Alteration of N-Terminal Phosphoesterase Signature Motifs Inactivates *Saccharomyces cerevisiae* Mre11

Debra A. Bressan, Heidi A. Olivares, Benjamin E. Nelms and John H. J. Petrini

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

Manuscript received March 5, 1998
Accepted for publication June 24, 1998

ABSTRACT

Saccharomyces cerevisiae Mre11, Rad50, and Xrs2 function in a protein complex that is important for nonhomologous recombination. Null mutants of *MRE11*, *RAD50*, and *XRS2* are characterized by ionizing radiation sensitivity and mitotic interhomologue hyperrecombination. We mutagenized the four highly conserved phosphoesterase signature motifs of Mre11 to create *mre11-11*, *mre11-2*, *mre11-3*, and *mre11-4* and assessed the functional consequences of these mutant alleles with respect to mitotic interhomologue recombination, chromosome loss, ionizing radiation sensitivity, double-strand break repair, and protein interaction. We found that *mre11* mutants that behaved as the null were sensitive to ionizing radiation and deficient in double-strand break repair. We also observed that these null mutants exhibited a hyperrecombination phenotype in mitotic cells, consistent with previous reports, but did not exhibit an increased frequency of chromosome loss. Differential ionizing radiation sensitivities among the hypomorphic *mre11* alleles correlated with the trends observed in the other phenotypes examined. Two-hybrid interaction testing showed that all but one of the *mre11* mutations disrupted the Mre11-Rad50 interaction. Mutagenesis of the phosphoesterase signatures in Mre11 thus demonstrated the importance of these conserved motifs for recombinational DNA repair.

RECOMBINATIONAL DNA repair in *Saccharomyces cerevisiae* is mediated by the gene products of the *RAD52* epistasis group (*RAD50-57*, *RAD59*, *MRE11*, and *XRS2*; Ivanov *et al.* 1992; Ajimura *et al.* 1993; Game 1993; Bai and Symington 1996). Mutants exhibit recombination defects and ionizing radiation (IR) sensitivity and can be subdivided into two subgroups according to their mitotic and meiotic recombination phenotypes. One subgroup, composed of the gene products of *RAD51*, *RAD52*, *RAD54*, *RAD55*, and *RAD57*, mediates homologous recombination (Game 1993), whereas Mre11, Rad50, and Xrs2 function in a protein complex that is important for nonhomologous end-joining (Schiestl *et al.* 1994; Johzuka and Ogawa 1995; Moore and Haber 1996; Tsukamoto *et al.* 1996).

S. cerevisiae mre11, *rad50*, and *xrs2* null mutants are proficient at homologous recombination-based repair and exhibit a hyperrecombination phenotype in mitotic cells (Malone *et al.* 1990; Ivanov *et al.* 1992; Ajimura *et al.* 1993; Schiestl *et al.* 1994). However, double-strand breaks (DSBs) to initiate meiotic recombination do not form in these null mutants (Alani *et al.* 1990; Ivanov *et al.* 1992), resulting in inviable spores (Game and Mortimer 1974; Ajimura *et al.* 1993). The sporulation defect in *mre11*, *rad50*, and *xrs2* null mutants is rescued in a *spo13* background through bypass of meiosis I, confirming a role for these proteins at an early stage of meiosis (Malone *et al.* 1991; Ivanov *et al.* 1992; Ajimura *et al.* 1993).

The specific biochemical functions of the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex in recombinational DNA repair have not been elucidated. One model for the function of this protein complex is suggested by the similarity to the *Escherichia coli* exonuclease SbcCD (Sharples and Leach 1995), which mediates single-strand endonuclease and ATP-dependent double-strand exonuclease activities (Connelly and Leach 1996; Connelly *et al.* 1997). The N-terminal portion of Mre11 contains four motifs that are similar to SbcD, a component of the SbcCD nuclease complex (Figure 1). Three of these motifs are conserved in a functionally diverse spectrum of proteins that cleave phosphoester bonds, including serine/threonine protein phosphatases, whereas the fourth is unique to exonucleases such as SbcD. Hence, the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex may function as a nuclease in recombinational DNA repair (Koonin 1994; Zhuo *et al.* 1994; Sharples and Leach 1995).

The phenotypic features of *mre11* mutants in both mitotic and meiotic cells support this hypothesis. In mitotic cells, the processing of double-stranded ends in mating-type switching and the resection of DSB ends during repair by single-strand annealing are delayed in *mre11*, *rad50*, and *xrs2* mutants (Ivanov *et al.* 1994, 1996; Nairz and Klein 1997; Tsubouchi and Ogawa 1998). The hypomorphic *mre11S* and *rad50S* alleles are proficient in the formation of DSBs in meiotic recombination, but are deficient in the resection of those breaks (Alani *et al.* 1990; Nairz and Klein 1997; Tsubouchi

Corresponding author: John H. J. Petrini, Laboratory of Genetics, University of Wisconsin Medical School, 445 Henry Mall, Madison, WI 53706. E-mail: jpetrini@facstaff.wisc.edu

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source
4053-5-2a[a]	*MAT***a** *his7 leu2 trp1 ura3*	L. Hartwell
6509-25b[a]	*MAT*α *his7 leu2 ura3 hom3 can1*	L. Hartwell
JPY39	*MAT***a**/*MAT*α diploid of 4053-5-21a × 6509-25b	This study
JPY40	*MAT*α *his7 leu2 trp1 ura3 hom3 can1* (Spore derived from JPY39)	This study
JPY41	*MAT***a**/*MAT*α diploid of 4053-5-2a × JPY40	This study
JPY33	*MAT***a** *his7 leu2 trp1 ura3 mre11::hisG*	This study
JPY44	*MAT*α *his7 leu2 trp1 ura3 hom3 can1 mre11::hisG*	This study
JPY45	*MAT***a**/*MAT*α diploid of JPY33 × JPY44 (*mre11::hisG/mre11::hisG*)	This study
JPY67	*MAT***a**/*MAT*α diploid of JPY33 × JPY40 (*mre11::hisG/+*)	This study
YPH500[b]	*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Sikorski and Hieter (1989)
YPH1124[b]	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 CFIII (CEN3.L.YPH983) URA3 SUP11*	P. Hieter
JPY30	*MAT***a**/*MAT*α diploid of YPH500 × YPH1124	This study
JPY35	*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 mre11::hisG*	This study
JPY36	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 mre11::HIS3 CFIII (CEN3.L.YPH983) URA3 SUP11*	This study
JPY37	*MAT***a**/*MAT*α diploid of JPY35 × JPY36 (*mre11::hisG/mre11::HIS3*)	This study
EGY48	*MAT*α *his3 trp1 ura3 6LexAop-LEU2*	Estojak *et al.* (1995)

[a] A364A strain background.
[b] S288C strain background.

and Ogawa 1998). In addition, the apparent mitotic cell cycle specificity of recombination defects (Moore and Haber 1996), as well as the hyperrecombination phenotypes associated with the *mre11*, *rad50*, and *xrs2* mutants, may indicate additional *in vivo* roles of the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex.

We mutagenized each of the four conserved motifs in Mre11 to create the *mre11* alleles, *mre11-11*, *mre11-2*, *mre11-3*, and *mre11-4*. Phenotypic characterizations of these mutants were carried out with respect to mitotic recombination, chromosome loss, IR sensitivity, DSB repair, and association with Rad50. We found that all *mre11* mutants exhibited increased IR sensitivity and explicit defects in DSB repair. These mutants also displayed a hyperrecombination phenotype in mitotic cells, the severity of which correlated with the degree of IR sensitivity and DSB repair deficiency. Finally, two-hybrid interaction testing showed that all but one of the *mre11* mutations disrupted the ability of Mre11 to interact with Rad50.

