The *Saccharomyces cerevisiae mre11(ts)* Allele Confers a Separation of DNA Repair and Telomere Maintenance Functions

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ABSTRACT

The yeast Mre11 protein participates in important cellular functions such as DNA repair and telomere maintenance. Analysis of structure-function relationships of Mre11 has led to identification of several separation-of-function mutations as well as N- and C-terminal domains essential for Mre11 meiotic and mitotic activities. Previous studies have established that there is a strong correlation between Mre11 DNA repair and telomere maintenance functions and that Mre11-Rad50-Xrs2 complex formation appears to be essential for both of these activities. Here we report that the *mre11(ts)* allele, previously shown to cause temperature-dependent defects in DNA repair and meiosis, confers a temperature-independent telomere shortening, indicating that *mre11(ts)* is a separation-of-function mutation with respect to DNA repair and telomere maintenance. In a yeast two-hybrid system, Mre11(ts) fails to form a homodimer or interact with Rad50 and Xrs2 irrespective of experimental temperatures. These observations collectively suggest that the $Pro_{162}Ser$ substitution in Mre11(ts) confers a novel separation of Mre11 mitotic functions. Moreover, we observed that while overexpression of the 5′-3′ exonuclease gene *EXO1* partially complements the MMS sensitivity of *mre11*, *rad50*, and *xrs2* null mutants, it has no effect on telomere shortening in these strains. This result provides additional evidence on possible involvement of distinctive mechanisms in DNA repair and telomere maintenance by the Mre11-Rad50-Xrs2 complex.

THE *Saccharomyces cerevisiae MRE11* gene plays key roles in a variety of mitotic and meiotic pathways. Among the many important mitotic functions of Mre11 are DNA repair (Johzuka and Ogawa 1995; Nairz and Klein 1997) and its requirement for the maintenance of chromosomal telomeric repeats (Boulton and Jackson 1998; Nugent *et al.* 1998). The *mre11* null mutant is highly sensitive to X and γ-rays and simple DNA alkylating agents such as methyl methanesulfonate (MMS; Johzuka and Ogawa 1995; Nairz and Klein 1997; Chamankhah and Xiao 1998). It also displays telomere shortening (Boulton and Jackson 1998), a phenotype that is attributed to its involvement in DNA replication at telomeres (Nugent *et al.* 1998). Sequence alignments suggest that Mre11 is a member of a family of nucleases including *Escherichia coli* SbcD (Sharples and Leach 1995) and subsequent *in vitro* biochemical studies revealed that purified Mre11 is a single-stranded DNA (ssDNA) endonuclease, 3′- to 5′-ssDNA exonuclease, and 3′- to 5′-dsDNA exonuclease (Furuse *et al.* 1998; Paull and Gellert 1998; Usui *et al.* 1998; Moreau *et al.* 1999).

In a multifunctional gene like *MRE11*, identification of mutations that could separate one activity from another often leads to important clues on the biochemical functions of the protein. Ogawa *et al.* (1995) provided the first evidence that Mre11 plays a role in both formation and processing of double-strand breaks (DSBs) during meiosis. When the temperature-sensitive *mre11-1 (ts)* diploid was allowed to proceed to meiosis at permissive temperature, DSBs formed, but upon a shift to restrictive temperature these breaks were not processed and accumulated (Ogawa *et al.* 1995). Later, a separation-of-function (*S*) mutant, *mre11S*, was isolated that was proficient in the formation of DSBs during meiosis, while being deficient in the processing step (Nairz and Klein 1997). Tsubouchi and Ogawa (1998) also demonstrated that a previously known *rad58* mutation is allelic to *mre11* and carries two amino acid substitutions, one of which ($His_{213}Tyr$) exhibits an *S* phenotype during meiosis. Subsequent site-directed mutagenesis of the conserved amino acids in phosphoesterase signature motifs (Furuse *et al.* 1998; Moreau *et al.* 1999) and deletion of DNA binding site A located within amino acids 407–421 (Usui *et al.* 1998) led to other *mre11S* mutants. These studies revealed that while the DNA binding site B comprising the C-terminal 49 amino acids is responsible for the formation of meiotic DSBs, the nuclease activity of Mre11 as well as its DNA binding at site A are essential for the processing of these breaks.

Contrary to its meiotic functions, no solid correlation has been established between different domains of Mre11 and its mitotic functions. However, recent experiments have provided valuable clues. For example, the Mre11 nuclease activity does not appear to be essential

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TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
DBY747	*MAT***a** *his3-Δ1 leu2-3,112 trp1-289 ura3-52*	D. Botstein
W303A	*MAT***a** *trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 ade2-1*	H. Klein
WXY9221	DBY747 but *rad50Δ::hisG-URA3-hisG*	Lab stock
MCY27	DBY747 but *mre11Δ::LEU2*	This study
L40	*MAT***a** *his3-Δ200 trp1-901 leu2-3,112 ade2 LYS::(LexA op)4-HIS3 URA3::(LexA op)8-LacZ-GAL4 gal4 gal80*	N. Hollingsworth
EI417	*MAT***a** *leu2-3,112 trp-1-289 ura3-5 lys1-1 his7-2*	J. Haber
EI425	EI417 but *xrs2-1*	J. Haber
EI447	EI417 but *xrs2Δ::LEU2*	J. Haber
E8-1	*MATα trp1 can1 ura3 cyh2 ade6 ade2 mre11-1(ts)*	H. Ogawa
EGY48	*MATα his3 trp1 ura3 6LexAop-LEU2*	E. Golemis

for such mitotic activities as nonhomologous end joining (NHEJ), telomere maintenance, or mating type switching (Moreau *et al.* 1999). Also, the C-terminal 134 amino acids do not seem to play a role in DNA repair or telomere maintenance (Usui *et al.* 1998; Chamankhah and Xiao 1999).

