Overlapping Functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 Nucleases in DNA Metabolism

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ABSTRACT

MRE11 functions in several aspects of DNA metabolism, including meiotic recombination, double-strand break repair, and telomere maintenance. Although the purified protein exhibits 3′ to 5′ exonuclease and endonuclease activities *in vitro*, Mre11 is implicated in the 5′ to 3′ resection of duplex ends *in vivo*. The *mre11-H125N* mutation, which eliminates the nuclease activities of Mre11, causes an accumulation of unprocessed double-strand breaks (DSBs) in meiosis, but no defect in processing HO-induced DSBs in mitotic cells, suggesting the existence of redundant activities. Mutation of *EXO1*, which encodes a 5′ to 3′ exonuclease, was found to increase the ionizing radiation sensitivity of both *mre11*Δ and *mre11-H125N* strains, but the *exo1 mre11-H125N* strain showed normal kinetics of mating-type switching and was more radiation resistant than the *mre11*Δ strain. This suggests that other nucleases can compensate for loss of the Exo1 and Mre11 nucleases, but not of the Mre11-Rad50-Xrs2 complex. Deletion of *RAD27*, which encodes a flap endonuclease, causes inviability in *mre11* strains. When *mre11-H125N* was combined with the leaky *rad27-6*, the double mutants were viable and no more γ-ray sensitive than the *mre11-H125N* strain. This suggests that the double mutant defect is unlikely to be due to defective DSB processing.

DNA double-strand breaks (DSBs) are potentially lethal lesions that arise spontaneously during normal cellular processes, such as replication, or by treatment of cells with DNA damaging agents. DSBs act to initiate several programmed genetic rearrangements, including mating-type switching in *Saccharomyces cerevisiae* (Strathern *et al.* 1982), meiotic recombination (Sun *et al.* 1989; Cao *et al.* 1990), and V(D)J rearrangement in B and T cells (Fugmann *et al.* 2000). DSBs are repaired either by homologous recombination or by nonhomologous end joining. Homologous recombination is considered to be an error-free process that requires the presence of a chromosome homolog or sister chromatid to template repair, whereas end-joining repair is homology independent and is potentially mutagenic. In yeast, DSBs are primarily repaired by homologous recombination and this process requires genes of the *RAD52* epistasis group (Paques and Haber 1999).

Mating-type switching, which is initiated by cleavage of the *MAT* locus by HO endonuclease, and meiotic recombination can be studied in synchronous populations of cells to identify DNA intermediates and follow the kinetics of repair. After the formation of HO-induced or meiosis-specific DSBs, the ends are processed to form long 3′ single-stranded tails (White and Haber 1990; Sun *et al.* 1991). The single-stranded tails are substrates for the pairing protein Rad51 (or Rad51 and Dmc1 in meiotic cells) to initiate strand invasion (Sung 1994; Shinohara *et al.* 1997). The Mre11-Rad50-Xrs2 (MRX) complex is thought to participate in the initial resection step because null alleles of any of the genes encoding these proteins result in reduced processing of HO-induced DSBs (Ivanov *et al.* 1994; Tsubouchi and Ogawa 1998). Meiosis-specific DSBs are not formed in *mre11*, *rad50*, and *xrs2* null mutants (Alani *et al.* 1990; Ivanov *et al.* 1992; Johzuka and Ogawa 1995). However, several separation-of-function alleles of *MRE11* and *RAD50* have been isolated that allow formation, but not processing, of meiosis-specific DSBs (Alani *et al.* 1990; Nairz and Klein 1997; Furuse *et al.* 1998; Tsubouchi and Ogawa 1998; Usui *et al.* 1998; Moreau *et al.* 1999). In *rad50S* mutants, the Spo11 protein remains covalently bound to the 5′ ends at meiotic break sites, preventing resection (Keeney *et al.* 1997). *In vitro*, Mre11 protein exhibits single-stranded endonuclease and 3′ to 5′ exonuclease activities. As the exonuclease is of the opposite polarity to that expected if Mre11 were the major resection activity, it seems more likely that the endonuclease activity of Mre11 is important for DSB processing and is targeted to the 5′ strand by an unknown mechanism. Mutations that abolish the endo- and exonuclease activities of Mre11 have been generated and shown to block processing of meiosis-specific DSBs (Furuse *et al.* 1998; Usui *et al.* 1998; Moreau *et al.* 1999). Strains containing the nuclease-deficient allele, *mre11-H125N*, show normal kinetics of mating-type switching, but the *mre11-58* strain, which is