MATERIALS AND METHODS

Yeast strains and growth media: The genotypes of the *S. cerevisiae* strains used in this study are described in Table 1. The *mre11::hisG* disruption strains, JPY33 and JPY44, were created by two-step gene replacement (Alani *et al.* 1987) in strains 4053-5-2a and JPY40, respectively. The *mre11::HIS3* disruption strains, JPY35 and JPY36, were created by one-step gene replacement (Morrow *et al.* 1995) in strains YPH500 (Sikorski and Hieter 1989) and YPH1124, respectively. YPH1124 is isogenic to YPH1015 (Connelly and Hieter 1996), except the chromosome fragment in YPH1015 is marked with *HIS3*. The *mre11::HIS3* disruption deleted the entire coding region; the *mre11::hisG* disruption deleted all but the N-terminal 105 amino acids. Both alleles conferred a similar degree of IR sensitivity. Disruptions were confirmed by Southern blotting. All strains were cultured at 30°. Yeast media and manipulations were carried out according to standard procedures (Ausubel *et al.* 1989; Guthrie and Fink 1991). Additional reagents were used at the following concentrations: 5-bromo-4-chloro-3-indolyl β-d-galactopyranoside (X-Gal, 40 μg/ml; Sigma Chemical, St. Louis, MO), l-canavanine (60 mg/liter; Sigma), and 5-fluoroorotic acid (5-FOA, 1 mg/ml; U. S. Biologicals).

Molecular biology: Plasmid construction, Southern blotting, DNA sequencing, PCR, and DNA isolation were carried out according to standard procedures (Ausubel *et al.* 1989). The pSK-*Scmre11*Δ disruption plasmid was constructed by insertion of the *hisG-URA3-hisG* cassette (Alani *et al.* 1987) at the *Hpa*I sites in pSK-*ScMRE11* [*MRE11*, cloned from a YCp50 genomic library (Rose *et al.* 1987) by complementation of the IR sensitivity phenotype of the *mre11*Δ mutant, inserted into pBluescript (Stratagene, La Jolla, CA) as a *Bam*HI restriction fragment].

For construction of *MRE11* expression constructs, the *ADH1* promoter and terminator from pDB20 (Becker *et al.* 1991) were inserted in pRS314 (Sikorski and Hieter 1989) as a *Bam*HI restriction fragment. A 3X HA epitope was fused to the C terminus of Mre11 on pRS314-derived plasmids by gap repair using a PCR product derived from pMPY-3X-HA (Schneider *et al.* 1995). The 3X HA tag did not affect the ability of the construct to complement the IR sensitivity phenotype of the *mre11*Δ strain (data not shown).

For construction of two-hybrid N-terminal fusion expression

constructs, *Eco*RI/*Xho*I fragments of *MRE11*, *RAD50*, *hMRE11*, and *hRAD50* were cloned into the *Eco*RI/*Xho*I restriction sites of pEG202 or pJG4-5 (Estojak *et al.* 1995). All constructs included the entire coding sequence, except the hMre11 fusions deleted the first four amino acids. Details of their constructions are available upon request.

Site-specific mutations in *MRE11* were generated by linear amplification using complementary 35-mer primers. Restriction fragments bearing the desired mutation(s) were confirmed by DNA sequencing. Western blotting confirmed that all four *mre11* alleles were expressed at similar levels. The allele bearing the mutation in motif I was named *mre11-11*, as *mre11-1* had been previously described and characterized (Ajimura *et al.* 1993).

Irradiation studies: Strains were grown to mid-log phase (typical OD = 0.5; 1×10^7 cells/ml), sonicated, and diluted to 4×10^4 cells/ml in sterile ddH_2O. Cells were γ-irradiated in a Mark I ^{137}Cs source at a dose rate of ~2.5 Gy/min and plated at a density of 2000 cells/plate. Plates were incubated at 30° and scored for colony formation for 5 days after irradiation.

Pulsed-field gel electrophoresis: Haploid JPY33 transformants were grown in liquid media lacking tryptophan (Trp) to maintain *mre11* expression constructs to mid-log phase (typical OD = 0.5; 1×10^7 cells/ml) and labeled with ^{32}P as described (Jong *et al.* 1995). Cells were γ-irradiated in 1× PBS [10 mm phosphate buffer (pH 7.4), 150 mm NaCl] and processed for pulsed-field gel electrophoresis (PFGE) immediately or after 3 hr postirradiation recovery in YPD as described (Friedl *et al.* 1995). Briefly, cells were washed in 50 mm EDTA (pH 8) and resuspended in 50 mm EDTA (pH 8), 10 mm Tris-Cl (pH 7.5), 1 mg/ml Zymolase 100T (U. S. Biologicals) buffer. An equal volume of 2% low-melting-point agarose (GIBCO, Grand Island, NY) at 55° was added to the cell suspension, and plugs were cast in plug molds (90 μl per plug). Plugs were transferred to 0.5 m EDTA (pH 8) and 10 mm Tris-Cl (pH 9) buffer; incubated at 37° for 8–16 hr; transferred to 0.5 m EDTA (pH 9), 10 mm Tris-Cl (pH 7.5), 20 mg/ml Sarkosyl, and 2 mg/ml proteinase K; and incubated at 50° for 24 hr. The plugs were washed once with cold rinsing buffer [10 mm EDTA (pH 8), 10 mm Tris-Cl (pH 7)] and rotated at 4° with three subsequent washing steps over the next 40 hr. Half of each plug was subjected to PFGE, and the other half counted in a scintillation counter. PFGE was carried out in 1% agarose gels (0.5 × TBE) on a Bio-Rad CHEF-DR II system (Bio-Rad Laboratories, Hercules, CA) at 14° for 22 hr at 6 V/cm, with an included angle of 120° and a switch time of 60–120 sec. After separation by PFGE, radiolabeled DNA was visualized and quantitated by phosphorimaging (Molecular Dynamics).

Mitotic recombination and chromosome loss: The rates of mitotic interhomologue recombination and endogenous chromosome loss were determined as described (Meeks-Wagner and Hartwell 1986), with the following exceptions: Single colonies were picked from plates lacking methionine (Met) and tryptophan (Trp), diluted to less than 200 cells/ml culture, grown to late-log phase (~20 doublings) in liquid media lacking Met and Trp, and plated onto solid canavanine-containing media lacking Met and/or Trp for analysis. A visual assay for ploidy of a nonessential chromosome fragment was performed essentially as described (Hieter *et al.* 1985). Telomeric sequences and the entire left arm of chromosome *III* were added onto the ends of a linear centromere-containing DNA fragment by homologous recombination, with endogenous chromosomes to generate the nonessential chromosome fragment (Spencer *et al.* 1990). Single colonies were picked from a Trp dropout plate into sterile water, sonicated, diluted, and plated onto solid Trp dropout media containing 5-FOA and limiting amounts of adenine (6 μg/ml) to score loss rate of the nonessential *URA3*-marked chromosome fragment. Dilutions were plated onto solid Trp dropout media to control for cell number in each assay. Colonies were scored after 2–5 days of incubation at 30°.

