The Mre11-Rad50-Xrs2 Protein Complex Facilitates Homologous Recombination-Based Double-Strand Break Repair in *Saccharomyces cerevisiae*†

DEBRA A. BRESSAN, BONNIE K. BAXTER, AND JOHN H. J. PETRINI*

Laboratory of Genetics, University of Wisconsin Medical School, Madison, Wisconsin 53706

*Saccharomyces cerevisiae mre11*Δ **mutants are profoundly deficient in double-strand break (DSB) repair, indicating that the Mre11-Rad50-Xrs2 protein complex plays a central role in the cellular response to DNA DSBs. In this study, we examined the role of the complex in homologous recombination, the primary mode of DSB repair in yeast. We measured survival in synchronous cultures following irradiation and scored sister chromatid and interhomologue recombination genetically.** *mre11*Δ **strains were profoundly sensitive to ionizing radiation (IR) throughout the cell cycle. Mutant strains exhibited decreased frequencies of IR-induced sister chromatid and interhomologue recombination, indicating a general deficiency in homologous recombination-based DSB repair. Since a nuclease-deficient** *mre11* **mutant was not impaired in these assays, it appears that the role of the** *S. cerevisiae* **Mre11-Rad50-Xrs2 protein complex in facilitating homologous recombination is independent of its nuclease activities.**

Repair of DNA double-strand breaks (DSBs) by homologous recombination requires a sister chromatid or a homologous chromosome as a template. In *Saccharomyces cerevisiae*, the sister chromatid is the preferred template for the repair of damaged DNA (20). Consequently, ionizing radiation (IR) resistance of wild-type haploid and diploid cells is maximal in the G_2 phase of the cell cycle when sister chromatids are present (8, 9). However, wild-type diploid strains are more resistant to IR-induced DNA damage than haploid strains, reflecting that chromosomal homologues can also serve as templates for repair (28, 33).

Genetic and biochemical analyses have implicated the Mre11-Rad50-Xrs2 protein complex in nonhomologous end joining (NHEJ) (14, 21, 29, 31). The rate of spontaneous heteroallelic recombination is increased in *mre11*Δ, *rad50*Δ, and *xrs2*Δ diploid strains relative to that of the wild type (1), indicating that deficiency in the complex does not abrogate homologous recombination. However, the extent to which DSB repair is impaired in *mre11*Δ mutants (7, 26) suggests that the impact of Mre11 deficiency extends beyond NHEJ. Therefore, we hypothesized that *mre11*Δ mutants are deficient in homologous recombination and that this defect results from a diminished ability to utilize the sister chromatid as a template for recombinational DNA repair.

We tested this hypothesis by measuring cell survival of synchronous cultures following irradiation and by scoring sister chromatid recombination (SCR) and interhomologue recombination genetically. We found that Mre11 deficiency leads to a decrease in homologous recombination-based DSB repair. Both SCR and interhomologue homologous recombination are affected by Mre11 deficiency, but the defect is most pronounced with respect to SCR. Since SCR and interhomologue recombination were normal in *hdf1*Δ mutants, the defects observed in *mre11*Δ strains are not a general feature of NHEJ mutants. Further, the data indicate that the nuclease activity of the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex is not required for homologous recombination, as SCR and interhomologue recombination were normal in a nuclease-deficient *mre11* strain.

The phenotypic features of *mre11*Δ mutants described herein are consistent with the hypothesis that the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex stabilizes chromatid interactions, and thus plays a structural role in the homologous recombination process. The data also suggest that the complex may regulate resection of DSB ends to facilitate homologous recombination.

MATERIALS AND METHODS

Yeast strains. The genotypes of yeast strains used in this study are listed in Table 1. *MAT*a/− diploid strains JPY145, JPY146, JPY260, and JPY264, capable of arresting the cell cycle in response to the mating pheromone α-factor, were constructed by transformation of strains JPY41, JPY45, JPY84, and JPY259, respectively, with plasmid pFP18 (a gift of Jim Haber) linearized with *Pvu*II. Integrative transformation of this construct results in replacement of the HO-site-containing 138-bp *Bgl*II/*Bsa*AI fragment of the *MAT*α locus with the *hisG-URA3-hisG* cassette (2). Disruption was assessed morphologically by response to α-factor and was confirmed by Southern blotting. The *hdf1*Δ disruption was introduced into diploid strains JPY45, JPY67, and JPY115 using plasmid pHSX-YKuLEU2 as described (5). Double mutant haploids were obtained by sporulation and tetrad dissection of double heterozygotes. For *XRS2* disruption in diploid strain JPY115 by one-step gene replacement, a *hisG-URA3-hisG* cassette (2) was inserted into the *Hinc*II/*Bgl*II site in *XRS2*, deleting all but 213 bp at the 5′ end and 383 bp at the 3′ end of the coding sequence.

SCR was monitored in haploid spores derived from strain JPY104, obtained by the integration of MFp102 (11) into diploid strain JPY102, which resulted in the replacement of the *TRP1* locus with the MFp102 SCR construct (see Fig. 3). The *his3-*Δ*200* mutation in JPY102 deletes *HIS3* sequences contained in the SCR construct so that histidine prototrophy can be generated only by an unequal SCR event. All strains were cultured at 30°C. Disruptions were confirmed by Southern blotting. Yeast media were prepared and strain manipulations were carried out according to standard procedures (4, 13).

Plasmids. For construction of pScM11-314, a 2.9-kb *Bam*HI/*Kpn*I *MRE11* fragment was subcloned from pSK-*MRE11*-BNX (pSK-*ScMRE11* [7] digested with *Nru*I and *Xho*I, blunted, and reclosed, deleting sequences 3′ of the *MRE11* stop codon) into the centromeric vector pRS314 (34). *MRE11* expression from this construct is under the control of the native *MRE11* promoter. The *ADH1* promoter-driven *MRE11* and *mre11-3* expression constructs have previously been described (7).