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also Mre11 nuclease defective, is delayed for mating-type switching (Tsubouchi and Ogawa 1998; Moreau *et al.* 1999). The more severe phenotype of the *mre11-58* strain could be due to failure of the mutant protein to interact with Rad50 and Xrs2 (Usui *et al.* 1998). It has been suggested that the MRX complex unwinds ends, providing a substrate for the endonuclease activity of Mre11 to remove Spo11 from meiosis-specific DSBs (Keeney *et al.* 1997; Nairz and Klein 1997; Furuse *et al.* 1998; Usui *et al.* 1998; Moreau *et al.* 1999). In the absence of a covalently bound protein, such as at HO-induced breaks, the 5′ ends could be processed by other nucleases (Moreau *et al.* 1999).

Exonucleases that act with a 5′ to 3′ polarity would appear to be the best candidates for factors redundant with Mre11 in mitotic cells. *S. cerevisiae* has five putative 5′ to 3′ nucleases with homology to the RNase H family of nucleases: Exo1, Din7, Rad2, Rad27 (FEN-1), and Yen1 (Mueser *et al.* 1996; Hosfield *et al.* 1998; Johnson *et al.* 1998). Rad2 and Rad27 act preferentially as structure-specific endonucleases with weak 5′ to 3′ exonuclease activity (Habraken *et al.* 1994; Harrington and Lieber 1994). Rad2 generates the endonucleolytic cleavage 3′ to bulky lesions and *rad2* mutants show high sensitivity to UV light, but not to ionizing radiation (Prakash and Prakash 2000). Rad27 functions during DNA synthesis to remove RNA primers from Okazaki fragments (Hosfield *et al.* 1998). Although *rad27* null mutants are viable, they are unable to grow at 37°, are hypermutagenic and hyperrecombinogenic, and are synthetically lethal with mutations of genes in the *RAD52* epistasis group (Tishkoff *et al.* 1997b; Symington 1998). Exo1 is a 5′ to 3′ exonuclease with a twofold preference for double-stranded over single-stranded DNA and also exhibits flap endonuclease activity (Szankasi and Smith 1992; Fiorentini *et al.* 1997; Lee and Wilson 1999). Exo1 interacts with Msh2 and *exo1* mutants exhibit a mutator phenotype as well as defects in mitotic and meiotic recombination (Szankasi and Smith 1995; Fiorentini *et al.* 1997; Tishkoff *et al.* 1997a; Khazanehdari and Borts 2000; Kirkpatrick *et al.* 2000; Tsubouchi and Ogawa 2000). The Din7 protein localizes to mitochondria and has no obvious role in nuclear DNA metabolism (Fikus *et al.* 2000). Yen1 is the least conserved member of the family and no DNA repair defects have been reported for *yen1* mutants (Johnson *et al.* 1998).

EXO1 present in high copy suppresses the methyl methanesulfonate (MMS) sensitivity of *mre11*, *rad50*, and *xrs2* null mutants, suggesting that *EXO1* is able to take over some functions of the MRX complex (Chamankhah *et al.* 2000; Tsubouchi and Ogawa 2000). Furthermore, an *exo1* mutation increases the MMS sensitivity of *mre11* and *rad50* strains, and *mre11 exo1* double mutants show an even longer delay in processing an HO-induced DSB than *mre11* mutants (Tsubouchi and Ogawa 2000). *EXO1* in high copy also suppresses the mutator phenotype of *rad27* and certain *msh2* mutants (Tishkoff *et al.* 1997a; Sokolsky and Alani 2000). In efforts directed at identifying the nuclease activity redundant with the Mre11 nuclease, we constructed strains deficient for *MRE11* and genes encoding members of the Rad2 family. Consistent with two recent reports, we find that *exo1* mutations intensify the DNA repair defect of *mre11* deletion strains (*mre11*Δ), and *EXO1* present in high copy suppresses the DNA repair defect of a *mre11*Δ strain (Chamankhah *et al.* 2000; Tsubouchi and Ogawa 2000). However, *mre11-H125N exo1* strains are more repair proficient than *mre11*Δ strains and show no defect in HO-induced break processing, suggesting that there are other nucleases that can compensate for loss of the Exo1 and Mre11 nucleases, but not of the MRX complex.

