

Xrs2p Regulates Mre11p Translocation to the Nucleus and Plays a Role in Telomere Elongation and Meiotic Recombination[□]

Yasumasa Tsukamoto,* Chikako Mitsuoka, Masahiro Terasawa, Hideyuki Ogawa, and Tomoko Ogawa

Iwate College of Nursing, Iwate 020-0151, Japan

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The Mre11-Rad50-Xrs2 (MRX) protein complex plays pivotal roles in meiotic recombination, repair of damaged DNA, telomere elongation, and cell cycle checkpoint control. Xrs2p is known to be essential for all the functions of the complex, but its role in the complex has not been clearly elucidated. A 32-amino acid region near the C terminus of Xrs2p was identified as an Mre11p-binding site. No more function of Xrs2p than translocation of Mre11p from the cytoplasm to the nucleus is necessary for response to DNA damage. However, domains in Xrs2p located both 49 amino acids upstream and 104 amino acids downstream of the Mre11p binding site are required for meiotic recombination and telomere elongation, respectively, in addition to the 32-amino acid region. These findings demonstrate that Xrs2p acts as a specificity factor that allows the MRX complex to function in meiotic recombination and in telomere elongation.

INTRODUCTION

The MRX complex in *Saccharomyces cerevisiae* acts to maintain genome stability by functioning in both DNA repair and genetic recombination pathways (for reviews, see Haber, 1998; D'Amours and Jackson, 2002). It is composed of Mre11p, Rad50p, and Xrs2p subunits (Johzuka and Ogawa, 1995; Usui *et al.*, 1998) and is necessary for phosphorylation and activation of both Rad53p and Chk1p checkpoint kinases on a Tel1p-dependent checkpoint pathway (Grenon *et al.*, 2001; Usui *et al.*, 2001). One the other hand, Mre11p and Xrs2p are phosphorylated in a Tel1p-dependent manner in response to DNA damage, indicating that the MRX complex functions as a sensor and a signal transducer for DNA double-strand breaks (DSBs). The complex also is involved in telomere elongation through a Tel1 pathway (Ritche and Petes 2000) and is suggested to recruit telomerase to telomeres (Tsukamoto *et al.*, 2001). In addition, it plays at least two roles in meiotic recombination. One is introduction of meiosis-specific DSBs with an aid of a meiosis-specific DNA endonuclease, Spo11p. The other is successive removal of Spo11p–DNA complexes and resection of the broken DNA ends (for reviews, see Haber, 1998; D'Amours and Jackson, 2002). There is separation of function alleles of the *rad50* and *mre11* mutants that are defective in the processing of meiotic DSBs; however, *xrs2* mutants that show similar phenotype to these mutants are not isolated. Null strains defective in any one of these subunits display the same phenotype, which includes defects in meiotic homologous recombina-

tion, nonhomologous end-joining, DNA damage repair, S-phase checkpoint control, and telomere elongation.

The Mre11p and Rad50p components of the complex have been characterized extensively. Mre11p displays several biochemical properties, including 3'-to-5' single-strand (ssDNA) and double-strand DNA (dsDNA) exonuclease activity, ssDNA endonuclease activity, a limited DNA unwinding activity, and binding to both ssDNA and dsDNA (Furuse *et al.*, 1998; Usui *et al.*, 1998). Rad50p exhibits ATP-dependent binding to dsDNA, and the binding involves dimer formation of the protein (Raymond and Kleckner, 1993). The dimerization is mediated through globular ATP binding domains at the N and C terminals, and the heptad repeat regions form intramolecular coiled-coil structure (Anderson *et al.*, 2001). Mre11p binds as a dimer to a region in Rad50p located between the two catalytic domains.

Xrs2p consists of 854 amino acids and binds to Mre11p, but not to Rad50p (Usui *et al.*, 1998). Xrs2p was recently shown to display DNA binding activity that is specific for DNA structure and enhances the Mre11p nuclease activity (Trujillo *et al.*, 2003). Similar enhancements of hMre11p activities have been reported in a study of the human homologue of the *XRS2* gene *NBS1*. Nbs1p binds at the C-terminal region to hMre11p and enhances the activities of hMre11p, as measured in *in vitro* DNA binding, ssDNA endonuclease, and DNA unwinding assays (Carney *et al.*, 1998; Paull and Gellert, 1999; Desai-Mehta *et al.*, 2001, Tauchi *et al.*, 2001). Interestingly, Nbs1p is located at the telomere region only in S phase when telomeres are elongated in HeLa cells, whereas hMre11p and hRad50p are associated with telomeres throughout the cell cycle (Zhu *et al.*, 2000), suggesting that Nbs1p acts a factor that allows hMre11p and hRad50p to function in telomere elongation. This result prompted us to speculate that Nbs1p and Xrs2p are regulators that permit the Mre11p and Rad50p complexes to function specifically when their respective activities are required. To test this possibility, we examined various mutants with specific func-

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* Corresponding author. E-mail address: tsukamot@iwate-nurse.ac.jp.

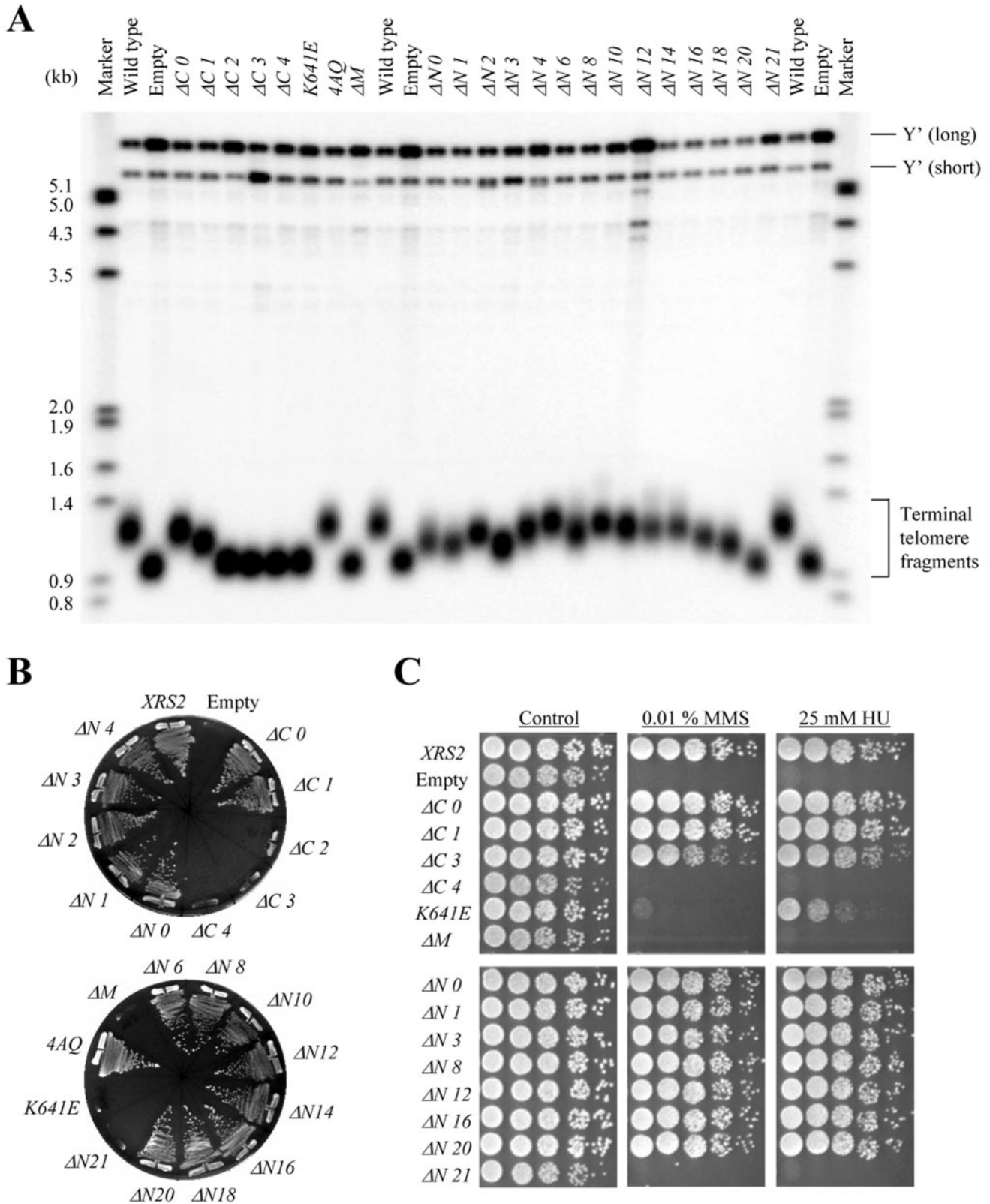


Figure 2. Characterization of the *xrs2* mutants. (A) SYT358a (*MATa xrs2 Δ ::URA3 ade2 ade3 trp1 Δ leu2-3,112 ura3 Δ lys2-801 can1*) containing integrations of the indicated *xrs2* alleles were examined for telomere length. (B) Complementation of the synthetic senescence phenotype exhibited in *xrs2 mec1 sml1 hsf1* mutants. The indicated *xrs2* alleles present on *CEN* plasmids (*ARSH4 CEN6 LEU2 CYH2*) were introduced into SYT301 harboring the plasmid pYT1501. The cells that are viable after loss of pYT1501 can grow on SC (+5-FOA -Leu) plates. (C) Sensitivity to MMS and HU in the *xrs2* mutants with indicated alleles. Fivefold serial dilutions of the indicated *xrs2* mutants were spotted onto YPAD plates containing 0.01% MMS or 25 mM HU. The *xrs2* mutants examined in this assay are the SYT358a derivatives in which the truncated *xrs2* genes were integrated into the genome.

