependent on the *RAD52* gene product. In contrast, we have observed no significant level of X-ray induction of sister chromatid recombination in a *rad52* background (L. C. KADYK, unpublished data), and we conclude that all X-ray induced mitotic recombination events are *RAD52* dependent, in agreement with others (PRAKASH *et al.* 1980; SAEKI, MACHIDA and NAKAI 1980).

The rate of spontaneous unequal sister chromatid recombination in the SCR::*URA3* substrate was measured using the method of LEA and COULSON (1948) to be 2.7×10^{-6} events per cell division, nearly the same as that reported by FASULLO and DAVIS (1987) for a similar substrate (2×10^{-6} per cell division). In addition, we find that the spontaneous frequency of sister chromatid recombination decreases about 10-fold in a *rad52-1* background (from 2×10^{-5} per viable cell to 2×10^{-6} per viable cell), in agreement with the 10-fold decrease in rate reported by FASULLO and DAVIS (1987). Because of their rarity, it was impossible to determine whether the events induced in a *rad52* background were reciprocal recombination or gene conversion.

A comparison of the inducibility of homolog recombination and sister chromatid recombination in G_1 and G_2: Levels of sister chromatid recombination and homolog recombination induced by X-ray damage at different parts of the cell cycle were compared in *MAT***a**/*MAT***a** diploid strain 8301. This strain is heterozygous for the SCR::*URA3* substrate so that sister chromatid recombination can be monitored, and heteroallelic for the mutations *leu1-1* and *leu1-12*, so that gene conversion between homologs can be monitored. Exponentially growing cultures of this strain were divided into two portions, arrested in either G_1 using α-pheromone or in G_2 using MBC, and irradiated with X-rays to induce recombination. Cells were then plated on -leu or -his plates to select recombinants, and on rich medium to determine viable cell number. In agreement with others (ESPOSITO 1968; FABRE, BOULET and ROMAN 1984), we found that homolog recombination was induced to a large extent in cells irradiated in G_1, but was induced to only 10 to 20% of G_1 levels when the cells were irradiated in G_2 (Figure 3A). Sister chromatid recombination was induced weakly in cells irradiated in G_1, (there was a significant difference between the spontaneous and the 8 krad induction levels, by the two-sample t-test, $P = 0.05$) and was induced to a greater extent in G_2-irradiated cells (Figure 3B).

Alternative arrests: To test the possibility that the particular drugs used to synchronize cells for these experiments (α-pheromone and MBC) in some way affect the levels of induced recombination, apart from the effect of being arrested at a specific cell-cycle stage, alternative methods to synchronize cells were used. Alternative forms of G_1 arrest were achieved in strain 8301 by growing cells until they reached sta-

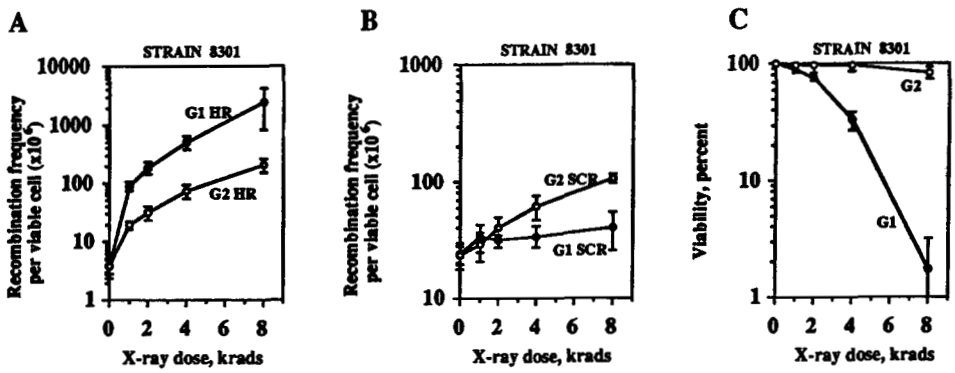


FIGURE 3.—Induction of homolog recombination and sister chromatid recombination in diploid strain 8301 by X-irradiation in G_1 and G_2. Growing cells were arrested, irradiated and plated for recombinants and viability as described in MATERIALS AND METHODS. (A) Homolog recombination events induced by irradiating G_1 or G_2 arrested cells. (B) Sister chromatid recombination events induced by irradiating G_1 or G_2 arrested cells. (C) Viability of G_1 and G_2 irradiated cells of strain 8301. Each data point represents the average values from six independent cultures.

tionary phase or by starving them for nitrogen (see MATERIALS AND METHODS). An alternative G_2 arrest was achieved using strain 8291, homozygous for *cdc13-1*, a mutation which causes a *RAD9*-dependent cell cycle arrest in G_2 at elevated temperatures (T. WEINERT, personal communication). Under the conditions used, each of these arrests gave a high level of synchrony (>95%), little loss in viability and no induction of recombination prior to irradiation. (Elevating the temperature of *cdc13* strains induces recombination; however, this occurs only in regions of the chromosome close to the telomere (KRANZLER 1986; CARSON 1987), and does not affect levels of recombination in either substrate used in these experiments, both of which are centromere-linked). These alternative methods of arresting cells showed patterns of X-ray-induced recombination similar to those seen in the original arrest protocols (Table 2). Our results are also consistent with other results using cells synchronized without use of drugs (ESPOSITO 1968; FABRE, BOULET and ROMAN 1984). We therefore believe that our conclusions are unaffected by the methods we have used to synchronize cells.

Normalization of the sister chromatid recombination assay to the homolog recombination assay: Since the net induction of homolog recombination in G_2-irradiated cells is much lower than in G_1-irradiated cells (Figure 3A), most of the lesions that resulted in homolog recombination when induced in G_1 must have an alternative fate when induced in G_2 cells. One possible fate for these lesions in G_2 might be repair by sister chromatid recombination rather than by homolog recombination. Alternatively, some lesions that are repaired recombinationally in G_1 may be repaired nonrecombinationally in G_2. To determine whether the amount of sister chromatid recombination induced in G_2 is equivalent to the difference in the net inductions of homolog recombination in G_1 and G_2, it was necessary to determine the factor by which the

TABLE 2

Effect of alternative methods of arrest on X-ray induced recombination

	Dose (krad)	Viability	*ADE3* frequency[a]	*LEU1* frequency
Alternative G_1 arrest				
Stationary	0	99%	18	5
Stationary	8	17%	11	950
α-Pheromone	0	100%	24	4
α-Pheromone	8	17%	40	2500
-N starvation	0	98%	22	20
-N starvation	8	30%	30	2300
Alternative G_2 arrest				
cdc13, asynchronous	0	83%	13	17
cdc13, 36°	0	88%	19	18
cdc13, 36°	8	80%	245	155
MBC	0	88%	24	4
MBC	8	74%	210	110

Data shown for all experiments are the average of two cultures, except for the α-pheromone and MBC arrests, which are the average of six cultures. Arrests and irradiation were as described in MATERIALS AND METHODS, except the *cdc13* strain was arrested in G_2 by shifting a growing culture to 36° for 2 hr.