MATERIALS AND METHODS

Media and growth conditions: Rich medium (YPD) and synthetic complete (SC) medium lacking the appropriate amino acid or nucleic acid base were prepared as described previously (Sherman *et al.* 1986). Sporulation medium contained 1% potassium acetate and the appropriate amino acids or nucleic acid bases at one-fifth of the concentration used in SC medium. Yeast cells were grown at 30°. Strains containing the conditional *rad27-6* allele were grown at the semipermissive temperature of 30°. Strains containing the *rad27*Δ allele were grown at room temperature.

Yeast strains and plasmids: All of the strains used for this study are derivatives of W303-1A or W303-1B and are listed in Table 1 (Thomas and Rothstein 1989). The *MRE11* locus of strain LSY716, described previously as containing the *mre11-H125N::URA3::mre11-D56N* allele, was amplified from genomic DNA, directly sequenced, and shown to be *mre11-H125N::URA3::mre11-D56N, H125N*. Thus all strains derived from LSY716 (LSY726, LSY967, LSY985, LSY845-2A, LSY845-5B, LSY865-7C, and LSY865-8D) contain the *mre11-H125N::URA3::mre11-D56N, H125N* allele. Where indicated, Ura− derivatives containing the *mre11-H125N* allele were used. Strains LSY716 and LSY716A have identical phenotypes in all of the assays described. Standard methods were used for crosses and tetrad dissection to generate the strains described in Table 1 (Sherman *et al.* 1986). Yeast transformation was by the lithium acetate method (Ito *et al.* 1983).

The *EXO1* gene was amplified by PCR from genomic DNA using two primers, one containing an *Xho*I site (5′ CCG CTCGAGACAACATCACAGTTCATTGC 3′) and the second one containing a *Sac*II site (5′ TCCCCGCGGCATCTACTTT TAATCTTTTC 3′). The restriction enzyme sites are underlined. The resulting PCR fragment was digested with *Xho*I and *Sac*II and cloned into the *CEN* vector, pRS414 (Sikorski and Hieter 1989), generating the plasmid pSM464. Plasmid pSM502 was generated by cloning the *Kpn*I-*Sac*II fragment from plasmid pSM464 into the high-copy-number 2μ vector, pRS424 (Christianson *et al.* 1992). The plasmid pSM502 was used for the construction of *EXO1* mutations with the Quick Change Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The oligonucleotides 5′ GCTATTTGGTCTTCGCTGGT GATGCCATTCC 3′ and 5′ GGAATGGCATCACCAGCGAAG ACCAAATACG 3′ were used to generate the *exo1-D78A* mutation, and oligonucleotides 5′ TATCCGAAGATTCTGCCCT CCTCGTCTTCGG 3′ and 5′ CCGAAGACGAGGAGGGCAGA ATCTTCGGATA 3′ were used to generate the *exo1-D173A* mutation. The resulting plasmids were named pSM506 (*exo1-D78A*) and pSM638 (*exo1-D173A*).