Screening of *xrs2* Point Mutants with a Defect in Telomere Elongation

XRS2 was randomly mutagenized by error prone PCR in the presence of 0.2–0.4 mM MnCl₂, by using *Taq*DNA polymerase (Promega, Madison, WI), the primers T3 (5'-AATTAACCTCACTAAAGGG-3') and T7 (5'-GTAAT-ACGACTACTATAGGG-3'), and the pYT1269 DNA template. PCR products were digested with *Bam*HI and cloned into the *Bam*HI site of pRS318. The resulting mutant pools were introduced into SYT301 harboring the plasmid pYT1501. The transformants were streaked onto plates containing 5-fluoroorotic acid (5-FOA) to screen *xrs2* mutants that are inviable after loss of pYT1501. *xrs2* mutants displaying an accelerated senescence phenotype in a *mec1 smf1 hdf1* mutant background were isolated by plasmid shuffling method. Then, *xrs2* mutant plasmids were recovered by transforming whole yeast DNA derived from an *xrs2* mutant into the *Escherichia coli* strain XL1-Blue (Stratagene, La Jolla, CA). The location of the *xrs2* mutation was determined by sequencing both strands of the *XRS2* gene.

Construction of *xrs2* Mutants with Truncations

A series of *xrs2* mutants with C-terminal truncations was constructed by replacing the specified C-terminal region of the *XRS2* gene on pYT1269 in SYT9a with a 9Myc-*klTRP1* unit (Knop *et al.*, 1999), by using PCR-mediated one-step gene replacement. Similarly, a series of N-terminal truncated *xrs2* mutants were constructed by replacing the specified N-terminal region with a 3Myc-*URA3*-3Myc fragment (Schneider *et al.*, 1995). Pop out events, which eliminated one 3Myc fragment and the *URA3* gene, were selected on 5-FOA media to obtain 3Myc-tagged *xrs2-ΔN* mutants. For construction of the *xrs2-ΔM* mutant, the amino acid residues 630–661 of *xrs2-ΔC0* on pRS318 was replaced with a 3HA-*URA3*-3HA fragment (Schneider *et al.*, 1995). The DNA sequences of the primers used for the truncations are described in the Supplemental Data. These sets of mutations were subcloned into the integration vectors and introduced into *xrs2* null strains.

Analysis of Telomere Length by Southern Blotting

For each mutant, genomic DNA was prepared after ~100 cell divisions after strain construction. The DNA from each strain was digested with *Xho*I, electrophoresed in a 0.9% TAE agarose gel, transferred to a nylon membrane (Roche Diagnostics, Indianapolis, IN), and hybridized with a 247-base pair probe bearing Y' element DNA. The probe was labeled with digoxigenin, by using a PCR DIG Probe Synthesis kit (Roche Diagnostics) and primer pairs 5'-CAGTTTAGCAGGCATCATCG-3' and 5'-CGAGAACTTCAACGTTT-GCC-3'. The Southern blot signals were quantified with a DIG Luminescent Detection kit (Roche Diagnostics) and a LAS1000 chemiluminescence image analyzer (Fuji Film, Tokyo, Japan). The digoxigenin-labeled molecular weight markers (Roche Diagnostics) were λDNA digested with *Eco*RI and *Hind*III.

Synchronous Entry of Cells into Meiosis

Cells from a frozen stock were grown on a YPG plate for 12 h to remove ρ⁻ cells. The cells were then streaked on a YPAD plate and incubated for 2 d. A single colony was incubated in YPAD liquid medium and grown for 24 h. The resulting culture was added to presporulation medium at a 1/100 dilution and incubated with vigorous aeration for 12 h. Cells were then harvested, washed twice with water, and transferred to sporulation medium to induce meiosis. All experiments were performed at 30°C.

Immunostaining

Immunostaining was carried out as described previously (Burke *et al.*, 2000). For staining of Myc-tagged Xrs2p or Mre11p, cells prepared on slides were first treated with α-Myc (9E10) mouse monoclonal antibody (mAb) (Sigma-Aldrich, St. Louis, MO) and then stained with Alexa-Fluor-488-conjugated α-mouse IgG (Molecular Probes, Eugene, OR). The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Immunofluorescence microscopy and image processing were carried out as described previously (Terasawa *et al.*, 1995).

Two-Hybrid Analysis

DNA fragments encoding the Mre11p-binding domain for Xrs2p (MBX) of Xrs2p, full-length Xrs2p and full-length Mre11p were cloned into both pG-BDU-c2 (2 μm, *URA3* and *GAL4* DNA binding domain fusion construct) and pGAD424 (2 μm, *LEU2* and *GAL4* activation domain fusion construct). The resulting plasmids were introduced into the PJ69-4A strain to examine two-hybrid interactions through *HIS3* expression after a 2-d incubation on SC plates (-His -Ura -Leu).

Immunoprecipitation

α-Mre11 rabbit serum was prepared by Tanpaku Seisei Kogyo (Gunma, Japan). The IgG fraction of α-Mre11 rabbit serum was affinity purified using a HiTrap affinity column (Amersham Biosciences, Piscataway, NJ). For each immunoprecipitation experiment, ~5 × 10⁸ cells were suspended in 300 μl of lysis buffer (20 mM HEPES, pH 7.5, 300 mM NaCl, 5 mM EDTA, 0.01% NP-40,

Table 1. Meiotic division and meiotic recombination in the *xrs2* mutants

Mutants	Spore formation (%)	Spore viability (%)	Fold increase of recombinant fraction	
			(24 h/0 h in SPM)	
			Arg ⁺	His ⁺
No tag	86	97	600	340
Empty	36	0	1.8	1.6
ΔC 0	88	99	1600	330
ΔC 1	87	100	620	260
ΔC 2	87	92	1300	270
ΔC 3	85	86	840	190
ΔC 4	47	0	2.2	1.1
ΔN 0	73	76	290	51
ΔN 1	18	3	30	22
ΔN 2	31	0	24	5.8
ΔN 3	60	21	140	25.1
ΔN 4	87	86	930	130
ΔN 8	84	88	1900	290
ΔN 11	80	96	470	150
ΔN 12	66	18	140	51
ΔN 13	82	8	110	53
ΔN 14	78	38	110	77
ΔN 15	74	54	160	57
ΔN 16	87	40	550	110
ΔN 17	82	78	100	80
ΔN 18	77	96	650	180
ΔN 20	69	0	9.4	2.4
ΔN 21	35	0	1.3	1.1
K641E	46	0	1.7	1.7
4AQ	81	100	1300	240
ΔM	45	0	2.2	1.3
E-empty	46	0	2.9	1.3
E 1	56	3	5.0	6.9
E 4	67	39	76	19
E 1-K641E	53	0	3.6	0.8
E 4-K641E	31	0	4.6	2.3

Spore formation indicates percentage of cells containing more than one spore in all visible cells after 24 h. Spore viability was measured by dissection of asci by micromanipulation, and the proportion of spores germinating to give visible colonies was assessed. Eighteen asci were dissected for each strain.

5 mM dithiothreitol, 0.04% 1-octanol, 10% glycerol, 0.8 mM phenylmethylsulfonyl fluoride, and Roche protease inhibitor cocktail [Complete, EDTA free]) and lysed by glass bead disruption by using a mini Bead-Beater (Bio-spec, Bartlesville, OK). After centrifugation at 20,000 × g for 10 min, α-Myc serum (9E10) agarose conjugate (Santa Cruz Biotechnology, Santa Cruz, CA) or affinity-purified α-Mre11 rabbit serum cross-linked to protein A-Sepharose 4FF (Amersham Biosciences) by dimethyl pimelimidate was mixed with the supernatant at 4°C for 45 min. The immunoprecipitates collected by centrifugation at 2000 × g for 15 s were washed with 600 μl of the lysis buffer four times, fractionated using 7 or 8.5% SDS-PAGE, and analyzed by Western blotting by using ECL Plus Western blotting detection reagents (Amersham Biosciences). For Western blotting, α-Mre11 guinea pig serum (Usui *et al.*, 1998) and α-Myc (9E10) mAb (Sigma-Aldrich) were used at a 1/1500 and 1/1000 dilutions, respectively. Horseradish peroxidase-conjugated anti-guinea pig (Chemicon International, Temecula, CA) and anti-mouse (Promega) IgGs were used, as instructed by the manufacturer.