[a] All frequency values listed are the number of events per 10^6 viable cells.

homolog recombination and sister chromatid recombination substrates differed in their response to X-rays, since the two substrates measure different recombination events. There are several reasons why these two substrates might differ in their response to X-ray damage. First, the size of the homologous region wherein a productive recombination event can occur might be quite different for the two assays. The SCR::*URA3* substrate has a 305-bp region of homology, whereas the distance separating the *leu1* heteroalleles may be considerably larger; the locations of the lesions in *leu1-1* and *leu1-12* are not known, but, if they were near opposite ends of the gene, the target for a productive recombination event could be up to 2.9 kb. A second factor affecting the relative sensitivities of the two assays is that the sister chromatid

recombination assay measures only unequal recombination events, whereas the homolog recombination assay measures equal events; most of the recombination events induced between sister chromatids are likely to be equal recombination and would not be detected. Finally, since the two assays are not in identical locations in the genome, a position effect might contribute to the difference in sensitivity to X-rays between the two assays. Examples of sequences that exhibit a position effect on recombination frequency include a mitotic recombination hot spot that has been mapped near the centromere of chromosome *XIV* (NEITZ and CARBON 1987), and a recombination enhancing sequence that has been identified in the rDNA (KEIL and ROEDER 1984).

To directly determine the factor by which the *LEU1* and *ADE3* recombination substrates differed in their response to X-rays, we measured the levels of induction of *ADE3* and *LEU1* in a second diploid, 8302. Diploid 8302 is identical to diploid 8301 except that it is homozygous for the SCR::*URA3* substrate, so that in G_1-irradiated cells, *ADE3* recombinants are produced by an unequal recombination event between homologs that is analogous to that between sister chromatids in strain 8301. By determining the relative homolog recombination responses of each of these substrates following X-irradiation of strain 8302 in G_1, it is possible to calculate a "normalization factor" to use when comparing the levels of induction of the two different substrates. We irradiated strain 8302 in G_1 with 4 krad, since this dose is in a range where the dose-response curve is fairly linear and the cell viability is still high, and observed that 13 times as many *LEU1* events/viable cell are induced as *ADE3* events (Table 3A). Assuming that both regions experience the same number of lesions per unit DNA, the *leu1* heteroalleles are 13 times as likely to undergo a detectable recombination event as is the sister chromatid recombination substrate, and we can use this factor to normalize induced *ADE3* events to induced *LEU1* events. In using this factor, we assume that the frequency of X-ray induced recombination in the SCR::*URA3* substrate is equivalent whether the recombination event occurs between homologs in G_1 or between sister chromatids in G_2. This assumption seems reasonable, since there is no evidence that sister chromatids have a different recombinational response to DNA damage than do homologs, nor that recombinational repair occurs to a different extent in G_2 than in G_1.

We then applied this factor to the measured G_2 induction of *ADE3* events in strain 8301 to determine if sister chromatids are used as alternatives to homologs for repair of G_2-induced X-ray damage. The X-ray induction of homolog and sister chromatid recombination in G_1- and G_2-arrested cells of strain 8301 was repeated in 10 independently irradiated samples, using a dose of 4 krad, to obtain statistically significant data (Table 3B). We calculate that the level of homolog recombination induced per 4-krad dose of damage in G_2 was about 14% (71.6/501) of that in G_1 cells. Since G_2 cells have twice as much DNA and therefore twice the target size of G_1 cells, this means that the amount of homolog recombination induced in G_2 is only about 7% of the amount induced for the same amount of damage incurred in a G_1 cell. It follows that 93% of the lesions that induce homolog recombination in G_1 must have an alternative fate in a G_2 cell, corresponding to a calculated value of 465 fewer homolog recombination events per 10^6 viable cells in G_2 cells than in G_1 cells irradiated with the same dose (Table 3C). In good agreement with this value, we find that multiplying the observed sister chromatid recombination events by the normalization factor of

TABLE 3

Sister chromatids are used as alternatives to homologs for G_2 repair

A. Calculation of "normalization factor" at 4 Krads in strain 8302, G_1[a]

Strain 8302, G_1:

Net induction of *LEU1* = 893.8 ± 261

Net induction of *ADE3* = 68.1 ± 26.9

"Normalization factor" = net *LEU1*/net *ADE3* = 13.1 ± 3.6

B. Net induction of *LEU1* and *ADE3* by 4 krad in strain 8301, G_1 and G_2:

	LEU1	*ADE3*
G_1	501 ± 74.9	14.6 ± 6.64
G_2	71.6 ± 12.3	37.4 ± 7.09
LEU1 G_2/G_1 =	0.14	

C. Sister chromatids are used as alternatives to homologs as substrates for repair in G_2-irradiated cells[b]

Number of lesions repaired by HR in G_1 which are repaired by alternative means in G_2 =

Net induction of *LEU1*(G_1) − 0.5[net induction of *LEU1*(G_2)] = (501 ± 74.9) − (35.8 ± 6.15) = **465 ± 75.2**

Net induction of SCR in G_2 (normalized) =

Net induction of *ADE3* in G_2 × normalization factor = (37.4 ± 7.09) × (13.1 ± 3.6) = **489 ± 166**

Fraction of alternative repair events in G_2 cells that can be accounted for by induced SCR =

$$\frac{489 \pm 166}{465 \pm 75.2} = \mathbf{105 \pm 39.5\%}$$

[a] All values listed in this table are the number of recombinants per 10^6 viable cells. The spontaneous (0 krad) levels of the events were subtracted from the 4 krad-induced levels to determine the net induction by X-rays. For the experiment in strain 8301, n = 10. For the experiment in strain 8302, n = 8. Values are listed ± one standard deviation. The standard deviation is the square root of the variance. The variance of sums and differences is: var $[X + Y]$ = var$[X]$ + var$[Y]$ + 2 cov$[X,Y]$. The variance of a product is: var$[XY] = \mu_y^2$ var$[X] + \mu_x^2$ var$[Y]$ + var$[X]$var$[Y]$. The variance of a quotient is var$[X/Y] = (\mu_x/\mu_y)^2$(var$[X]/\mu_y^2$ + var$[Y]/\mu_y^2$ − 2 cov$[XY]/\mu_x\mu_y$).

[b] HR = homolog recombination, SCR = sister chromatid recombination.