Data analysis: Calculation of mitotic recombination and chromosome loss rates using the method of the median and the maximum likelihood method (Lea and Coulson 1947) do not take into account differences in colony sizes, which may introduce error into the calculation of rates. We performed data analysis using a computer program written by B. E. Nelms (nelms@cms-stl.com). This program calculates mitotic recombination and chromosome loss rates using the method of the median and the maximum likelihood method, but it additionally uses a corrected maximum likelihood method that takes into account the variability introduced by unequal colony size. The maximum likelihood method is first used to approximate the mitotic recombination or chromosome loss rate based on the average colony size. This estimated rate is then used in a scaling procedure to account for additional mutations (if scaling up) or to subtract out recent mutations (if scaling down), based on the difference in individual colony sizes compared with the average. The corrected data represented by mutant and colony counts for colonies of equal size are then used to recalculate the mitotic recombination or chromosome loss rate by the maximum likelihood method. The corrected values thus obtained generally fell within 10% of those derived by the method of the median (Lea and Coulson 1947).

The statistical significance of differences observed in mitotic interhomologue recombination rate was determined using Wilcoxon Rank Sum and chi-square analyses. Pairwise comparisons of mutant fractions (corrected number of mutants divided by average colony size) for wild-type and *mre11* mutant strains were carried out using data from all five independent transformants per strain.

Two-hybrid analysis: The EGY48 strain, containing either a pJK103 or pSH18-34 lacZ reporter plasmid, was cotransformed with pEG202-DNA-binding domain (LexA) and pJG4-5-activation domain hybrid constructs (Estojak *et al.* 1995) in the configurations shown in Figure 5. Transformants were examined for protein interaction by galactose-dependent β-galactosidase production and leucine prototrophy, as described (Ausubel *et al.* 1989).

Immunoblot analysis: Protein extracts were prepared as described (Nasmyth 1977), and protein concentration was quantitated using the Bio-Rad Protein Assay. Western blotting was carried out according to standard procedures (Harlow and Lane 1988) using the 12CA5 mAb (Boehringer Mannheim, Indianapolis, IN), α-LexA antibody (gift from R. Brent), or with polyclonal antiserum directed against hMre11 or hRad50 (Dolganov *et al.* 1996). Chemiluminescent detection of antigen-antibody complexes was carried out with horseradish peroxidase-conjugated protein G, plus protein A (Pierce Chemical, Rockford, IL), or HRP-conjugated α-mouse (Santa Cruz) secondary antibodies in conjunction with Super Signal Luminol reagent (Pierce). A portion of each filter was stained using the Bio-Rad Biotin-Blot Protein Detection Kit to control for protein loading according to manufacturer's instructions.

RESULTS

***mre11* alleles:** The four conserved N-terminal phosphoesterase motifs of Mre11 contain a number of residues that are invariant over vast phylogenetic distances (Figure 1). Our mutagenesis to create *mre11-11*, *mre11-2*, *mre11-3*, and *mre11-4* was focused on those residues in

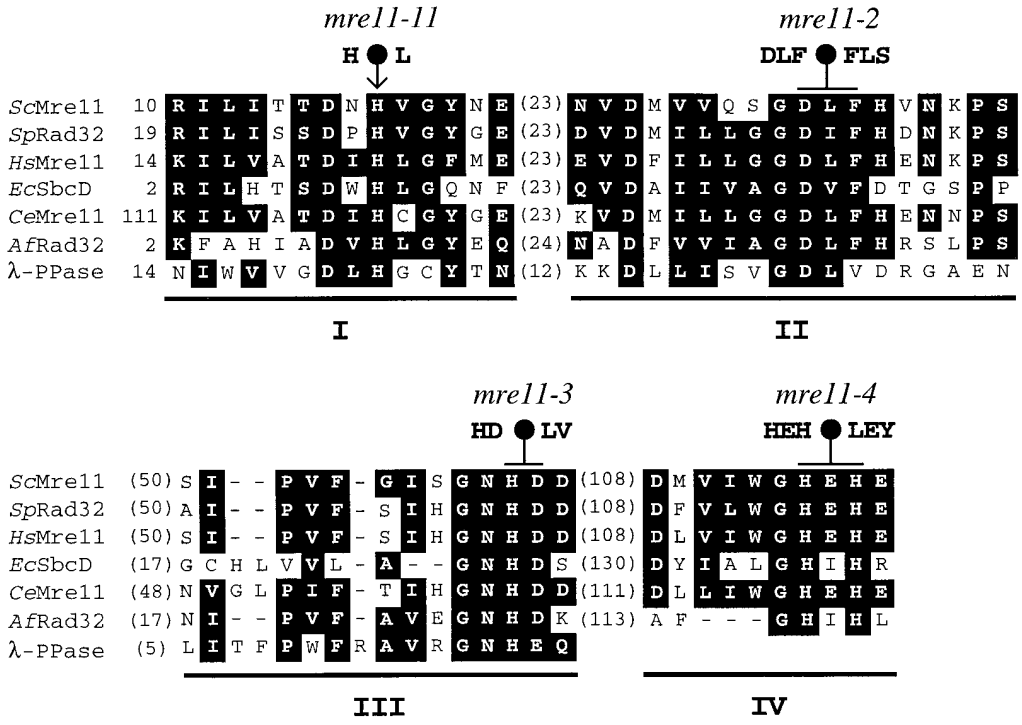

Figure 1.—Mutagenesis of conserved Mre11 motifs. Identical or similar amino acids are shaded in black. The following amino acids are considered similar: D, E, N, and Q; F, W, and Y; K and R; A and G; I, V, and L; M; S, and T; C; H; and P. *Scmre11* alleles are indicated above the sequence alignment; the wild-type and mutant residues are left and right of the symbol, respectively. Motifs are indicated below the sequence alignment. Residue numbers at the left indicate the number of the first residue in each species. Numbers in parentheses indicate the number of amino acids between each motif. Organisms, GenBank/SwissProt accession numbers, and references are as follows: *Sc*, *Saccharomyces cerevisiae* (D11463; Johzuka and Ogawa 1995); *Sp*, *Schizosaccharomyces pombe* (X82322; Tavassoli *et al.* 1995); *Hs*, *Homo sapiens* (U37359; Petrini *et al.* 1995); *Ec*, *Escherichia coli* (P13457; Naom *et al.* 1989); *Ce*, *Caenorhabditis elegans* (Z73978; Wilson *et al.* 1994); *Af*, *Archaeoglobus fulgidus* (AF1031; Klenk *et al.* 1997); and λPPase, bacteriophage lambda protein phosphatase (P03772; Cohen *et al.* 1988).

motifs I–IV, respectively. The invariant histidine residues in motifs I and III (altered in *mre11-11* and *mre11-3*) and the invariant aspartate in motif II (altered in *mre11-2*) occupy positions analogous to critical active site residues in the catalytic subunit of mammalian and bacteriophage serine/threonine phosphatases (Zhuo *et al.* 1994; Goldberg *et al.* 1995; Griffith *et al.* 1995). Motif IV, which does not appear to be present in those protein phosphatases, similarly contains histidine residues (altered in *mre11-4*) that are invariant in the Mre11 homologues from archaea to humans (Figure 1). Phenotypic analysis of the engineered *mre11* mutants was carried out in an *mre11*Δ deletion strain transformed with each of the four *mre11* expression constructs. Control strains consisted of an *mre11*Δ deletion strain transformed with either the wild-type *MRE11* expression construct or an empty vector. Expression was confirmed by Western blotting with the 12CA5 mAb (Figure 2).

Spontaneous mitotic interhomologue recombination is increased in *mre11* mutants: We assessed the impact of alterations in the Mre11 phosphoesterase motifs on the rates of mitotic interhomologue recombination and endogenous chromosome loss in JPY45. This strain is heterozygous for linked positive selection (*HOM3* by methionine) and negative selection (*can1* by canavanine) markers on opposite arms of chromosome *V*. Acquisition of canavanine resistance by chromosome loss results in methionine auxotrophy through concomitant loss of the *HOM3*+ allele, whereas interhomologue recombinants at *CAN1* retain the *HOM3*+ allele, and the strain remains a methionine prototroph (Hartwell and Smith 1985). Recombination and loss events were scored as *HOM3*+ *can1* and *hom3*− *CAN1*, respectively, and frequencies were then calculated based on the total number of cells plated, determined from control platings.