RESULTS

Identification of a Minimum Region of Xrs2p Necessary for Telomere Elongation

To determine the functional regions associated with the respective functions of Xrs2p, we first constructed a system

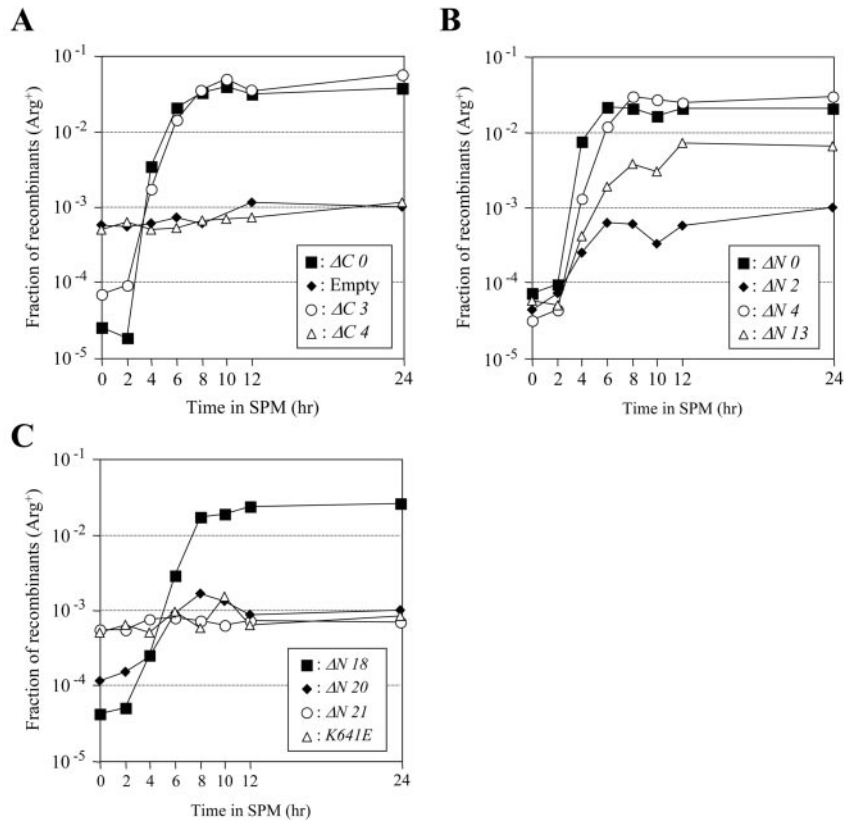


Figure 3. Commitment to meiotic recombination in the constructed *xrs2* mutants; Return to growth experiment. Meiosis was induced in diploid strains containing the indicated *xrs2* alleles. At various times after induction, cells were returned to vegetative growth media, and the fraction of Arg⁺ and His⁺ prototrophs was measured.

to screen *xrs2* mutants that are defective in telomere elongation. *mec1* strains lacking any one of the MRX components display an ever shorter telomeres (*est*) phenotype, which is referred to as senescence (Ritchie and Petes, 2000). This phenotype is accelerated in an *hdf1* mutant background (Tsukamoto *et al.*, 2001) and therefore *xrs2* mutants with a telomere elongation defect can be isolated in a *mec1 hdf1* background. The *mec1 hdf1* strain we constructed also contains *sml1* to suppress the lethality of the *mec1* null mutation. From the 2160 clones with an *xrs2* mutant gene that was mutagenized randomly by error prone PCR in the presence of MnCl₂, 33 mutants were isolated by screening for the senescence phenotype. The DNA sequences of mutation sites were then analyzed, and 31 were found to be frameshift mutations or nonsense mutations that caused deletion of the C-terminal region, suggesting that the region necessary for telomere elongation is located near the C terminus. Moreover, 15 mutants showed resistance to DNA damaging agents, indicating that the region of Xrs2p necessary for telomere elongation partially overlaps with, but is separable from, that the region required for response to DNA damage (our unpublished data).

To better understand the Xrs2p domain associated with telomere elongation, we constructed a series of *xrs2* mutants with various lengths of C-terminal or N-terminal truncations (Figure 1A). The truncation points were determined by the location of the *xrs2* frameshift mutations and nonsense mutations described above, as well as by changes in secondary structure predicted by Chou-Fasman analysis (Chou and Fasman, 1978). The wild-type and truncated mutant proteins were tagged with 9Myc at their C termini or with 3Myc at their N termini. The *xrs2-ΔC0* strain, expressing wild-type Xrs2p with 9Myc, displayed telomere lengths that were

indistinguishable from wild type (Figure 2A). The length of telomeres in the *xrs2-ΔC1* mutant strain, which encodes an Xrs2p lacking 89 C-terminal residues, was slightly shorter (~50 base pairs). In contrast, the *xrs2-ΔC2* to *-ΔC12* mutant strains displayed telomere elongation defects similar to that observed in the null strain (Figure 2A; unpublished data for *xrs2-ΔC5* to *-ΔC12*). Consistent with this, *xrs2-ΔC2* to *-ΔC12*, but not the *xrs2-ΔC0* and *-ΔC1* mutants, displayed a senescent phenotype in the *mec1 hdf1* strain background (Figure 2B; unpublished data for *xrs2-ΔC5* to *-ΔC12*). The XRS2 construct containing a Myc epitope tag at the N terminus caused a moderate telomere length defect (~100 base pairs shorter than wild type; *xrs2-ΔN0*, Figure 2A). The lengths of telomeres in the *xrs2-ΔN1* to *-ΔN20* mutant strains were similarly shorter than in the wild type, but longer than in the null strain. This observation, in conjunction with the fact that these mutants did not show senescence in the *mec1 hdf1* strain background, suggests that the *xrs2-ΔN1* to *-ΔN20* mutants are functional for telomere elongation (Figure 2B). In contrast, telomere length in the *xrs2-ΔN21* mutant strain was similar to that in the null strain, and the *xrs2-ΔN21* mutant allele conferred a senescence phenotype in the *mec1 hdf1* strain background. Hence, the results obtained from the analysis of the N- and C-terminal truncation alleles identify amino acid residues 630–765 as the region that is important for the telomere elongation function of Xrs2p.

A 32-Amino Acid Region of Xrs2p Is Necessary for Response to DNA Damage

The domain of Xrs2p necessary for response to DNA damage is separable from that for telomere elongation, as shown in the previous section. To identify the regions of Xrs2p necessary for response to DNA damage, the *xrs2* truncation

mutants were analyzed for sensitivity to the alkylating agent MMS and the ribonucleotide reductase inhibitor HU. HU reversibly disrupts the progression of replication forks from early-firing origins and also inhibits the firing of late origins (Santocanale and Diffley, 1998). Epitope tagging at either the N or C termini of Xrs2p did not affect function, as measured by complementation of *xrs2* null strains by the tagged *XRS2* constructs in the MMS and HU sensitivity assays (Figure 2C). As shown in Figure 2C, the *xrs2*- $\Delta C0$ to $\Delta C3$ and *xrs2*- $\Delta N0$ to $\Delta N20$ mutant strains showed wild-type resistance to both HU and MMS. N- and C-terminal mutants containing truncations larger than *xrs2*- $\Delta N21$ and *xrs2*- $\Delta C4$, respectively, displayed sensitivities that were similar to the *xrs2* null strain (Figure 2C; unpublished data for *xrs2*- $\Delta C5$ to $\Delta C12$). These results predict that only 32 amino acids located at positions 630–661 and included in the region required for telomere elongation are sufficient for the Xrs2p DNA damage response.