13 gives the equivalent of 489 normalized sister chromatid recombination events induced per 10^6 viable cells in G_2 cells. We did not divide the net increase in sister chromatid recombination in G_2 by two as we did for homolog recombination since the sister chromatid recombination substrate was only present on one of the two homologs. Thus, lesions that were recombinogenic in G_1 were equally recombinogenic in G_2, but the distribution of the events at the two stages of the cell cycle was very different. Most of the recombination induced in G_1 cells was homolog recombination, whereas over 90% of G_2-induced events were sister chromatid recombination. Because normally only about 95% of cells treated with MBC appear to be in G_2 (large budded with a single nucleus), it is possible that the homolog recombination measured in G_2 irradiated cells was due to cells that are actually in G_1 at the time of irradiation. Therefore, it is possible that essentially 100% of recombination events induced in G_2 are sister chromatid recombination. These results mean that there is a great preference in G_2 cells for repair using sister chromatids rather than homologs, since if repair were to occur with an equal probability of using either the sister chromatid or one of the two chromatids from the homologous chromosome, only 33% of the events would occur by sister chromatid recombination.

We have assumed that the sister chromatid recombination observed would be the same even if the SCR::*URA3* substrate had an homologous partner in G_2. We believe this is a fair assumption because we have measured the G_2 induction of *ADE3* events in strain 8302, homozygous for the SCR::*URA3* construct, and find that similar levels of induction of *ADE3* were observed in strain 8302 whether it was irradiated in G_1 or in G_2. Although it is impossible in that strain to genetically distinguish *ADE3* events arising via sister chromatid recombination from those arising via homolog recombination, we assume that, as for the *leu1* heteroalleles, many fewer events occur between homologs following G_2-irradiation than following G_1-irradiation. Therefore, we infer that high levels of sister chromatid recombination were induced following G_2-irradiation of strain 8302, even though a competing substrate was present on the homologous chromatids.

We note that some sister chromatid recombination was induced by X-irradiation of G_1-arrested cells (14.6 events per 10^6 viable cells, Table 3B). It is unlikely that these events can be explained as popouts followed by reintegration of the nonreplicating episome, as we have previously discussed. Another possible explanation for these events, that a large percentage of cells were not in G_1 at the time of irradiation, is eliminated because greater than 99% of the cells arrested with α-pheromone appear to be unbudded and have a G_1 DNA content by flow cytometry. We have evidence suggesting that UV-lesions induced in G_1 can stimulate high levels of sister chromatid recombination during replication (L. C. KADYK and L. H. HARTWELL, manuscript in preparation), and we presume that the events induced by X-rays in G_1 cells are due to lesions similar to those left by UV-irradiation.

Effect of chromosomal location on frequency of sister chromatid recombination: One possible explanation for the observed preference for using sister chromatids rather than homologs to repair in G_2 is that sister chromatids are closer together than are homologous chromatids and therefore find each other more easily during the search for a homologous molecule with which to repair. However, there is no physical evidence that sister chromatids are held together along their entire lengths, and it may be that telomeric sequences on sister chromatids are no closer to each other than they are to homologous chromatids. Both the sister chromatid recombination and the homolog recombination substrates used in the above experiments were centromere-linked, thus the observation that sister chromatids were preferred over homologs for recombinational repair in G_2 might only apply to centromere-proximal regions of a chromosome arm. To test this possibility, we constructed diploid strain 8317, which is *MAT***a**/*MAT*α, heteroallelic for *leu1*, and contains the sister chromatid recombination substrate integrated at the telomere of chromosome III. G_2-arrested cells of this strain were irradiated with 4 krad, levels of induced sister chromatid recombination and homolog recombination were monitored, and the percentage of G_2 events that were sister chromatid recombination was calculated. To determine the normalization factor to apply to the measured induction of sister chromatid recombination at the telomere in strain 8317, we constructed a strain homozygous for the SCR::*URA3* substrate at the telomeric location, 8318 (*MAT***a**/*MAT*α). Following X-irradiation of this strain with 4 krad in G_1 (stationary phase), we calculated a normalization factor of 5.0, as the ratio of induction of *LEU1* and *ADE3* (Table 4B). We then used this factor to calculate the fraction of total G_2 events induced by 4 krad in strain 8317 that were sister chromatid recombination. Even when the sister chromatid recombination events were measured at the telomere, over 80% of G_2 events were calculated to be sister chromatid recombination, indicating that even telomere-proximal sequences are repaired preferentially by sister chromatid rather than homolog recombination (Table 4C). For comparison, we made the same calculation using strain 8285, which is also *MAT***a**/*MAT*α and heteroallelic for *leu1* but contains the SCR::*URA3* construct at the centromeric location used in strain 8301 for the first set of calculations. To calculate the fraction of G_2 events that

TABLE 4

Effect of chromosomal location on induced sister chromatid recombination

A. Net induction of *ADE3* and *LEU1* by 4 krad of X-rays in G_2[a]

Strain:	*ADE3*	*LEU1*
8317 **a**/α SCR::*URA3* (TEL)	48 ± 6.4	90 ± 11.4
8285 **a**/α SCR::*URA3* (CEN)	61 ± 13.6	102 ± 8.9

B. Calculation of "normalization factor" at 4 krad of X-rays in strain 8318, G_1

Net induction of *LEU1* = 245 ± 29.2
Net induction of *ADE3* = 48.6 ± 11.0
"Normalization factor" = net *LEU1*/net ADE3 = 5.0 ± 1.1

C. Calculation of the fraction of total G_2-induced recombination events that occur by SCR[b]

1) 8317 (SCR::*URA3*/TEL): $\frac{(40 \pm 6.4) \times (5.0 \pm 1.1)}{(40 \pm 6.4) \times (5.0 \pm 1.1) + (45 \pm 5.7)} = 82 \pm 30\%$

2) 8285 (SCR::*URA3*/CEN): $\frac{(61 \pm 13.6) \times (13.1 \pm 3.6^c)}{(61 \pm 13.6) \times (13.1 \pm 3.6) + (51 \pm 4.5)} = 94 \pm 46\%$

[a] All values listed in this table are the number of recombinants per 10^6 viable cells. The spontaneous (0 krad) levels of the events were subtracted from the 4 krad-induced levels to determine the net induction by X-rays.

[b] This calculation is [(the net induction of SCR in G_2) × (normalization factor)] ÷ [(the net induction of SCR in G_2) × (normalization factor)] + [(0.5) × (the net induction of HR in G_2)].

[c] The normalization factor used in this calculation is that determined in Table 3B.

were sister chromatid recombination using the data from strain 8285, we used the normalization factor of 13.1 determined in Table 3B. The observation that 94% of G_2 events were sister chromatid recombination is consistent with the conclusions reached using *MAT***a**/*MAT***a** strain 8301.