The engineered *mre11* alleles fell into two general classes with respect to these phenotypic parameters; one class behaved essentially as the null *mre11*Δ mutant, and the other displayed an intermediate phenotype (Table 2). *mre11-2* and *mre11-4* transformants exhibited mitotic interhomologue recombination rates of 4.4×10^{-3} and 3.4×10^{-3}, respectively. These values were increased about sevenfold relative to the wild-type transformant (6.4×10^{-4}) and were not significantly different from the *mre11*Δ mutant (4.0×10^{-3}). In contrast, *mre11-11* was somewhat less affected, with a rate of 2.5×10^{-3} (a fourfold increase over wild type). The increases in mitotic interhomologue recombination rates observed between these *mre11* transformants and the wild-type transformant are statistically significant at a confidence level >99%. The recombination frequency in *mre11-3* transformants was 1.1×10^{-3}, a twofold increase over the wild-type transformant. The rate in the heterozygous *MRE11*/*mre11*Δ strain JPY67 (6.4×10^{-4}) was indistinguishable from the wild-type rate (Table 2).

The frequency of endogenous chromosome loss measured in the chromosome *V* system was not increased in any of the *mre11* transformants (data not shown). This result was unexpected given the dramatic impact

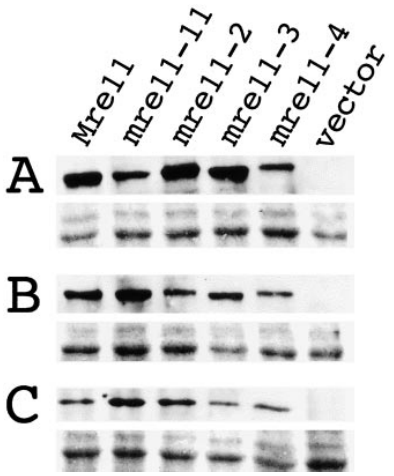

Figure 2.—Expression of *mre11*. Immunoblot analysis of yeast protein extracts (10 μg of total protein per lane) was performed using the 12CA5 mAb (A and B, top rows) or with polyclonal antiserum directed against hMre11 (C, top row). Loading controls shown for each filter (bottom rows) were performed as described in materials and methods. (A) JPY33 strains transformed with wild-type or *mre11* mutant expression constructs or empty vector. (B) EGY48 strains transformed with AD-ScMre11 or AD-Scmre11 fusion constructs. (C) EGY48 strains transformed with AD-hMre11 or AD-hmre11 fusion constructs.

of the *Sprad32*Δ mutation (*S. pombe MRE11* homologue) on chromosome loss. In that context, spontaneous loss of a nonessential minichromosome was elevated 300-fold over the wild-type rate (Tavassoli *et al.* 1995). To ascertain whether loss of a nonessential chromosome fragment would be similarly increased in *S. cerevisiae*, we used the system developed by Spencer *et al.* (1990) in JPY37 (Gerring *et al.* 1990; Spencer *et al.* 1990).

JPY37 harbors a nonessential chromosome fragment marked with the *SUP11* and *URA3* genes. We plated JPY37 transformants on solid media containing 5-FOA to select for cells that had undergone chromosome fragment loss events and then scored 5-FOA-resistant colonies to calculate loss rate based on control platings. As above, the *mre11* mutants did not dramatically increase the rate of chromosome loss in this assay system (Table 2). The *mre11*Δ strain exhibited a loss rate of 1.64×10^{-3}, a fourfold increase over the wild type (3.87×10^{-4}), and *mre11-11*, *mre11-2*, and *mre11-4* were each threefold higher than wild type. Although we consistently observed at least a twofold increase in chromosome fragment loss rate in these *mre11* transformants, this increase was not striking considering the relative impact of the *Sprad32*Δ mutation on chromosome loss described above. The nonessential chromosome fragment was as stable in *mre11-3* as in the wild-type transformant, with a loss rate of 3.07×10^{-4} (Table 2).

This system also permits distinction between chromosome fragment loss and nondisjunction. JPY37 is homozygous for the *ade2-101* mutation, which results in the accumulation of red pigment in cells plated on solid media containing limiting amounts of adenine. This pigmented phenotype is suppressed by *SUP11* in a dosage-dependent manner. The presence of one copy of the *SUP11* gene results in pink colonies, and that of two copies results in white colonies. When grown under conditions that allow for loss of the chromosome fragment, nondisjunction (2:0) produces colonies with red and white sectors, whereas loss of the nonessential chromosome fragment (1:0) results in red- and pink-sectored colonies. All of the *mre11* transformants displayed a bias toward chromosome fragment loss events over nondisjunction in a ratio of about 9:1, similar to the wild-type strain (data not shown).

In addition to the mitotic interhomologue recombination phenotypes observed, the null class of *mre11* transformants also exhibited decreased plating efficiency of at least 2.5- to 3-fold relative to wild type. The relative plating efficiency of untransformed wild-type and *mre11*Δ strains plated on YPD was 2.3-fold, similar to that observed for the transformants plated on solid Trp dropout media (Table 2, footnote *d*).

***mre11* mutants are sensitive to ionizing radiation:** Having established the phenotypes of *mre11-11*, *mre11-2*, *mre11-3*, and *mre11-4* under normal growth conditions, we next assessed the impact of these mutations in cells treated with IR. *mre11* transformants were γ-irradiated in liquid suspension at 0, 100, 200, and 300 Gy and plated in triplicate for scoring of colony formation. As we observed in normally growing cells, the *mre11* mutants fell into two phenotypic classes, in this case according to severity of their IR sensitivity phenotypes relative to the wild-type transformant. The LD37 of the wild-type strain was >300 Gy. In contrast, the *mre11-2* and *mre11-4* mutants exhibited LD37s of 35 and 50 Gy, respectively (Figure 3A), indistinguishable from the *mre11*Δ strain (LD37 = 40 Gy). *mre11-11* and *mre11-3* transformants were more resistant to IR, exhibiting LD37s of 105 and 285 Gy, respectively.

Previous studies have shown that diploid *rad50*Δ and *xrs2*Δ strains exhibit increased radiation resistance in comparison to the corresponding mutant haploids (Ivanov *et al.* 1992). This diploid effect is not seen in *rad52* mutants, presumably because homologous recombination is blocked in these strains. Consistent with the observation that the *mre11*Δ mutant is competent to undergo mitotic recombination (Ajimura *et al.* 1993) (Table 2), we observed the same diploid-specific increase in radiation resistance upon comparison of haploid and diploid *mre11*Δ strains (Figure 3B).