Regions in Xrs2p Necessary for Spore Formation, Spore Viability, and Meiotic Recombination

The series of *xrs2* truncation mutations analyzed above were introduced into an SK1 *xrs2/xrs2* null mutant strain to identify regions in Xrs2p necessary for meiotic events. The resulting strains were induced to enter meiosis and then examined for spore formation and viability. Meiotic functions were impaired in the *xrs2*- $\Delta C4$ mutant strain, but not in the *xrs2*- $\Delta C0$ to $\Delta C3$ mutant strains (Table 1). Therefore, a region necessary for both spore formation and meiotic viability is located between the N terminus and the 32-amino acid region. To identify this region, the N-terminal *xrs2* truncation mutants were examined. Mutants *xrs2*- $\Delta N1$, $\Delta N2$, $\Delta N3$, $\Delta N20$, and $\Delta N21$ displayed severe meiotic defects. These defects were not caused by a lack of expression of the mutant genes, because a normal level of protein expression was observed in meiotic extracts obtained from each mutant strain (our unpublished data). Furthermore, the meiotic defects in the *xrs2*- $\Delta N1$ to $\Delta N3$ mutant strains seemed not to be caused by the loss of a specific region of Xrs2p, because some mutant strains with deletions longer than *xrs2*- $\Delta N1$ to $\Delta N3$ were proficient in meiotic function. In support of this idea, we found that *xrs2*- $\Delta N12$ to $\Delta N16$ mutant strains were partially defective in meiotic function, but that the *xrs2*- $\Delta N17$ and $\Delta N18$ mutant strains, which have extensive deletions in this region, were functional.

Because defects in spore formation and viability are often associated with a deficiency in meiotic recombination, we examined recombination functions by using the return-to-growth protocol (Sherman and Roman, 1963). *xrs2* mutant strains bearing the *arg4-nsp/arg4-bgl* and *his4X::LEU2/his4B* heteroalleles (Cao *et al.*, 1990) were constructed, and their recombination functions were examined by measuring the frequency of Arg⁺ and His⁺ recombinants. In the wild-type strain, the fractions of recombinants increased with incubation time and reached a maximal level of ~1000-fold at 6 h postmeiotic induction, relative to uninduced cultures (Figure 3). The recombination frequency in the *xrs2* null strain was ~10 times higher than in wild type, consistent with null mutations in the MRX complex conferring a hyperrecombination phenotype in vegetative growth (Figure 3A; Alani *et al.*, 1990; Malone *et al.*, 1990; Ivanov *et al.*, 1992; Ajimura *et al.*, 1993). As predicted by the spore formation and viability assays, meiotic recombination was poorly induced in *xrs2*- $\Delta N1$, $\Delta N2$, $\Delta N3$, $\Delta N20$, and $\Delta N21$ strains (Figure 3, B and C). A less severe defect in meiotic recombination was observed in *xrs2*- $\Delta N12$ to $\Delta N16$ mutant strains, which showed moderate defects in spore viability. In meiotic recombina-

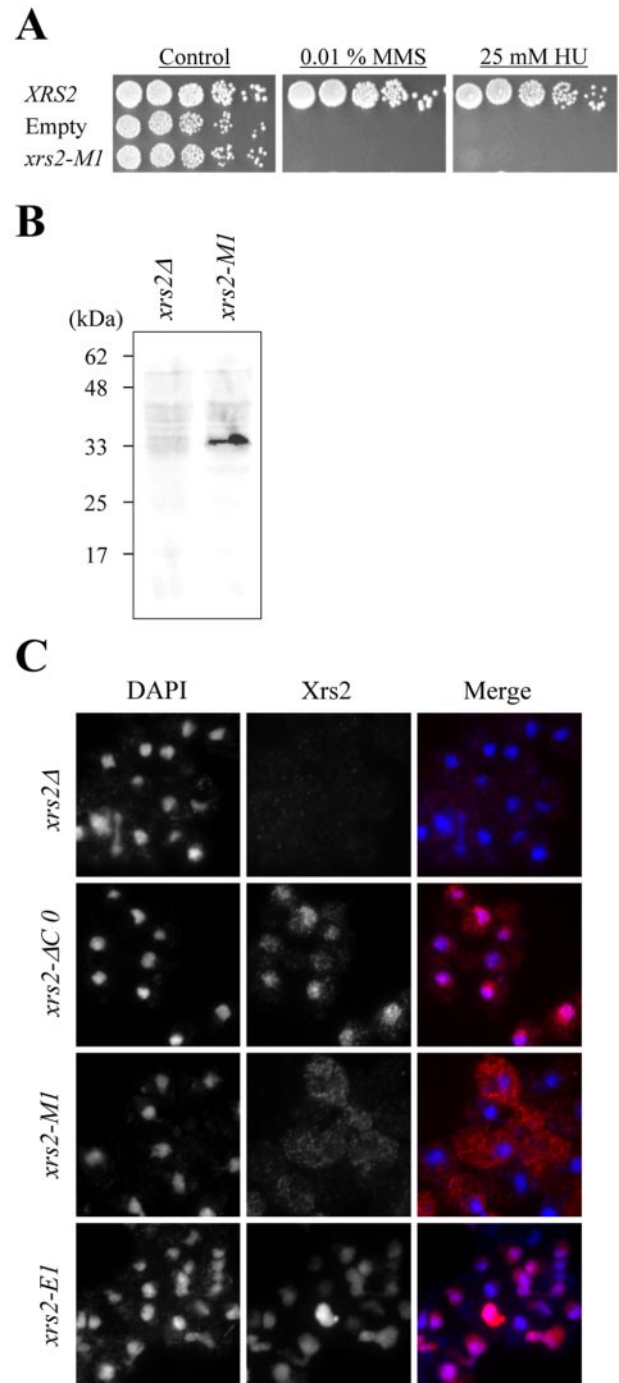


Figure 4. Cellular localization of the 32-amino acid peptide of Xrs2p. (A) Sensitivity to MMS and HU in the *xrs2-M1* mutants with truncations. Fivefold serial dilutions of the indicated *xrs2* mutants were spotted onto YPAD plates containing 0.01% MMS or 25 mM HU. The *xrs2-M1* is SYT358a derivative in which the truncated *xrs2* mutant gene was integrated into the genome. (B) Expression of the mutant Xrs2-M1 peptide was detected in a Western blot by immunostaining with α -Myc antibody. (C) Cellular localization of mutant Xrs2p. *xrs2* mutant cells in vegetative growth were immunostained with α -Myc antibody to examine the localization of the mutant Xrs2 protein. The middle panels are images of the localization of Xrs2p, obtained by staining with Alexa-Fluor-488-conjugated α -mouse IgG (specific for α -Myc antibody). The left panels are images of the localization of chromosomes, obtained by DAPI staining. The right panels show the merged images of the left (blue) and middle (red) images.

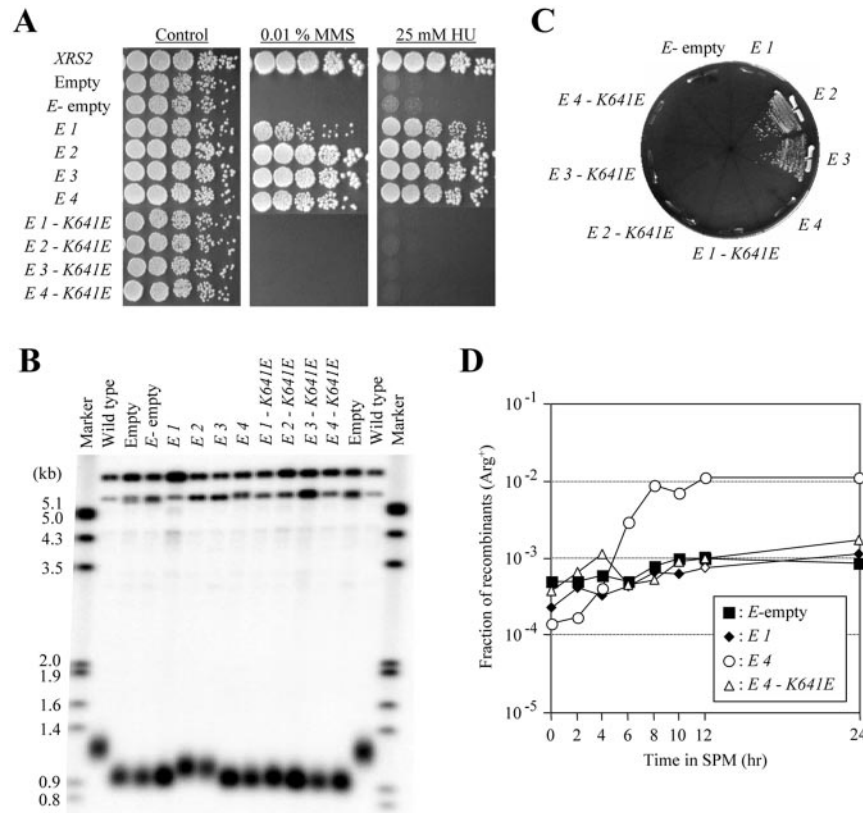


Figure 5. Characteristics of the *xrs2-E1* to *-E4* mutants and their derivatives. (A) Sensitivity to MMS and HU in the strain with *xrs2-E1* to *-E4* mutations and their derivatives. Fivefold serial dilutions of the indicated *xrs2* alleles were spotted onto YPAD plates containing 0.01% MMS or 25 mM HU. The *xrs2* mutants examined in this assay are SYT358a derivatives in which mutant *xrs2* genes were integrated into the genome. (B) The mutants were examined for telomere length. Southern blot analysis was performed as described in Figure 2. (C) Complementation of the synthetic senescence phenotype exhibited in *xrs2 mec1 hdf1* mutants with *xrs2-E1* to *-E4* mutations and their derivatives. The synthetic senescence assay was performed as described in Figure 2. (D) Meiosis was induced in diploid strains with the *xrs2-E1*, *-E4*, and *-E4-K641E* mutations. At various times after induction, cells were returned to vegetative growth media, and the fraction of Arg⁺ and His⁺ prototrophs was measured.

tion, the MRX complex is involved in at least two steps of the processes. One is the recognition step of the hot-spots, where introduction of DSBs are followed by Spo11p, and the other is the resection step at the broken ends for formation of 3' overhang (Usui *et al.*, 1998; for reviews, see Haber 1998; D'Amours and Jackson 2002). The formation of meiotic DSBs was not detected in the *xrs2-ΔN1* to *-ΔN3*, *-ΔN13*, and *-ΔN20* similar to null strain (our unpublished data).