G_1 *vs.* G_2 viability differences: We observed that G_1 diploid cells are much more sensitive to the lethal effects of X-ray damage than are G_2 diploid cells (Figure 3C), in agreement with previous reports (HATZFELD and WILLIAMSON 1974; BRUNBORG and WILLIAMSON 1978; BRUNBORG, RESNICK and WILLIAMSON 1980). Diploid cells can repair at least some X-ray damage incurred in G_1 using recombination between homologs (Figure 3A; ESPOSITO 1968; FABRE 1978; FABRE, BOULET and ROMAN 1984), so it is unclear why this cell-cycle difference should exist. Several possible explanations were tested.

One possible explanation for the low resistance of G_1 cells to X-ray damage is that they may achieve saturation of their ability to repair at lower doses because they have fewer templates available for repair than do G_2 cells. For example, it may be that damage to both homologs in a given region is lethal in a G_1 diploid, but that damage to all four of the chromatids in the same region must occur in a G_2 cell before the damage is irreparable. This possibility was addressed experimentally by comparing the sensitivity of a G_1 tetraploid, strain 8298, with that of G_2 diploid strain 8285. If the number of homologous molecules alone could account for the difference in resistance between G_1 and G_2 diploids, a G_1 tetraploid should be as resistant as a G_2 diploid. Instead, it was found that a G_1 tetraploid was much more sensitive than a G_2 diploid (Figure 4A). In fact, tetraploid G_1 cells were even less resistant than were diploid G_1 cells, in agreement with a previous report (MORTIMER 1958). These results show that the number of homologous molecules alone cannot account for resistance to X-ray damage.

Another possible explanation for the high X-ray resistance of G_2 diploids relative to G_1 diploids is the existence of the *RAD9*-mediated cell-cycle delay at the G_2 stage to allow repair following X-ray damage (WEINERT and HARTWELL 1988). X-irradiation of cells in G_1 may not stimulate an analogous cell-cycle arrest before replication to allow repair, and unrepaired lesions could be lethal during replication. To test the idea that the *RAD9* function is the source of the increased X-ray resistance of G_2 cells, strain 8247, a *rad9*/*rad9* diploid, was irradiated in G_1 and G_2. If the *RAD9*-dependent delay in G_2 were solely responsible for the increased viability of G_2 cells over G_1 cells, then one would expect the cell-cycle difference to disappear in *rad9*/*rad9* diploids. Instead, it was found that even without *RAD9* function, G_2 diploids were more resistant to X-ray damage than were G_1 diploids (Figure 4B). We conclude that the relative X-ray resistance of G_2 cells cannot be attributed to the presence of the *RAD9* function in G_2. One possible caveat to this experiment is that a G_2 arrest might be simulated by the MBC used to synchronize the cells in G_2; although the drug is removed before irradiation, it is possible that there is some delay in recovery from the arrest. Since the DNA content of an asynchronously growing culture is roughly the average of an equal number of G_1 and G_2 cells, we irradiated such a culture and compared the resulting viability curve with that predicted from an average of the G_1 and G_2-arrested viability curves. The predicted curve

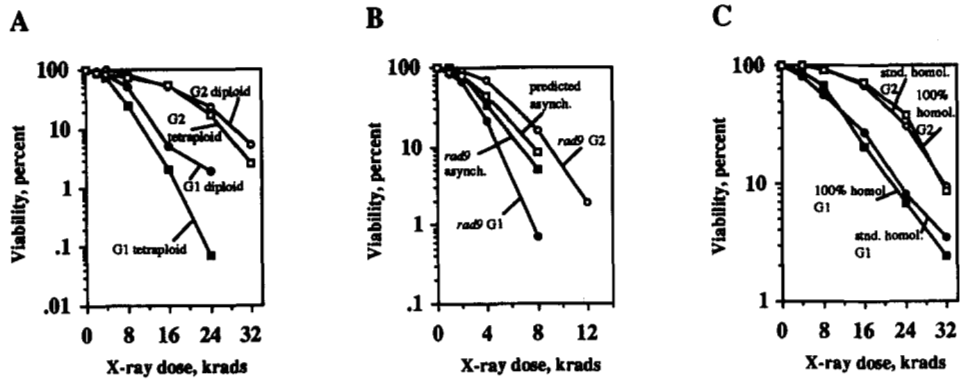


FIGURE 4.—The effect of ploidy, *rad9* or homology level on X-ray resistance. (A) The survival curves following X-irradiation of strains 8285 (diploid, **a**/α) and 8298 (tetraploid, **a**/**a**/α/α) were determined following arrest in G_1 by nitrogen starvation or in G_2 by treatment with MBC. Each data point represents the average of three independent irradiations. (B) The survival curves of strain 8247 (**a**/α,*rad9*/*rad9*) were determined as in panel (A). The predicted asynchronous viability curve is the average of the G_1 and G_2 viability curves. Each data point is the average of two independent irradiations. (C) The survival curves of **a**/α strains 8285 (standard level of homology between the homologs) and 8202A10 (which is a diploid selected from a haploid clone) were determined as in panel (A).

is very close to the actual curve (Figure 4B), thus it is unlikely that MBC pretreatment is significantly rescuing the viability of the G_2-irradiated cells. We note that *rad9*/*rad9* cells are more sensitive than *RAD9*/*RAD9* cells to X-irradiation in either G_1 or G_2 (*e.g.*, compare Figure 4, A and B). This result suggests that either *RAD9* has some role in ensuring repair in G_1, or else that some X-ray damage induced in G_1 is not normally repaired until G_2, and thus requires the *RAD9*-mediated cell cycle delay in G_2. The fact that some sister chromatid recombination is induced following X-ray damage in G_1 (Table 3B), is further evidence for the latter possibility.

Since neither the greater number of molecules present in G_2 nor the presence of *RAD9* function in G_2 can explain the difference in X-ray resistance of G_1 and G_2 cells, we hypothesize that the increased resistance of G_2 cells is a reflection of the superiority of sister chromatids over homologs as substrates for repair. That is, not only are sister chromatids used preferentially over homologs as substrates for recombinational repair, but they are used more effectively.

Role of homology in the preference for sister chromatid recombination: We can imagine two reasons why sister chromatids may be preferentially used over homologs as substrates for repair of G_2-induced DNA damage: one is that sister chromatids may be in closer proximity to each other than to homologous chromatids, perhaps because of their attachment at the centromere or because of some other physical interaction; the second is that sister chromatids have 100% homology between them, whereas some heterology may exist between homologous chromosomes. It is known that lowered levels of homology can influence the frequency of recombination events (WALDMAN and LISKAY 1988; SHEN and HUANG 1986; BAILIS and ROTHSTEIN 1990; AHN *et al.* 1988; SMOLIK-UTLAUT and PETES 1983). Although our strains are highly inbred (all strains used have been backcrossed to the original strain background at least 10 times), the ability of cells to repair recombinationally might be affected by the low levels of heterology remaining. Therefore, we selected a diploid (8202A10 *MAT***a**/*MAT*α) which shares essentially 100% homology between its homologs (save for the *MAT* locus) because it arose due to a self mating within a haploid clone. We compared the X-ray resistance of this diploid with that of a standard diploid, strain 8285 (*MAT***a**/*MAT*α). In either G_1 or G_2, 8202A10 and 8285 have identical sensitivities to X-irradiation (Figure 4C), showing that the heterology present between homologs in our strains does not play a significant role in the reduced efficiency of repair of X-ray damage in G_1 cells compared to G_2 cells. Therefore, it seems likely that sister chromatids are preferentially used as substrates for repair because of their proximity to each other.