***mre11* mutants are defective in DSB repair:** We used PFGE to assay DSB repair directly. Chromosomal DNA of *mre11* transformants was metabolically labeled by growth in the presence of ^{32}P-containing inorganic phosphate (Jong *et al.* 1995). ^{32}P-labeled DNA from unirradiated and γ-irradiated cells was separated into distinct chromosome bands by PFGE, and DSB repair was monitored by autoradiography (Figure 4A). Immediately

TABLE 2

Effect of *mre11* mutations on spontaneous mitotic recombination,[a] chromosome loss,[a] and plating efficiency

Strain	Mitotic recombination $\times 10^4$/division[b]	Chromosome fragment loss $\times 10^4$/division[c]	Plating efficiency[d] (%)
MRE11	6.4 (1)	3.87 ± 2.1 (1)	21 (1)
*mre11*Δ/+	6.4 (1)[e]	ND	20 (1)
mre11-11	25 (4)	11.1 ± 3.2 (3)	10 (0.5)
mre11-2	44 (7)	10.2 ± 3.4 (2.6)	8 (0.4)
mre11-3	11 (2)	3.07 ± 0.82 (0.8)	17 (0.8)
mre11-4	34 (5)	11.9 ± 5.6 (3)	7 (0.3)
*mre11*Δ	40 (6)	16.4 ± 6.4 (4)	7 (0.3)

[a] Data were analyzed using the corrected maximum likelihood method (materials and methods); fold wild-type values are given in parentheses.

[b] Rate calculated from five independent JPY45 transformants per strain; *P* values of 0.00397 and 0.000312 were determined according to statistical analyses of wild-type *vs. mre11* mutant transformants as described (materials and methods).

[c] Rate ± standard deviation calculated from three independent experiments of 10 independent JPY37 transformants per strain per experiment.

[d] Percent-plating efficiency was determined in the course of the ionizing radiation sensitivity experiments and was calculated as the number of colony forming units scored after 5 days divided by the number of cells plated. Data represent the average plating efficiency of five independent experiments, using JPY33 and JPY36 haploid transformants plated on solid Trp dropout media; average plating efficiency of untransformed *MRE11* and *mre11*Δstrains plated on YPD were 63 and 28%, respectively. Percent-plating efficiency of *mre11*Δ/+ was determined in the diploid JPY67. Fold wild-type values are given in parentheses.

[e] Rate calculated from three independent experiments of five independent JPY67 cultures per experiment.

after γ-irradiation with 600 Gy (t = 0 hr), a dose at which a wild-type *MRE11* strain exhibits 27% survival (data not shown), bands corresponding to individual chromosomes diminished in intensity, and a heterogeneous population of lower molecular weight DNA became evident (Figure 4A, lanes 2 and 5).

Quantitation of radiolabeled chromosomal DNA in irradiated *mre11* transformants was effected by phosphorimaging analysis (Figure 4B). In the wild-type transformant, we observed restoration of the three most prominent chromosome band intensities (migration distances of 25, 34, and 43 mm) to 42–49% of the unirradiated control by 3 hr postirradiation (t = 3 hr) and a decrease in the abundance of low molecular weight DNA (Figure 4A, lane 3, and Figure 4B, graphs 1–3). The *mre11*Δ, *mre11-2*, and *mre11-4* strains exhibited severe defects in the repair of IR-induced chromosome breaks. The intensities of the three most prominent bands diminished to 10% of the unirradiated control values during the 3-hr recovery period (Figure 4B, graphs 7 and 9). In contrast, the *mre11-11* mutants exhibited an intermediate DSB repair capacity, with postrecovery peak intensities of 31–34% of the control values (Figure 4B, graphs 4 and 6). The *mre11-3* transformants were essentially indistinguishable from the wild-type transformants by this criterion (data not shown).

mre11 mutants affect interaction with Rad50: Epistasis and two-hybrid analyses have demonstrated that Mre11, Rad50, and Xrs2 function as a protein complex (Johzuka and Ogawa 1995; Petrini *et al.* 1997). To address whether null and hypomorphic alleles impair protein function by disrupting complex formation, we examined the ability of mre11 mutant proteins to interact with Rad50 by two-hybrid interaction testing. Analysis of EGY48 transformants revealed that only the Mre11 (WT) and mre11-3 proteins were capable of interacting with Rad50, as determined by galactose-dependent lacZ activation (Figure 5A). The mutants that behaved as the null (*mre11-2* and *mre11-4*) or had an intermediate phenotype (*mre11-11*) according to previous analyses did not exhibit this interaction.

Conservation of the Mre11/Rad50 protein complex in human cells (Dolganov *et al.* 1996) prompted us to generate corresponding mutations in *hMRE11* (Petrini *et al.* 1995). We tested the ability of the hmre11 mutant proteins to interact with hRad50 in EGY48 and found that *hmre11* mutations that abolished or impaired interaction with hRad50 corresponded to null *Scmre11* mutations (Figure 5B). Scmre11-11 did not interact with ScRad50, whereas hmre11-11 did interact with hRad50. These data were confirmed by galactose-dependent activation of the *LEU2* reporter gene (data not shown), although the interaction between ScMre11 and ScRad50 detected by this method was relatively weak.

DISCUSSION

We mutagenized the conserved phosphoesterase signature motifs in the N terminus of Mre11 and assessed the impact of these mutations by several phenotypic

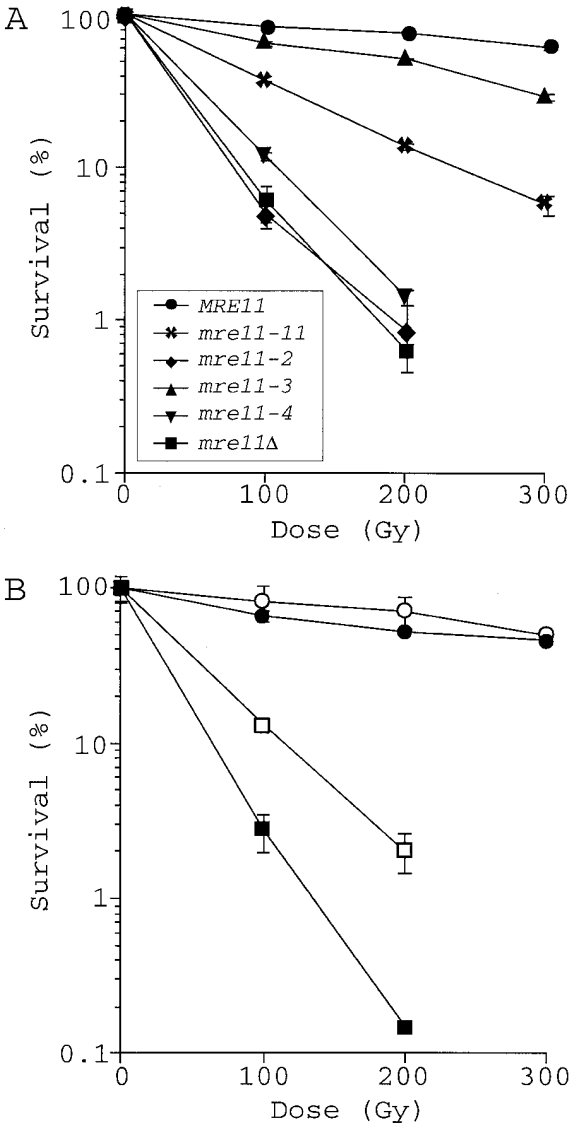

Figure 3.—Radiation sensitivity of *mre11* mutants. Strains were γ-irradiated at the doses indicated and scored for colony formation 5 days after irradiation. Plotted values indicate average of triplicate platings. Error bars represent standard deviation. (A) Haploid JPY33 transformants. (B) Haploid *vs.* diploid wild-type and *mre11*Δ strains. Open circles, JPY41 (*MRE11*/*MRE11*); solid circles, JPY23 (*MRE11*); open squares, JPY45 (*mre11*Δ/*mre11*Δ); solid squares, JPY44 (*mre11*Δ).

parameters. The residues altered in this study correspond to those found at the catalytic center of proteins containing the canonical phosphoesterase motifs (Zhuo *et al.* 1994; Goldberg *et al.* 1995; Griffith *et al.* 1995). Our findings demonstrate the importance of these motifs for recombination and DSB repair. The severity of spontaneous and radiation-induced phenotypes observed fell into two classes. One class behaved as the null mutant, exhibiting spontaneous interhomologue hyperrecombination, sensitivity to killing by IR, and DSB repair deficiency. The second class was less severe, but exhibited similar defects. Aside from the mutation in phosphoesterase motif III, mutations in this region also disrupted interaction with Rad50. The relative importance of impaired enzymatic function and reduced protein association are not distinguished in this study. Nonetheless, our results support the hypothesis that Mre11 functions require association with Rad50.