* Corresponding author. Mailing address: Laboratory of Genetics, University of Wisconsin Medical School, 445 Henry Mall, Madison, WI 53706. Phone: (608) 265-6043. Fax: (608) 262-2976. E-mail: jpetrini @facstaff.wisc.edu.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference[a]
JPY27	*MAT**a** ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	
JPY35	*MATα ura3-52 lys2-801 ade2-101 trp1-63 his3-200 leu2-1 mre11::hisG*	7
JPY41	*MAT**a**/MATα his7/his7 leu2/leu2 ura3/ura3 trp1/trp1 hom3/+ can1/+*	7
JPY45	*MAT**a**/MATα his7/his7 leu2/leu2 ura3/ura3 trp1/trp1 hom3/+ can1/+ mre11::hisG/mre11::hisG*	7
JPY67	*MAT**a**/MATα his7/his7 leu2/leu2 ura3/ura3 trp1/trp1 hom3/+ can1/+ mre11::hisG/+*	7
JPY69	*MAT**a** his7 leu2 ura3 trp1 mre11::hisG* (spore of JPY67)	
JPY70	*MAT**a** his7 leu2 ura3 trp1* (spore of JPY67)	
JPY84	*MAT**a**/MATα his7/his7 leu2/+ ura3/ura3 trp1/trp1 hom3/+ can1/+ mre11::hisG/mre11::hisG*	
JPY92	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1* (spore of JPY104)	
JPY94	*MAT**a** ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1* (spore of JPY104)	
JPY97	*MATα ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 mre11::hisG* (spore of JPY104)	
JPY98	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 mre11::hisG* (spore of JPY104)	
JPY102	*MAT**a**/MATα ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 mre11::hisG/+* (JPY27 × JPY35)	
JPY104	Same as JPY102 except *trp1-Δ63/trp1::SCE*	
JPY115	*MAT**a**/MATα ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1-Δ63/trp1::SCE his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1* (JPY92 × JPY94)	
JPY145	Same as JPY45 except *MAT**a**/matα::hisG*	
JPY146	Same as JPY41 except *MAT**a**/matα::hisG*	
JPY154	Same as JPY115 except *xrs2::hisG-URA3-hisG/+*	
JPY155	*MATα ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 xrs2::hisG-URA3-hisG* (spore of JPY154)	
JPY156	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 xrs2::hisG-URA3-hisG* (spore of JPY154)	
JPY169	Same as JPY67 except *hdf1::LEU2/+*	
JPY170	Same as JPY115 except *hdf1::LEU2/+*	
JPY174	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 hdf1::LEU2* (spore of JPY170)	
JPY176	*MATα ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 hdf1::LEU2* (spore of JPY170)	
JPY177	*MAT**a**/MATα ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1::SCE/trp1::SCE his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 mre11::hisG/+ hdf1::LEU2/+* (JPY97 × JPY174)	
JPY181	*MAT**a** his7 leu2 ura3 trp1 hdf1::LEU2* (spore of JPY169)	
JPY202	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1* (spore of JPY177)	
JPY205	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 mre11::hisG hdf1::LEU2* (spore of JPY177)	
JPY206	*MAT**a** ura3-52 lys2-801 ade2-101 trp1::SCE his3-Δ200 leu2-Δ1 mre11::hisG hdf1::LEU2* (spore of JPY177)	
JPY247	Same as JPY45 except *hdf1::LEU2/+*	
JPY250	*MAT**a** his7 leu2 ura3 trp1 mre11::hisG* (spore of JPY247)	
JPY254	*MAT**a** his7 leu2 ura3 trp1 mre11::hisG hdf1::LEU2* (spore of JPY247)	
JPY255	*MAT**a** his7 leu2 ura3 trp1 mre11::hisG hdf1::LEU2* (spore of JPY247)	
JPY259	*MAT**a**/MATα his7/his7 leu2/leu2 ura3/ura3 trp1/trp1 mre11::hisG/mre11::hisG hdf1::LEU2/hdf1::LEU2* (JPY254 × JPY255)	
JPY260	Same as JPY259 except *MAT**a**/matα::hisG-URA3-hisG*	
JPY264	Same as JPY84 except *MAT**a**/matα::hisG-URA3-hisG*	

[a] All strains listed were generated during this study except where indicated by a reference.

Cell cycle arrest. Cultures were grown to a density of approximately 5×10^6 cells/ml and arrested in G_1 with α-factor (3 μM final concentration, incubated for 2 to 3 h) (U.S. Biologicals) or in G_2 with carbendazim (150 μg/ml final concentration, incubated for 2 to 3 h) (Aldrich). Synchronization was assessed morphologically and by flow cytometry (see below).

Flow cytometry. Cells were fixed in 70% ethyl alcohol at 4°C for at least 12 h, were pelleted, were resuspended in 1 ml of 50 mM sodium citrate (pH 7.5), and were sonicated for 15 s. Cells were resuspended in sodium citrate containing 0.25 mg of RNase A per ml, were incubated at 50°C for 1 h or at 37°C overnight, and were resuspended in sodium citrate containing 1 μM Sytox Green (1:5,000 dilution) (Molecular Probes). Samples were kept in the dark at room temperature for at least 1 h prior to flow cytometric analysis.

Irradiation studies. Strains were irradiated in mid-log phase (approximately 10^7 cells/ml) or following cell cycle arrest (see above) as previously described (7).

Sister chromatid recombination. To monitor spontaneous SCR, approximately 100 cells from an overnight culture were used to inoculate fresh 50-ml yeast extract-peptone-dextrose (YEPD) cultures, and the cultures were then grown to a density of approximately 5×10^7 cells/ml. Approximately 5×10^7 cells per synthetic complete (SC) medium plate lacking His (SC-His) and 500 cells per nonselective plate were plated in triplicate. The rate of spontaneous SCR was determined from at least nine independent cultures per strain by fluctuation analysis (23) with modifications as previously described (7).

To measure IR-induced SCR, cultures were grown to mid-log phase (approximately 10^7 cells/ml) in YEPD. Approximately 3×10^8 cells were harvested for each strain and were resuspended in 900 μl of double-distilled water (ddH$_2$O). Each cell suspension was split into two aliquots, one of which was irradiated on ice with 50 Gy while the other served as the unirradiated control. Cells were then diluted 10-fold into fresh YEPD, were allowed to recover for 30 min at 30°C, and were plated as described above. The number of IR-induced SCR events was determined by subtracting the ratio of histidine prototrophs to total viable cells in the unirradiated sample from the same value in the irradiated sample.