The above-mentioned analysis suggests that two regions, comprising amino acids 581–661 and 5–123, respectively, are important for meiotic function in Xrs2p, in particular for formation of meiotic DSBs. A third region, amino acids 330–518, seems to play an important but less essential role.

Complementation of Defects in the *xrs2* Null Strain by the Selected Domains

We examined whether the minimum regions of Xrs2p identified above complement the defects of the null strain. The 32 amino acids located at positions 630–661 are suggested to be sufficient for the Xrs2p DNA damage response, based on the analysis of truncation mutants (Figure 2C). To examine whether this 32-amino acid region can complement the MMS and HU sensitivity exhibited by *xrs2* null strains, we constructed an *xrs2-M1* mutant that expresses amino acids 630–661 of Xrs2p fused to the Myc tag. The *xrs2-M1* allele, which expressed Xrs2-M1 protein at wild-type levels, but did not complement the HU and MMS sensitivity of *xrs2* null strains (Figure 4, A and B). Because the failure in complementation may be caused by absence of a nuclear localization signal in the 32-amino acid region, we examined the cellular localization of Xrs2-M1 protein, which was shown to remain in the cytoplasm (Figure 4C). We then made a fusion construct in which amino acids 630–661 of

Xrs2p were fused to the Gal4p activation domain (Gal4_{AD}), which contains a nuclear localization signal (*xrs2-E1*; Figure 1B). The resulting Xrs2-E1 peptide was able to localize to the nucleus (Figure 4C), and the *xrs2-E1* construct, but not a control construct (*xrs2-E-empty*) containing only the nuclear localization signal, complemented the MMS and HU sensitivity of the *xrs2* null, as shown in Figure 5A. Collectively, these experiments suggest that amino acids 630–661 of Xrs2p, when localized to the nucleus, can reproduce the Xrs2p DNA damage response.

Next, to test whether a peptide spanning amino acids 630–765 is sufficient to complement the telomere elongation defects of *xrs2* mutants, we made a fusion construct containing Gal4_{AD} and amino acids 630–765 of Xrs2p (*xrs2-E3*). *xrs2-E3* strains displayed a longer telomere length than null strains, and furthermore, the *xrs2-E3* allele suppressed the senescence of the *xrs2 mec1 hdf1* mutant (Figure 5, B and C).

Amino acids 581–661 of Xrs2p, the predicted minimum region for meiotic function, were then tested to determine whether this region alone is sufficient for meiotic function. The *xrs2-E4* construct (Gal4_{AD} with amino acids 581–661 of Xrs2p) was examined to determine whether it was able to complement the meiotic defects observed in the *xrs2* null strain. As shown in Table 1 and Figure 5D, *xrs2-E4* significantly restored the meiotic function of *xrs2* null strains.

Hence, the above-mentioned results indicate that peptides comprising the amino acid regions 630–765, 630–661, and 581–661 of Xrs2p are functional for telomere elongation, response to DNA damage, and meiotic function, respectively, provided that they are localized to the nucleus.

The 32-Amino Acid Peptide of Xrs2p Binds to Mre11p

The 32 amino acids located at positions 630–661 of Xrs2p are required commonly for response to DNA damage, telomere elongation, and meiotic functions. A *K641E* point mutation in this region, which was identified in initial screening, causes defects in all three functions (Figures 2 and 3C), and the introduction of the corresponding point mutation into the *xrs2-E3*, *-E1*, and *-E4* alleles resulted in mutants that failed to complement defects in telomere elongation, response to DNA damage, and meiotic events, respectively (Figure 5). This suggested that K641 is essential for each function of Xrs2p. Strains bearing full-length Xrs2p but lacking amino acid residues 630–661 displayed defects similar to the null strain (*xrs2-ΔM*; Figures 2 and 3C), and collectively these experiments suggest that amino acid residues 630–661 of Xrs2p are indispensable for Xrs2p function.

Xrs2p binds to Mre11p but not to Rad50p (Usui *et al.*, 1998). Hence, we tested whether the Xrs2-E1 peptide, containing amino acids 630–661 of Xrs2p and a nuclear localization signal, can bind to Mre11p, by using coimmunoprecipitation (see *Materials and Methods*). Extracts obtained from log-phase cell lysates of the *xrs2-E1* strain were treated with agarose-conjugated α -Myc antibody to precipitate the Myc-tagged Xrs2-E1 peptide. As shown in Figure 6A, Mre11 protein was detected in coimmunoprecipitates prepared from *xrs2-E1* cell extracts, but the protein was not detected in immunoprecipitates from *xrs2-E1-K641E* extracts. Conversely, Xrs2-E1 peptide, but not Xrs2-E1-K641E, was detected in immunoprecipitates by using α -Mre11 rabbit antibody. An interaction between the 32-amino acid region of Xrs2p and Mre11p also was observed in a two-hybrid analysis (Figure 6B; see *Materials and Methods*), and introduction of the *K641E* mutation disrupted the two-hybrid interaction. Collectively, these data indicate that amino acids 630–661 of Xrs2p can bind to Mre11p, and consequently we have named this region MBX. The results also suggest that binding of Mre11p to the MBX site of Xrs2p is necessary for the MRX complex to function in telomere elongation, DNA damage response, and meiotic events.

xrs2 DNA Repair Defects, but Not Defects in Telomere Elongation and Meiotic Recombination, Can Be Suppressed by Fusing a Nuclear Localization Signal to Mre11p

Mutation of the *NBS1* gene causes mislocalization of hMre11p to the cytoplasm (Carney *et al.*, 1998; Maser *et al.*, 2001; Tauchi *et al.*, 2001). Similarly, the nuclear localization of Mre11p is dependent on Xrs2p in yeast (Figure 7A), indicating that one of the roles of Xrs2p is to translocate Mre11p from the cytoplasm to the nucleus. To test this, we made a construct in which Gal4_{AD} bearing a nuclear localization signal was fused to Mre11p (*NLS-MRE11*). *xrs2* null strains including this construct displayed resistance to DNA damage at levels nearly similar to wild type (Figure 7B), indicating that Xrs2p is not required for the DNA damage response if Mre11p is present in the nucleus. In contrast, the DNA damage sensitivity exhibited by *rad50* null strains was not suppressed by the *NLS-MRE11*, indicating that Rad50p is required for the DNA damage response, even if Mre11p is present in the nucleus. Finally, we tested whether the *NLS-MRE11* was able to suppress the telomere elongation and meiosis defects observed in *xrs2* null strains. As shown in Figure 7, C–F, the fusion construct did not suppress these defects, indicating that Xrs2p has other function(s) that are required for these processes. *xrs2-E3* constructs lacking Gal4_{AD}, with the MBX domain deleted, or containing muta-

