Role of the Nuclease Activity of *Saccharomyces cerevisiae* Mre11 in Repair of DNA Double-Strand Breaks in Mitotic Cells

L. Kevin Lewis,*[,1] Francesca Storici,[†] Stephen Van Komen,[‡] Shanna Calero,* Patrick Sung[‡] and Michael A. Resnick[†]

**Department of Chemistry and Biochemistry, Texas State University, San Marcos, Texas 78666, [†]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709 and [‡]Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06520*

Manuscript received November 17, 2003
Accepted for publication December 22, 2003

ABSTRACT

The Rad50:Mre11:Xrs2 (RMX) complex functions in repair of DNA double-strand breaks (DSBs) by recombination and nonhomologous end-joining (NHEJ) and is also required for telomere stability. The Mre11 subunit exhibits nuclease activities *in vitro*, but the role of these activities in repair in mitotic cells has not been established. In this study we have performed a comparative study of three mutants (*mre11-D16A*, *-D56N*, and *-H125N*) previously shown to have reduced nuclease activities *in vitro*. In ends-in and ends-out chromosome recombination assays using defined plasmid and oligonucleotide DNA substrates, *mre11-D16A* cells were as deficient as *mre11* null strains, but defects were small in *mre11-D56N* and *-H125N* mutants. *mre11-D16A* cells, but not the other mutants, also displayed strong sensitivity to ionizing radiation, with residual resistance largely dependent on the presence of the partially redundant nuclease Exo1. *mre11-D16A* mutants were also most sensitive to the S-phase-dependent clastogens hydroxyurea and methyl methanesulfonate but, as previously observed for *D56N* and *H125N* mutants, were not defective in NHEJ. Importantly, the affinity of purified Mre11-D16A protein for Rad50 and Xrs2 was indistinguishable from wild type and the mutant protein formed complexes with equivalent stoichiometry. Although the role of the nuclease activity has been questioned in previous studies, the comparative data presented here suggest that the nuclease function of Mre11 is required for RMX-mediated recombinational repair and telomere stabilization in mitotic cells.

EUKARYOTIC organisms repair broken chromosomes by at least two distinct DNA repair pathways, homologous recombination and nonhomologous end-joining (NHEJ). The conserved *Saccharomyces cerevisiae* Rad50, Mre11, and Xrs2 proteins (referred to as RMX) play a unique role in that they function in both recombination and NHEJ repair. Yeast cells containing inactivated RMX genes are defective in NHEJ assays (*e.g.*, homology-independent plasmid recircularization, sensitivity to *in vivo* expression of *Eco*RI endonuclease, deletion formation within dicentric plasmids, etc.) and also exhibit reduced efficiency of DSB-induced homologous recombination (Lewis and Resnick 2000; Sung *et al.* 2000; Symington 2002). RMX mutants also have greatly increased frequencies of spontaneous chromosome rearrangements, shortened telomeres, defects in S-phase checkpoint responses to DNA damage, hypersensitivity to clastogenic chemicals and ionizing radiation and reduced recombination in meiosis (Chen and Kolodner 1999; Kouprina *et al.* 1999; Lewis and Resnick 2000; Grenon *et al.* 2001; Usui *et al.* 2001; Chang *et al.* 2002; D'Amours and Jackson 2002; Myung and Kolodner 2002).

Several of the metabolic defects described for yeast RMX mutants are also observed in mammalian cells upon inactivation of the corresponding gene orthologs. For example, mutations within the human genes hMRE11 and hNBS1 (hNBS1 is the apparent human equivalent of yeast *XRS2*) cause the human disorders Nijmegen breakage syndrome and ataxia telangiectasia-like disorder, respectively (Stewart *et al.* 1999). Cells derived from individuals with these disorders display multiple DNA damage response defects, including hypersensitivity to ionizing radiation and defective checkpoint responses. In addition, individuals with these disorders have an increased incidence of cancer (Petrini 1999; D'Amours and Jackson 2002). Further evidence suggesting a role for inactivation of the complex in cancer development has been obtained from directed sequencing of hMRE11 genes from random (Fukuda *et al.* 2001) and mismatch repair-deficient tumor cells (Giannini *et al.* 2002).

The Mre11 subunit of RMX has manganese-dependent 3′-to-5′ dsDNA exonuclease and ssDNA endonuclease activities that are active on a number of linear and circular DNA structures, including the tops of hairpin structures formed by inverted repeat sequences *in*

[1]*Corresponding author:* Department of Chemistry and Biochemistry, Texas State University, 601 University Dr., San Marcos, TX 78666. E-mail: ll18@txstate.edu

vitro and *in vivo* (Hopfner *et al.* 2000; Trujillo and Sung 2001; Lobachev *et al.* 2002). The purified enzyme also has DNA strand annealing and dissociation activities (D'Amours and Jackson 2002). Sequence comparisons have indicated that Mre11 contains five conserved sequence motifs found in many phosphodiesterase enzymes (although some reports recognize only four motifs) and these regions appear to harbor the nuclease activities of the enzyme (Baum 1995; Sharples and Leach 1995; Bressan *et al.* 1998; Tsubouchi and Ogawa 1998; Usui *et al.* 1998; Moreau *et al.* 1999; Hopfner *et al.* 2001, 2002).

The Rad50 subunit of RMX is a large ATP-binding protein whose sequence contains typical Walker A and B ATPase motifs on either side of two extended coiled-coil domains (Alani *et al.* 1990; Hopfner *et al.* 2000). The function of Xrs2 remains unclear, although recent work demonstrating protein:protein interactions between this subunit and Lif1, a component of the DNA ligase IV complex, suggest an important role in RMX-mediated repair by NHEJ (Chen *et al.* 2001). Studies of the equivalent protein in higher eukaryotes (NBS1) indicate that some activities of the complex, *e.g.* duplex DNA unwinding, are also dependent upon the presence of this subunit (Paull and Gellert 1999).

Structural studies of archaebacterial, yeast, and human Rad50 and Mre11 suggest that these proteins combine to form multimers whose unit structure consists of two molecules of each polypeptide (Anderson *et al.* 2001; Chen *et al.* 2001; De Jager *et al.* 2001; Hopfner *et al.* 2002). According to recent models, each Rad50 subunit forms a folded, antiparallel structure that places the N- and C-terminal Walker A and B motifs in proximity with each other. Association of two Mre11 molecules with the joined ends of two folded Rad50 subunits then forms the DNA-binding portion of the complex. Larger multimeric structures that can potentially form bridges between broken DNA ends or between adjacent sister chromatids have also been suggested, possibly resulting from Zn^{2+}-mediated joining of Rad50 molecules at a "hinge" or "hook" region (De Jager *et al.* 2001; Hopfner *et al.* 2002).

The specific mechanism(s) by which the RMX nuclease complex mediates repair by recombination and NHEJ, activates checkpoints, inhibits chromosome rearrangements, and stabilizes telomeres is unknown. We and others established that some DSB repair phenotypes of RMX mutants can be suppressed by overexpression of the gene encoding Exo1, a 5′-to-3′ exonuclease (and also by telomerase RNA; Lewis *et al.* 2002), suggesting that a critical function that has been lost in these mutants is DSB end-processing (Chamankhah *et al.* 2000; Symington *et al.* 2000; Tsubouchi and Ogawa 2000; Moreau *et al.* 2001; Lewis *et al.* 2002). More specifically, these experiments revealed that the nuclease activity of Exo1 could partially substitute for RMX in recombinational repair, but not repair by the NHEJ pathway. The results with Exo1 are inconsistent with recent analyses of mutants with substitutions in the conserved phosphoesterase motifs of Mre11 (*e.g.*, -D56N, -H125N, and -H125L/D126V), which suggested that nuclease activity is not required for several major functions of RMX in mitotic cells, including recombination, NHEJ, and telomere stabilization (Bressan *et al.* 1999; Moreau *et al.* 1999; Symington *et al.* 2000; Tsukamoto *et al.* 2001; Lobachev *et al.* 2002).

While the Mre11 nuclease is clearly required for processing of special DNA structures, such as meiotic DSBs containing attached proteins or certain DNA secondary structures in mitotic cells (Rattray *et al.* 2001; Lobachev *et al.* 2002; Symington 2002), previous studies of known nuclease-defective alleles observed only minor effects on repair of DSBs induced by ionizing radiation, chemicals, or site-specific endonucleases during mitotic growth. We report here that cells expressing a mutant Mre11 protein (Mre11-D16A; motif I), which is deficient in endonuclease and exonuclease activities, but which retains the ability to bind DNA and to form multimers with Mre11, has multiple DNA metabolic defects that are consistent with a role for its nuclease function(s) in recombinational repair of DSBs and telomere stabilization, but not NHEJ in mitotic cells. Several phenotypes of *mre11-D16A* cells differ only in severity from cells expressing two other Mre11 variants shown to have reduced nuclease activities *in vitro*. Together with past observations of recombination-specific suppression by *EXO1*, these results suggest that catalytic activities established for Mre11 *in vitro* are in fact important for major functions of the enzyme *in vivo* such as repair of DSBs by homologous recombination and stabilization of telomeres.