In a previous study, we demonstrated that the *mre11(ts)* allele carries a $Pro_{162}Ser$ missense mutation (Chamankhah and Xiao 1998), which results in a temperature-dependent meiotic defect and DNA repair deficiency (Ajimura *et al.* 1993). In this study, we further characterized *mre11Δ* mutant cells carrying the *mre11(ts)* allele. To our surprise, under conditions where the *mre11(ts)* mutant is resistant to killing by DNA damaging agents, its telomere sequences are shortened irrespective of experimental temperatures. Furthermore, the ability of Mre11(ts) to interact with itself, Rad50, and Xrs2 is severely compromised under all experimental temperatures, suggesting that these Mre11 mitotic functions can be separated by a single amino acid substitution. The separation of DNA repair and telomere maintenance functions is also demonstrated by overexpression of the *EXO1* gene encoding a yeast Exo1 5′-3′ exonuclease.

MATERIALS AND METHODS

Yeast strains, cell culture, and transformation: *S. cerevisiae* strains used in this study are listed in Table 1. Strains L40 and EGY48 were used for the two-hybrid and Western analyses, respectively. Yeast cells were grown in either complete YPD medium or minimal synthetic dextrose (SD) medium supplemented with amino acids and bases (Kaiser *et al.* 1994). Cells were grown at 30° unless otherwise specified. The permissive and nonpermissive temperatures for the temperature-sensitive *mre11(ts)* mutant were 24° and 34°, respectively. Genetic manipulations were as described (Kaiser *et al.* 1994). Transformation of yeast cells was performed by a modified DMSO protocol (Hill *et al.* 1991).

Cell killing and gradient plate assays: Liquid killing experiments were carried out as described (Chamankhah and Xiao 1998). Briefly, yeast cells harboring autonomously replicating plasmids were grown overnight in 5 ml of selective media. Next day, a 200- to 500-μl aliquot was transferred to 5 ml of fresh media. Cultures were incubated until the cell titer was approximately $2–5 \times 10^7$ cells/ml. MMS was added to the given concentration and samples were taken every 20 min during incubation, treated with fresh solution of sodium thiosulfate (5% w/v) to neutralize MMS, and washed once with sterile distilled water. Cells were resuspended in sterile distilled water and serial dilutions were made and plated on YPD or selective plates. Colonies were scored after 3–5 days of incubation. The rate of plasmid loss was determined by plating cells on complete and selective media and scoring for the number of colonies that had lost the plasmid. The plasmid loss rate was found to be <5%. The gradient plate assay was performed as previously described (Xiao *et al.* 1996). Briefly, an MMS gradient was formed by pouring a bottom layer of YPD + MMS medium in a tilted square petri dish. After agar solidification, the petri dish was returned flat and a top layer of YPD medium was added. Cell cultures were printed onto each plate across the gradient using a microscope slide, the plates were incubated for 2–3 days as specified, and the relative growth of cells was measured. Each experiment was repeated at least two times and comparisons were always restricted to the same set of plates.

Plasmids and plasmid construction: Restriction endonucleases and modifying enzymes were purchased from New England Biolabs (Beverly, MA) or Gibco-BRL (Gaithersburg, MD) and used according to the manufacturer's instructions. *E. coli* strain DH5α was used for plasmid manipulation. All plasmids were constructed by standard procedures (Sambrook *et al.* 1989). Plasmids pLexA-MRE11NN, pGAD-Mre11, pGAD-Rad50, pLexA-Xrs2, pGAD-Xrs2, pLex-M11(1-559), and pGAD-M11(1-559) were all described previously (Chamankhah and Xiao 1999). Single-copy YCp-MRE11 and multicopy YEp-MRE11 plasmids carry the *MRE11* gene in a 4.3-kb *Bam*HI fragment inserted into the same sites of YCplac33 and YEplac195 (Gietz and Sugino 1988), respectively. YCp-mre11(ts) and YEp-mre11(ts) are derivatives of YCp-MRE11 and YEp-MRE11, respectively, but contain the *mre11(ts)* allele resulting in the $Pro_{162}Ser$ substitution. To clone the *mre11(ts)* allele in two-hybrid vectors, the 0.6-kb *Pml*I-*Dra*III fragment of pGAD-MRE11 was replaced with the corresponding fragment isolated from YCp-mre11(ts) to give pGAD-mre11(ts). The 2.3-kb *Eco*RI-*Bam*HI fragment of pGAD-mre11(ts) was cloned into the same sites of pBTM116 to give pLexA-mre11(ts). The 1.7-kb *Eco*RI-*Bst*YI fragment of pLexA-mre11(ts) was cloned into *Eco*RI-*Bam*HI sites of pBTM116 and pGAD424 to form plasmids pLexA-M11(ts)1-559 and pGAD-M11(ts)1-559, respectively. To construct pEG-M11, pEG-M11(ts), and pEG-M11(ts)1-559, the *Eco*RI-*Sal*I fragments of

pLexA-MRE11NN, pLexA-M11(ts), and pLexA-M11(ts)1-559 were cloned into the *Eco*RI-*Xho*I sites of pEG202 (Estojak *et al.* 1995; a gift from Dr. E. A. Golemis, Fox Chase Cancer Center, Philadelphia). All *mre11(ts)* clones were sequenced to confirm the presence of the *ts* allele in the corresponding constructs. Functional analysis of all plasmids was performed using a gradient plate assay.