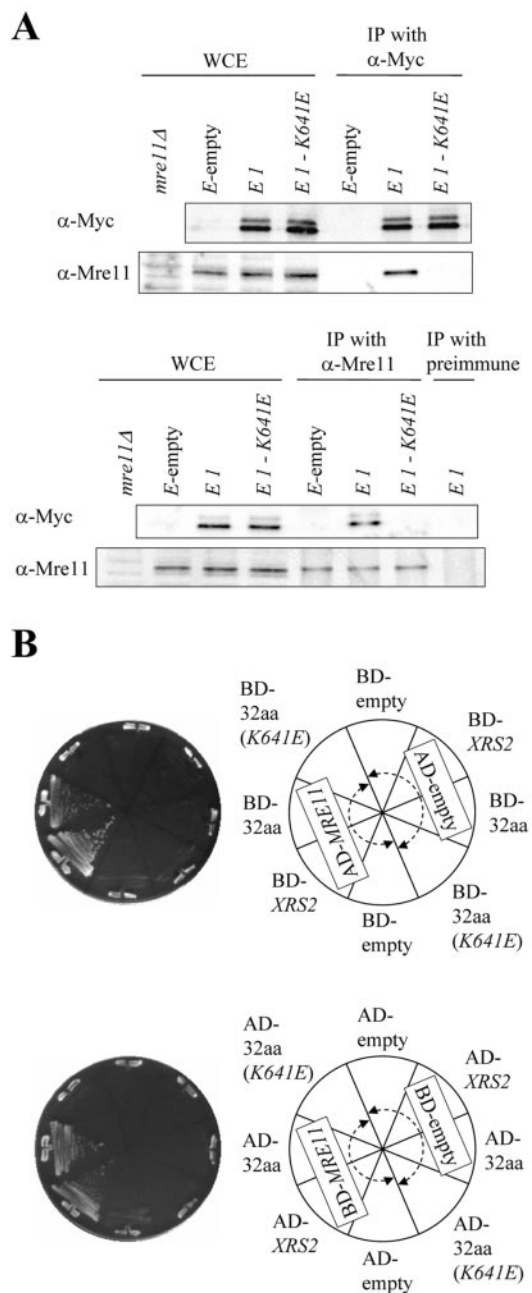
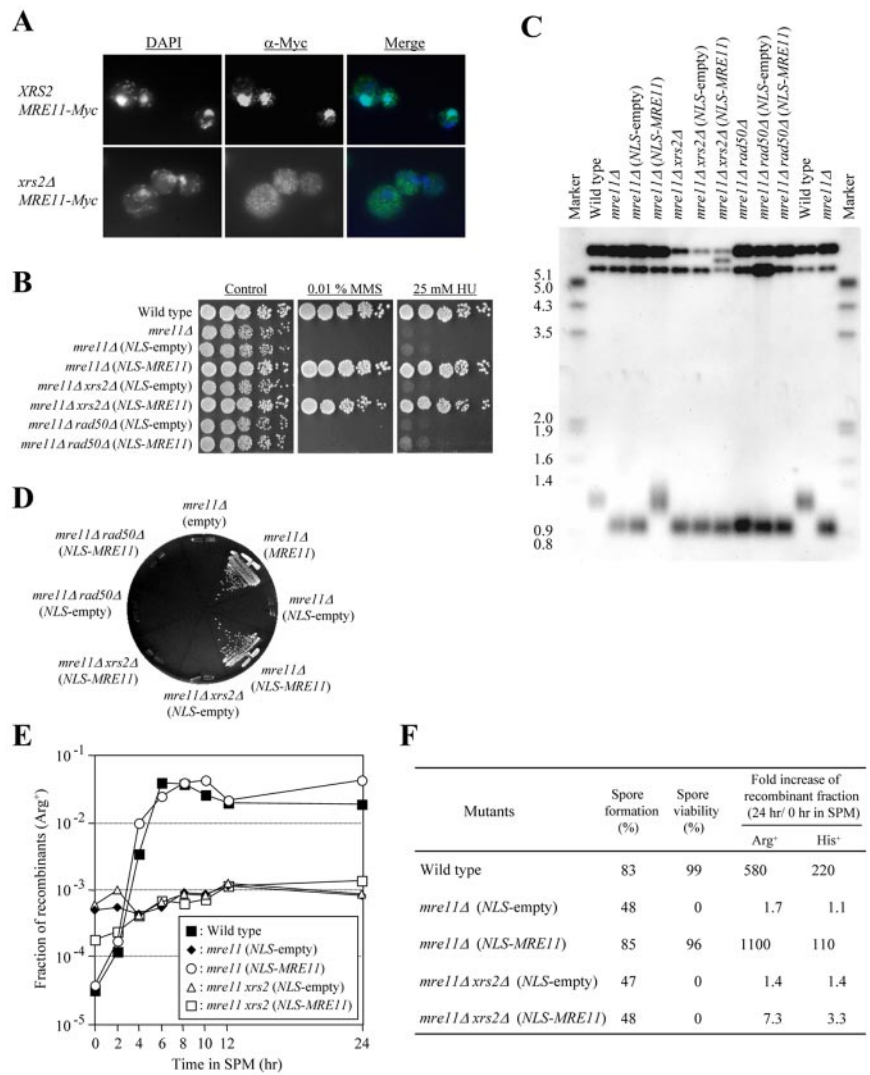


Figure 6. The 32-amino acid peptide of Xrs2p is responsible for binding to Mre11p. (A) Western blotting analyses of immunoprecipitates from cell extracts of mutant strains. The whole cell extracts (WCE) and the immunoprecipitates (IP) with α -Myc antibody were electrophoresed using 7 or 8.5% SDS-PAGE for detection of Mre11 or Xrs2-E1 proteins, respectively (top). The whole cell extracts, the α -Mre11 precipitates, and the preimmune serum precipitates were fractionated by SDS-PAGE for detection of Mre11 or Xrs2-E1 proteins (bottom). The blots were immunostained with α -Myc or α -Mre11 antibodies. (B) Two-hybrid interaction between the 32-amino acid region of Xrs2p and Mre11p. The yeast strain PJ69-4A containing the GAL4-DNA-binding-fusion plasmids and the GAL4-activation-domain-fusion plasmids was streaked onto SC (–His –Ura –Leu) plates. The physical interaction was examined after a 2-d incubation on SC plates at 30°C. BD-, fusion with the GAL4 DNA binding domain; AD, fusion with the GAL4 activation domain; empty, no insertion; *XRS2*, full-length *XRS2* gene; *MRE11*, full-length *MRE11* gene; 32aa, 32-amino acid region of Xrs2p; 32aa (*K641E*), 32-amino acid region of Xrs2p with the *K641E* mutation.

Figure 7. The *MRE11* gene with a heterologous nuclear localization signal suppresses *xrs2* DNA damage sensitivity but not meiotic or telomere elongation defects. (A) Mitotic cells of the *xrs2* mutant were immunostained with α -Myc antibody to examine localization of the Mre11 protein tagged with 9Myc at the C terminus. The middle panels are images of the localization of Mre11p, obtained by staining with Alexa-Fluor-488-conjugated α -mouse IgG (specific for α -Myc antibody). Left, images of the localization of chromosomes, obtained by DAPI staining. Right, merged images of the left (blue) and middle (green) images. (B) Sensitivity to MMS and HU in the *mre11*, *mre11 xrs2*, and *mre11 rad50* null strains with the NLS-MRE11 fusion constructs. The fusion bears a nuclear localization signal (NLS) of the Gal4 activation domain at the N-terminal of Mre11p. Fivefold serial dilutions of yeast cells were spotted onto YPAD plates containing 0.01% MMS or 25 mM HU. (C) Lengths of telomeres in the *mre11*, *mre11 xrs2*, and *mre11 rad50* null strains with the NLS-MRE11 fusion constructs. Southern blot analysis was performed as described in Figure 2. (D) Complementation of the synthetic senescence phenotype exhibited by *mec1 sml1 hdf1* mutants with the *mre11*, *mre11 xrs2*, and *mre11 rad50* mutations by the NLS-MRE11 fusion constructs. The NLS-MRE11 plasmid was introduced into SYT301a harboring the plasmid pYT1501. The senescence assay was performed as described in Figure 2. (E and F) Meiosis was induced in the indicated diploid strains. At various times after induction, cells were returned to vegetative growth media, and the fraction of Arg⁺ and His⁺ prototrophs was measured. Fractions of spore formation, spore viability, and recombinants were measured as described in Table 1.



tions that disrupt Mre11p binding, conferred a null-like phenotype in the telomere elongation assay, even when NLS-Mre11p was coexpressed (our unpublished data). Collectively, these data suggest that binding of Xrs2p to Mre11p is necessary for telomere elongation after the two proteins are translocated to the nucleus.

DISCUSSION

In this study, we have identified a 32-amino acid region of Xrs2p that is required for binding to Mre11p, and we refer to this as the MBX region. This binding is necessary for translocation of Mre11p from the cytoplasm to the nucleus. We showed that Xrs2p is not required in repair of DNA damage when Mre11p is localized in the nucleus. However, two other defined domains, which are upstream and downstream of the MBX region, respectively, are absolutely required for meiotic recombination and telomere elongation, respectively, in addition to the Mre11p binding domain (Figure 8).