Because a sharply elevated frequency of minichromosome loss was observed in mutants of the *MRE11* homologue in *S. pombe*, *RAD32* (Tavassoli *et al.* 1995), we expected, but did not observe, increased chromosome loss in the *mre11* mutants. Similarly, discordant phenotypic outcomes have been observed in mutants of the *S. cerevisiae* and *S. pombe RAD52* homologues. Despite the relatively high degree of conservation between *S. cerevisiae* and *S. pombe* gene products, mutation of the *S. pombe rad22* gene does not significantly impair homologous recombination, whereas *S. cerevisiae rad52* mutations completely abolish that process (Game 1993; Fortunato *et al.* 1996).

Mre11 is important for repair of spontaneous DSBs: Colinearity in the trends exhibited by *mre11* mutants with respect to mitotic interhomologue hyperrecombination, IR sensitivity, and DSB repair deficiency suggests that these phenotypic endpoints arose from similar DNA lesions. The role of the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex in the metabolism of DSBs induced by exogenous agents has been well established. Considerable evidence now exists to support the notion that DSBs occur spontaneously in bacterial, as well as eukaryotic cells (Kuzminov 1995). In null mutants of *HDF1*, the *S. cerevisiae* Ku70 homologue, the presence of spontaneous DSBs is indicated by the induction of transcriptional and cell-cycle DNA damage responses when cells are shifted to growth conditions that inactivate residual Ku activity (Barnes and Rio 1997). Further, inducible inactivation of hRad51 in DT-40 chicken B lymphocyte cells revealed that DSBs form spontaneously in essentially all cells upon traversal through S phase (Sonoda *et al.* 1998). Hence, it is reasonable to suggest the spontaneous *mre11* phenotypes are attributable to spontaneously forming DSBs. Failure to efficiently repair such spontaneous lesions may account for the lethality of *Mumre11* mutations in murine embryonic stem cells, where the persistence of DSBs may trigger apoptotic cell death or terminal cell cycle arrest (Xiao and Weaver 1997).

Haploid *mre11* mutants exhibit global defects in DSB repair: The primary role of *MRE11* appears to be in nonhomologous end-joining (Schiestl *et al.* 1994; Moore and Haber 1996). The mitotic interhomologue hyperrecombination observed in *mre11* mutants suggests that homologous recombination is not grossly affected in *mre11* strains. However, the severity of DSB repair defects observed in PFGE analysis of the engineered *mre11* mutants supports the hypothesis that the Mre11/Rad50/Xrs2 protein complex mediates sister chromatid cohesion in addition to its role in DSB repair (Moore and Haber 1996). Haploid yeast strains were used in the PFGE experiments. Therefore, cells irradi-

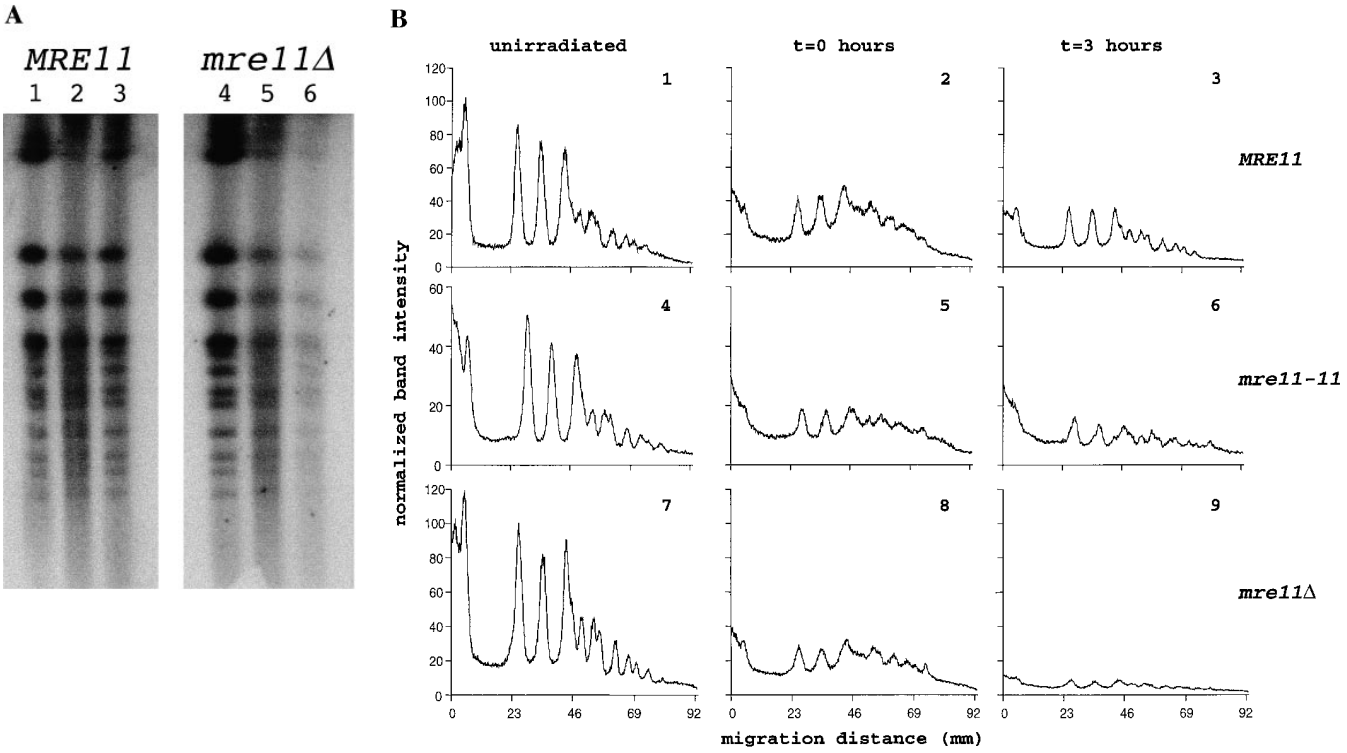

Figure 4.—Detection of DSB repair in *mre11* strains by pulsed-field gel electrophoresis. (A) Scanned image of pulsed-field agarose gel with ^{32}P-labeled DNA from JPY33 transformants: *MRE11* (lanes 1–3); *mre11*Δ (lanes 4–6). Unirradiated samples (lanes 1 and 4); irradiated samples (600 Gy) prepared at $t = 0$ hr (lanes 2 and 5) and at $t = 3$ hr (lanes 3 and 6) of recovery incubation. (B) Quantitative analysis of band intensities, normalized to the total number of radioactive counts loaded per lane, for *MRE11* (graphs 1–3), *mre11-11* (graphs 4–6), and *mre11*Δ (graphs 7–9) strains. Unirradiated samples (graphs 1, 4, and 7); irradiated samples (600 Gy) prepared at $t = 0$ hr (graphs 2, 5, and 8) and at $t = 3$ hr (graphs 3, 6, and 9) of recovery incubation. Normalized band intensity scale for the *mre11-11* strain is half that of the *MRE11* and *mre11*Δ strains due to differences in the total number of radioactive counts loaded per lane.

ated in G1 would lack a homologous template and would thus be restricted to DSB repair by nonhomologous end-joining, which is impaired by Mre11 deficiency. The profound DSB repair defect observed in *mre11*Δ mutants argues that both end-joining and sister chromatid recombination-based DSB repair in G2 cells are defective.