MATERIALS AND METHODS

Strains and plasmids: Yeast strains used for this work are shown in Table 1. *rad50::hisG-URA3-hisG* disruptions were generated using pNKY83 (a generous gift from N. Kleckner) digested with *Eco*RI + *Bgl*II and *exo1::URA3* disruptions were created using plasmid p244 cut with *Hin*dIII + *Kpn*I (Tran *et al.* 1999). Geneticin/G418 (BRL) and Hygromycin B (Boehringer Mannheim, Indianapolis) were added to plates for selection of resistant strains at concentrations of 200 and 300 μg/ml, respectively. 5-Fluoroorotic acid (5-FOA) used for selection of Ura^- cells was purchased from United States Biological. Methyl methanesulfonate (MMS) was obtained from Fluka (Buchs, Switzerland) and hydroxyurea (HU) was purchased from Sigma (St. Louis).

Plasmids used for expression studies were as follows: pRS314 (*CEN/ARS, TRP1*; Sikorski and Hieter 1989), pMre11-D16A (*CEN/ARS, TRP1 mre11-D16A*; this work), pSM258 (*CEN/ARS, TRP1, MRE11*; a kind gift from L. Symington), pSM304 (as pSM258, but *mre11-H125N*), pSM312 (as PSM258, but *mre11-D56N*), and pRS316Gal (Lewis *et al.* 1998).

Site-specific mutagenesis of chromosomal and plasmid loci: A recently developed technique (*delitto perfetto*; Storici *et al.* 2001) was employed to create substitutions in *MRE11* at its natural locus on chromosome XIII (Figure 1A). A DNA frag-

ment containing selectable and counterselectable markers for G418^r and *URA3* flanked by *MRE11* sequences was generated using primers MRE11.G and MRE11.U to amplify DNA in the cassette plasmid pCORE (Storici *et al.* 2001). Sequences of these and all other primers are available upon request. This fragment was used to insert the cassette into the *MRE11* gene on chromosome XIII between nucleotides (nt) G46 and A47 of the coding region. Cells were subsequently transformed with the 80-mer MRE11.a and MRE11.b and 5-FOA^r G418-sensitive cells were selected. Genomic *MRE11* DNAs from three independent transformants were sequenced and found to contain a single mutation at codon 16 from GAT to GCT, changing the coding from aspartate to alanine. Using the same approach plasmid pSM258 (*CEN/ARS, TRP1, MRE11*) was modified in an *MRE11*-deleted strain background (YLKL-555) to create pMre11-D16A. All PCR reactions utilized Platinum Pfx enzyme (GIBCO/Invitrogen).

Ends-in and ends-out chromosome recombination and NHEJ assays: Plasmid NHEJ assays were performed by LiAc transformation as previously described (Lewis *et al.* 2002) using uncut or *Bam*HI-cut pRS314 with strains VL6α (*MRE11*), YLKL503 (*mre11*Δ), and YLKL641 (*mre11-D16A*). In these experiments the uncut pRS314 DNA serves as a control for variability in transformation efficiencies among different strains.

Ends-in recombination proficiencies of cells expressing mutant *mre11* alleles were assessed using strain YLKL503 (*mre11*Δ) containing pRS314, pSM258, pSM304, pSM312, or pMre11-D16A. Cells were transformed with pLKL37Y that had been cut inside *URA3* with *Nco*I. pLKL37Y was created in the following way: A 1.2-kb *Hin*dIII *URA3* gene fragment obtained from YEp24 was made blunt with T4 DNA polymerase and cloned into *Sal*I/*Not*I-cut pRS303 that had also been made flush by extension of sticky ends with T4 DNA polymerase. The resulting plasmid, pLKL37Y, is an integrating vector containing *URA3* and *HIS3*. After digestion with *Nco*I and transformation, Ura$^+$ colonies formed by recombinational integration of the plasmid into the *ura3-52* locus on chromosome V were scored. In this assay most transformants are Ura$^+$ His$^+$ integrants (see Figure 3B), with a small fraction (≤1%) of Ura$^+$ His$^-$ cells presumed to arise by conversion of *ura3-52* on the chromosome. All transformation efficiencies (transformants per microgram of DNA) were normalized to those for uncut *CEN/ARS* plasmid DNA (pRS316Gal) transformed into the same competent cell preparations on the same day. Results presented are the mean ±SD of 3–5 experiments for each strain.

Ends-out gene conversion assays were performed using derivatives of the strain BY4742-TRP5-HP53 (Table 1). This strain contains a selectable-counterselectable HygB^r + GALp::p53-V122A CORE cassette inserted into nucleotides 1002 and 1003 of the *TRP5* gene in strain BY4742. This strain is used for quantitative analysis of oligonucleotide-mediated recombination events that result in perfect excision of the CORE cassette. The cassette used for these studies differs from the cassette previously described in Storici *et al.* (2001) in that hygromycin B resistance is selectable and resistance to the growth inhibitory effects of p53-V122A expression can be counterselected (Storici and Resnick 2003). p53-V122 is a variant of human p53 that is highly toxic to yeast cells when expressed from the *GAL1* promoter (Storici and Resnick 2003). *MRE11*, *mre11*Δ, and *mre11-D16A* cells (BY4742-TRP5-HP53, YLKL770, and YLKL771, respectively) were transformed with complementary 95-nt oligonucleotides TRP5.e and TRP5.f and frequencies of HygB^s p53$^-$ cells quantitated as described previously for recombination-dependent *delitto perfetto* mutagenesis (Storici *et al.* 2001). The *rad52*Δ control cells used for Figure 4 were identical to the above strains except that an alternative cassette, *URA3* + G418^r, was employed. BY4742-TRP5-CORE and YLKL769 were used for the latter assays.

Binding of Rad50 and Xrs2 to wild-type and mutant Mre11 and Mre11-D16A proteins: 6His-Mre11 and 6His-Mre11-D16A were purified from *Escherichia coli* strains tailored to express these proteins (Furuse *et al.* 1998). Nontagged Rad50, Mre11, and Xrs2 were overexpressed in yeast and purified to near homogeneity as described previously (Trujillo and Sung 2001; Trujillo *et al.* 2003). The concentrations of Rad50, Mre11, 6His-Mre11, 6His-Mre11-D16A, and Xrs2 were determined by densitometric scanning of 7.5% SDS-PAGE gels containing multiple loadings of the purified proteins against known amounts of bovine serum albumin run on the same gel (Trujillo *et al.* 2003).

Binding studies were conducted by incubating purified Rad50 (5 μg, 1.1 μM) or Xrs2 (2.3 μg, 0.8 μM) with and without purified Mre11 (3.5 μg, 1.5 μM) or Mre11-6His (3.5 μg, 1.5 μM) at 0° in 30 μl of B buffer (20 mM KH_2PO_4, pH 7.4, 0.5 mM EDTA, 1 mM dithiothreitol) containing 150 mM KCl, 5 μg BSA, 10 mM imidazole, and 0.01% Igepal (Sigma). After 60 min of incubation, 10 μl of nickel-NTA-agarose beads (QIAGEN, Valencia, CA) were added and the reaction mixtures were left at 0° for another 60 min, with gentle tapping every 2 min. The beads were washed twice with 30 μl of B buffer containing 20 mM imidazole before eluting the bound proteins from the nickel matrix with 30 μl of 200 mM imidazole in B buffer.

Cell survival assays: Survival after treatment with gamma radiation was monitored after exposure to a ^{137}Cesium source emitting at a dose rate of 2.7 krad/min. Two or three independent log phase cultures containing YLKL503 (*mre11*Δ) cells with pRS314 or different *MRE11* plasmids (see above) were irradiated and placed on ice and mean fractions of surviving cells were calculated after dilutions were spread onto synthetic glucose plates without tryptophan. Hydroxyurea survival assays were performed by dilution pronging and fivefold dilutions of cells as described (Lewis *et al.* 2002). Strains used for the assays were YLKL503 containing pRS314 and *MRE11* plasmids as above. Control strains were YLKL532 (Δ*rad51*) and YLKL-593 (Δ*yku70*) containing pRS314. Cells were propagated on synthetic glucose plates minus tryptophan with increasing concentrations of hydroxyurea.

RESULTS

The *mre11-D16A* mutation greatly increases sensitivity to ionizing radiation: The endo- and exonuclease activities of Mre11 reside in conserved phosphodiesterase motifs located in the amino terminus of the protein (Figure 1B; Hopfner *et al.* 2001; D'Amours and Jackson 2002). The aspartic acid residue in phosphoesterase domain I (D16) is associated with a manganese ion in the crystal structure of *Pyrococcus furiosus* Mre11 (the corresponding aspartic acid in P. f. Mre11 is the eighth residue of the protein; Hopfner *et al.* 2001). Conversion of this negatively charged residue to a neutral alanine produces a protein that has no detectable nuclease activities *in vitro*, but which retains the ability to bind to DNA and to other Mre11 molecules (Furuse *et al.* 1998). Our study was designed to assess the precise consequences of this and other substitutions known to produce proteins with no detectable nuclease activity on DSB repair capabilities in mitotic cells.