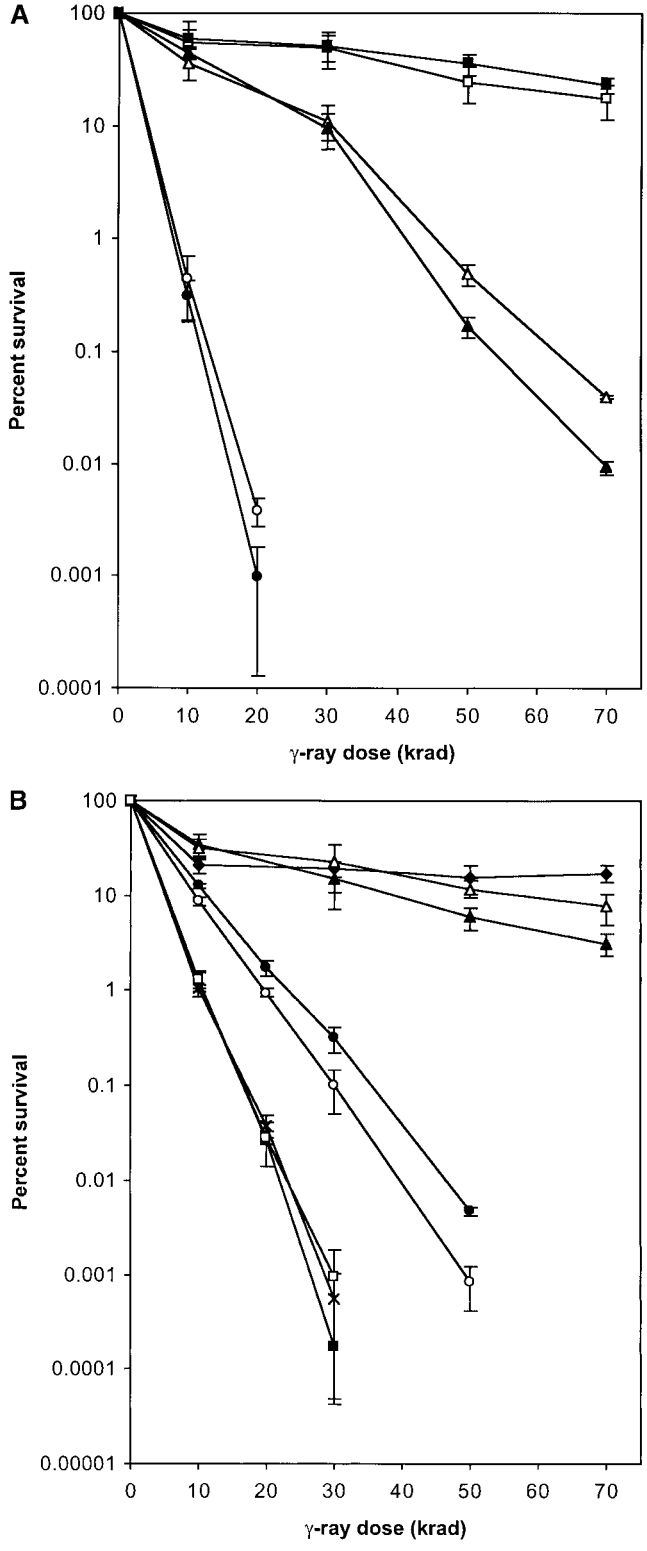
TABLE 1

Yeast strains

Name	Relevant genotype or description[a]	Reference
W303-1A	*MAT***a**	Thomas and Rothstein (1989)
W303-1B	*MAT*α	Thomas and Rothstein (1989)
LSY380	*MAT*α *rad2::TRP1*	Fiorentini *et al.* (1997)
LSY485-2C	*MAT***a** *yen1::HIS3*	Fiorentini *et al.* (1997)
LSY491-3B	*MAT***a** *rad2::TRP1 yen1::HIS3*	This study
LSY496-20A	*MAT*α *exo1::HIS3*	Fiorentini *et al.* (1997)
LSY509	*MAT***a** *exo1::HIS3 din7::LEU2*	Fiorentini *et al.* (1997)
LSY543	*MAT***a** *rad57::LEU2*	This study
LSY568	*MAT***a** *mre11::LEU2*	Moreau *et al.* (1999)
LSY569	*MAT*α *mre11::LEU2*	Moreau *et al.* (1999)
LSY716	*MAT***a** *mre11-H125N::URA3::mre11-D56N, H125N*	Moreau *et al.* (1999)
LSY716A	*MAT***a** *mre11-H125N*	Moreau *et al.* (1999)
LSY726	*MAT*α *mre11-H125N::URA3::mre11-D56N, H125N*	Moreau *et al.* (1999)
LSY726A	*MAT*α *mre11-H125N*	Moreau *et al.* (1999)
LSY968	*MAT***a** *exo1::HIS3*	This study
LSY615-1A	*MAT***a** *mre11::LEU2 exo1::HIS3*	This study
LSY615-1D	*MAT*α *mre11::LEU2 exo1::HIS3*	This study
LSY967	*MAT*α *mre11-H125N::URA3::mre11-D56N, H25N exo1::HIS3*	This study
LSY967A	*MAT*α *mre11-H125N exo1::HIS3*	This study
LSY720	*MAT*α *rad52::LEU2 met17-sna ADE2*	Bärtsch *et al.* (2000)
LSY984	*MAT***a** *mre11::LEU2 rad52::LEU2*	This study
LSY985	*MAT***a** *mre11-H125N::URA3::mre11-D56N, H125N rad52::LEU2*	This study
LSY486-2A	*MAT*α *rad27::URA3*	Symington (1998)
LSY702-2A	*MAT***a** *rad27::TRP1*	Symington (1998)
LSY702-3B	*MAT*α *rad27::TRP1*	Symington (1998)
LSY706-3A	*MAT***a** *rad27-6*	Symington (1998)
LSY845-2A	*MAT*α *mre11-H125N::URA3::mre11-D56N, H125N rad27-6*	This study
LSY845-12A	*MAT*α *exo1::HIS3 rad27-6*	This study
LSY845-5B	*MAT*α *mre11-H125N::URA3::mre11-D56N, H125N exo1::HIS3 rad27-6*	This study
LSY865-7C	*MAT*α *mre11-H125N::URA3::mre11-D56N, H125N rad2::TRP1 yen1::LEU2*	This study
LSY865-8D	*MAT*α *mre11-H125N::URA3::mre11-D56N, H125N rad27-6 rad2::TRP1 yen1::LEU2*	This study
LSY1030-4C	*MAT***a** *mre11-H125N::URA3::mre11-D56N, H125N din7::LEU2*	This study
LSY1030-7D	*MAT***a** *mre11-H125N::URA3::mre11-D56N, H125N din7::LEU2 exo1::HIS3*	This study
LSY739	*MAT*α *rad50S::URA3 leu2-K::ADE2-URA3::leu2R*	Moreau *et al.* (1999)