Interhomologue recombination. To measure IR-induced interhomologue recombination, cultures of JPY264 transformants were grown to early log phase

(approximately 5×10^6 cells/ml) in SC-Trp-Met media. Cultures were then split into three aliquots for asynchronous and G_1- and G_2-synchronized samples as described above. Each was then resuspended in ddH$_2$O and split into three aliquots, two of which were irradiated on ice with 50 Gy and 150 Gy while the third served as the unirradiated control. Cells were then diluted 10-fold into fresh SC-Trp media, were allowed to recover for 30 min at 30°C, and were plated onto SC-Trp and SC-Trp-Met canavanine plates. The number of IR-induced interhomologue recombination events was determined by subtracting the ratio of canavanine-resistant methionine prototrophs to total viable cells in the unirradiated sample from the same value in the irradiated sample.

RESULTS

Synchronous cultures of haploid and diploid strains provide a means to examine the ability of cells to utilize different homologous templates for recombinational DSB repair. In G_1-synchronous cultures, cells must rely on NHEJ to repair DSBs in the absence of homology (Fig. 1). Haploid cells contain a homologous template, in the form of a sister chromatid, only during the G_2 phase. In contrast, diploid cells contain homologous templates, in the form of homologous chromosomes, throughout the cell cycle. Additionally, sister chromatids are present in the G_2 phase and are the preferred template for repair (20). We monitored the contribution of each of these templates to the survival of *mre11*Δ cells following irradiation using synchronous cultures of haploid and diploid strains.

Radiation sensitivity of haploid *mre11*Δ strains. Given the DSB repair deficiency observed in *mre11*Δ strains (7, 26), we
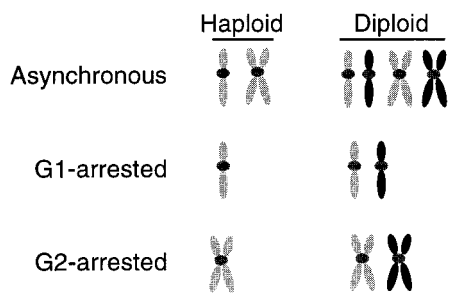
	Haploid	Diploid

FIG. 1. Schematic representation of asynchronous and synchronous cultures. The genotype shows that cells in G_1 rely on NHEJ (haploids) or interhomologue recombination (diploids) for recombinational DNA repair. Cells in G_2 can also undergo sister chromatid recombination (haploids and diploids). The petal-shaped figures represent chromosome arms; the gray dots represent the centromeres.

asked whether homologous recombination was impaired by Mre11 deficiency, as suggested by previous work using $rad50\Delta$ and $xrs2\Delta$ strains (18). In order to determine whether $mre11\Delta$ strains were defective in SCR, we examined the IR sensitivity of synchronous cultures of wild-type (JPY70) and $mre11\Delta$ (JPY69) haploid strains. Cells were grown to early log phase and arrested in G_1 or G_2 by treatment with α-factor or carbendazim, respectively. Synchronization of cultures was confirmed morphologically and by flow cytometry. Cells were irradiated in suspension at 150 Gy and were plated onto rich media to score cell survival relative to that of unirradiated cultures.

The wild-type haploid strain JPY70 exhibited 37% survival following irradiation during asynchronous growth, and the $mre11\Delta$ haploid strain JPY69 exhibited 0.7% survival (Fig. 2). Whereas wild-type cells arrested in G_1 exhibited 1.5% survival, cells in G_2 exhibited 70% survival, indicating that the presence of a sister chromatid in G_2-synchronous cultures increases the survival of wild-type cells.

In contrast to the wild-type strain, the presence of the sister chromatid in the G_2-arrested $mre11\Delta$ cells did not increase survival following IR. G_2-synchronous $mre11\Delta$ cells exhibited less than a twofold increase in survival upon irradiation relative to that of G_1-synchronous cells (1 and 0.6% survival, respectively) (Fig. 2). Hence, the IR sensitivity of asynchronous hap-

loid $mre11\Delta$ strains can primarily be attributed to the increased sensitivity of the G_2 population. These data suggest that use of the sister chromatid for recombinational repair is substantially reduced in $mre11\Delta$ mutants.

The IR sensitivity of wild-type haploid cells in G_1 demonstrates that NHEJ does not contribute significantly to cell survival following IR. Indeed, a substantial fraction of the surviving cells are unlikely to have received any DSBs at the IR dose used. A dose of 150 Gy is predicted to impart four to six DSBs per cell (32). Since IR-induced breaks are distributed stochastically, 0.25 to 2% of cells were predicted to sustain less than one DSB at the level of irradiation employed in this experiment—a dose at which we observed approximately 1.5% survival. Hence, most of the surviving cells in the G_1-synchronous populations may not have sustained DSBs.

Radiation-induced sister chromatid recombination. To assess SCR genetically, we used a chromosomal substrate consisting of a tandemly repeated *HIS3* gene in which the first repeat is truncated at the 5′ end and the second is truncated at the 3′ end (11). In this configuration, a functional *HIS3* gene can be generated only by an unequal SCR event (Fig. 3). The wild-type (JPY92 and JPY202) and $mre11\Delta$ (JPY97 and JPY98) haploid strains exhibited similar rates of spontaneous SCR ([1.4 to 2.0] $\times 10^{-6}$ per generation).

We next assessed radiation-induced unequal SCR following irradiation at 50 Gy. The wild-type strains exhibited an IR-induced increase in SCR frequency of 13.3×10^{-6} (Table 2). We consistently observed significantly smaller increases in SCR frequency following irradiation of the $mre11\Delta$ strains JPY97 and JPY98 (4.8×10^{-6}). Similar data were obtained with a mutant of another member of the complex. The $xrs2\Delta$ strains JPY155 and JPY156 exhibited an IR-induced increase in SCR frequency of 5.4×10^{-6} (Table 2). At this IR dose, the decrease in survival of these mutant strains relative to the survival of the wild type (1.5-fold) is of similar magnitude to the decrease in the number of recombinants scored (2.6-fold).