Plasmid pDB-EXO1 (Qiu *et al.* 1999) carrying the *EXO1* gene under the control of a constitutive *ADH1* promoter and a *URA3* selectable marker was a gift from Dr. B. Shen (City of Hope National Medical Center, Duarte, CA). A 4.1-kb *Sac*I-*Xba*I fragment containing the P_{ADH1}-*EXO1*-T_{ADH1} cassette was isolated from pDB-EXO1 and cloned into a multicopy plasmid pRS423 (Christianson *et al.* 1992) to form YEpH-EXO1 with a *HIS3* selectable marker.

Yeast two-hybrid system: A filter assay was employed to determine the β-galactosidase (β-gal) activity (Bartel and Fields 1995). Briefly, 5–10 independent L40 transformants harboring both LexA DNA binding and Gal4 activation domain fusion constructs were grown on selective plates for 1–2 days at different temperatures (24° and 34° for permissive and nonpermissive temperatures, respectively). Cells were transferred to Whatman No. 1 filter paper, immersed in liquid nitrogen for 10 sec to permeabilize cells, and placed on top of another filter, which was presoaked in a mixture of 1.8 ml Z-buffer containing 5 μl β-mercaptoethanol and 45 μl of 20 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-d-galactoside) in *N*,*N*-dimethylformamide (Guarente 1983). Plates were sealed with parafilm and incubated at 30°. Color development was monitored within the first hour, and at 4, 8, 16, and 24 hr after incubation. L40 cells transformed with vectors alone were used as negative controls. The β-gal activity was quantitated as described previously (Chamankhah and Xiao 1999).

Western analysis: EGY48 cells transformed with pEG-M11, pEG-M11(ts), and pEG-M11(ts)1-559 were cultured at 24° and 34° and protein extracts were analyzed for the expression of LexA fusion proteins using a polyclonal rabbit anti-LexA antibody (a gift from Dr. E. A. Golemis). Chemiluminescent detection of antigen-antibody complexes was carried out with horseradish peroxidase-conjugated α-rabbit secondary antibodies (from Bio-Rad Laboratories, Richmond, CA) in conjunction with ECL detection reagent (from Amersham Pharmacia Biotech, Braunschweig, Germany).

Analysis of telomere length: Telomere length in wild-type and mutant strains was analyzed as described (Ausubel *et al.* 1991; Chamankhah and Xiao 1999). Briefly, genomic DNA from stationary phase cells was isolated and treated with *Xho*I, separated in a 0.8% agarose gel, transferred to a Hybond-XL membrane (Amersham Pharmacia Biotech) using an alkaline transfer method, and hybridized with a [α-^{32}P]dCTP-labeled telomere fragment, which was obtained by PCR using plasmid pYT14 (a gift from Dr. T. Petes, University of North Carolina) as a template. The restriction enzyme *Xho*I cleaves in the conserved Y′ repeat located at the ends of most yeast chromosomes, generating terminal restriction fragments of ~1.3 kb in wild-type strains, which includes ~400 bp of the telomeric poly $G_{1\text{-}3}$T repeats.

RESULTS

Temperature-dependent MMS sensitivity of the *mre11(ts)* mutant: Consistent with the previously reported results (Ajimura *et al.* 1993; Johzuka and Ogawa 1995), we demonstrated earlier that strain E8-1 displays a temperature-sensitive defect in DNA repair (Chamankhah and Xiao 1998). The *mre11(ts)* allele

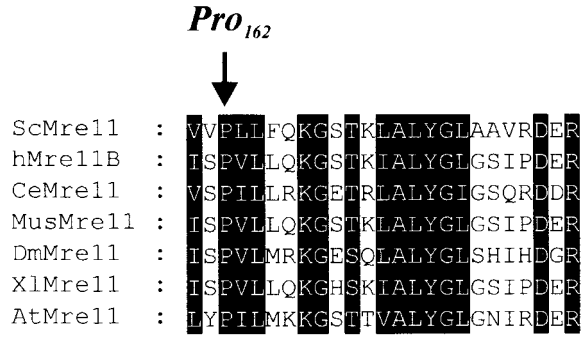

Figure 1.—Location of *mre11(ts)* mutation and alignment of *MRE11*s around the Pro_{162}Ser mutation.