Roles of Xrs2p Domains Adjacent to the MBX Region

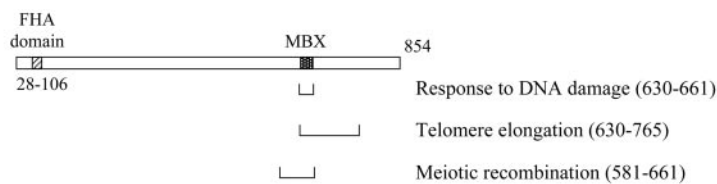
The MBX region and a downstream region (amino acids 662–765) of Xrs2p were identified as essential for telomere

elongation function. Recently, Tel1p, an ATM homologue that phosphorylates Xrs2p in response to DNA double-strand breaks, was shown to bind to Xrs2p (D'Amours and Jackson, 2001; Usui *et al.*, 2001; Nakada *et al.*, 2003). This binding is eliminated in an Xrs2p mutant protein that lacks the C-terminal 162 amino acid residues (Nakada *et al.*, 2003). This truncated version of Xrs2p is only two amino acids shorter than Xrs2- Δ C2, which is defective in telomere elongation (Figures 1 and 2), and therefore these data suggest that the telomere elongation defect observed in *xrs2- Δ C2* to - Δ C12 mutants is caused by a defect in binding to Tel1p.

The MBX region and a upstream region (amino acids 581–629) are both required for meiotic recombination and may regulate the activity of Mre11p. In support of this idea, we showed that meiotic processing of DSBs by MRX requires a stable MRX complex (Usui *et al.*, 1998). In addition, Xrs2p binds to the N-terminal half of Mre11p, in which its nuclease domains are located (our unpublished data). These data imply that not only the domain for meiotic recombination but also that for telomere elongation may induce conformational changes of Mre11p or act to regulate Mre11p activity. An analysis of whether the Xrs2p domains modify Mre11p properties such as nuclease activity and DNA binding activity, and screening for transacting factors to these

A

	Response to DNA damage	Telomere elongation	Spore viability	Meiotic recombination
No tag 854	+++	+++	+++	+++
Empty (No fragment)	-	-	-	-
<i>AC 0</i> 854	+++	+++	+++	+++
<i>AC 1</i> 765	+++	++	+++	+++
<i>AC 2</i> 694	+++	-	+++	+++
<i>AC 3</i> 661	+++	-	+++	+++
<i>AC 4</i> 629	-	-	-	-
<i>AN 0</i> 4	+++	++	++	+++
<i>AN 1</i> 28	+++	++	-	-
<i>AN 2</i> 51	+++	+++	-	-
<i>AN 4</i> 124	+++	+++	+++	+++
<i>AN 13</i> 385	+++	+++	+	++
<i>AN 18</i> 581	+++	++	+++	+++
<i>AN 20</i> 630	+++	++	-	-
<i>AN 21</i> 662	-	-	-	-
<i>K641E</i>	-	-	-	-
<i>ΔM</i> Δ630-661	-	-	-	-
<i>E-empty</i> NLS- (No fragment)	-	-	-	-
<i>E 1</i> NLS- 630-661	++	-	-	-
<i>E 1 - K641E</i> NLS- 630-661	-	-	-	-
<i>E 2</i> NLS- 581-765	+++	++	ND	ND
<i>E 2 - K641E</i> NLS- 581-765	-	-	ND	ND
<i>E 3</i> NLS- 630-765	+++	++	ND	ND
<i>E 3 - K641E</i> NLS- 630-765	-	-	ND	ND
<i>E 4</i> NLS- 581-661	++	-	++	++
<i>E 4 - K641E</i> NLS- 581-661	-	-	-	-

B

regions, would provide valuable information regarding their function.

We showed that amino acids 581–661 and 630–765 of Xrs2p complement defects in meiotic functions and telomere elongation, respectively, when they are fused to a nuclear localization signal (Figure 5). However, other regions located in the N terminus of Xrs2p also have some effect on meiosis and telomere elongation (Figures 4A and 5 and Table 1). In particular, the *xrs2-ΔN1*, *-ΔN2*, and *-ΔN13* mutants are severely defective in meiosis, whereas the *xrs2-ΔN4*, *-ΔN8*, *-ΔN11*, and *-ΔN18* mutants display meiotic function, even though they contain deletions in *XRS2* that are more extensive than in other truncation mutants that are strongly defective in meiosis (Figures 2 and 3 and Table 1). These differences are unlikely to be due to protein expression, because on the whole the expression of the mutant proteins is comparable with wild type in vegetative growth and during meiosis (our unpublished data). We favor the idea that certain truncations at the N-terminal region of

Xrs2p might induce conformational changes that impair the meiotic functions of the Mre11 complex, whereas more extensive truncations in Xrs2p remove such impairment.

A Comparison of Xrs2p with Its Human Homologue Nbs1p

Xrs2p and Nbs1p share several features, in addition to the enhancement of the in vitro DNA binding and DNA nuclease activities of Mre11p (Paull and Gellert, 1999; Trujillo *et al.*, 2003). For both proteins, their binding to Mre11p seems essential for MRX or Mre11p–Rad50p–Nbs1p complex function (Figures 2, 3, 5, and 6; Desai-Mehta *et al.*, 2001, Tauchi *et al.*, 2001). The Mre11p binding site in both Xrs2p and Nbs1p is located near the C terminus, and the amino acid sequence of the binding site is conserved between Xrs2p and Nbs1p and also in other species (Figure 2; Tauchi *et al.*, 2001). The K641 residue of Xrs2p, mutation of which causes an almost null phenotype, is highly conserved across species, and this

Figure 8. Summary of the domain analyses of Xrs2p. (A) Summary of the effects of the various *xrs2* mutations on DNA damage response, telomere elongation, spore viability, and meiotic recombination. The symbols indicate the level of function: +++, comparable to wild-type; ++, slight defect; +, moderate defect; –, null. (B) Minimum regions of Xrs2p required for DNA damage response, telomere elongation, and meiotic recombination. The MBX region and the FHA-like domain of Xrs2p are indicated.

strongly suggests that Xrs2p and its homologues bind to a conserved structure in Mre11p.

Nbs1p is suggested to affect nuclear location of hMre11p because hMre11p is mislocalized to the cytoplasm in *nbs1* mutants with the 657del5 allele, which is found in ~90% of Nijmegen breakage syndrome patients (Carney *et al.*, 1998; Tauchi *et al.*, 2001). In this study, Mre11p was shown not to be localized to the nucleus in the *xrs2* null strain (Figure 7A). Furthermore, Mre11p fused to a nuclear localization signal completely suppresses the MMS sensitivity of the *xrs2* null strain. Hence, these results show that Xrs2p has a role in translocation of Mre11p from the cytoplasm to nucleus. Three nuclear localization signal motifs have been located near the hMre11 binding site of Nbs1p, one of which is located at the C-terminal side of the site (Tauchi *et al.*, 2002). Xrs2p is also suggested to have such signals that located at both sides of the MBX, in regions amino acids 320–629 and 766–854 (Tsukamoto, Ogawa, and Ogawa; unpublished data). Alternatively, Xrs2p might have interaction sites with another protein, which possesses a nuclear localization signal, at both sides of the MBX.

An FHA motif, corresponding to a phosphopeptide recognition motif found in many proteins that act in nuclear signaling, is located at the N-terminal regions of both Nbs1p (amino acids 24–100) and Xrs2p (amino acids 28–106). The 70-kDa Nbs1 mutant protein, which the *nbs1-657del5* mutant cells express, lacks the N-terminal region, including the FHA domain, but still possesses the hMre11 binding domain and a nuclear localization signal (Maser *et al.*, 2001). The absence of this motif in Nbs1p causes sensitivity to DNA damage, radioresistant DNA synthesis, and less foci formation of the hMre11 complex in response to DNA damage (Petrini, 2000; Cerosaletti and Concannon 2003). On the other hand, analogous truncation mutations on the *XRS2* gene do not cause deficiency in terms of sensitivity to DNA damage (Figure 2C). Expression of the 70-kDa Nbs1 mutant protein in *nbs1-657del5* mutant cells is reduced (Maser *et al.*, 2001), and the x-ray sensitivity of the *nbs1* mutant is greatly compensated for by overexpression of Nbs1 mutant proteins lacking the N-terminal regions, although properties of radioresistant DNA synthesis and reduced foci formation of the hMre11 complex were not recovered (Desai-Mehta *et al.*, 2001; Tauchi *et al.*, 2001). Therefore, a reduced amount of the N-terminal truncated Nbs1p may cause different effects of similar mutations in human and yeast cells, in terms of response to DNA damage. To validate this possibility, an examination of radioresistant DNA synthesis and foci formation of the Mre11 complex in response to DNA damage in yeast is required.