Previous studies have shown that $mre11\Delta$ strains are deficient in NHEJ (14, 21, 31). We asked whether the decrease in SCR in $mre11\Delta$ cells was a general, and presumably indirect, outcome of NHEJ deficiency. We analyzed the IR sensitivity of synchronous cultures and the IR-induced frequency of SCR in
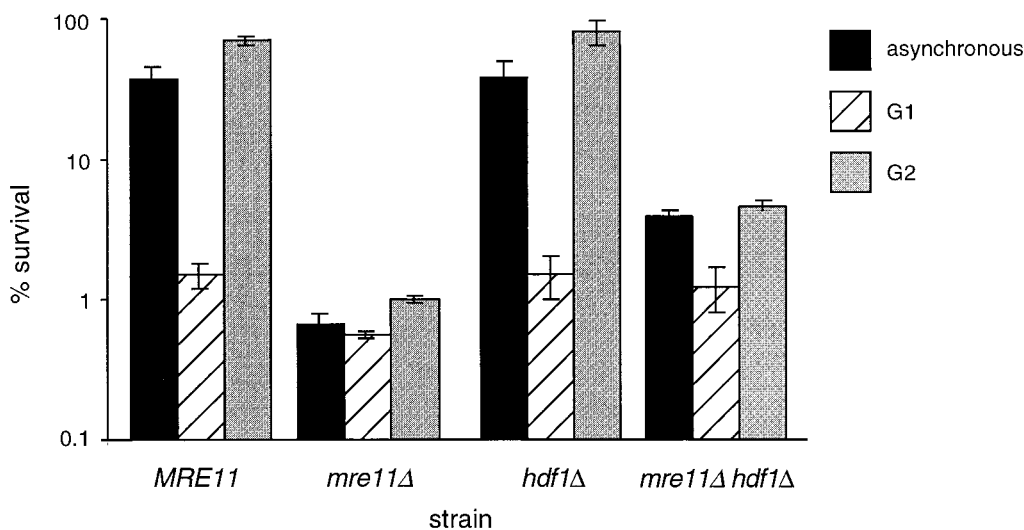

FIG. 2. Radiation sensitivity of haploid $mre11\Delta$ and $hdf1\Delta$ strains. Asynchronous cultures and cells synchronized with α-factor (G_1 synchronous) or carbendazim (G_2 synchronous) were irradiated at a dose of 150 Gy as described in Materials and Methods. Cell survival was scored for 5 days following irradiation. Values plotted represent the average of triplicate platings from at least three experiments. Error bars represent standard deviations. Haploid strains were JPY70 (*MRE11*), JPY69 (*mre11Δ*), JPY181 (*hdf1Δ*), and JPY254 (*mre11Δ hdf1Δ*).
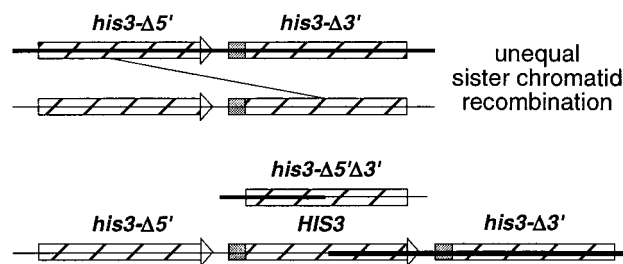
FIG. 3. Sister chromatid recombination assay. Integrative transformation of MFp102 (11) results in the replacement of the *TRP1* locus with the SCR substrate. Striped bars indicate the location of *his3* fragments (*his3-Δ5'* and *his3-Δ3'*) in a head-to-tail arrangement along the sister chromatids (solid horizontal lines). The arrowhead and shaded box represent regions of *HIS3* deleted in the opposite allele. A functional *HIS3* gene can be generated only by an unequal sister chromatid recombination event as indicated by the diagonal line. An intrachromatid recombination event will produce a *HIS3* circle (not shown).

the haploid *hdf1Δ* strains JPY181, JPY174, and JPY176, which are also impaired in NHEJ (6, 25). Haploid *hdf1Δ* cells were no more IR sensitive than wild-type cells, exhibiting survival of 38% in asynchronous cultures, 1.5% in G_1-synchronous cultures, and 81% in G_2-synchronous cultures (Fig. 2). The rate of spontaneous SCR in the haploid *hdf1Δ* strains JPY174 and JPY176 was 1.5×10^{-6} per generation, not significantly different from those of the other mutant and wild-type strains examined in this assay (data not shown). However, in contrast to the *mre11Δ* and *xrs2Δ* strains, the *hdf1Δ* strains showed an IR-dependent increase in SCR frequency equivalent to that of the wild type (16.3×10^{-6}) (Table 2).

The IR sensitivity of haploid *mre11Δ* cells was partially suppressed by the *hdf1Δ* mutation. Survival of the *mre11Δ hdf1Δ* strain JPY254 was 5.7-fold greater than that of the *mre11Δ* strain JPY69 following irradiation of asynchronous cultures, with G_1- and G_2-synchronous cultures displaying 2.2- and 4.6-fold increases in survival, respectively (Fig. 2).

Radiation sensitivity of diploid *mre11Δ* strains. We previously showed that diploid *mre11Δ* strains exhibit higher radiation resistance than their haploid counterparts (7), consistent with the behavior of *rad50Δ* and *xrs2Δ* strains (18). As in wild-type cells, this effect is presumably due to the presence of homologous chromosomes for repair of DSBs (33). To assess the ability of Mre11-deficient diploid cells to use interhomologue recombination for DSB repair, *MAT***a**/*matα::hisG* disruptions were established in wild-type (JPY41) and *mre11Δ* (JPY45) diploid strains to allow G_1 synchronization with α-factor. The IR sensitivity of synchronous populations of these diploid strains was examined as described above.