was isolated by gap repair and the nucleotide alteration that results in the *ts* phenotype was previously determined and illustrated in Figure 1. This mutation results in a single Pro_{162}Ser amino acid substitution between the proposed phosphoesterase motifs III and IV, and the corresponding proline residue is conserved in all known Mre11 proteins (Figure 1). To investigate phenotypes of the *mre11(ts)* mutant in an isogenic genetic background, we rescued the *ts* allele to a single-copy centromeric plasmid and used it to transform the *mre11*Δ strain MCY27 for further complementation and telomere maintenance studies. Figure 2 shows the MMS sensitivity of *mre11*Δ transformants harboring YCp-mre11(ts). Clearly the *mre11*Δ strain carrying the *mre11(ts)* allele displays a temperature-dependent MMS sensitivity. At 24°, cells are DNA repair competent. At 34°, however, they behave like the *mre11* null mutant. It is noteworthy that after 60 min even at 24°, *mre11*Δ cells harboring single-copy *mre11(ts)* display a slightly decreased survival rate compared to the wild-type cells, indicating that the *ts* mutation leads to a partial loss of DNA repair function. This observation was further confirmed by using higher concentrations of MMS on a gradient plate assay (data not shown). Compared to our previously reported results (Chamankhah and Xiao 1998), data shown in Figure 2 demonstrate a greater MMS sensitivity for the *mre11*Δ and a lower survival rate for the *mre11(ts)* mutant at both permissive and nonpermissive temperatures. This could be attributed to different genetic backgrounds as we used DBY747 strain in the present study.

Partial loss-of-function mutations are frequently associated with deficiencies in protein-protein interactions and, therefore, overexpression of such mutant alleles often leads to functional complementation of the null defect. This prompted us to study the effect of the overexpression of the *ts* allele to complement the MMS sensitivity in the *mre11*Δ mutant. Figure 2 shows that at the permissive temperature, overexpression of the *ts* allele via multicopy *mre11(ts)* plasmids results in MMS resistance at a level comparable to the isogenic wild-type cells. At the restrictive temperature, this overexpression

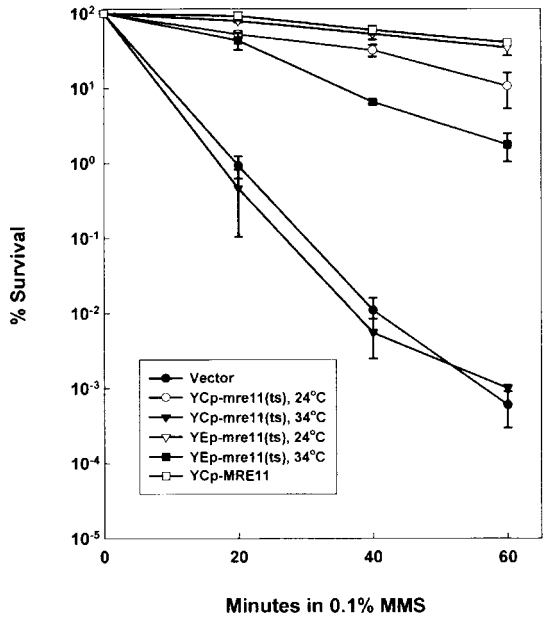

Figure 2.—DNA repair deficiency of the *mre11(ts)* mutant is temperature dependent. MCY27 (*mre11*Δ) cells were transformed with YCp-mre11(ts) and YEp-mre11(ts), cultured at 24° or 34°, and killing experiments were performed as described in materials and methods. *mre11*Δ cells transformed with vector alone or YCp-MRE11 were used as negative and positive controls, respectively. Transformants with YEp-MRE11 exhibited the same survival rate as YCp-MRE11 (data not shown). Results are the average of three independent experiments. The rate of plasmid loss was measured to be within ±5%.

gives rise to an intermediate MMS sensitivity. This result is consistent with the assertion that $Pro_{162}Ser$ substitution in Mre11 leads to a partial temperature-dependent defect in DNA repair, possibly due to compromised Mre11 structure alterations, protein-protein, or protein-DNA interactions.

Telomere shortening by the *mre11(ts)* allele is temperature independent: The *mre11* null mutant has been shown to have substantially shorter telomeres than its wild-type strain (Boulton and Jackson 1998; Nugent *et al.* 1998). Having demonstrated the effect of single- and multicopy expression of *mre11(ts)* on DNA repair deficiency of *mre11*Δ strain, we investigated whether or not the *mre11(ts)* allele also exhibits telomere shortening in a temperature-dependent manner. *mre11*Δ cells transformed with the single-copy YCp-mre11(ts) were grown in selective media at permissive (24°), semipermissive (30°), and nonpermissive (34°) temperatures until saturation (~20 doubling times), subcultured once more, and allowed to grow to the stationary phase. Total yeast genomic DNA was then subjected to Southern analysis using a specific probe for Y′ telomeres (Figure 3). The restriction enzyme *Xho*I cuts yeast DNA in the subtelomeric Y′ repeat, generating a terminal restriction fragment in wild-type yeast strains of ~1.3 kb, ~400 bp representing the terminal poly $G_{1\text{-}3}T$ tract (Walmsley and Petes 1985). Interestingly, when a single-copy *mre11(ts)* was expressed in *mre11*Δ strain, telomeric repeats were shorter compared to the wild type regardless of the temperature employed (Figure 3). In fact, telomeres of *mre11(ts)* transformants at all permissive, semipermissive, and nonpermissive temperatures were at the same length as the *mre11*Δ strain, which shows that the $Pro_{162}Ser$ mutation in the *mre11(ts)* allele causes a specific temperature-independent defect in telomere maintenance.

