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

Mahmood Chamankhah, Treena Fontanie and Wei Xiao

Department of Microbiology and Immunology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada

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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 mitotic 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 Pro162Ser 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 mre1, 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; Paul 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 mre1 and carries two amino acid substitutions, one of which (His213Tyr) 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 mre11 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...
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 Pro162Ser 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 (Hiil 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 × 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 BamHI 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 Pro162Ser substitution. To clone the mre11(ts) allele in two-hybrid vectors, the 0.6-kb PmlI-OraiI fragment of pGAD-MRE11 was replaced with the corresponding fragment isolated from YCp-mre11(ts) to give pGAD-mre11(ts). The 2.3-kb EcoRI-BamHI fragment of pGAD-mre11(ts) was cloned into the same sites of pBTM116 to give pLexA-mre11(ts). The 1.7-kb EcoRI-BstEII fragment of pLexA-mre11(ts) was cloned into EcoRI-BamHI 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 EcoRI-Sall fragments of

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**TABLE 1**

*S. cerevisiae* strains

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pLexA-MRE11NN, pLexA-M11(ts), and pLexA-M11(ts)-1-559 were cloned into the EcoRI-XhoI 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 SacI-XbaI fragment containing the P_adh1::EXO1-T_dhaI 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 Fielis 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 pre-soaked in a mixture of 1.8 ml 2× Z-buffer containing 5 μl β-mercaptoethanol and 45 μl of 2000 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-d-galactoside) in N,N-dimethylformamide (Guar et al. 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 Xhol, 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-32P]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 XhoI 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 G12T repeats.

**RESULTS**

**Temperature-dependent MMS sensitivity of the mre11(ts) mutant:** Consistent with the previously reported results (Ajimura et al. 1993; Jozuka and Ogasawara 1995), we demonstrated earlier that strain E8-1 displays a temperature-sensitive defect in DNA repair (Chamankhah and Xiao 1998). The mre11(ts) allele 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 Pro162Ser 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 mre11Δ strain MCY27 for further complementation and telomere maintenance studies. Figure 2 shows the MMS sensitivity of mre11Δ transformants harboring YCP-mre11(ts). Clearly the mre11Δ 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°, mre11Δ 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 mre11Δ 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 mre11Δ 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...
Interestingly, when a single-copy mre11(ts) was expressed in mre11Δ 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, semi-permissive, and nonpermissive temperatures were at the same length as the mre11Δ strain, which shows that the Pro162Ser 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 mre11Δ 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 mre11Δ. Figure 3 shows that compared to isogenic wild-type and mre11Δ mutants, mre11Δ cells transformed with multicopy mre11(ts) have an intermediate length of telomeres regardless of growing at restrictive, semi-permissive, or permissive temperatures. Hence, the telomere length effect of mre11(ts) appears to be tem-

gives rise to an intermediate MMS sensitivity. This result is consistent with the assertion that Pro162Ser 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 mre11Δ strain, we investigated whether or not the mre11(ts) allele also exhibits telomere shortening in a temperature-dependent manner. mre11Δ cells transformed with the single-copy YCp-mre11(ts) were grown in selective media at permissive (24°C), semi-permissive (30°C), and non-permissive (34°C) 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 XhoI 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 G13T tract (Wal et al. 1985). Interestingly, when a single-copy mre11(ts) was expressed in mre11Δ 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, semi-permissive, and non-permissive temperatures were at the same length as the mre11Δ strain, which shows that the Pro162Ser mutation in the mre11(ts) allele causes a specific temperature-independent defect in telomere maintenance.

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Separation of Mre11 Functions by mre11(ts) 573

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.

Fig. 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 Gal4AD 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 Pro162Ser 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 Pro162Ser is compromised 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 mre11Δ, rad50Δ, and xrs2Δ mutants: S. cerevisiae EXO1 was previously cited as a multicopy suppressor of the MMS sensitivity in mre11Δ and rad50Δ 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 mre1, 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 mre1, 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.
**Protein interactions of Mre11(ts)**

<table>
<thead>
<tr>
<th>Gal4&lt;sub&gt;α&lt;/sub&gt; fusions</th>
<th>pGAD424</th>
<th>Homodimerization</th>
<th>Rad50</th>
<th>Xrs2</th>
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<tr>
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<td>Mre11(ts)</td>
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<td>Mre11(ts) 1-559</td>
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The permissive temperature was 24°C. No interactions were observed at 34°C for Mre11(ts).

<sup>a</sup> 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 mre15 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<sub>162</sub>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<sub>162</sub> 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<sub>162</sub>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 mamillar 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
Separation of Mre11 Functions by mre11(ts)

Figure 5.—Effect of EXO1 overexpression by gradient plate assays. (A) Overexpression of EXO1 partially complements the MMS sensitivity of mre11Δ, rad50Δ, 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 (mre11Δ; lanes 1 and 2), WXY9221 (rad50Δ; 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 rad50Δ 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 (mre11Δ) strain transformed with vector alone. (B) Overexpression of EXO1 in mre11(ts). YCp-mre11(ts) and YEpH-EXO1 were cotransformed into Mre11-Rad50-Xrs2 complex 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 (mre11Δ) cells transformed with YCp-MRE11 and YEpH-EXO1.

nuclease-dependent repair pathway is conditionally defective due to the Pro→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 mre11Δ, rad50Δ, and xrs2Δ mutations from telomere shortening. EI447 (xrs2Δ), WX9221 (rad50Δ), and MCY27 (mre11Δ) 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.

First, since Exo1 is a 5'-3' exo-nuclease and the Mre11-Rad50 complex has a 3'-5' exo-nuclease as well as other nuclease activities, the role of Mre11-Rad50-Xrs2 complex 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 rad27Δ 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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LITERATURE CITED


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