Xrs2p Is Unique in Eukaryotes

The Mre11p and Rad50p components of MRX are conserved from bacteria to vertebrates. The bacterial SbcD proteins of *E. coli*, *Bacillus subtilis*, and *Rhodobacter capsulatus*, and gp47 of bacteriophage T4 are homologues of Mre11p, and the SbcC proteins of these bacteria and gp46 of the bacteriophage are homologues of Rad50p (Sharples and Leach, 1995). Similarly to yeast Mre11p and Rad50p, *E. coli* SbcD and SbcC proteins form a complex and have 3'-to-5' exonuclease and ssDNA endonuclease activities, which are important for DNA repair and recombination functions mediated by the complex (Connelly and Leach, 1996). However, homologues of Xrs2p are not found in bacteria. We hypothesize that bacteria do not require such homologues because they have no need to translocate Mre11 homologues into a nuclear compartment. Also, the functions of Xrs2p in telomere elongation and meiotic processes are unlikely to be

required in organisms that contain circular genomes and do not have a meiotic recombination pathway analogous to that observed in eukaryotes.

In conclusion, our results suggest that Xrs2p has important roles in regulation of the extensive activities of the Mre11 complex, as well as for a role in the translocation of Mre11p into the nucleus. We anticipate that an understanding of the yeast MRX complex will aid in studies on the functions of the human MRN complex in telomere elongation and meiotic recombination, which are difficult to analyze in humans. Our results also should contribute to the understanding of genetic disorders associated with defects in this complex, such as Nijmegen breakage syndrome and ataxia-telangiectasia-like disease (Carney *et al.*, 1998; Mat-suura *et al.*, 1998; Varon *et al.*, 1998; Stewart *et al.*, 1999).

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REFERENCES

- Ajimura, M., Leem, S. H., and Ogawa, H. (1993). Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics* 133, 51–66.
- Alani, E., Padmore, R., and Kleckner, N. (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., Trujillo, K. M., Sung, P., and Erickson, H. P. (2001). Structure of the Rad50-Mre11 DNA repair complex from *Saccharomyces cerevisiae* by electron microscopy. *J. Biol. Chem.* 276, 37027–37033.
- Burke, D., Dawson, D., and Stearns, T. (2000). *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Cao, L., Alani, E., and Kleckner, N. (1990). A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* 61, 1089–1101.
- Carney, J. P., Maser, R. S., Olivares, H., Davis, E. M., Le Beau, M., Yates, J. R., 3rd, Hays, L., Morgan, W. F., and Petrini, J. H. (1998). The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 93, 477–486.
- Cerosaletti, K. M., and Concannon, P. (2003). Nibrin forkhead-associated domain and breast cancer C-terminal domain are both required for nuclear focus formation and phosphorylation. *J. Biol. Chem.* 278, 21944–21951.
- Chou, P. Y., and Fasman, G. D. (1978). Prediction of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol. Relat. Areas Mol. Biol.* 47, 45–148.
- Connelly, J. C., and Leach, D. R. (1996). The *sbcC* and *sbcD* genes of *Escherichia coli* encode a nuclease involved in palindrome inviability and genetic recombination. *Genes Cells* 1, 285–291.
- D'Amours, D., and Jackson, S. P. (2001). The yeast Xrs2 complex functions in S phase checkpoint regulation. *Genes Dev.* 15, 2238–2249.
- D'Amours, D., and Jackson, S. P. (2002). The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. *Nat. Rev. Mol. Cell. Biol.* 3, 317–327.
- Desai-Mehta, A., Cerosaletti, K. M., and Concannon, P. (2001). Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization. *Mol. Cell. Biol.* 21, 2184–2191.
- Furuse, M., Nagase, Y., Tsubouchi, H., Murakami-Murofushi, K., Shibata, T., and Ohta, K. (1998). Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. *EMBO J.* 17, 6412–6425.
- Gietz, R. D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene* 74, 527–534.

- Grenon, M., Gilbert, C., and Lowndes, N. F. (2001). Checkpoint activation in response to double-strand breaks requires the Mre11/Rad50/Xrs2 complex. *Nat. Cell Biol.* 3, 844–847.
- Haber, J. E. (1998). The many interfaces of Mre11. *Cell* 95, 583–586.
- Ivanov, E. L., Korolev, V. G., and Fabre, F. (1992). XRS2, a DNA repair gene of *Saccharomyces cerevisiae*, is needed for meiotic recombination. *Genetics* 132, 651–664.
- James, P., Halladay, J., and Craig, E. A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144, 1425–1436.
- Johzuka, K., and Ogawa, H. (1995). Interaction of Mre11 and Rad 50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. *Genetics* 139, 1521–1532.
- Kato, R., and Ogawa, H. (1994). An essential gene, ESRI, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 22, 3104–3112.
- Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K., and Schiebel, E. (1999). Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast* 15, 963–972.
- Malone, R. E., Ward, T., Lin, S., and Waring, J. (1990). The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. *Curr. Genet.* 18, 111–116.
- Maser, R. S., Zinkel, R., and Petrini, J. H. (2001). An alternative mode of translation permits production of a variant NBS1 protein from the common Nijmegen breakage syndrome allele. *Nat. Genet.* 27, 417–421.
- Matsuura, S., *et al.* (1998). Positional cloning of the gene for Nijmegen breakage syndrome. *Nat. Genet.* 19, 179–181.
- Nakada, D., Matsumoto, K., and Sugimoto, K. (2003). ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev.* 17, 1957–1962.
- Paull, T. T., and Gellert, M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev.* 13, 1276–1288.
- Petrini, J. H. (2000). The Mre11 complex and ATM: collaborating to navigate S phase. *Curr. Opin. Cell Biol.* 12, 293–296.
- Raymond, W. E., and Kleckner, N. (1993). RAD50 protein of *S. cerevisiae* exhibits ATP-dependent DNA binding. *Nucleic Acids Res.* 21, 3851–3856.
- Ritchie, K. B., and Petes, T. D. (2000). The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. *Genetics* 155, 475–479.
- Santocanale, C., and Diffley, J. F. (1998). A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. *Nature* 395, 615–618.
- Schneider, B. L., Seufert, W., Steiner, B., Yang, Q. H., and Futcher, A. B. (1995). Use of polymerase chain reaction epitope tagging for protein tagging in *Saccharomyces cerevisiae*. *Yeast* 11, 1265–1274.
- Schulz, V. P., and Zakian, V. A. (1994). The *Saccharomyces* PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell* 76, 145–155.
- Sharples, G. J., and Leach, D. R. (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.
- Sherman, F., and Roman, H. (1963). Evidence for two types of allelic recombination in yeast. *Genetics* 48, 255–261.
- Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122, 19–27.
- Sikorski, R. S., and Boeke, J. D. (1991). In vitro mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. *Methods Enzymol.* 194, 302–318.
- Stewart, G. S., Maser, R. S., Stankovic, T., Bressan, D. A., Kaplan, M. I., Jaspers, N. G., Raams, A., Byrd, P. J., Petrini, J. H., and Taylor, A. M. (1999). The DNA double-strand break repair gene *hMRE11* is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 99, 577–587.
- Tauchi, H., Kobayashi, J., Morishima, K., Matsuura, S., Nakamura, A., Shi-raishi, T., Ito, E., Masnada, D., Delia, D., and Komatsu, K. (2001). The fork-head-associated domain of NBS1 is essential for nuclear foci formation after irradiation but not essential for hRAD50-hMRE11-NBS1 complex DNA repair activity. *J. Biol. Chem.* 276, 12–15.
- Tauchi, H., Matsuura, S., Kobayashi, J., Sakamoto, S., and Komatsu, K. (2002). Nijmegen breakage syndrome gene, NBS1, and molecular links to factors for genome stability. *Oncogene* 21, 8967–8980.
- Terasawa, M., Shinohara, A., Hotta, Y., Ogawa, H., and Ogawa, T. (1995). Localization of RecA-like recombination proteins on chromosomes of the lily at various meiotic stages. *Genes Dev.* 9, 925–934.
- Trujillo, K. M., Roh, D. H., Chen, L., Van Komen, S., Tomkinson, A., and Sung, P. (2003). Yeast xrs2 binds DNA and helps target rad50 and mre11 to DNA ends. *J. Biol. Chem.* 278, 48957–48964.
- Tsukamoto, Y., Taggart, A. K., and Zakian, V. A. (2001). The role of the Mre11-Rad50-Xrs2 complex in telomerase-mediated lengthening of *Saccharomyces cerevisiae* telomeres. *Curr. Biol.* 11, 1328–1335.
- Usui, T., Ogawa, H., and Petrini, J. H. (2001). A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol. Cell* 7, 1255–1266.
- Usui, T., Ohta, T., Oshiumi, H., Tomizawa, J., Ogawa, H., and Ogawa, T. (1998). Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell* 95, 705–716.
- Varon, R., *et al.* (1998). Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 93, 467–476.
- Zhu, X. D., Kuster, B., Mann, M., Petrini, J. H., and de Lange, T. (2000). Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. *Nat. Genet.* 25, 347–352.