Effects of *mre11(ts)* overexpression on telomere shortening: Since at permissive temperature overexpression of the *mre11(ts)* allele in the *mre11*Δ null mutant displayed an MMS-resistant phenotype indistinguishable from wild-type cells (Figure 2), we examined whether or not overexpression of *mre11(ts)* under the same condition can complement the shortened telomeres in *mre11*Δ. Figure 3 shows that compared to isogenic wild type and *mre11*Δ mutant, *mre11*Δ cells transformed with multicopy *mre11(ts)* have an intermediate length of telomeres regardless of growing at restrictive, semipermissive, or permissive temperatures. Hence, the telomere length effect of *mre11(ts)* appears to be tem-

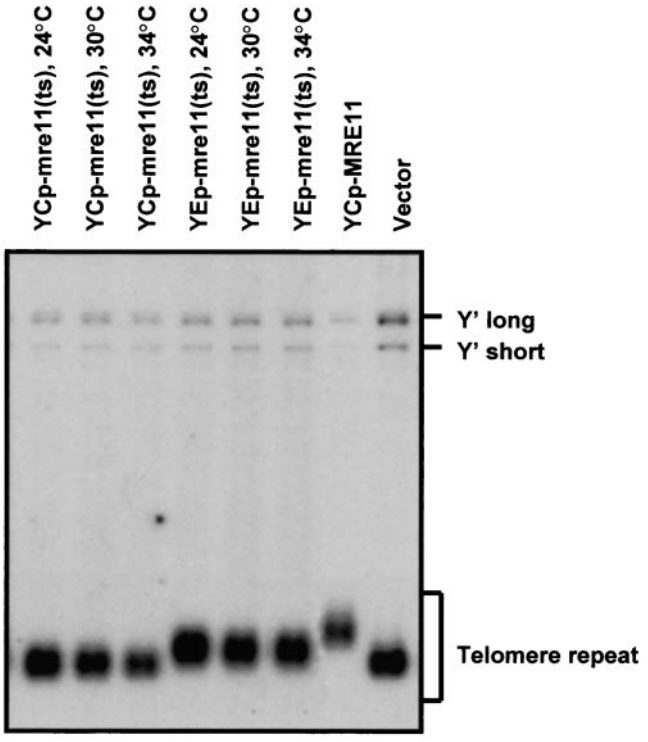

Figure 3.—Telomere shortening of the *mre11(ts)* mutant is temperature independent. MCY27 (*mre11*Δ) cells were transformed with plasmids YCp-MRE11, YCp-mre11(ts), and YEp-mre11(ts) and the transformants were grown at 24° or 34° until saturation. Cells were subcultured once more until saturation. *Xho*I-cleaved genomic DNA isolated from transformants was hybridized with the 0.8-kb telomere probe that recognizes a characteristic 1.3-kb fragment in wild-type cells and shorter fragments in mutants.

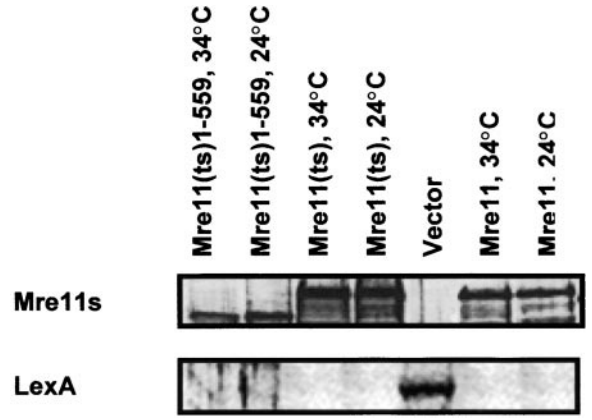

Figure 4.—The Mre11(ts) protein is present at both permissive and nonpermissive temperatures. EGY48 cells were transformed with pEG-M11, pEG-M11(ts), and pEG-M11(ts) 1-559 and the transformants were cultured at 24° or 34° for 2 days. Protein extracts were prepared using a glass bead method (Ausubel *et al.* 1991). Protein (10 μg) was loaded in each lane and subjected to Western analysis using an anti-LexA antibody (Estojak *et al.* 1995). EGY48 cells transformed with vector alone were used as a control.

perature independent, which further supports the hypothesis that the *mre11(ts)* allele possesses a telomere maintenance defect biochemically distinct from the temperature-dependent DNA repair deficiency conferred by the same allele.