The wild-type diploid strain JPY146 was more resistant to IR than its haploid counterpart JPY70, exhibiting survival of 51% following irradiation of asynchronous cultures (Fig. 4). As with the wild-type haploid strain, G_2-synchronous diploid cultures exhibited increased IR resistance relative to the resistance of G_1-arrested cells (90 and 59%, respectively). Although asynchronous cultures of the *mre11Δ* JPY145 strain did exhibit an increase in IR resistance relative to its haploid counterpart JPY69 (compare Fig. 2 and Fig. 4) (7), the G_2-synchronous *mre11Δ* diploid culture did not show increased survival relative to that of the G_1-synchronous culture (Fig. 4). Instead, we reproducibly observed a two- to threefold decrease in survival of *mre11Δ* diploid cells in G_2 (1.5% survival) relative to that of G_1-synchronous cultures (3.3% survival) (Fig. 4). This effect was not due to mating-type hemizygosity, as *MAT***a**/*MATα* strains synchronized in G_1 by growth to saturation exhibited an even more dramatic increase in survival following IR (data not shown).

As in haploid strains, the *hdf1Δ* mutation partially suppressed the IR sensitivity of the diploid *mre11Δ* strain JPY145. Survival of the homozygous *mre11Δ hdf1Δ* diploid strain JPY260 was 6.3-fold greater than that of the diploid *mre11Δ* strain following irradiation of asynchronous cultures (Fig. 4). *mre11Δ hdf1Δ* diploid cells arrested in G_1 and G_2 exhibited 1.7- and 4-fold increases in survival, respectively, relative to that of *mre11Δ* cells.

The IR sensitivity of *mre11Δ* diploid cells suggested that *mre11Δ* strains are defective in IR-induced interhomologue recombination, as shown previously for *rad50Δ* strains (33). We measured the induction of interhomologue recombination by IR in the diploid strain JPY264 transformed with either a wild-type *MRE11* expression construct (pScM11-314) or an empty vector. JPY264 is an Mre11-deficient *MAT***a**/*matα::hisG* diploid strain heterozygous for *can1* and *hom3* on opposite arms of chromosome V. In this strain, the frequency of interhomologue recombination can be determined by scoring the frequency of canavanine-resistant methionine prototrophs (7, 16). Heteroallelic (intragenic) recombination cannot be distinguished from intergenic recombination by this assay.

As in previous studies, we found that spontaneous interhomologue recombination was increased in *mre11Δ* diploid cells relative to that of the wild type, with frequencies of 4.0×10^{-3} and 3.7×10^{-4} recombinants per viable cell, respectively (1). We observed greater IR induction of interhomologue recombination in both wild-type and *mre11Δ* G_1-synchronous cells relative to that of asynchronous and G_2-synchronous cultures (Table 3), consistent with the observation that sister chromatids are preferred over homologous chromosomes as templates for recombinational repair (20). However, the IR-induced frequency of interhomologue recombination in the *mre11Δ* strain was indistinguishable from that of the wild type at a dose of 50 Gy (Table 3).

We found that IR induction of interhomologue recombination following irradiation at 150 Gy was reduced in asynchronous cultures of the *mre11Δ* strain relative to that of the wild-type (pScM11-314) transformants (Table 3), with frequencies of -4.7×10^{-4} and 7.3×10^{-4} recombinants per viable cell, respectively. In contrast, the frequency of IR-induced interhomologue recombination observed in G_1-synchronous *mre11Δ* cultures (31.5×10^{-4}) was not different from that of the wild type (35.0×10^{-4}) following irradiation at 150 Gy (Table 3). The extent to which IR-induced interhomologue recombina-

TABLE 2. Effect of *mre11* and *hdf1* mutations on radiation-induced sister chromatid recombination

Genotype	Strain	IR-induced frequency $(n)^a$	% Survival
MRE11	JPY92 and JPY202	13.3 ± 5.9 (13)	48
mre11Δ	JPY97 and JPY98	4.8 ± 2.8 (8)	31
xrs2Δ	JPY155 and JPY156	5.4 ± 2.2 (8)	35
hdf1Δ	JPY174 and JPY176	16.3 ± 5.3 (12)	46
mre11Δ hdf1Δ	JPY205 and JPY206	6.1 ± 4.7 (15)	52
mre11Δ transformants[b]	*mre11Δ*:DB-MRE11-TRP	13.1 ± 5.2 (7)	58
	mre11Δ:DB-mre11-3-TRP	14.6 ± 6.5 (7)	51
	mre11Δ:DB-P-TRP	3.7 ± 2.6 (7)	43

[a] Frequencies were calculated by subtracting the spontaneous frequency of histidine prototrophs in the unirradiated sample from the frequency in the irradiated sample. Values are given as 10^{-6} recombinants per viable cell ± standard deviations. Numbers in parentheses indicate the number of independent cultures used to determine the given value. IR dose = 50 Gy.

[b] Haploid *mre11Δ* strains JPY97 and JPY98 were transformed with an *ADH1* promoter-driven *MRE11* or *mre11-3* expression construct (DB-MRE11-TRP or DB-mre11-3-TRP) or an empty vector (DB-P-TRP). Details of plasmid construction are given in Materials and Methods.
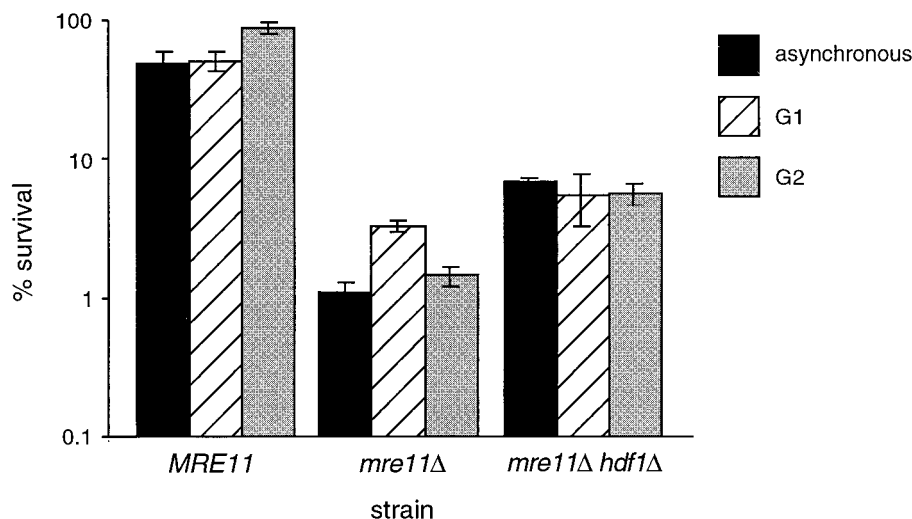
FIG. 4. Radiation sensitivity of diploid $mre11\Delta$ and $mre11\Delta$ $hdf1\Delta$ strains. Methods of synchronization and irradiation are as described in the legend to Fig. 3 and in Materials and Methods. IR dose = 150 Gy. Values plotted represent the averages of triplicate platings from at least three experiments. Error bars represent standard deviations. Diploid strains were JPY146 ($MRE11$), JPY145 ($mre11\Delta$), and JPY260 ($mre11\Delta$ $hdf1\Delta$).