To determine the impact of the D16A substitution on DNA repair in mitotic cells, the *MRE11* locus on

TABLE 1

Yeast strains used in this study

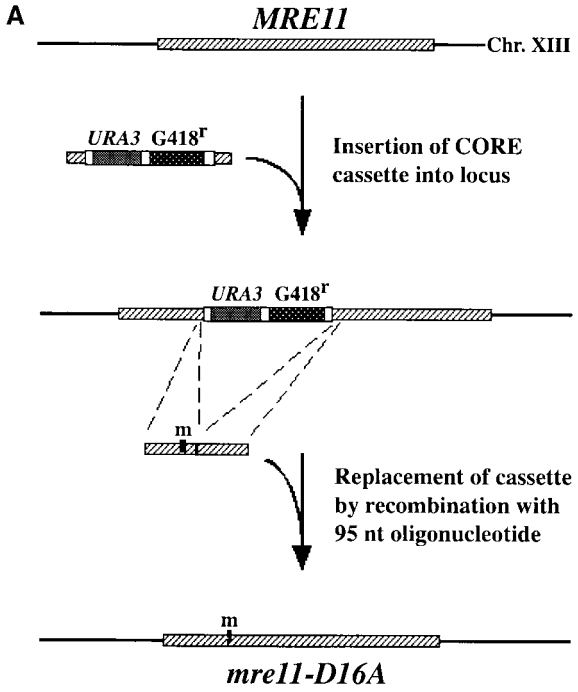
Strain	Genotype	Reference
VL6α	*MATα ura3-52 his3-Δ200 trp1-Δ63 lys2-801 ade2-101 met14*	Larionov *et al.* (1994)
YLKL499	VL6α, Δ*rad50::hisG*	Lewis *et al.* (2002)
YLKL503	VL6α, Δ*mre11::G418*	Lewis *et al.* (2002)
YLKL532	VL6α, Δ*rad51::hisG*	Lewis *et al.* (2002)
YLKL546	VL6α, Δ*exo1::G418*	Lewis *et al.* (2002)
YLKL640	VL6α, *rad50-K40A*	This work
YLKL641	VL6α, *mre11-D16A*	This work
YLKL724	YLKL641, *exo1::URA3*	This work
YLKL725	YLKL503, *exo1::URA3*	This work
VL6-48α	VL6α, Δ*leu2::G418*	V. Larionov
YLKL555	VL6-48α, Δ*mre11::HygB*	Lewis *et al.* (2002)
YLKL684	VL6-48α, Δ*rad50::hisG*	This work
YLKL593	VL6-48α, Δ*yku70::HIS3*	This work
BY4742	*MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Brachmann *et al.* (1998)
YLKL649	BY4742, Δ*rad50::G418*	This work
BY4742-TRP5-CORE	BY4742, *trp5::[G418^r KlURA3]*	Storici *et al.* (2001)
YLKL769	BY4742-TRP5-CORE, Δ*rad52:LEU2*	This work
BY4742-TRP5-HP53	BY4742, *trp5::[HygB^r p53]*	Storici and Resnick (2003)
YLKL770	BY4742-TRP5-HP53, Δ*mre11*	This work
YLKL771	BY4742-TRP5-HP53, *mre11-D16A*	This work

chromosome XIII of strain VL6α was altered by the *delitto perfetto* method of oligonucleotide-mediated, site-specific mutagenesis (Storici *et al.* 2001; Storici and Resnick 2003) as shown in Figure 1A. Initially, a two-gene "CORE" cassette was integrated into *MRE11* by PCR fragment-mediated gene targeting. One of the CORE genes provides for selection by resistance to G418 and the other for counterselection against *URA3* after subsequent transformation with oligonucleotides. After transformation of cassette-containing cells with long, complementary oligonucleotides containing one or more sequence changes, transformants containing perfectly excised cassettes were identified by 5-FOA counterselection of Ura$^-$ cells and confirmation of loss of the selectable marker (G418^r) along with sequencing of the resulting DNA locus (see materials and methods). A plasmid-borne version of *MRE11* on pSM258 was similarly converted to *mre11-D16A* after propagation in an *MRE11*-deleted strain background, producing the plasmid pMre11-D16A.

mre11 null cells are hypersensitive to killing by many physical and chemical agents that induce DSBs, including ionizing radiation. For example, haploid *mre11* mutants are fully as sensitive to ionizing radiation as strongly recombination-defective *rad51*, *rad52*, and *rad54* strains (Saeki *et al.* 1980; Lewis and Resnick 2000; Bennett *et al.* 2001). Survival of logarithmically growing cells containing *mre11-D16A* was found to be reduced at all doses tested, although cells were not as sensitive as *mre11*Δ strains (Figure 2A). In contrast, the widely studied phosphoesterase motif II and III mutants *mre11-D56N* and *-H125N* displayed near-wild-type resistance up to 20 krad, corresponding to ~10–15 DSBs per haploid genome (Resnick and Martin 1976). This result is consistent with a recent report demonstrating that the latter two mutants have a weak radiation sensitivity that becomes apparent at relatively high doses (30–70 krad; Moreau *et al.* 2001).

Past experiments have established that the 5′-to-3′ exonuclease encoded by *EXO1* can partially substitute for the RMX complex in recombinational repair of DSBs (Chamankhah *et al.* 2000; Tsubouchi and Ogawa 2000; Moreau *et al.* 2001; Lewis *et al.* 2002). Haploid *exo1* mutants are not sensitive to radiation, but *exo1 rmx* double mutants exhibit slightly more gamma sensitivity than *rmx* single mutants and reduced repair proficiency in plasmid DSB repair assays (Symington *et al.* 2000; Lewis *et al.* 2002). To assess the possibility that the residual radiation resistance of nuclease-defective *mre11-D16A* cells is due to basal level expression of Exo1, double-mutant strains were constructed and tested for radiation sensitivity. *exo1 mre11-D16A* double mutants exhibited ~10-fold more killing at 20 krad than *mre11-D16A* cells did (Figure 2B). This suggests that a large fraction of radiation-induced DSBs in *mre11-D16A* cells are processed by the 5′-to-3′ exonuclease activity of Exo1. However, killing did not reach the level of *exo1 mre11*Δ double mutants, which were slightly more sensitive than *mre11* single mutants.

Radiation-induced DSBs are repaired primarily by homologous recombinational mechanisms and current models propose that RMX initiates recombination by processing DSB ends to generate 3′ single-strand overhangs (Sung *et al.* 2000; Symington 2002). The gamma



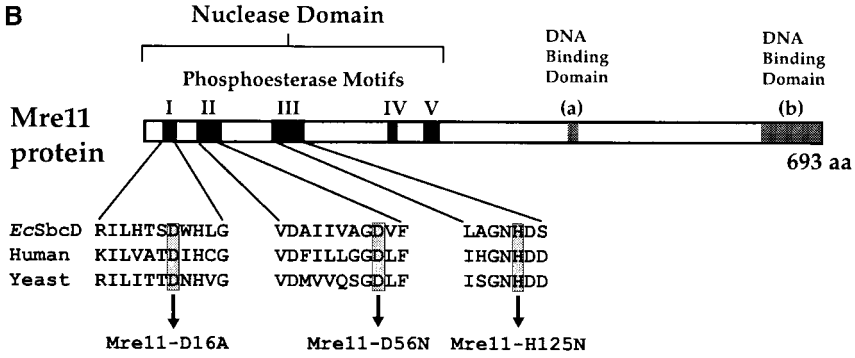


FIGURE 1.—Site-specific mutagenesis of the *MRE11* gene using oligonucleotides. (A) Schematic of the *delitto perfetto* method of oligonucleotide-mediated mutagenesis. (B) Phosphoesterase motifs within Mre11 and three substitutions resulting in proteins with reduced activity in *in vitro* assays.

sensitivity of the *mre11-D16A* mutants is in agreement with this model and may also be an indication that Mre11-D16A protein has reduced nuclease activity relative to the Mre11-D56N and Mre11-H125N enzymes (see DISCUSSION).

***mre11-D16A* cells are unable to repair a site-specific DSB by homologous recombination, but are proficient in NHEJ repair:** To address the consequences of the *MRE11* mutations on repair by the two pathways we utilized separate assays that each relied on repair of a defined DSB structure created in a plasmid (shown schematically in Figure 3, A and B). For each assay a single, cohesive-ended DSB with 5′ overhangs that were four bases long served as substrate for repair (see MATERIALS AND METHODS). Cells lacking Rad50, Mre11, or Xrs2 have reduced ability to recircularize linear plasmids *in vivo* after cell transformation if the DSB is in a region that lacks homology with chromosomal DNA. This reduction in recombination-independent repair by NHEJ, typically ~10- to 100-fold, is not observed in mutants deficient only in the recombination pathway (*e.g.*, *rad51* or *rad52*). NHEJ repair events were scored as transformant cells that had recircularized the broken plasmid under conditions where repair by homologous recombination was not possible. Repair of the DSB by NHEJ was reduced 20-fold in *mre11*Δ strains (Figure 4A). Similar to a previous report for *mre11-D56N* and *mre11-H125N* mutants (MOREAU *et al.* 1999), *mre11-D16A* strains exhibited approximately wild-type levels of NHEJ repair. This observation reinforces the idea that the nuclease functions of the complex are not required for RMX-mediated NHEJ repair. The proficiency at NHEJ also implies that each of the mutants is able to form productive RMX complexes *in vivo*.