Mre11(ts) is defective in protein-protein interactions: As shown in Figures 2 and 3, the *mre11(ts)* allele displays different responses to MMS damage and maintenance of telomeric sequences. To investigate whether these phenotypes could be correlated with the protein interaction properties of the Mre11(ts) protein, we studied the homodimerization of Mre11(ts) and its interactions with Rad50 and Xrs2 using a yeast two-hybrid system. L40 cells were cotransformed with LexA and $Gal4_{AD}$ fusion proteins, grown on selective media for 2–4 days at permissive or nonpermissive temperatures, and analyzed for protein-protein interactions using a β-gal filter assay by scoring the time it required to develop blue colonies. Table 2 summarizes the results of our two-hybrid analyses. Interestingly, at both permissive and restrictive temperatures, the full-length Mre11(ts) protein did not homodimerize, nor did it interact with Rad50 or Xrs2. Western analysis showed that the reduced protein interaction was not due to decreased level of cellular Mre11(ts) fusion protein (Figure 4), ruling out the possibility that $Pro_{162}Ser$ substitution simply affects protein stability. It remains possible that the Mre11(ts)-Rad50-Xrs2 protein complex may not be stable enough to drive the expression of the β-gal to a detectable level. Thus, we took advantage of our previous finding that the C-terminal 134 amino acid truncation of Mre11 displays a sevenfold increase in β-gal activity compared to the full-length Mre11 when interaction with Rad50 or Xrs2 is examined (Chamankhah and Xiao 1999). The C-terminal 134 amino acids were removed from Mre11(ts) and its self-association as well as interactions with Rad50 and Xrs2 was examined in the hope of enhancing the sensitivity of detection. While this truncated Mre11(ts) protein was still unable to homodimerize or interact with Xrs2, it exhibited a weak temperature-dependent interaction with Rad50 (Table 2).

To confirm that Mre11(ts) is unable to interact with other proteins, we examined whether the *mre11(ts)* allele displayed a dominant negative effect in wild-type strain. If a mutated protein is still able to self-interact or form a complex with other proteins, this mutant allele often exhibits dominant negative effect. The *mre11(ts)* allele on a single-copy, multicopy, and under strong *ADH1* promoter (cloned in pBTM116) was expressed in two different wild-type strains (DBY747 and W303A), and the MMS sensitivity as well as telomere lengths in these transformants was examined. The *mre11(ts)* allele did not exhibit any detectable dominant negative effects at various temperatures (data not shown). These results support our speculation that $Pro_{162}Ser$ is compromised with Mre11-Rad50-Xrs2 complex formation (Table 2) and are consistent with the observation that Mre11(ts) is also unable to interact with wild-type Mre11 in a similar two-hybrid assay (data not shown).

The above results taken together suggest that the mutated Mre11(ts) protein may be compromised to different degrees with its ability to form homodimers or to interact with Rad50 and Xrs2. However, we cannot rule out the possibility that one distinct mode of DNA repair activity of Mre11 may be independent of its protein-interaction properties, while being absolutely required for telomere maintenance.

***EXO1* overexpression does not suppress telomere shortening in *mre11*Δ, *rad50*Δ, and *xrs2*Δ mutants:** *S. cerevisiae EXO1* was previously cited as a multicopy suppressor of the MMS sensitivity in *mre11*Δ and *rad50*Δ mutants (Tsubouchi and Ogawa 1998). To determine whether the two Mre11 mitotic functions examined above are correlated in the case of *EXO1* overexpression, we analyzed the effect of overexpression of *EXO1* in *mre11*, *rad50*, and *xrs2* null mutants. By a gradient plate assay (Figure 5A), we confirmed that *EXO1* overexpression was able to rescue each of the above mutants from killing by MMS; however, it is evident that it only partially complements the MMS sensitivities of the mutant strains compared to the wild-type background. *EXO1* overexpression was also able to complement the *mre11(ts)* mutant at nonpermissive temperature (Figure 5B). Under the same *EXO1* overexpression conditions, the *mre11*, *rad50*, and *xrs2* null mutants displayed shortened telomeres indistinguishable from those of their respective null mutants (Figure 6). Hence, these results provide additional evidence for the existence of distinct mechanisms in Mre11-mediated DNA repair and telomere maintenance.

TABLE 2

Protein interactions of Mre11(ts)

	$Gal4_{AD}$ fusions			
LexA fusions	pGAD424	Homodimerization	Rad50	Xrs2
pBTM116	−[a]	−	−	−
Mre11	−	+++++	++	+/−
Mre11 1-559	−	++	+++++	++
Mre11(ts)	−	−	−	−
Mre11(ts) 1-559	−	−	+	−

The permissive temperature was 24°. No interactions were observed at 34° for Mre11(ts).

[a] β-Gal activities were determined by a filter assay as described in materials and methods. Color development was scored as follows: +++++, <15 min; ++++, 15 min–1 hr; +++, 1–4 hr; ++, 4–8 hr; +, 8–12 hr; +/−, 12–24 hr. Colonies remaining white after 24 hr were considered negative.

DISCUSSION

Mre11 is a multifunctional protein with pivotal roles in meiosis and mitosis (Haber 1998). Dissecting the functional domains of Mre11 as well as characterizing *mre11S* mutations that separate the two consecutive and key steps of "formation" and "processing" of DSBs during meiosis has yielded important clues on its various biochemical activities. In this article, we present evidence that the *mre11(ts)* allele is a novel separation-of-function mutation with respect to two important mitotic properties of Mre11, *i.e.*, DNA repair and telomere maintenance. We previously identified the *mre11(ts)* mutation as a $Pro_{162}Ser$ substitution (Chamankhah and Xiao 1998). This mutation does not fall into any of the putative phosphoesterase motifs based on the alignment between Mre11 and the *E. coli* SbcD. However, the Pro_{162} residue is absolutely conserved among all known Mre11 homologs and is probably important to maintain the Mre11 secondary and tertiary structure. Therefore, we investigated the mechanism of temperature sensitivity of this allele. Surprisingly we found that *mre11(ts)* is a novel *S* allele in two aspects. First, at the permissive temperature, the *mre11(ts)* mutant is normal in DNA repair but is defective in telomere maintenance. Second, its DNA repair function is temperature dependent, while the defect in telomere maintenance is independent of our experimental temperatures.