Effect of mating type on induced recombination and X-ray sensitivity: In our experiments measuring the induction of recombination in G_1 and G_2, we used *MAT***a**/*MAT***a** cells to facilitate synchronization in G_1. Since it has been reported that homozygosity at the *MAT* locus leads to lower levels of spontaneous and UV-induced recombination (FRIIS and ROMAN 1968) and greater sensitivity to lethal X-ray damage than does heterozygosity (MORTIMER 1958; GAME 1983), we decided to test whether our results could be generalized to *MAT***a**/*MAT*α cells. We first tested whether *MAT***a**/*MAT***a** diploids had lower levels of X-ray-induced recombination than *MAT***a**/*MAT*α diploids. We arrested growing cultures of strains 8301 (*MAT***a**/*MAT***a**) and 8285 (*MAT***a**/*MAT*α) in G_1 by nitrogen starvation, or in G_2 with MBC, irradiated with X-rays, and plated for recombinants. Induced sister chromatid and homolog recombination levels among the surviving cells were very similar for the two strains, in both G_1 and G_2 (Figure 5).

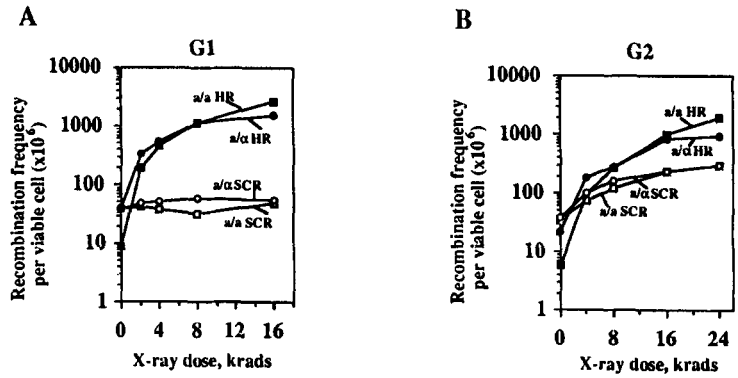


FIGURE 5.—X-ray induced homolog recombination and sister chromatid recombination in **a**/**a** and **a**/α diploids. Growing cells of strain 8301 (**a**/**a**) or 8285 (**a**/α) were arrested in G_1 by nitrogen starvation (A) or in G_2 by using MBC (B), irradiated, and plated for recombination frequency. Values shown represent the average of two independent cultures for each strain.

We also confirmed that cells homozygous at the *MAT* locus are more sensitive to X-irradiation than those heterozygous at *MAT*, using congenic *MAT***a**/*MAT*α, *MAT***a**/*MAT***a**, and *MAT*α/*MAT*α diploids (Figure 6A). To test whether this increased resistance of *MAT***a**/*MAT*α cells was likely due to improved recombinational repair, diploid *MAT***a**/*MAT***a** cells and haploid *MAT***a** cells were transformed with a centromere-bearing plasmid, pLK22, containing *LEU1* and *MAT*α. The X-ray sensitivity of the transformants was compared to that of the parental strains. *MAT***a**/*MAT***a** cells containing a *MAT*α centromere plasmid had equivalent X-ray resistance to *MAT***a**/*MAT*α cells when irradiated in G_1 (Figure 6B). However, haploid *MAT***a** cells transformed with the same *MAT*α plasmid did not change in their sensitivity to X-irradiation in G_1, presumably because they lack a homolog for recombinational repair in G_1. We then compared *MAT***a**/*MAT***a** and *MAT***a**/*MAT*α diploids with regard to differences in sensitivity between G_1 and G_2 stage cells (Figure 6C). The difference between G_1 and G_2 cells is seen in *MAT***a**/*MAT*α as well as *MAT***a**/*MAT***a** cells. In both stages of the cell cycle *MAT***a**/*MAT***a** cells were more sensitive than *MAT***a**/*MAT*α cells, although the difference between *MAT***a**/*MAT***a** and *MAT***a**/*MAT*α cells was more pronounced in G_1 than in G_2. It is therefore possible that the effect of heterozygosity for mating type is primarily to improve repair by homolog recombination rather than by sister chromatid recombination.

DISCUSSION

We show that cells X-irradiated in G_1 repair recombinogenic damage primarily by homolog recombination, whereas cells X-irradiated in G_2 repair almost exclusively by sister chromatid recombination. Taking into account the twofold difference in target size between the two stages of the mitotic cell cycle, we calculate that the total number of recombination events per viable cell induced per dose of damage is similar in G_1 and in G_2 irradiated cells. This result suggests that damage which is recombinogenic in G_1 is equally recombinogenic in G_2, but that there is preferential use of sister chromatids for repair in G_2, as suggested by FABRE, BOULET and ROMAN (1984).

Why are sister chromatids preferred substrates for repair in G_2? To repair DNA damage by recombination, a damaged molecule must find and pair with a homologous DNA sequence. The efficiency of recombinational repair depends on the efficiency with which a damaged molecule can be paired with a homologous sequence and on the degree of sequence identity shared between the recombining molecules. The preferential use of sister chromatids over homologs as substrates for recombinational repair could be due to differences in either one or both of these factors. To test whether sister chromatids might be preferred over homologs as substrates for repair because they, unlike homologous chromosomes, share perfect DNA sequence homology, we determined the X-ray resistance of a diploid strain in which the homologous chromosomes should be identical (save for the *MAT* locus). This strain was no more resistant to X-irradiation in G_1 or G_2 than was an ordinary diploid containing homologs with some sequence heterology (Figure 4C). Therefore, whatever heterology is present between homologs in our inbred strain does not significantly influence the ability of cells to repair X-ray damage when irradiated in G_1.