RMX mutants exhibit reduced frequencies of ends-in (CROMIE and LEACH 2000; SYMINGTON 2002) DSB-induced plasmid:chromosome recombination. For assessment of DSB repair by recombination, cells were

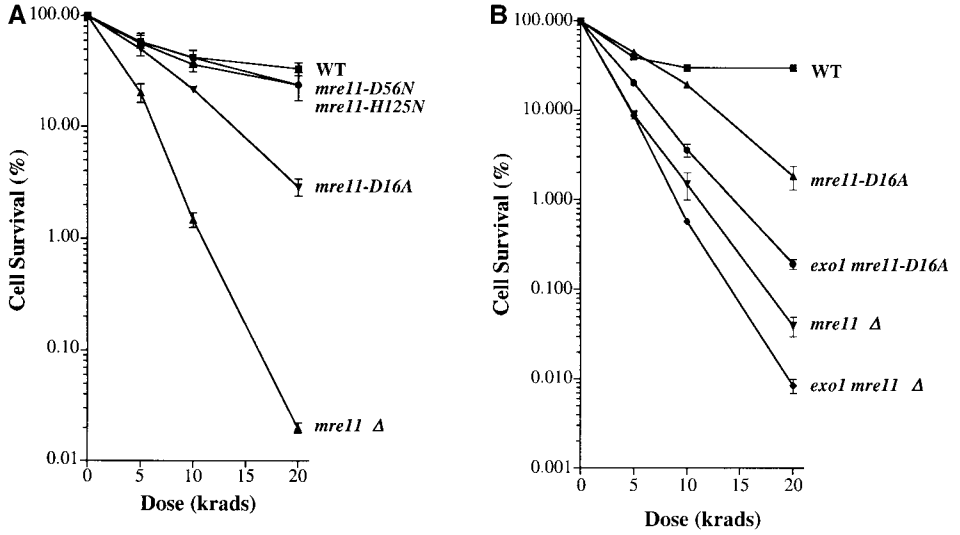


FIGURE 2.—Radiation-induced killing in three nuclease-defective mutant strains. (A) Dose response curves obtained using logarithmically growing haploid cells expressing Mre11-D16A, Mre11-D56N, or Mre11-H125N proteins. (B) Impact of inactivation of the 5′-to-3′ exonuclease activity of Exo1 on radiation sensitivities of *mre11* null and *mre11-D16A* cells.

transformed with a linearized integrating plasmid that could undergo recombination with a homologous chromosomal locus (shown schematically in Figure 3B). Cells were transformed with a *HIS3 URA3* DNA fragment (cut within *URA3* using *Nco*I) that could recombine with the chromosomal *ura3-52* locus. Nearly all Ura^+ recombinants arose from integration of the entire cut plasmid into the chromosome by ends-in recombination to produce Ura^+ His^+ cells, but a small fraction (typically ~0.5–1% in wild-type cells) of DSBs were repaired by gene conversion of the chromosomal locus to produce Ura^+ His^- colonies (see below). For all experiments, transformation efficiencies (recombinants formed per microgram of DNA) were normalized to those for uncut *CEN/ARS* plasmids transformed into the same competent cell preparations on the same day.

The efficiency of ends-in recombinational repair was reduced ~20-fold in *mre11*Δ strains (Figure 4B). Interestingly, *mre11-D16A* cells were as defective in recombinational repair of the plasmid DSBs as *mre11*Δ strains. In contrast, recombination was much higher in strains expressing the *mre11-D56N* and *-H125N* mutants (25 and 33% of wild-type levels, respectively). Over 99% of transformant colonies from wild-type cells contained integrated plasmids and were phenotypically Ura^+ His^+, with the remainder being Ura^+ His^- gene convertants. The corresponding numbers for *mre11*Δ and *mre11-D16A* cells were 99 and 96%, suggesting that crossover and noncrossover frequencies were not greatly affected.

We also determined if the severe recombination defect observed in the *mre11-D16A* cells was restricted to the types of ends-in plasmid:chromosome targeting events analyzed in Figure 4B. The chromosome mutagenesis procedure employed to create *mre11-D16A* involved replacement of a selectable-counterselectable cassette with homologous DNA contained within an oligonucleotide. This process requires a functional *RAD52* gene (STORICI *et al.* 2001) and involves the alternative, "ends-out" form of DSB-induced recombination (CROMIE and LEACH 2000; SYMINGTON 2002). The scheme used for the assays is depicted in Figure 3C. Briefly, wild-type and mutant cells containing a *HygB^r GALp::p53-V122* cassette integrated into *TRP5* were transformed with 95-mer DNA composed of upstream and downstream *TRP5* sequences as described (STORICI *et al.* 2001). Correct recombinational repair events resulted in cells that were *HygB^s p53*$^-$ and *TRP5*$^+$. As shown in Figure 4C, no recombinants were observed when *rad52* cells were assayed. Recombination frequencies in *mre11*Δ and *mre11-D16A* strains (recorded as integration events per 0.5 nmol of oligonucleotide DNA) were decreased to 2.1 and 1.3% of wild-type levels, respectively. Thus, *mre11-D16A* mutants are approximately as deficient as *mre11* null cells in both classes of recombination events.

The nuclease mutants are differentially sensitive to the S-phase clastogens HU and MMS: Exposure of cells to high levels of the ribonucleotide reductase inhibitor HU leads to replication inhibition and formation of DSBs in chromosomal DNA (MERRILL and HOLM 1999; D'AMOURS and JACKSON 2001). In contrast, low levels of HU produce few DSBs, but do result in activation of the S-phase checkpoint and killing of cells that are deficient in this checkpoint response. Early checkpoint activation events such as phosphorylation of Rad53 are inhibited and survival of RMX mutants is reduced after exposure to low levels of HU (D'AMOURS and JACKSON 2001). Like HU, the DNA-methylating agent MMS induces DSBs during replication and is lethal to mutants defective in DSB repair and the S-phase checkpoint (LEWIS and RESNICK 2000; USUI *et al.* 2001; CHANG *et al.* 2002).

We examined sensitivities of several repair-deficient mutant strains to a range of HU and MMS concentrations (Figure 5). Growth inhibition was apparent in *mre11*Δ strains at concentrations of HU as low as 5.0