Undoubtedly, the temperature-independent telomere shortening points to an unconditional failure of Mre11(ts) protein to function at telomeres. The following four hypotheses are consistent with the observed *S* phenotype. First, the Mre11 participation in replication at telomere sequences may require a rapid Mre11 turnover and the $Pro_{162}Ser$ substitution may reduce Mre11 stability in a temperature-dependent manner. Although attractive, our Western analysis of LexA-Mre11(ts) fusion constructs at both permissive and nonpermissive temperatures does not lend support to this hypothesis. Second, the *ts* mutation may affect the ability of Mre11 to interact with itself and with other proteins that are critical for telomere maintenance. Our data support this notion as we observed that the *ts* mutation abolishes the Mre11 protein interactions. It was recently demonstrated that, in the presence of Nbs1, a putative human homolog of *S. cerevisiae* Xrs2, the hMre11-hRad50 complex acquires new biochemical activities such as partial unwinding of the DNA duplex and an ATP-dependent switch in endonuclease specificity that allows hMre11-hRad50-Nbs1 to cleave a 3′-protruding strand (Paull and Gellert 1999). Additionally, Nbs1 seems to be essential for a cell-cycle-independent phosphorylation of hMre11 upon DNA damage (Dong *et al.* 1999). Since we observed a weak interaction between C-terminally truncated Mre11(ts) and Rad50 at the permissive temperature, we speculate that Mre11 alone or a compromised Mre11-Rad50 complex may function only in one of the Mre11-dependent pathways of DNA repair while the activity at telomeres may require the ternary Mre11-Rad50-Xrs2 complex. Third, epistasis analysis has placed *mre11* in the telomere replication machinery (Nugent *et al.* 1998). Failure of Mre11(ts) to cooperate with members of the replication complex at telomeres may have caused the observed separation of function. Recent findings also showed an interaction between mammalian Mre11 and Ku70 (Goedecke *et al.* 1999). Ku70 and Mre11 both have DNA binding properties and participate in the NHEJ pathway of DSB repair. Although the Ku70-dependent telomere maintenance seems to be distinct from the Mre11/Rad50/Xrs2 pathway, this interaction may have significant roles in Mre11-mediated telomere maintenance. Hence, it is interesting to test if Mre11(ts) can function in NHEJ. These hypotheses are currently under investigation in our laboratory. Finally, analysis of MMS sensitivities of *mre11* mutant alleles has demonstrated that one mode of Mre11-mediated DNA repair involves its nuclease activities and that this mode shares common mechanisms with meiotic recombination (Usui *et al.* 1998). The fact that the *mre11(ts)* allele also displays temperature-dependent meiotic defects (Ajimura *et al.* 1993) suggests that the