tion was reduced in the asynchronous and G_2-synchronous $mre11\Delta$ cells is consistent with the decrease in cell survival at this dose. The negative IR induction of recombinants in the $mre11\Delta$ strain reflects that the degree of cell killing at this dose exceeds the frequency of viable recombinants.

Mre11 nuclease activity in homologous recombination. The data described above indicates that Mre11 deficiency profoundly impairs homologous recombination. Since nucleolytic processing is required for homologous recombination (15, 35), we asked whether the role of the Mre11-Rad50-Xrs2 complex in homologous recombination was dependent upon the nuclease activity of Mre11 (12, 27, 29, 36, 37). For this analysis, we used the $mre11$-3 mutant, in which the conserved histidine residue at position 125 is altered (7). Alteration of this residue in the $Scmre11$ H125N allele inactivates the nuclease function of Mre11 and disrupts the early stages of meiotic recombination (27). Like $Scmre11$ H125N, $mre11$-3 mutants are unable to produce viable spores (data not shown).

The haploid $mre11\Delta$ strains JPY69, JPY97, and JPY98 were each transformed with a centromeric plasmid containing no insert (empty vector) or $MRE11$ or $mre11$-3 (both expressed from the $ADH1$ promoter) (7). Transformants were examined with respect to cell survival of synchronous cultures following irradiation and IR-induced SCR as described above.

As with the wild-type JPY69 transformants, the presence of the sister chromatid in the G_2-synchronous $mre11$-3 transformants increased cell survival following IR relative to that of

G_1-synchronous cultures. Asynchronous and G_2-synchronous cultures of $mre11$-3 transformants exhibited 37 and 42% survival, respectively, whereas G_1-synchronous cells exhibited 2% survival (Fig. 5). Consistent with cell survival, we found that the frequency of IR-induced SCR in the haploid JPY69 $mre11$-3 transformants was 14.6×10^{-6} (Table 2), indistinguishable from that of the wild type (13.1×10^{-6}).

DISCUSSION

We examined the response of $mre11\Delta$ mutants to DSBs using cell survival, SCR, and interhomologue recombination assays. We found that Mre11 deficiency leads to a dramatic reduction in homologous recombination, as evidenced by decreased survival following irradiation of synchronous cultures and by decreased frequencies of IR-induced sister chromatid and interhomologue recombination. Although previous studies have implicated the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex in NHEJ, this study clearly indicates that the primary role of the complex in the cellular DNA damage response is in facilitating homologous recombination. This role in the damage response does not appear to depend upon the nuclease activities exhibited by the complex, as a nuclease-deficient allele of $mre11$ did not affect homologous recombination in our assays.

It is likely that deficiency in the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex destabilizes the association of homologous chromatids during recombinational DNA repair, thereby

TABLE 3. Radiation-induced interhomologue recombination in $mre11\Delta/mre11\Delta$ transformants

Plasmid[a]	IR-induced frequency (n)[b]					
	50 Gy			150 Gy		
	Asynchronous	G_1 arrested	G_2 arrested	Asynchronous	G_1 arrested	G_2 arrested
pScM11-314	7.7 ± 2.5 (14)	33.5 ± 16.7 (16)	7.1 ± 3.5 (16)	7.3 ± 1.4 (5)	35.0 ± 8.4 (6)	4.8 ± 2.2 (6)
pRS314	11.1 ± 12.1 (10)	49.0 ± 15.6 (8)	8.5 ± 15.9 (8)	−4.7 ± 1.9 (6)	31.5 ± 6.9 (5)	−3.2 ± 6.9 (6)

[a] Diploid $mre11\Delta$ strain JPY264 was transformed with a native promoter-driven $MRE11$ expression construct (pScM11-314) or an empty vector (pRS314). Details of plasmid construction are given in Materials and Methods.

[b] IR-induced frequencies were calculated by subtracting the spontaneous frequency of canavanine-resistant methionine prototrophs in the unirradiated sample from the frequency in the irradiated sample. Values are given as 10^{-4} recombinants per viable cell ± standard deviations. Numbers in parentheses indicate the number of independent cultures used to determine the given value.
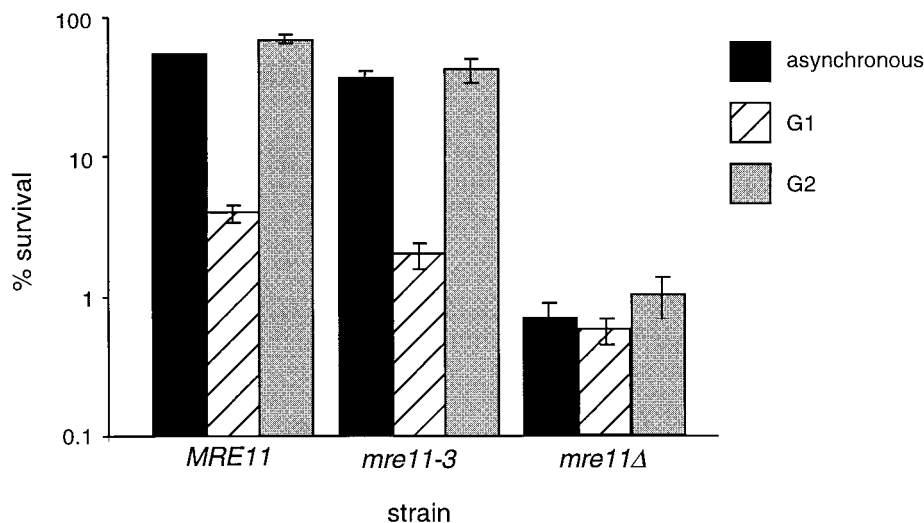
FIG. 5. Radiation sensitivity of synchronous *mre11-3* cultures. Methods of synchronization and irradiation are as described in the legend to Fig. 3 and Materials and Methods. IR dose = 150 Gy. Haploid *mre11*Δ strain JPY69 was transformed with an *ADH1* promoter-driven *MRE11* or *mre11-3* expression construct (DB-MRE11-TRP or DB-mre11-3-TRP) or an empty vector (DB-P-TRP) (7). Asynchronous, G_1-synchronous, and G_2-synchronous cultures of JPY69 transformants were plated onto SC-Trp media and scored for cell survival for 5 days following irradiation. Values plotted represent the averages of triplicate platings. Error bars represent standard deviations. The standard deviation for the asynchronous *MRE11* culture (1.2%) is not visible at the scale of this plot.