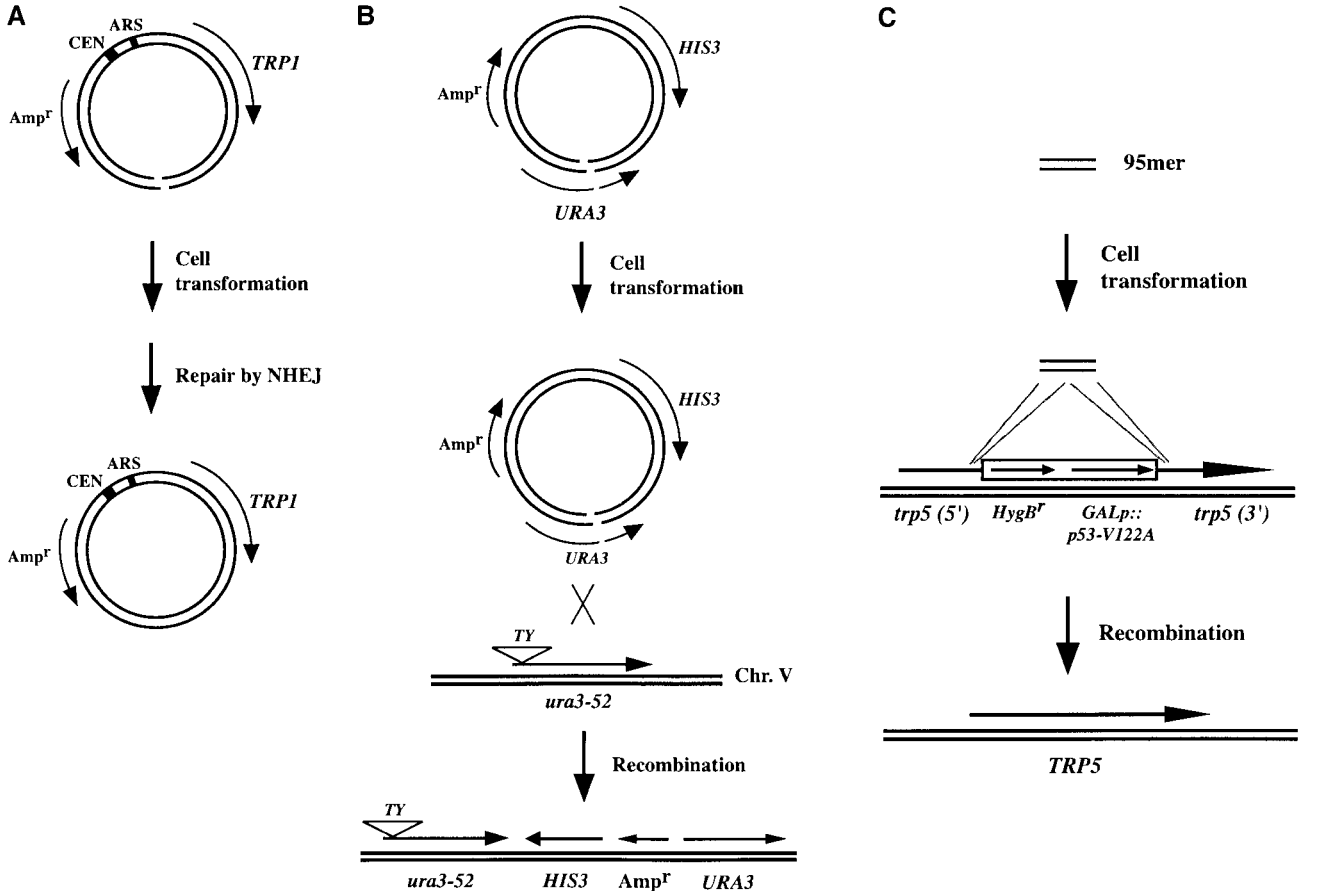


FIGURE 3.—Plasmid assay systems used to monitor repair of a single, cohesive-ended DSB by (A) NHEJ, (B) ends-in plasmid:chromosome homologous recombination, or (C) ends-out recombination using 95-mer oligonucleotide substrates.

mM. These cells were moderately more sensitive than Rec⁻ *rad51* cells and much more sensitive than NHEJ-deficient *yku70* cells. Cells expressing the phosphoesterase mutants *Mre11-D16A*, *-D56N*, and *-H125N* required much higher doses of HU to detect loss of viability than did *mre11* null cells. The *mre11-D16A* strains exhibited killing at a lower dose (40 mM) than that of either of the other nuclease mutants. A similar general pattern of survival was observed when cells were exposed to MMS (Figure 5B). Relative sensitivities could again be ordered as *mre11*Δ > *rad51*Δ > *mre11-D16A* > *mre11-D56N* or *mre11-H125N* (most sensitive to least sensitive). The greater killing of *mre11-D16A* cells compared to the other two mutants is qualitatively consistent with the radiation survival curves (Figure 2A).

Purified Mre11-D16A protein binds efficiently to Rad50 and Xrs2: Mre11 interacts with Rad50 and Xrs2 to form a trimeric complex (SUNG *et al.* 2000; SYMINGTON 2002). To ask whether Mre11-D16A protein retains the ability to bind Rad50 and Xrs2, purified six-histidine-tagged Mre11-D16A was mixed with purified Rad50 or Xrs2, and the complexes formed between the protein pairs were isolated using nickel-NTA-agarose beads, which have high affinity for the histidine tag on Mre11-D16A. We included as positive control six-histidine-tagged wild-type Mre11 protein. As shown in Figure 6, A and B, while Rad50 and Xrs2 have no affinity for the nickel-NTA-agarose beads, a substantial portion of these two proteins became associated with the beads when tagged Mre11-D16A was present, indicating complex formation. Importantly, the histidine-tagged Mre11-D16A protein has the same affinity for Rad50 and Xrs2 as histidine-tagged wild-type Mre11 (Figure 6, A and B, lanes 4 and 8). Consistent with the affinity pulldown results, Mre11-D16A forms a trimeric complex with Rad50 and Xrs2 that has a component stoichiometry indistinguishable from that assembled with wild-type Mre11 (CHEN *et al.* 2001; data not shown). These results, in conjunction with the previous work of FURUSE *et al.* (1998), demonstrate that Mre11-D16A protein is proficient at both DNA binding and RMX complex formation.

DISCUSSION

The RMX complex is required for successful completion of several specific DNA metabolic processes in mitotic cells. These functions include repair by recombination and end-joining, telomere length maintenance, DNA replication-associated cell cycle checkpoints, inhi-

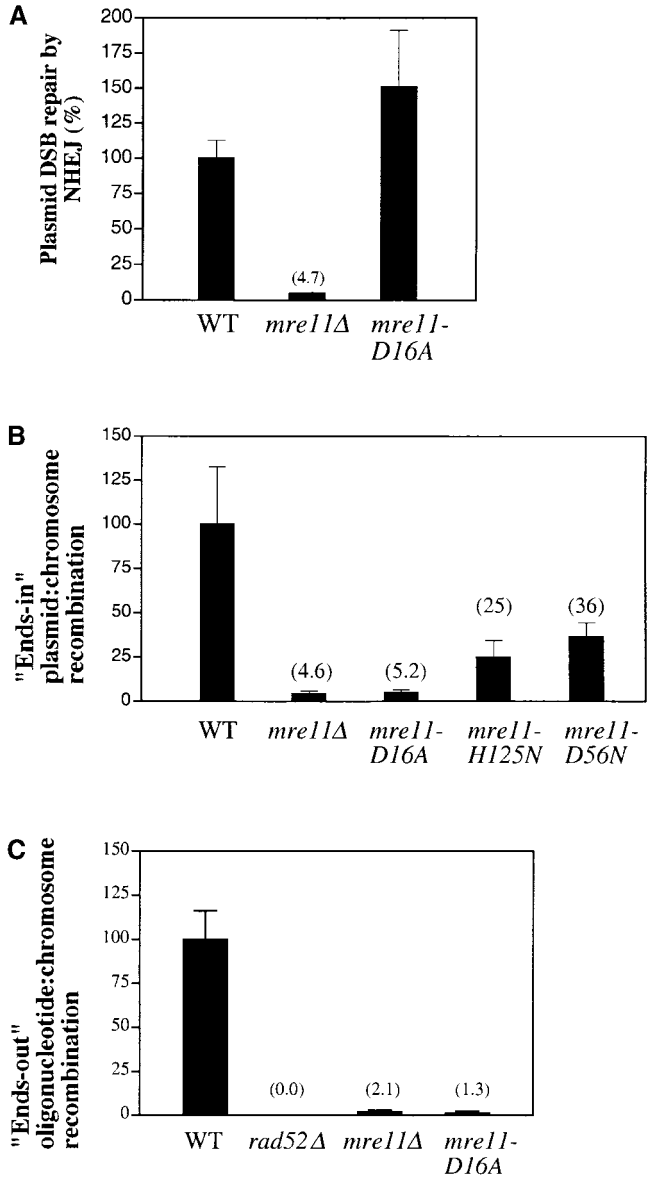


FIGURE 4.—DSB repair proficiencies of haploid cells expressing altered Mre11 proteins with reduced nuclease activity. Efficiencies of repair in wild-type cells by (A) end-joining, (B) ends-in recombination, and (C) ends-out recombination. Transformation efficiencies (repair events per microgram of DNA) in A and B were normalized to those for uncut *CEN/ARS* plasmids transformed into the same competent cell preparations on the same day. Numbers in parentheses indicate means derived from 3–5 assays for each strain. No recombinants were detected for *rad52*Δ strains in C. Error bars indicate standard deviations.

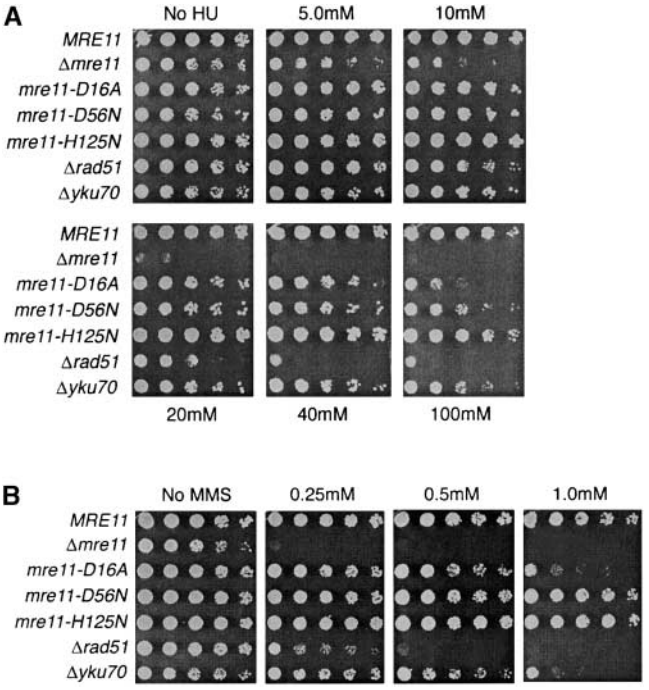


FIGURE 5.—Assessment of S-phase clastogen sensitivities of *mre11-D16A*, *mre11-D56N*, and *mre11-H125N* mutants. Haploid yeast cells were pronged to synthetic glucose plates lacking tryptophan and containing increasing concentrations of (A) hydroxyurea or (B) MMS.

bition of gross chromosomal rearrangements and processing of transiently formed DNA secondary structures such as hairpins (summarized in D'AMOURS and JACKSON 2002; SYMINGTON 2002). Several recent studies have examined possible correlations between the nuclease activities of the purified complex detected *in vitro* and the multiple roles *in vivo*. Most experiments focused on expression and characterization of mutant Mre11 proteins containing alterations within one or more conserved phosphoesterase motifs in the nuclease domain. Four mutant proteins described in the literature, Mre11-D16A (motif I, FURUSE *et al.* 1998), Mre11-D56N and Mre11-H125N (motifs II and III, MOREAU *et al.* 1999; SYMINGTON *et al.* 2000; D'AMOURS and JACKSON 2001; RATTRAY *et al.* 2001; TSUKAMOTO *et al.* 2001; LOBACHEV *et al.* 2002), and Mre11-H213Y (motif IV, TSUBOUCHI and OGAWA 1998; USUI *et al.* 1998; CHAMANKHAH and XIAO 1999; LEE *et al.* 2002), have been evaluated for both *in vitro* nuclease activities and multiple *in vivo* consequences. The D16, D56, and H213 residues are each associated with an Mn^{2+} ion in the crystal structure of Mre11, while the histidine at position 125 is thought to be involved in the phosphodiester hydrolysis reaction (HOPFNER *et al.* 2001).