In wild-type cells, we found that the intensity of chromosomal bands was restored to <50% of control values at 3 hr following irradiation. Based on flow cytometric analyses of asynchronously growing cultures of this strain, ~60% of cells are in G1 during mid-log phase (data not shown). The commensurate reduction in intact chromosomes may reflect that even in wild-type haploid cells, nonhomologous end-joining-based repair of damage induced in G1 is inefficient. Consistent with this idea, we have observed that the ability of irradiated cells to reconstitute intact chromosomes is increased in diploid cells (data not shown). Because the PFGE assay we used relied upon measurement of intact chromosomes, misrepair events and degradation of unrepaired DNA do not appear in our calculations, although to some extent, the reduction in chromosome band intensity may be attributed to those causes. Because the values obtained for intact chromosomes are normalized to the amount of DNA loaded, restoration of intact chromosomes at 3 hr postirradiation indicates repair, rather than reduction in the background of intermediately sized broken DNA.

Scmre11 mutations do not appear to affect interhomologue recombination. This notion is supported by the increased IR resistance of diploid *mre11*Δ cells. The increased spontaneous mitotic interhomologue recombination observed in *mre11*Δ transformants may thus reflect increased reliance upon the homologue for repair of spontaneous damage. Genetic evidence for a distinct mitotic interhomologue recombination pathway has recently been obtained (Klein 1997).

Phosphoesterase signature motifs are important for Mre11 function in mitotic cells: In previous mutational analyses of *MRE11*, intragenic complementation between N- and C-terminal mutations was observed (Nairz and Klein 1997). These data suggest that Mre11 contains two functionally distinct domains. The N-terminal *mre11S* mutations, which alter residues outside the conserved phosphoesterase motifs, affect DSB processing in meiotic recombination and confer relatively mild phenotypes in mitotic cells (Nairz and Klein 1997; Tsubouchi and Ogawa 1998). Mutations in the C ter-

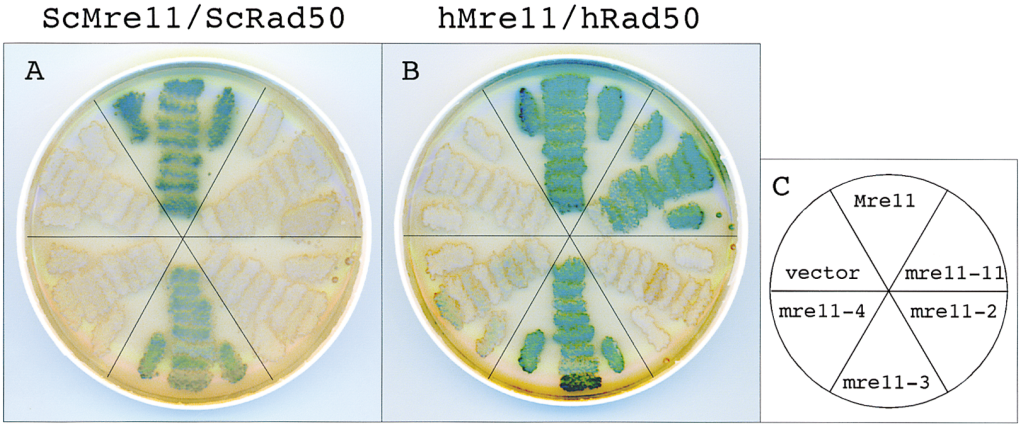

Figure 5.—Two-hybrid interaction between Mre11 and Rad50. Ten independent transformants per strain were picked onto solid media lacking uracil, histidine, and tryptophan. Cell patches were replica plated onto solid dropout media containing X-Gal and scored after 7 days of incubation at 30°. (A) EGY48 strains cotransformed with the pSH18-34 lacZ reporter plasmid, LexA-ScRad50 and AD-ScMre11, or AD-Scmre11 mutants. (B) EGY48 strains cotransformed with the JK103 lacZ reporter plasmid, LexA-hRad50 and AD-hMre11, or AD-hmre11 mutants. The mutations generated in *hMRE11* are identical to those made in *ScMRE11*, except hmre11-2 changes DLF to GLS in motif II. (C) Configuration of AD-Mre11 or AD-mre11 fusion construct transformants.

minus of Mre11 impair the creation of DSBs in meiotic cells. Hence, this region of the protein was hypothesized to control the assembly of a protein complex required for the initiation of meiotic recombination. C-terminal mutants also exhibit relatively severe mitotic phenotypes, suggesting that this Mre11 domain is important for function of the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex in mitotic cells as well.

The similarity between Mre11/Rad50 and SbcD/SbcC (Sharples and Leach 1995) suggests that these proteins constitute the catalytic core of the *S. cerevisiae* Mre11/Rad50/Xrs2 protein complex. Our data show that alteration of conserved residues in the phosphoesterase motifs affects interaction with Rad50. If the Mre11 C terminus is indeed important for protein interaction, it is conceivable that it contains the Xrs2 interaction domain. The Mre11 homologues are not well conserved at the C termini. We have shown that the human hMre11/hRad50 protein complex does not contain a conserved Xrs2 homologue (Carney *et al.* 1998), suggesting that Xrs2 is conserved to a much lesser extent than Mre11 and Rad50. Based on the residues altered in the conserved phosphoesterase signature motifs, it is likely that the observed *mre11* phenotypes may be explained by loss of enzymatic activity. However, these mutations also impair interaction between Mre11 and Rad50. Assessment of the relative contributions of impaired enzymatic activity and disrupted protein interactions to the phenotypes observed requires biochemical analysis of the mutant proteins.

We thank B. Garvik, L. Hartwell, C. Connelly, P. Hieter, our laboratory, and members of the Culbertson and Craig laboratories at the University of Wisconsin for materials, assistance, or support, and N. Drinkwater for helpful discussions. We also thank D. Bishop, D. Brow, M. Cox, and T. Prolla for critical reviews of the manuscript. This work was supported by grants from the March of Dimes, the Milwaukee Foundation, the American Cancer Society (grant NP-918), the Howard Hughes Medical Institute (J.H.J.P.), and the National Institutes of Health predoctoral training grant 5T32GM-07133 (D.A.B.). This is manuscript no. 3511 from the University of Wisconsin, Madison, Laboratory of Genetics.

LITERATURE CITED

Ajimura, M., S.-H. Leem and H. Ogawa, 1993 Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. Genetics **133:** 51–66.

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.

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1989 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Bai, Y., and L. Symington, 1996 A *RAD52* homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*. Genes Dev. **10:** 2025–2037.

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.

Becker, D. M., J. D. Fikes and L. Guarente, 1991 A cDNA encoding a human CCAAT-binding protein cloned by functional complementation in yeast. Proc. Natl. Acad. Sci. USA **88:** 1968–1972.

Carney, J. P., R. S. Maser, H. Olivares, E. M. Davis, M. Le Beau *et al.*, 1998 The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell **93:** 477–486.

Cohen, P. T., J. F. Collins, A. F. Coulson, N. Berndt and O. B. da Cruz e Silva, 1988 Segments of bacteriophage lambda (orf 221) and phi 80 are homologous to genes coding for mammalian protein phosphatases. Gene **69:** 131–134.

Connelly, C., and P. Hieter, 1996 Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell **86:** 275–285.