We argue, therefore, that the preferential use of sister chromatids rather than homologous chromosomes for repair in G_2 is probably due to a higher probability of encountering a sister chromatid than a homolog during the search for homology. The importance of proximity of recombining sequences has been shown by ROEDER, SMITH and LAMBIE (1984) and LICHTEN and HABER (1989). LICHTEN and HABER (1989) showed that spontaneous mitotic recombination between heteroalleles located 20 kb apart on the same chromosome occurred more frequently than did

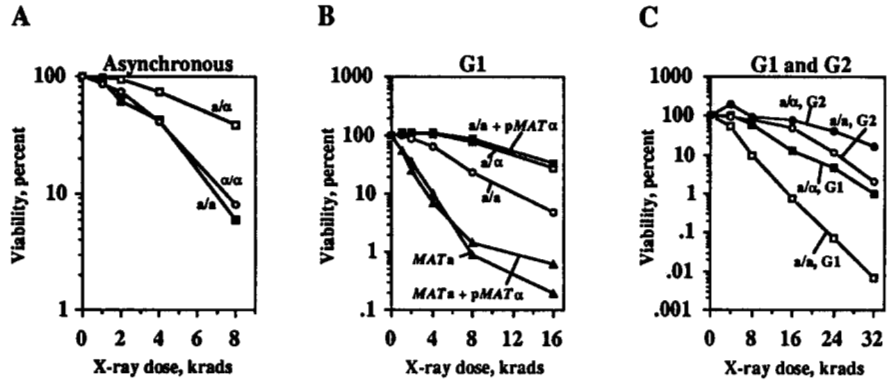


FIGURE 6.—The effect of mating type on X-ray resistance. (A) Asynchronously growing cultures of strains 8260 (**a**/α), 8261 (**a**/α), and their derivatives 8260-1 (**a**/**a**) and 8261-1 (α/α) were concentrated, plated, and irradiated. Viabilities were determined by microscopically scoring growth of microcolonies on rich medium. At least 200 colonies were scored for each culture at each dose. These data represent the average values of two independent cultures of each strain. (B) Cultures of strains 8285 (diploid **a**/α), 8301 (diploid **a**/**a**), 8301 carrying pLK22, (a *MAT*α plasmid), 8202 (haploid *MAT***a**), and 8202 carrying pLK22 were irradiated after arrest in G_1 by nitrogen starvation and viabilities were determined as in panel (A). (C) Cultures of strains 8285 or 8301 were arrested in G_1 using nitrogen starvation or in G_2 using MBC before irradiation. Viabilities were determined as in panel (A).

recombination between the same sequences when located further apart on the same chromosome, and these latter events were more frequent than recombination between the same heteroalleles located on homologous chromosomes. In addition, recombination between heteroalleles on homologs occurred no more frequently than did recombination between heteroalleles located ectopically on different chromosomes; similar results were obtained for recombination between Ty elements (KUPIEC and PETES 1988). These results indicate that the mechanism which searches for homology can search throughout the genome but prefers sequences located in close proximity on the same chromatid (or on sister chromatids) and further indicates that the search for homology acts by pairing short lengths of DNA rather than whole chromosomes.

Recombination involving sequences duplicated on the same chromatid may occur intramolecularly, in either G_1 or G_2 (Figure 2A), or intermolecularly, between sister chromatids (Figure 2, B and C). One possibility was that the effect of proximity observed in the above experiments was primarily on intramolecular recombination; however, LICHTEN and HABER (1989) showed that almost all reciprocal recombination between duplicated sequences on the same chromosome (10–20% of the total recombination) occurred by intermolecular recombination between sister chromatids. Our data suggest that sequences located on sister chromatids are in a preferred relationship for pairing and recombination, even though the recombination events occur intermolecularly. Furthermore, this relationship appears to be maintained along the whole length of the chromatids (Table 4). This preferred relationship is most likely due to a physical proximity maintained between sister chromatids. Sister chromatids are topologically interwound following replication (HOLM *et al.* 1985), become unwound prior to anaphase and then remain paired at the centromere until the onset of anaphase (KOSHLAND and HARTWELL 1987). Staining of yeast cells with an immunofluorescent probe of the amplified *CUP1* locus lights up only a single spot in haploids arrested in G_2 with the microtubule-inhibitor drug nocodazole, whereas two spots are seen in diploids (D. KOSHLAND, personal communication). It is inferred that sister chromatids, unlike homologs, are too close to be distinguished at this stage. In contrast to sister chromatids, it appears that there is no special relationship between homologs in vegetatively growing yeast cells. Although pairing of homologous chromosomes does occur in the polytene chromosomes of Drosophila larvae (PAINTER 1934), and in adult flies as well (WU and GOLDBERG 1989), there is little evidence for somatic pairing of homologs in other organisms.

Sister chromatids are more effective for repair than homologous chromosomes: Since G_2-irradiated diploids are much more resistant than G_1-irradiated diploids to high doses of X-ray damage, we argue that sister chromatids are not only preferred over homologs as substrates for recombinational repair, but are also more effective. Comparision of the survival curves for G_1 and G_2 diploids (Figure 3C) shows that cells at both stages repair small amounts of damage efficiently but that G_2 diploid cells are much more effective at repairing large amounts of damage. The ability of G_1 diploids to survive low levels of damage is due to successful recombinational repair since diploids unable to repair recombinationally (*rad52*/*rad52*) die at much lower doses of X-irradiation (RESNICK and MARTIN 1976). We tested whether the existence of more homologous molecules in G_2 than in G_1 or the existence of the *RAD9*-mediated delay in G_2 could explain the greater X-ray resistance of G_2 cells

and found that neither hypothesis survived the tests. Therefore, we suggest that for a given dose of damage, more lesions can be repaired by sister chromatid recombination when the cells are irradiated in G_2 than can be repaired by homolog recombination when they are irradiated in G_1. This hypothesis does not contradict the fact that we measure approximately the same number of recombination events per viable cell in G_1 and G_2 irradiated cells, if cells which fail to repair enough recombinogenic lesions also fail to survive. We imagine two reasons why sister chromatids might be more effective substrates for the repair of high levels of X-ray damage than are homologs. One is that sister chromatids may share a physical interaction along their lengths that enables them to repair a greater number of potentially lethal hits per molecule than can homologs. A second possibility is that stable pairing between homologs requires proteins that are not required by sister chromatids, and that these are in limited quantity in vegetatively growing cells. Although we favor the possibility that G_2 cells are more X-ray resistant than G_1 cells because sister chromatids are superior substrates for repair, we cannot rule out the possibility that G_2 cells simply have a greater capacity for repair than do G_1 cells, for example by having higher levels of repair enzymes.