Characteristics of cells expressing each of the mutant proteins are summarized in Table 2. An additional less well-characterized mutant, *mre11-H125L/D126V*, was included in the table because of its similarity to the *mre11-H125N* allele, although nuclease activities of this protein have not been measured *in vitro*. One of the mutants listed in the table, *mre11-H213Y*, behaves essentially like a null mutation in most *in vivo* assays and is also defective in protein:protein interactions. Thus, this protein is deficient in nuclease activities and also in other functions of the enzyme.

Three of the mutant proteins depicted in Table 2 (D56N, H125N, and D16A) are particularly useful for

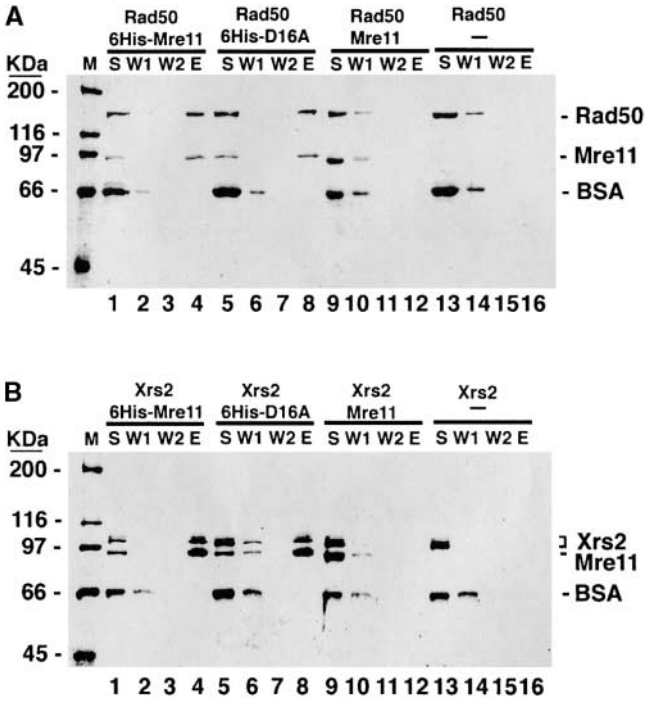


FIGURE 6.—Analysis of binding of wild-type Mre11 and mutant Mre11-D16A protein to Rad50 and Xrs2. (A) Rad50 alone, Rad50 with nontagged Mre11, Rad50 with 6His-Mre11, or Rad50 with 6His-Mre11-D16A (6His-D16A) were incubated with nickel-NTA-agarose beads and washed twice with buffer before the bound proteins were eluted with 200 mM imidazole. The supernatant (S), first wash (W1), second wash (W2), and eluate (E) fractions were run in a 7.5% denaturing polyacrylamide gel followed by staining with Coomassie blue. (B) Xrs2 alone, Xrs2 with Mre11, Xrs2 with 6His-Mre11, and Xrs2 with 6His-Mre11-D16A (6His-D16A) were incubated with nickel-NTA-agarose and bound proteins were eluted as in A. Note that binding of Rad50 and Xrs2 to the nickel-NTA matrix is dependent on the histidine tag on the Mre11 protein (compare lanes 4 and 12 in both A and B).

analysis of cellular requirements for the Mre11 nuclease activities. Each of these proteins has been reported to have no detectable nuclease activities *in vitro*, but the mutant proteins retain many Mre11 functions. For example, each of the proteins is proficient for DNA repair by NHEJ and the purified proteins are capable of RMX complex formation *in vitro* (SYMINGTON 2002; Figure 6; Table 2).

Several common DNA repair and chromosome stability defects are found in cells expressing the altered proteins. For example, all of the mutants are unable to complete meiotic DSB processing. In addition, each mutant is more sensitive than wild-type cells to ionizing radiation, MMS, and HU. *mre11-D16A* cells consistently demonstrated a stronger sensitivity to the clastogens than did the *D56N* and *H125N* mutants. In the two assays of recombinational repair of a defined DSB presented here, the *D16A* mutant behaved as a null while the *D56N* and *H125N* mutants displayed modest reductions. This result is qualitatively consistent with the relative radiation, MMS, and HU sensitivities. Another property of *mre11-D16A* strains is that telomeres are shortened in these mutants, unlike *mre11-D56N* or *mre11-H125N* cells (FURUSE *et al.* 1998; MOREAU *et al.* 1999). This property has previously been observed in RMX-deleted cells and in strains containing deletions of other NHEJ genes, including *YKU70*, *YKU80*, *SIR2*, *SIR3*, and *SIR4* (LEWIS and RESNICK 2000). If this defect in telomere maintenance is due to a greater reduction in nuclease activity in the Mre11-D16A protein (discussed below), it would be supportive of models that postulate a role for RMX in processing of chromosome ends to generate single-stranded DNA overhangs (DIEDE and GOTTSCHLING 2001).

Of central importance is the question of why the *D16A* mutant has more severe defects in mitotic cells than the other phosphoesterase mutants do. The RMX complex has ssDNA endonuclease and 3′-to-5′ dsDNA exonuclease activities, as well as a weak DNA helicase activity. In addition, the Mre11 subunit forms specific associations with DNA, Rad50, Xrs2, and possibly other proteins (Sae2?) and may also be subject to post-translational modification in mitotic cells (D'AMOURS and JACKSON 2002; SYMINGTON 2002). Although each of these activities and associations may vary in the three nuclease mutants, we favor the simplest explanation; *i.e.*, the D16A protein is more defective than the other mutants in nuclease processing of DSBs *in vivo*.

Support for this proposal comes from several considerations. First, many phenotypic differences between the mutants are simply a matter of degree. For example, radiation, MMS, and HU sensitivities and plasmid:chromosome recombination are reduced in all of the mutants and *mre11-D16A* cells are simply more defective than the others.

Second, studies utilizing either overexpression or inactivation of *EXO1* in RMX mutants also provide support. Overexpression of the 5′-to-3′ exo activity of Exo1 partially rescues repair of DSBs induced by radiation, MMS, *Eco*RI, and HO in RMX mutants, as well as the mitotic recombination defects of the mutants (LEE *et al.* 2002; LEWIS *et al.* 2002; SYMINGTON 2002 and references within). This effect is likely due to enhanced processing of the broken DNA ends by Exo1 to create 3′ tailed substrates for the Rad51/Rad52 strand exchange complex. We note, however, that *EXO1* overexpression does not rescue meiotic DSB repair (involving removal of DNA ends containing attached protein by Mre11 endonuclease activity), inverted repeat-stimulated recombination (thought to involve endonuclease cleavage of hairpin loops), or shortening of telomeres (which might also involve endo cleavage of T-loop structures; DIEDE and GOTTSCHLING 2001; SYMINGTON 2002; K. LOBACHEV and M. RESNICK, unpublished results). These latter results clearly point to the importance of the endonuclease activity of Mre11 *in vivo*. The endo activity may also be important in resection of damage-induced DSB ends in mitotic cells, possibly in conjunction with the

TABLE 2

Impact of Mre11 proteins with reduced *in vitro* nuclease activities on DNA repair and stability

Allele	*In vitro* nuclease activities		Associations			Plasmid NHEJ	Spontaneous diploid recombination	Survival		DSB repair by recombination		Telomere stability	Meiotic recombination
	Endo	Exo	DNA	Mre11	R/X[a]			Radiation	MMS	Ends-in	Ends-out		
MRE11	+	+	+	+	+	+	+	+	+	+	+	+	+
mre11Δ	NA	NA	NA	NA	NA	−−−	+++	−−−	−−−	−−−	−−−	−−−	−−−
mre11-D16A	−−−	−−−	+	+	+	+	+	−−	−−	−−−	−−−	−−	−−−
mre11-D56N	−−−	−−−	ND	ND	+	+	+	+/−[b]	+/−[b]	−	ND	+	−−−
mre11-H125N	−−−	−−−	ND	ND	+	+	+	+/−[b]	+/−[b]	−	ND	+	−−−
mre11-H125L/D126V (*mre11-3*)	ND	ND	ND	ND	+	ND	+	+/−[b]	+	ND	ND	+	−−−
mre11-H213Y (*mre11-58*)	−−−	−−−	ND	−−−	−−−	ND	+++	ND	−−/−−−[c]	ND	ND	−−	−−−

References are as follows: Mre11-D56N and Mre11-H125N (Moreau *et al.* 1999; Symington 2002; this work), Mre11-H125L/D126V (Bressan *et al.* 1998; Lee *et al.* 2002), Mre11-D16A (Furuse *et al.* 1998; this work), and Mre11-H213Y (Mre11-58; Tsubouchi and Ogawa 1998; Usui *et al.* 1998; Chamankhah and Xiao 1999; Lee *et al.* 2002). NA, not applicable; ND, no data; +, wild-type efficiency; +++, higher than wild type; −, slight deficiency; −−, moderate deficiency; −−−, strong deficiency.