Connelly, J. C., E. S. de Leau, E. A. Okely and D. R. F. Leach, 1997 Overexpression, purification, and characterization of the SbcCD protein from *Escherichia coli*. J. Biol. Chem. **272:** 19819–19826.

Connelly, J. C., and D. R. F. Leach, 1996 The *sbcC* and *sbcD* genes of *Escherichia coli* encode a nuclease involved in palindrome inviability and genetic recombination. Genes to Cells **1:** 285–291.

Dolganov, G. M., R. S. Maser, A. Novikov, L. Tosto, S. Chong *et al.*, 1996 Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. Mol. Cell. Biol. **16:** 4832–4841.

Estojak, J., R. Brent and E. A. Golemis, 1995 Correlation of two-hybrid affinity data with *in vitro* measurements. Mol. Cell. Biol. **15:** 5820–5829.

Fortunato, E. A., F. Osman and S. Subramani, 1996 Analysis of

spontaneous and double-strand break-induced recombination in *rad* mutants of *S. pombe*. Mutat. Res. **364:** 147–160.

Friedl, A. A., A. Kraxenberger and F. Eckardt-Schupp, 1995 Use of pulsed-field gel electrophoresis for studies of DNA double-strand break repair in the yeast *Saccharomyces cerevisiae*. Methods: A Companion to Methods in Enzymology **7:** 205–218.

Game, J. C., 1993 DNA double strand breaks and the *RAD50-RAD57* genes in *Saccharomyces*. Sem. in Cancer Biol. **4:** 73–83.

Game, J. C., and R. K. Mortimer, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24:** 281–292.

Gerring, S. L., F. Spencer and P. Hieter, 1990 The *CHL1 (CTF1)* gene product of *Saccharomyces cerevisiae* is important for chromosome transmission and normal cell cycle progression in G2/M. EMBO J. **9:** 4347–4358.

Goldberg, J., H. Huang, Y. Kwon, P. Greengard, A. C. Nairn *et al.*, 1995 Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. Nature **376:** 745–753.

Griffith, J. P., J. L. Kim, E. E. Kim, M. D. Sintchak, J. A. Thomson *et al.*, 1995 X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK50 complex. Cell **82:** 507–522.

Guthrie, C., and G. R. Fink, (Editors), 1991 Guide to yeast genetics and molecular biology. Methods Enzymol. **194:** 1–933.

Harlow, E., and D. Lane, 1988 *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hartwell, L. H., and D. Smith, 1985 Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. Genetics **110:** 381–395.

Hieter, P., C. Mann, M. Snyder and R. W. Davis, 1985 Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40:** 381–392.

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.

Ivanov, E. L., N. Sugawara, J. Fishman-Lobell and J. E. Haber, 1996 Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics **142:** 693–704.

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.

Johzuka, K., and H. Ogawa, 1995 Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. Genetics **139:** 1521–1532.

Jong, A. Y., B. Wang and S. Q. Zhang, 1995 Pulsed field gel electrophoresis labeling method to study the pattern of *Saccharomyces cerevisiae* chromosomal DNA synthesis during the G1/S phase of the cell cycle. Anal. Biochem. **227:** 32–39.

Klein, H. L., 1997 *RDH54*, a *RAD54* homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. Genetics **147:** 1533–1543.

Klenk, H. P., R. A. Clayton, J. F. Tomb, O. White, K. E. Nelson *et al.*, 1997 The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature **390:** 364–370.

Koonin, E. V., 1994 Conserved sequence pattern in a wide variety of phosphoesterases. Protein Science **3:** 356–358.

Kuzminov, A., 1995 Collapse and repair of replication forks in *Escherichia coli*. Mol. Microbiol. **16:** 373–384.

Lea, D. E., and C. A. Coulson, 1947 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

Malone, R. E., S. Bullard, M. Hermiston, R. Reiger, M. Cool *et al.*, 1991 Isolation of mutants defective in the early steps of meiotic recombination in the yeast *Saccharomyces cerevisiae*. Genetics **128:** 79–88.

Malone, R. E., T. Ward, S. Lin and J. Waring, 1990 The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr. Genet. **18:** 111–116.

Meeks-Wagner, D., and L. H. Hartwell, 1986 Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44:** 43–52.

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.

Morrow, D. M., D. A. Tagle, Y. Shiloh, F. S. Collins and P. Hieter, 1995 *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. Cell **82:** 831–840.

Nairz, K., and F. Klein, 1997 *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. **11:** 2272–2290.

Naom, I. S., S. J. Morton, D. R. F. Leach and R. G. Lloyd, 1989 Molecular organisation of *sbcC*, a gene that affects genetic recombination and the viability of DNA palindromes in *Escherichia coli* K-12. Nucleic Acids Res. **17:** 8033–8045.

Nasmyth, K. A., 1977 Temperature-sensitive lethal mutants in the structural gene for DNA ligase in the yeast *Schizosaccharomyces pombe*. Cell **12:** 1109–1120.

Petrini, J. H. J., D. A. Bressan and M. S. Yao, 1997 The *RAD52* epistasis group in mammalian double strand break repair. Sem. in Immunol. **9:** 181–188.

Petrini, J. H. J., M. E. Walsh, C. Di Mare, J. R. Korenberg, X.-N. Chen *et al.*, 1995 Isolation and characterization of the human *MRE11* homologue. Genomics **29:** 80–86.

Rose, M. D., P. Novick, J. H. Thomas, D. Botstein and G. R. Fink, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

Schiestl, R. H., J. Zhu and T. D. Petes, 1994 Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 4495–4500.

Schneider, B. L., W. Seufer, B. Steiner, Q. H. Yang and A. B. Futcher, 1995 Use of polymerase chain reaction epitope tagging for protein tagging in *Saccharomyces cerevisiae*. Yeast **11:** 1265–1274.

Sharples, G. J., and D. R. Leach, 1995 Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the Rad50 and Mre11 (Rad32) recombination and repair proteins of yeast. Mol. Microbiol. **17:** 1215–1217.

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.

Sonoda, E., M. S. Sasaki, J.-M. Buerstedde, O. Bezzubova, A. Shinohara *et al.*, 1998 Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J. **17:** 598–608.

Spencer, F., S. L. Gerring, C. Connelly and P. Hieter, 1990 Mitotic chromosome transmission fidelity mutants in *Saccharomyces cerevisiae*. Genetics **124:** 237–249.

Tavassoli, M., M. Shayeghi, A. Naism and F. Z. Watts, 1995 Cloning and characterization of the *Schizosaccharomyces pombe rad32* gene: a gene required for repair of double strand breaks and recombination. Nucleic Acids Res. **23:** 383–388.

Tsubouchi, H., and H. Ogawa, 1998 A novel *mre11* mutation impairs processing of double-strand breaks of DNA during both mitosis and meiosis. Mol. Cell. Biol. **18:** 260–268.

Tsukamoto, Y., J. Kato and H. Ikeda, 1996 Effects of mutations of *RAD50*, *RAD51*, *RAD52*, and related genes on illegitimate recombination in *Saccharomyces cerevisiae*. Genetics **142:** 383–391.

Wilson, R., R. Ainscough, K. Anderson, C. Baynes, M. Berks *et al.*, 1994 2.2Mb of contiguous nucleotide sequence from chromosome *III* of *C. elegans*. Nature **368:** 32–38.

Xiao, Y., and D. T. Weaver, 1997 Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. Nucleic Acids Res. **25:** 2985–2991.

Zhuo, S., J. C. Clemens, R. L. Stone and J. E. Dixon, 1994 Mutational analysis of a Ser/Thr phosphatase. J. Biol. Chem. **269:** 26234–26238.

Communicating editor: M. Lichten