[a] All strains are derivatives of W303 (*leu2-3, 112 trp1-1 ura3-1 can1-100 ade2-1 his3-11, 15*); only mating type and differences in genotype are given.

The *RAD27* gene was amplified by PCR from genomic DNA using two primers, one containing a *Sac*I site 5′ TAGCGAGCTCTACGATGGTTCCGATATGCCA 3′ and the second one containing an *Eco*RI site 5′ CCGGAATTCCTTGTGAAATTGCAAATATGG 3′. The resulting PCR fragment was digested with *Eco*RI/*Sac*I and cloned into the high-copy-number 2μ vector, pRS423 (Christianson *et al.* 1992), generating the plasmid pSM476.



γ-Irradiation survival assays: Cells were grown in liquid medium to midlog phase. The cultures were serially diluted and aliquots of each dilution were plated on solid medium. The plates were irradiated in a Gammacell-220 containing ^{60}Co (Atomic Energy of Canada) for the designated dose. The dose rate of the Gammacell-220 was 50 rad/sec. The plates were incubated for 3–4 days before survivors were counted. Each strain was assayed at least three times and mean values are presented.

Physical analysis of mating-type switching and telomere length: Physical analyses of mating-type switching and telomere length were performed as described previously (Moreau *et al.* 1999).

End-joining assay: Yeast strains containing a *GAL-HO* plasmid were grown in SC medium to midlog phase and dilutions were plated on medium containing either 2% glucose or 2% galactose. The number of colonies obtained from growth on galactose divided by the number of colonies obtained from growth on glucose provides a measure of the efficiency of end joining of the chromosomal HO-induced break (Moore and Haber 1996).

RESULTS

Mutation of *EXO1* increases the radiation sensitivity of the *mre11*Δ and *mre11-H125N* strains: Genetic analysis of yeast strains lacking the Mre11 nuclease activity (*mre11-H125N*) revealed weak sensitivity to ionizing radiation and no defects in processing HO-induced DSBs, telomere maintenance, or end-joining repair (Moreau *et al.* 1999). However, the *mre11-H125N* strain was sporulation defective due to the accumulation of unprocessed meiosis-specific DSBs (Moreau *et al.* 1999). This observation led to the idea that redundant nucleases in mitotic cells can process ends in the absence of the Mre11 nuclease, but are unable to act on Spo11-bound breaks generated during meiotic recombination. To determine whether any member of the Rad2 family of nucleases is redundant with Mre11, we performed crosses to generate haploid progeny containing either the *mre11*Δ or *mre11-H125N* allele with mutations in *RAD2* family genes (*rad2*, *rad27*, *exo1*, *din7*, and *yen1*). The resulting strains were then tested for increased radiation sensitivity compared to the *mre11* strains. The *rad2*, *din7*, and *yen1* mutations caused no increase in radiation sensitivity to *mre11*Δ or *mre11-H125N* strains (data not shown). We have previously shown lethality caused by combining the *rad27*Δ and *mre11*Δ or *rad27*Δ and *mre11-H125N* mutations. How-