[a] Binding of mutant Mre11 protein to Rad50/Xrs2.

[b] Mutants exhibit more killing than wild-type cells only at high doses of radiation (>30 krad) or high concentrations of MMS (this work; Moreau *et al.* 1999); survival of *mre11-H125L/D126V* mutants was also near wild type over the dose range 0–30 krad (Bressan *et al.* 1998).

[c] Different levels of MMS sensitivity were reported (Tsubouchi and Ogawa 1998; Usui *et al.* 1998; Lee *et al.* 2002).

weak helicase activity of the complex (Trujillo and Sung 2001; Symington 2002). The major point here is that increased levels of a nuclease (Exo1) rescues clastogen sensitivities and recombination defects of RMX mutants during mitotic growth, suggesting that nuclease processing is the function that is missing.

Analyses of the ionizing radiation sensitivities of *mre11-D16A* and *mre11-H125* mutants with and without a functional *EXO1* gene present also lend support to this premise. *mre11-D16A* strains were more sensitive than the other nuclease mutants and *mre11-D16A exo1* double mutants exhibited a linear, dose-dependent reduction in survival that was greater than that of *mre11-D16A* single mutants (~10-fold difference at 20 krad). This indicates that much of the resistance in the *D16A* single mutants was due to basal levels of Exo1. The strong sensitivity of these cells and its dependence on Exo1 seem most consistent with the idea that very little or no nuclease activity is retained in the Mre11-D16A complex *in vivo*, although other factors may also be involved.

In contrast to results with D16A, radiation survival was high in *mre11-H125N* mutants and was not reduced further in *mre11-H125N exo1* double mutants at doses up to 30 krad (Moreau *et al.* 2001; Figure 2), which corresponds to ~40 DSBs per G_2 cell (Resnick and Martin 1976). If the Mre11-H125N protein is nuclease deficient, then this result would indicate that cells lacking both RMX and Exo1 nuclease activities are largely proficient at processing of radiation-induced DSBs for recombinational repair. Put another way, this would mean that the major enzymatic activities defined for Mre11 (and Exo1) *in vitro* are not essential for a major function of the complex *in vivo* (repair of chemically and physically induced DSBs). It seems more likely that survival is high in *mre11-H125N* cells lacking the "backup" Exo1 nuclease activity because the mutant RMX complex has residual nuclease activity *in vivo*.

Another question that must be addressed is the following: If the nuclease activity of *mre11-D16A* mutants is absent (or greatly reduced), why is radiation resistance not reduced to the level of *mre11* null strains? We suggest that an important difference here is the presence or absence of the RMX complex bound to DSB ends. Structural studies have indicated that two Mre11 molecules bind to the proximal ends of two folded, fibrous Rad50 subunits to form the DNA-binding portion of the complex (Anderson *et al.* 2001; Chen *et al.* 2001; De Jager *et al.* 2001; Hopfner *et al.* 2002). The structures imply that RMX might potentially form a bridge between two DNA ends in a broken molecule or between adjacent sister chromatids in a replicated chromosome. The latter structure would be consistent with the observation that Rad50 is structurally similar to SMC proteins required for sister chromatid cohesion (Hopfner *et al.* 2000). It is possible that this "tethering" function of RMX is retained in the mutant Rad50/Mre11-D16A/Xrs2 complex, although nuclease activities are reduced. We infer that this tethering, combined with redundant nuclease activities, provides an explanation for the observation that the radiation sensitivity of *mre11-D16A* cells did not reach that of *mre11*Δ cells. After exposure to ionizing radiation, the tethering function would keep sister chromatids (or possibly broken DNA ends) in proximity and enhance the likelihood that a break is processed by Exo1 or another partially redundant nuclease and repaired by the dominant pathway of radiation repair in yeast, homologous recombination.

mre11-D16A mutants were not as radiation sensitive as *mre11*Δ cells, but they were as defective as null cells in the ends-in and ends-out recombination assays. It is possible that the impact of RMX DNA bridging is less in the plasmid:chromosome and oligonucleotide:chromosome DSB repair assays than in the radiation survival assays, since the latter are almost completely dependent on sister chromatid exchanges. DNA tethering by mutant RM*X complexes may also explain why spontaneous recombination rates of diploid cells are not elevated in the three mutants with reduced nuclease activities (Table 2). Unlike other RAD52 group mutants, diploid strains lacking RMX display increased spontaneous recombination between homologous chromosomes, possibly because of a reduced preference for interactions between sister chromatids (Symington 2002). The absence of high spontaneous recombination rates in the three nuclease mutants may be an indication that RMX complexes containing Mre11-D16A, Mre11-D56N, and Mre11-H125N are still capable of forming bridges between sister chromatids, and therefore the strong preference for sister-sister recombination has been retained.

mre11-D56N and *mre11-H125N* mutants have only slight reductions in mitotic DSB repair, but they show strong defects in assays of inverted repeat-stimulated recombination in mitotic cells and DSB processing in meiotic cells (this work; Rattray *et al.* 2001; Lobachev *et al.* 2002; Symington 2002). The latter two processes are likely to involve endonucleolytic cleavage of transiently formed hairpin structures and protein-bound DNA ends, respectively, and they cannot be rescued by overexpression of *EXO1*. It is possible that the mutant D56N and H125N complexes have a reduced level of endonuclease activity *in vivo* and that the type of end-processing required for these structures cannot be supplied by backup enzymes such as Exo1. If this is true, then the reduced levels of RMX endonuclease activity in the mutants might be limiting for these repair events, but not for others that can also be performed by redundant nucleases. Other possibilities, such as impacts on helicase activity or postendonucleolytic processing by the exonuclease cannot be ruled out, however.

Finally, we note that D16 of *S. cerevisiae* Mre11 is completely conserved among many related yeasts (H125 also), but D56 is changed to a valine in the yeast *S. kluyveri* (Saccharomyces Genome Database; http://www.

yeastgenome.org/). The reduced evolutionary conservation of this aspartic acid, one of several residues found in association with Mn^{2+} ions in the *P. furiosus* Mre11 crystal structure, suggests that its contributions to the phosphodiesterase reaction may be less critical than those of other residues such as D16.

In summary, cells expressing Mre11-D16A exhibit several dramatic mitotic DNA repair defects that are more severe than those seen in two widely studied phosphoesterase mutants with reduced *in vitro* nuclease activities. The mutant protein exhibits normal RMX complex formation and DNA binding *in vitro* and *mre11-D16A* cells are proficient at NHEJ repair *in vivo*. We suggest that the strong radiation sensitivity and recombination defects are due primarily to lack of nuclease processing by the mutant Rad50/Mre11-D16A/Xrs2 complex. This conclusion is contrary to those of previous mutant studies proposing a limited role for the Mre11 nuclease activity in mitotic cells (*e.g.*, Bressan *et al.* 1999; Moreau *et al.* 1999; Symington *et al.* 2000; Tsukamoto *et al.* 2001; Lobachev *et al.* 2002) and suggests the possibility that some mutants such as *mre11-D56N* and *-H125N* may have residual nuclease activity *in vivo*. We note that more subtle distinctions are also possible. For example, the endo- and exonuclease activities, whose precise roles in DNA processing *in vivo* remain unclear, may be differentially affected in the mutants. It is likely that the residual radiation resistance in haploid *mre11-D16A* mutants and the absence of high spontaneous recombination in *mre11-D16A* diploids arise, at least in part, because the mutant complex retains the ability to tether sister chromatids and/or DSB ends. It is intriguing that telomeres are shortened in *mre11-D16A* cells. This result might also be due to a greater loss of nuclease activities in this mutant and supports the idea that chromosome ends (possibly forming T-loop structures) may require processing by RMX to create substrates for DNA replication by telomerase (Diede and Gottschling 2001).

The authors thank James Mason and Kirill Lobachev for critical reviews of the manuscript and Kunihiro Ohta for His6-tagged Mre11 constructs. We also thank Brian Wasko for expert technical assistance. K. Lewis was supported by DOE grant 8333777. S. Van Komen and P. Sung were supported by National Institutes of Health grant ES07061.

LITERATURE CITED

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.