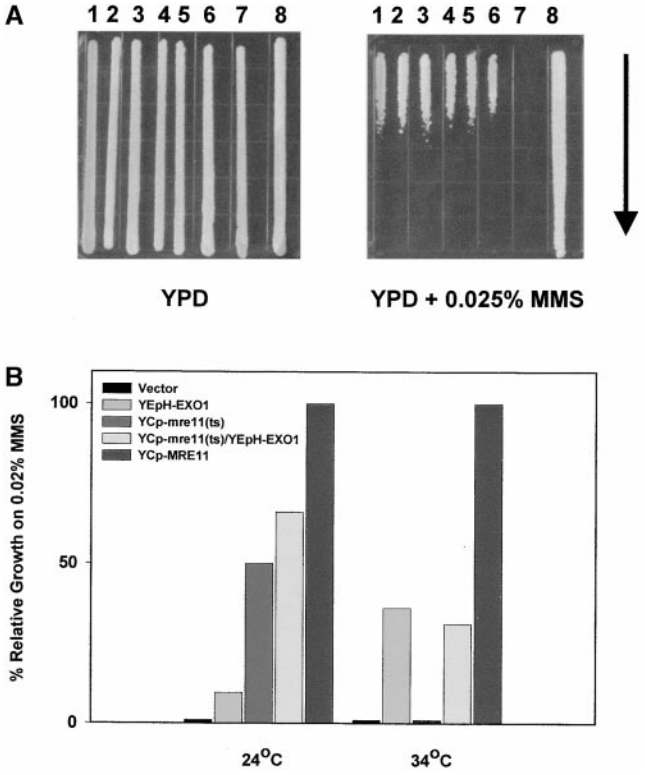

Figure 5.—Effect of *EXO1* overexpression by gradient plate assays. (A) Overexpression of *EXO1* partially complements the MMS sensitivity of *mre11*Δ, *rad50*Δ, and *xrs2* mutants. The above mutants were transformed with plasmid pDB-EXO1 or vector alone and the transformants were grown in selective media overnight at 30° until saturation. Gradient plate assay was performed essentially as described previously (Xiao *et al.* 1996). The results shown are from two independent transformants of MCY27 (*mre11*Δ; lanes 1 and 2), WXY9221 (*rad50*Δ; lanes 3 and 4), and EI425 (*xrs2*; lanes 5 and 6). MCY27 cells transformed with vector alone (lane 7) and with YCp-MRE11 (lane 8) were used as negative and positive controls, respectively. The MMS sensitivity of *rad50*Δ and *xrs2* mutants transformed with vector alone was indistinguishable from MCY27 (data not shown). Percentage of relative growth for all transformants was measured to be 31% compared to 100% for the mutant strains transformed with their corresponding wild-type genes. At this concentration of MMS, no growth was observed for MCY27 (*mre11*Δ) strain transformed with vector alone. (B) Overexpression of *EXO1* in *mre11(ts).* YCp-mre11(ts) and YEpH-EXO1 were cotransformed into MCY27 (*mre11*Δ) cells and the transformants were subjected to the MMS gradient plate assay at 24° for 60 hr and 34° for 48 hr. Percentage of relative growth was determined by the length of growth on the MMS plate in comparison to MCY27 (*mre11*Δ) cells transformed with YCp-MRE11 and YEpH-EXO1.

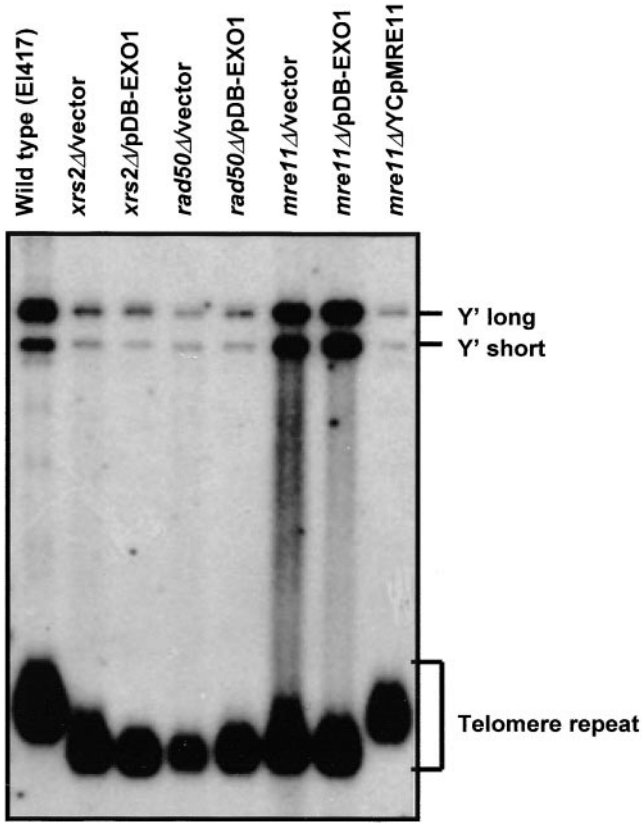

Figure 6.—*EXO1* overexpression fails to rescue *mre11*Δ, *rad50*Δ, and *xrs2*Δ mutants from telomere shortening. EI447 (*xrs2*Δ), WX9221 (*rad50*Δ), and MCY27 (*mre11*Δ) cells were transformed with the corresponding wild-type genes (data shown only for *MRE11* wild-type control), pDB-EXO1, or vector alone and the transformants were analyzed for telomere shortening as described in Figure 3.

nuclease-dependent repair pathway is conditionally defective due to the $Pro_{162}Ser$ substitution. This hypothesis needs further *in vitro* biochemical characterization of the Mre11(ts) protein and has to accommodate the observed lack of protein-protein interactions.

In this study, separation of DNA repair and telomere maintenance functions was also achieved by overexpression of the *EXO1* gene. The *EXO1* overexpression partially rescues *mre11*, *rad50*, and *xrs2* null mutants with respect to MMS sensitivity, but is unable to alter the shortened telomeres in these strains. Two explanations could accommodate the failure of excess Exo1 to complement the telomere shortening observed in *mre11*, *rad50*, and *xrs2* mutants. First, since Exo1 is a 5′-3′ exonuclease and the Mre11-Rad50 complex has a 3′-5′ exonuclease as well as other nuclease activities, the role of Mre11-Rad50-Xrs2 in maintaining the telomeric sequences may be due to its strict requirement(s) for such activities. Alternatively, as the Mre11-Rad50-Xrs2 complex acts on a wide range of DNA substrates, the specificity of the substrate structure may be a determining factor in telomere maintenance. The failure of Mre11(ts) to interact with Xrs2 may be a key finding that points to the importance of this interaction at the telomeres. The enhanced specificity of the hMre11-hRad50-Nbs1 complex for 3′-overhang structures (Paull and Gellert 1999) could be especially important for telomere maintenance, because during telomere replication in yeast, chromosome ends acquire a long single-stranded extension of the strand making the 3′ end, which is generated late in S phase (Dionne

and Wellinger 1998). *EXO1* overexpression has been shown to complement the temperature-dependent growth defect observed in the *rad27*Δ strain, but the telomere heterogeneity and the appearance of single-stranded DNA were not prevented by the overexpression of *EXO1* (Parenteau and Wellinger 1999). These observations collectively suggest that the multifunctionality of proteins like Mre11 and Rad27 in DNA repair and at telomeres could be related to their distinct biochemical activities.

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