impairing homologous recombination as well as NHEJ (26). In homologous recombination, the effect is more severe for SCR than for interhomologue recombination. For example, we observed that G_1-synchronous *mre11*Δ diploid cultures were more IR resistant than G_2-synchronous diploid cultures (Fig. 4).

The mechanistic basis for the putative reduction in chromatid interactions in *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex-deficient cells is not clear. In *mre11*Δ cells, the induction of interhomologue recombination by IR treatment is reduced in G_2-synchronous cultures relative to that of G_1-synchronous cultures (Table 3), as shown previously for wild-type cells (10). This suggests that the bias toward the use of sister chromatids for homologous recombination is intact in *mre11*Δ cells. The increased survival of G_1-synchronous cultures relative to that of G_2-synchronous diploid cultures (Fig. 4) is consistent with this interpretation. The increased sensitivity of G_2 cultures suggests that the completion, rather than the initiation, of SCR events is affected by Mre11 deficiency and that reduced survival relative to that of G_1 cultures may be caused by abortive SCR events.

The spontaneous hyperrecombination phenotype observed in *mre11*Δ, *rad50*Δ, and *xrs2*Δ strains is seemingly paradoxical in light of the homologous recombination defects in these mutant strains (1, 3, 18). Two factors may interact to influence the frequency of spontaneous interhomologue recombination. First, Mre11 deficiency may result in an increased steady-state level of recombinogenic lesions and thereby lead to an elevated frequency of interhomologue recombination. Second, the slow resection of DSB ends in *mre11*Δ strains may lead to shorter heteroduplex tracts during gene conversion. The result would be an apparent increase in gene conversion between heteroalleles in a diploid strain (14, 30). This scenario can also explain the observation that at low doses of IR (50 Gy), both wild-type and *mre11*Δ mutants exhibited an induction of *can1* recombinants (Table 3), despite a relative decrease in survival of the *mre11*Δ strain (data not shown). That is, although IR-induced DNA damage is repaired inefficiently in the *mre11*Δ strain, the decreased number of successful recombination events that do occur do so with DNA ends that are minimally resected and are thus more likely to result in a conversion of the heteroallele. At higher doses of IR (150 Gy), the frequency of cell death

in *mre11*Δ cells exceeds the frequency of successfully completed interhomologue recombination events. Consequently, we observed a negative induction of interhomologue recombination in *mre11*Δ cells at this dose (Table 3).

In contrast, the spontaneous rates of SCR among null mutants of the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex are indistinguishable from each other and from rates in wild-type cells. However, the frequency of IR-induced SCR events is reduced in *mre11*Δ cells relative to that in the wild type (Table 2). Although the magnitude of the observed effects is subtle, these data offer evidence that the repair of IR-induced DNA damage may be mechanistically distinct from the repair of DNA lesions that lead to spontaneous SCR. In this regard, it is important to consider the possibility that spontaneous SCR events occur in close proximity to the replication fork to repair spontaneously occurring DSBs (22), whereas IR-induced events occur at essentially random locations.

The homologous recombination defects we observed may not be fully explained by the hypothesized reduction in chromatid association. Rather, data presented here and elsewhere suggest that the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex also plays a role in facilitating end resection at DSB sites. Resection in the 5′ to 3′ direction to create a protruding 3′ end is a requisite first step in the homologous recombination process (15, 35). A number of studies have shown that the rate of 5′ to 3′ end resection at HO-induced DSBs is reduced in *mre11*, *rad50*, and *xrs2* deletion mutants (14). The magnitude of IR sensitivity we observed is disproportionate to the relatively modest end resection defects at HO-induced DSBs in these mutants (19, 24). However, IR-induced DSBs exhibit a significant degree of chemical and structural heterogeneity (17); therefore the end resection defects associated with Mre11 deficiency may be much more pronounced at IR- than at HO-induced DSBs.

In contrast to Mre11 deficiency, Hdf1 deficiency is associated with a sharp increase in the rate of end resection at HO-induced DSBs. Mre11 deficiency is apparently epistatic to Hdf1 deficiency in this regard, since resection rates are similar in *mre11*Δ and *mre11*Δ *hdf1*Δ mutants (24). However, we observed partial suppression of *mre11*Δ IR sensitivity in *mre11*Δ *hdf1*Δ mutants. We have hypothesized that homologous re-

combination defects in *mre11Δ* mutants result from impaired DSB end resection. We infer that the partial suppression of IR sensitivity in *mre11Δ hdf1Δ* double mutants may indicate that Hdf1 deficiency does indeed lead to a subtle increase in the rate of end resection at IR-induced DSBs. Given the relatively subtle degree of suppression observed, the increased rate of end resection imparted by Hdf1 deficiency in the *mre11Δ* background would not necessarily have been detectable by the physical methods employed by Lee et al. (24).