Anderson, D. E., K. M. Trujillo, P. Sung and H. P. Erickson, 2001 Structure of the Rad50-Mre11 DNA repair complex from *Saccharomyces cerevisiae* by electron microscopy. J. Biol. Chem. **276:** 37027–37033.

Baum, B., 1995 Mre11 and *S. pombe* Rad32 are phosphoesterases. Yeast Update Newsl. **3** (7): 1.

Bennett, C. B., L. K. Lewis, G. Karthikeyan, K. S. Lobachev, Y. H. Jin *et al.*, 2001 Genes required for ionizing radiation resistance in yeast. Nat. Genet. **29:** 426–434.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Bressan, D. A., H. A. Olivares, B. E. Nelms and J. H. Petrini, 1998 Alteration of N-terminal phosphoesterase signature motifs inactivates *Saccharomyces cerevisiae* Mre11. Genetics **150:** 591–600.

Bressan, D. A., B. K. Baxter and J. H. Petrini, 1999 The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 7681–7687.

Chamankhah, M., and W. Xiao, 1999 Formation of the yeast Mre11-Rad50-Xrs2 complex is correlated with DNA repair and telomere maintenance. Nucleic Acids Res. **27:** 2072–2079.

Chamankhah, M., T. Fontanie and W. Xiao, 2000 The *Saccharomyces cerevisiae mre11(ts)* allele confers a separation of DNA repair and telomere maintenance functions. Genetics **155:** 569–576.

Chang, M., M. Bellaoui, C. Boone and G. W. Brown, 2002 A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage. Proc. Natl. Acad. Sci. USA **99:** 16934–16939.

Chen, C., and R. D. Kolodner, 1999 Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. Nat. Genet. **23:** 81–85.

Chen, L., K. Trujillo, W. Ramos, P. Sung and A. E. Tomkinson, 2001 Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. Mol. Cell **8:** 1105–1115.

Cromie, G. A., and D. R. Leach, 2000 Control of crossing over. Mol. Cell **6:** 815–826.

D'Amours, D., and S. P. Jackson, 2001 The yeast Xrs2 complex functions in S phase checkpoint regulation. Genes Dev. **15:** 2238–2249.

D'Amours, D., and S. P. Jackson, 2002 The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. Nat. Rev. Mol. Cell. Biol. **3:** 317–327.

De Jager, M., J. Van Noort, D. C. Van Gent, C. Dekker, R. Kanaar *et al.*, 2001 Human Rad50/Mre11 is a flexible complex that can tether DNA ends. Mol. Cell **8:** 1129–1135.

Diede, S. J., and D. E. Gottschling, 2001 Exonuclease activity is required for sequence addition and Cdc13p loading at a de novo telomere. Curr. Biol. **11:** 1336–1340.

Fukuda, T., T. Sumiyoshi, M. Takahashi, T. Kataoka, T. Asahara *et al.*, 2001 Alterations of the double-strand break repair gene MRE11 in cancer. Cancer Res. **61:** 23–26.

Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata *et al.*, 1998 Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J. **17:** 6412–6425.

Giannini, G., E. Ristori, F. Cerignoli, C. Rinaldi, M. Zani *et al.*, 2002 Human MRE11 is inactivated in mismatch repair-deficient cancers. EMBO Rep. **3:** 248–254.

Grenon, M., C. Gilbert and N. F. Lowndes, 2001 Checkpoint activation in response to double-strand breaks requires the Mre11/Rad50/Xrs2 complex. Nat. Cell Biol. **3:** 844–847.

Hopfner, K. P., A. Karcher, D. S. Shin, L. Craig, L. M. Arthur *et al.*, 2000 Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell **101:** 789–800.

Hopfner, K. P., A. Karcher, L. Craig, T. T. Woo, J. P. Carney *et al.*, 2001 Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell **105:** 473–485.

Hopfner, K. P., L. Craig, G. Moncalian, R. A. Zinkel, T. Usuit *et al.*, 2002 The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature **418:** 562–566.

Kouprina, N., N. Nikolaishvili, J. Graves, M. Koriabine, M. A. Resnick *et al.*, 1999 Integrity of human YACs during propagation in recombination-deficient yeast strains. Genomics **56:** 262–273.

Larionov, V., N. Kouprina, N. Nikolaishvili and M. A. Resnick, 1994 Recombination during transformation as a source of chimeric mammalian artificial chromosomes in yeast (YACs). Nucleic Acids Res. **22:** 4154–4162.

Lee, S. E., D. A. Bressan, J. H. J. Petrini and J. E. Haber, 2002 Complementation between N-terminal *Saccharomyces cerevisiae mre11*

alleles in DNA repair and telomere length maintenance. DNA Repair **1:** 27–40.

Lewis, L. K., and M. A. Resnick, 2000 Tying up loose ends: nonhomologous end-joining in *Saccharomyces cerevisiae*. Mutat. Res. **451:** 71–89.

Lewis, L. K., J. M. Kirchner and M. A. Resnick, 1998 Requirement for end-joining and checkpoint functions, but not *RAD52*-mediated recombination after *Eco*RI endonuclease cleavage of *Saccharomyces cerevisiae* DNA. Mol. Cell. Biol. **18:** 1891–1902.

Lewis, L. K., G. Karthikeyan, J. W. Westmoreland and M. A. Resnick, 2002 Differential suppression of DNA repair deficiencies of yeast *rad50, mre11* and *xrs2* mutants by *EXO1* and *TLC1* (the RNA component of telomerase). Genetics **160:** 49–62.

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

Merrill, B. J., and C. Holm, 1999 A requirement for recombinational repair in *Saccharomyces cerevisiae* is caused by DNA replication defects of *mec1* mutants. Genetics **153:** 595–605.

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.

Moreau, S., E. A. Morgan and L. S. Symington, 2001 Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. Genetics **159:** 1423–1433.

Myung, K., and R. D. Kolodner, 2002 Suppression of genome instability by redundant S-phase checkpoint pathways in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **99:** 4500–4507.

Paull, T. T., and M. Gellert, 1999 Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. Genes Dev. **13:** 1276–1288.

Petrini, J. H., 1999 The mammalian Mre11-Rad50-Nbs1 protein complex: integration of functions in the cellular DNA-damage response. Am. J. Hum. Genet. **64:** 1264–1269.

Rattray, A. J., C. B. McGill, B. K. Shafer and J. N. Strathern, 2001 Fidelity of mitotic double-strand-break repair in *Saccharomyces cerevisiae*: a role for *SAE2/COM1*. Genetics **158:** 109–122.

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.

Saeki, T., I. Machida and S. Nakai, 1980 Genetic control of diploid recovery after gamma-irradiation in the yeast *Saccharomyces cerevisiae*. Mutat. Res. **73:** 251–265.

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.

Stewart, G. S., R. S. Maser, T. Stankovic, D. A. Bressan, M. I. Kaplan *et al.*, 1999 The DNA double-strand break repair gene hMRE11 is mutated in individuals with an Ataxia-Telangiectasia-like disorder. Cell **99:** 577–587.

Storici, F., and M. A. Resnick, 2003 *Delitto perfetto* targeted mutagenesis in yeast with oligonucleotides, pp. 191–209 in *Genetic Engineering*, Vol. 25, edited by J. K. Setlow. Kluwer Academic/Plenum Publishers, Dordrecht, The Netherlands.

Storici, F., L. K. Lewis and M. A. Resnick, 2001 *In vivo* site-directed mutagenesis using oligonucleotides. Nat. Biotechnol. **19:** 773–776.

Sung, P., K. M. Trujillo and S. Van Komen, 2000 Recombination factors of *Saccharomyces cerevisiae*. Mutat. Res. **451:** 257–275.

Symington, L. S., 2002 Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. **66:** 630–670.

Symington, L. S., L. E. Kang and S. Moreau, 2000 Alteration of gene conversion tract length and associated crossing over during plasmid gap repair in nuclease-deficient strains of *Saccharomyces cerevisiae*. Nucleic Acids Res. **28:** 4649–4656.

Tran, H. T., D. A. Gordenin and M. A. Resnick, 1999 The 3′→5′ exonucleases of DNA polymerases delta and epsilon and the 5′→3′ exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 2000–2007.

Trujillo, K. M., and P. Sung, 2001 DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50:Mre11 complex. J. Biol. Chem. **276:** 35458–35464.

Trujillo, K. M., D. H. Roh, L. Chen, S. Van Komen, A. Tomkinson *et al.*, 2003 Yeast Xrs2 binds DNA and helps target Rad50 and Mre11 to DNA ends. J. Biol. Chem. **278:** 48957–48964.

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.

Tsubouchi, H., and H. Ogawa, 2000 Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. Mol. Biol. Cell **11:** 2221–2233.

Tsukamoto, Y., A. K. Taggart and V. A. Zakian, 2001 The role of the Mre11-Rad50-Xrs2 complex in telomerase-mediated lengthening of *Saccharomyces cerevisiae* telomeres. Curr. Biol. **11:** 1328–1335.

Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa *et al.*, 1998 Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell **95:** 705–716.

Usui, T., H. Ogawa and J. H. Petrini, 2001 A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol. Cell **7:** 1255–1266.

Communicating editor: A. Nicolas