Role of the *MAT* locus in DNA repair: The genotype at the *MAT* locus also affects the ability of cells to repair X-ray damage (Figure 6; MORTIMER 1958; GAME 1983). This effect is more pronounced in G_1-irradiated diploids than in G_2 irradiated diploids (Figure 6C). It may be that the effect of heterozygosity at the *MAT* locus on X-ray resistance is to enhance the efficiency of pairing and recombination between homologous molecules. Since cells heterozygous for *MAT* have the possibility of entering meiosis, perhaps they maintain a constitutively higher level of pairing and recombination activities than do cells which are homozygous for *MAT*. If this were true, heterozygosity at *MAT* would have a greater effect on repair between homologs in G_1 than on repair between sister chromatids in G_2 if sister chromatids are already in close proximity. One might expect to see higher levels of induced recombination in cells heterozygous rather than homozygous for *MAT*, especially since the latter have lower levels of spontaneous and UV-induced recombination (FRIIS and ROMAN 1968). Although we were unable to observe a difference in X-ray induced recombination rates per viable cell between *MAT***a**/*MAT***a** and *MAT***a**/*MAT*α diploids (Figure 5), it should be noted that a difference in the efficiency of recombination in *MAT***a**/*MAT***a** *vs.* *MAT***a**/*MAT*α cells would be undetectable if only those cells which have successfully recombined survive. In support of the possibility that the mating genotype does affect the efficiency of X-ray-induced recombination is the fact that heterozygosity at the *MAT* locus has no effect on G_1 haploid survival following X-irradiation (Figure 6B) but does improve G_1 diploid survival (Figure 6, B and C); we infer that the improved resistance requires the interaction of homologous molecules. In addition, GAME and MORTIMER have found that many mutations in recombinational repair abolish the effect of mating type heterozygosity on X-ray resistance (cited in GAME 1983), although *rad55* and *rad57* do not (LOVETT and MORTIMER 1987).

Consistent with the results of FRIIS and ROMAN (1968), the spontaneous frequency of homolog recombination in *MAT***a**/*MAT***a** strain 8301 ($4.0 \pm 2.0 \times 10^{-6}$) was significantly lower (at the 0.02 level of the two-sample *t*-test) than in *MAT***a**/*MAT*α strain 8285 ($10 \pm 0.3 \times 10^{-6}$). However, the spontaneous frequency of sister chromatid recombination in strain 8301 ($2.3 \pm 0.6 \times 10^{-5}$) was not significantly different at the 0.1 level from the frequency in strain 8285 ($1.9 \pm 0.25 \times 10^{-5}$). This observation is expected if heterozygosity at *MAT* improves the efficiency of pairing and recombination between homologous molecules, which is severely rate-limiting for homolog interactions, but less so for interactions between sister chromatids. The fact that heterozygosity at *MAT* affects spontaneous but not induced levels of homolog recombination per viable cell is explained if unrepaired X-ray lesions are lethal but unrepaired spontaneous lesions are not.

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LITERATURE CITED

AHN, B.-Y., K. J. DORNFELD, T. J. FAGRELIUS and D. M. LIVINGSTON, 1988 Effect of limited homology on gene conversion in a *Saccharomyces cerevisiae* plasmid recombination system. Mol. Cell. Biol. **8:** 2442–2448.

BAILIS A. M., and R. ROTHSTEIN, 1990 A defect in mismatch repair in *Saccharomyces cerevisiae* stimulates ectopic recombination between homeologous genes by an excision repair dependent process. Genetics **126:** 535–547.

BRUNBORG, G., M. A. RESNICK and D. H. WILLIAMSON, 1980 Cell-cycle-specific repair of DNA double-strand breaks in *Saccharomyces cerevisiae*. Radiat. Res. **82:** 547–558.

BRUNBORG, G., and D. H. WILLIAMSON, 1978 The relevance of the nuclear division cycle to radiosensitivity in yeast. Mol. Gen. Genet. **162:** 277–286.

CARSON, M. J., 1987 *CDC17*, the structural gene for DNA polymerase I of yeast: mitotic hyper-recombination and effects on telomere metabolism. Ph.D. Thesis, University of Washington.

ESPOSITO, M. S., 1978 Evidence that spontaneous mitotic recombination occurs at the two-strand stage. Proc. Natl. Acad. Sci. USA **75:** 4436–4440.

ESPOSITO, R. E., 1968 Genetic recombination in synchronized cultures of *Saccharomyces cerevisiae*. Genetics **59:** 191–210.

FABRE, F., 1978 Induced intragenic recombination in yeast can occur during the G_1 mitotic phase. Nature **272:** 795–798.

FABRE, F., A. BOULET and H. ROMAN, 1984 Gene conversion at different points in the mitotic cycle of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **195:** 139–143.

FASULLO, M. T., and R. W. DAVIS, 1987 Recombinational substrates designed to study recombination between unique and repetitive sequences *in vivo*. Proc. Natl. Acad. Sci. USA **84:** 6215–6219.

FASULLO, M. T., and R. W. DAVIS, 1988 Direction of chromosome rearrangements in *Saccharomyces cerevisiae* by use of *his3* recombinational substrates. Mol. Cell. Biol. **8:** 4370–4380.

FITZGERALD-HAYES, M. L., L. CLARKE and J. CARBON, 1982 Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell **29:** 235–244.

FRIEFELDER, D., 1968 Rate of production of single-strand breaks in DNA by X-irradiation *in situ*. J. Mol. Biol. **35:** 303–309.

FRIIS, J., and H. ROMAN, 1968 The effect of the mating-type alleles on intragenic recombination in yeast. Genetics **59:** 33–36.

GAME, J. C., 1983 Radiation-sensitive mutants and repair in yeast, pp. 109–137 in *Yeast Genetics: Fundamental and Applied Aspects*, Ch. 4, edited by J. F. T. SPENCER, D. M. SPENCER and A. R. W. SMITH. Springer-Verlag, New York.

GOLIN, J. E., and M. S. ESPOSITO, 1981 Mitotic recombination: mismatch correction and replicational resolution of Holliday structures formed at the two-strand stage in *Saccharomyces*. Mol. Gen. Genet. **183:** 252–263.

HARTWELL, L. H., 1967 Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:** 1662–1670.

HATZFELD, J., and D. H. WILLIAMSON, 1974 Cell-cycle dependent changes in sensitivity to γ-rays in synchronously dividing yeast culture. Exp. Cell Res. **84:** 431–435.

HAYNES, R. H., and B. A. KUNZ, 1981 DNA repair and mutagenesis, pp. 371–414 in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

HENIKOFF, S., 1987 Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155:** 156–165.

HOLLIDAY, R., 1961 Induced mitotic crossing-over in *Ustilago maydis*. Genet. Res. **2:** 231–248.

HOLM, C., T. GOTO, J. C. WANG and D. BOTSTEIN, 1985 DNA topoisomerase II is required at the time of mitosis in yeast. Cell **41:** 553–563.

HSU, Y.-P., and P. SCHIMMEL, 1984 Yeast *Leu1*: repression of mRNA levels by leucine and relationship of 5′-noncoding region to that of *LEU2*. J. Biol. Chem. **259:** 3714–3719.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JACKSON, J. A., and G. R. FINK, 1981 Gene conversion between duplicated genetic elements in yeast. Nature **292:** 306–311.