In this context, it is noteworthy that *mre11-3*-expressing strains, in which Mre11 nuclease activity is presumably abolished, are not grossly deficient in the resection of HO-induced DSBs (23a), do not exhibit mitotic homologous recombination defects, and do not show markedly increased IR sensitivity (references 7 and 27 and this study). Hence, although deficiency in the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex reduces the rate of DSB end resection in vivo, the complex may not be directly responsible for resection activity. The complex does specify nuclease activity, although Mre11 homologues from *S. cerevisiae* and humans exhibit 3′ to 5′ rather than 5′ to 3′ exonuclease activity in vitro. The reduction of 5′ to 3′ exonuclease activity in *mre11Δ*, *rad50Δ*, and *xrs2Δ* mutants suggests the possibility that the complex regulates or otherwise facilitates the activity of a bona fide 5′ to 3′ exonuclease in vivo (12, 27, 29, 36, 37).

In summary, these data illustrate the central importance of the *S. cerevisiae* Mre11-Rad50-Xrs2 protein complex in homologous recombination. The phenotypic features of mutations affecting the complex suggest that the complex is required to establish chromatid interactions and suggest a structural, rather than enzymatic, role in the recombinational DNA repair process. The data are also compatible with a substantial role for the complex in DSB end resection to facilitate homologous recombination.

ACKNOWLEDGMENTS

REFERENCES

1. **Ajimura, M., S.-H. Leem, and H. Ogawa.** 1993. Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. Genetics **133:**51–66.
2. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
3. **Alani, E., and S. Subbiah, and N. Kleckner.** 1989. The yeast *RAD50* gene encodes a predicted 153 kD protein containing a purine nucleotide-binding domain and two large heptad repeat regions. Genetics **122:**47–57.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology, vol. 1–3. John Wiley & Sons, New York, N.Y.
5. **Barnes, G., and D. Rio.** 1997. DNA double-strand-break sensitivity, DNA replication, and cell cycle arrest phenotypes of Ku-deficient *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **94:**867–872.
6. **Boulton, S. J., and S. P. Jackson.** 1996. Saccharomyces cerevisiae Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. EMBO J. **15:**5093–5103.
7. **Bressan, D. A., H. A. Olivares, B. E. Nelms, and J. H. J. Petrini.** 1998. Alteration of N-terminal phosphoesterase motifs inactivates *Saccharomyces cerevisiae* Mre11. Genetics **150:**591–600.
8. **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.
9. **Brunborg, G., and D. H. Williamson.** 1978. The relevance of the nuclear division cycle to radiosensitivity in yeast. Mol. Gen. Genet. **162:**277–286.
10. **Esposito, R. E.** 1968. Genetic recombination in synchronized cultures of Saccharomyces cerevisiae. Genetics **59:**191–210.
11. **Fasullo, M. T., T. Bennett, P. AhChing, and J. Koudelik.** 1998. The *Saccharomyces cerevisiae RAD9* checkpoint reduces the DNA damage-associated stimulation of directed translations. Mol. Cell. Biol. **18:**1190–1200.
12. **Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata, and K. Ohta.** 1998. Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J. **17:**6412–6425.
13. **Guthrie, C., and G. R. Fink.** 1991. Methods in enzymology, vol. 194. Academic Press, Inc., San Diego, Calif.
14. **Haber, J. E.** 1998. The many interfaces of Mre11. Cell **95:**583–586.
15. **Haber, J. E.** 1998. Mating-type gene switching in Saccharomyces cerevisiae. Annu. Rev. Genet. **32:**561–599.
16. **Hartwell, L. H., and D. Smith.** 1985. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. Genetics **110:**381–395.
17. **Henner, W. D., S. M. Grunberg, and W. A. Haseltine.** 1982. Sites and structures of γ radiation-induced DNA strand breaks. J. Biol. Chem. **257:**11750–11754.
18. **Ivanov, E. L., V. G. Korolev, and F. Fabre.** 1992. *XRS2*, a DNA repair gene of *Saccharomyces cerevisiae*, is needed for meiotic recombination. Genetics **132:**651–664.
19. **Ivanov, E. L., N. Sugawara, C. I. White, F. Fabre, and J. E. Haber.** 1994. Mutations in XRS2 and RAD50 delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**3414–3425.
20. **Kadyk, L. C., and L. H. Hartwell.** 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics **132:**387–402.
21. **Kanaar, R., and J. H. J. Hoeijmakers.** 1997. Recombination and joining: different means to the same ends. Genes Funct. **1:**165–174.
22. **Kuzminov, A.** 1995. Collapse and repair of replication forks in *Escherichia coli*. Mol. Microbiol. **16:**373–384.
23. **Lea, D. E., and C. A. Coulson.** 1947. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:**264–285.
23a.**Lee, S., D. Bressan, J. Petrini, and J. Haber.** Unpublished data.
24. **Lee, S. E., J. K. Moore, A. Holmes, K. Umezu, R. Kolodner, and J. E. Haber.** 1998. Saccharomyces Ku70, Mre11/Rad50, and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell **94:**399–409.
25. **Milne, G. T., S. Jin, K. B. Shannon, and D. T. Weaver.** 1996. Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**4189–4198.
26. **Moore, J. K., and J. E. Haber.** 1996. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**2164–2173.
27. **Moreau, S., J. R. Ferguson, and L. S. Symington.** 1999. The nuclease activity of mre11 is required for meiosis but not for mating-type switching, end joining, or telomere maintenance. Mol. Cell. Biol. **19:**556–566.
28. **Mortimer, R. K.** 1958. Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. Radiat. Res. **9:**312–326.
29. **Paull, T. T., and M. Gellert.** 1998. The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. Mol. Cell **1:**969–979.
30. **Petes, T. D., R. E. Malone, and L. S. Symington.** 1991. Recombination in yeast, p. 407–521. *In* J. R. Broach, J. Pringle, and E. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics, vol. I. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Petrini, J. H. J., D. A. Bressan, and M. S. Yao.** 1997. The *RAD52* epistasis group in mammalian double strand break repair. Semin. Immunol. **9:**181–188.
32. **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.
33. **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.
34. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:**19–27.
35. **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.
36. **Trujillo, K. M., S. S. Yuan, E. Y. Lee, and P. Sung.** 1998. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. J. Biol. Chem. **273:**21447–21450.
37. **Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa, and T. Ogawa.** 1998. Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell **95:**705–716.