JAMES, A. P., 1955 A genetic analysis of sectoring in ultraviolet-induced variant colonies of yeast. Genetics **40:** 204–213.

JAMES, A. P., and B. LEE-WHITING, 1955 Radiation-induced genetic segregations in vegetative cells of diploid yeast. Genetics **40:** 826–831.

JONES, E. W., 1977 Bipartite structure of the *ade3* locus of *Saccharomyces cerevisiae*. Genetics **85:** 209–223.

KEIL, R. L., and G. S. ROEDER, 1984 *Cis*-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell **39:** 370–386.

KOSHLAND, D., and L. H. HARTWELL, 1987 The structure of sister minichromosome DNA before anaphase in *Saccharomyces cerevisiae*. Science **238:** 1713–1716.

KOSHLAND, D., J. C. KENT and L. H. HARTWELL, 1985 Genetic analysis of the mitotic transmission of minichromosomes. Cell **40:** 393–403.

KRANZLER, D. L., 1986 Induction of localized mitotic recombination in *CDC* mutants of *Saccharomyces cerevisiae*. Master's Thesis, University of Washington.

KUPIEC, M., and T. D. PETES, 1988 Allelic and ectopic recombination between Ty elements in yeast. Genetics **119:** 549–559.

LEA, D. E., and C. A. COULSON, 1948 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–284.

LICHTEN, M., and J. HABER, 1989 Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. Genetics **123:** 261–268.

LOVETT, S. T., and R. K. MORTIMER, 1987 Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength and mating type. Genetics **116:** 547–553.

MCKENZIE, K. Q., and E. W. JONES, 1977 Mutants of the formyltetrahydrofolate interconversion pathway of *Saccharomyces cerevisiae*. Genetics **86:** 85–102.

MORTIMER, R. K., 1958 Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. Radiat. Res. **9:** 312–326.

NASMYTH, K. A., and K. TATCHELL, 1980 The structure of transposable yeast mating type loci. Cell **19:** 753–764.

NEITZ, M., AND J. CARBON, 1987 Characterization of a centromere-linked recombination hot spot in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 3871–3879.

NEWLON, C. S., L. R. LIPSCHITZ, I. COLLINS, A. DESHPANDE, R. J. DEVENISH, R. P. GREEN, H. L. KLEIN, T. G. PALZKILL, R. REN, S. SYNN and S. T. WOODY, 1991 Analysis of a circular derivative of *Saccharomyces cerevisiae* chromosome *III*: a physical map and identification and location of ARS elements. Genetics **129:** 343–357.

PAINTER, T. S., 1934 Salivary chromosomes and the attack on the gene. J. Hered. **25:** 465–476.

PONTECORVO, G., and E. KAFER, 1958 Genetic analysis based on mitotic recombination. Adv. Genet. **9:** 71–104.

PRAKASH, L., and P. TAILLON-MILLER, 1981 Effect of the *rad52* gene on sister chromatid recombination in *S. cerevisiae*. Curr. Genet. **3:** 247–250.

PRAKASH, S., L. PRAKASH, W. BURKE and B. A. MONTELONE, 1980 Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. Genetics **94:** 31–50.

RESNICK, M. A., 1976 The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **54:** 97–106.

RESNICK, M. A., and P. MARTIN, 1976 The repair of double-strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. **143:** 119–129.

ROEDER, G. S., M. SMITH and E. J. LAMBIE, 1984 Intrachromosomal movement of genetically marked *Saccharomyces cerevisiae* transposons by gene conversion. Mol. Cell. Biol. **4:** 703–711.

ROMAN, H., 1956 Studies of gene mutation in *Saccharomyces*. Cold Spring Harbor Symp. Quant. Biol. **21:** 175–185.

ROMAN, H., and F. FABRE, 1983 Gene conversion and associated reciprocal recombination are separable events in vegetative cells of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80:** 6912–6916.

ROMAN, H., and F. JACOB, 1958 A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. Cold Spring Harbor Symp. Quant. Biol. **23:** 155–160.

SAEKI, T., I. MACHIDA and S. NAKAI, 1980 Genetic control of diploid recovery after γ-irradiation in the yeast *Saccharomyces cerevisiae*. Mutat. Res. **73:** 251–265.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS (Editors), 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold

Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCHIESTL, R. H., S. IGARASHI and P. J. HASTINGS, 1988 Analysis of the mechanism for reversion of a disrupted gene. Genetics **119:** 237–247.

SEIFERT, H. S., E. Y. CHEN, M. SO and F. HEFFRON, 1986 Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **83:** 735–739

SHEN, P., and H. V. HUANG, 1986 Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. Genetics **112:** 441–457.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1981 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SKOGERSON, L., C. MCLAUGHLIN and E. WAKATAMA, 1973 Modification of ribosomes in cryptopleurine-resistant mutants of yeast. J. Bacteriol. **116:** 818–822.

SMOLIK-UTLAUT, S., and T. D. PETES, 1983 Recombination of plasmids into the *Saccharomyces cerevisiae* chromosome is reduced by small amounts of sequence heterogeneity. Mol. Cell. Biol. **3:** 1204–1211.

STABEN, C., and J. C. RABINOWITZ, 1986 Nucleotide sequence of the *Saccharomyces cerevisiae ADE3* gene encoding C_1-tetrahydrofolate synthase. J. Biol. Chem. **261:** 4629–4637.

STERN, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster.* Genetics **21:** 625–730.

STRUHL, K., D. T. STINCHCOMB, S. SCHERER and R. W. DAVIS, 1979 High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76:** 1035–1039.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

SZOSTAK, J. W., and R. WU, 1980 Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae.* Nature **284:** 426–430.

VAN SOLINGEN, P., and J. B. VAN DER PLAAT, 1977 Fusion of yeast spheroplasts. J. Bacteriol. **130:** 946–947.

WALDMAN, A. S., and R. M. LISKAY, 1988 Dependence of intrachromosomal recombination in mammalian cells on uninterrupted homology. Mol. Cell. Biol. **8:** 5350–5357.

WEINERT, T. A., and L. H. HARTWELL, 1988 The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae.* Science **241:** 317–322.

WILDENBERG, J., 1970 The relation of mitotic recombination to DNA replication in yeast pedigrees. Genetics **66:** 291–304.

WU, C. T., and M. L. GOLDBERG, 1989 The *Drosophila zeste* gene and transvection. Trends Genet. **5:** 189–194.

ZAMB, T. J., and T. D. PETES, 1981 Unequal sister-strand recombination within yeast ribosomal DNA does not require the *RAD52* gene product. Curr. Genet. **3:** 125–132.

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