Figure 1.—Genetic interaction between *mre11* and *exo1* in the repair of ionizing radiation-induced DNA damage. (A) Radiation sensitivity of wild type (W303-1A), *exo1*Δ (LSY968), *mre11-H125N* (LSY716A), *mre11*Δ (LSY568), and double mutants (LSY615-1A and LSY967). (□) Wild type, (■) *exo1*, (△) *mre11-H125N*, (▲) *mre11-H125N exo1*, (○) *mre11*Δ, and (●) *mre11*Δ *exo1*. (B) *EXO1* on either a centromere (*CEN*) or 2μ plasmid, but not the *exo1-D78A* or *exo1-D173A* alleles, suppresses the DNA repair defect of the *mre11*Δ strain (LSY568). (◆) Wild type + pRS424, (▲) wild type + 2μ *EXO1*, (△) wild type + 2μ *exo1-D173A*, (●) *mre11*Δ + 2μ *EXO1*, (○) *mre11*Δ + *CEN EXO1*, (×) *mre11*Δ + pRS424, (■) *mre11*Δ + 2μ *exo1-D78A*, and (□) *mre11*Δ + 2μ *exo1-D173A*.

ever, viable spores were recovered from a cross between a strain containing the *rad27-6* allele and *mre11-H125N*. Even using the leaky *rad27-6* allele we were unable to recover double mutants with *mre11*Δ. The *rad27-6 mre11-H125N* strain showed no increase in ionizing radiation sensitivity compared to the *mre11-H125N* strain (data not shown). The addition of *rad2* and *yen1* mutations to the *mre11-H125N rad27-6* strain caused no additional increase in ionizing radiation sensitivity (data not shown). A high-copy-number plasmid containing the *RAD27* gene was unable to suppress the ionizing radiation sensitivity, growth, or sporulation defects of *mre11*Δ or *mre11-H125N* strains.

Consistent with results of Tsubouchi and Ogawa (2000), we found the *exo1*Δ mutation increased the radiation sensitivity of the *mre11*Δ strain and caused a growth defect more severe than that observed for the *mre11*Δ mutation alone (Figure 1A). The *exo1*Δ mutation alone caused no increase in radiation sensitivity, even at 70 krad (Figure 1A). The *exo1*Δ *mre11-H125N* strains showed near normal growth rates, but an increased sensitivity to ionizing radiation compared to the *mre11-H125N* strain. However, the *exo1*Δ *mre11-H125N* double mutant was still more resistant to ionizing radiation than the *mre11*Δ strain. Because Din7 and Exo1 are the most closely related members of the Rad2 family, a *din7*Δ *exo1*Δ *mre11-H125N* triple mutant was also tested, but was found to be as radiation sensitive as the *exo1*Δ *mre11-H125N* double mutant (data not shown). We constructed an *exo1*Δ *mre11-H125N rad27-6* triple mutant by crossing a *mre11-H125N rad27-6* strain to an *exo1*Δ strain, but found that its poor viability prevented further study. Preliminary tests showed similar γ-ray sensitivity to the *exo1*Δ *mre11-H125N* double mutant, suggesting that the severe growth defect of the triple mutant is not due to elimination of redundant DSB-processing activities. The growth deficiency of the triple mutant is primarily due to the combination of *exo1*Δ and *rad27-6* mutations; the *exo1*Δ *mre11-H125N* and *rad27-6 mre11-H125N* double mutants grow reasonably well, whereas the *exo1*Δ *rad27-6* double mutant forms small heterogeneously sized colonies. This growth defect presents in diploids as well as haploids, suggesting that it is not due to the accumulation of recessive mutations.

***EXO1* present in high copy reduces the ionizing radiation sensitivity but not the telomere length or end-joining defects of the *mre11*Δ strain:** *EXO1* was identified in a screen for high-copy suppressors of the MMS sensitivity of *mre11* mutants (Tsubouchi and Ogawa 2000). When present on a high-copy-number plasmid, *EXO1* could reduce the ionizing radiation sensitivity of the *mre11*Δ strain (Figure 1B), but not the *mre11-H125N* strain. Even when present on a *CEN* plasmid, *EXO1* reduced the ionizing radiation sensitivity of the *mre11*Δ strain, indicating that just one extra copy of *EXO1* is sufficient to improve the radiation resistance of this strain. This result provides further support for the hypothesis that Exo1 is partially redundant with Mre11 in DNA repair. To confirm the requirement for the Exo1 nuclease, two point mutations, *exo1-D78A* and *exo1-D173A*, were constructed within the putative catalytic site of Exo1 (on the basis of mutational studies of FEN-1; Shen *et al.* 1996; Hosfield *et al.* 1998) and tested for suppression of the *mre11* repair defect. In this case the *exo1* mutant alleles were unable to reduce the ionizing radiation sensitivity of the *mre11*Δ strain (Figure 1B) or *exo1*Δ *mre11*Δ strain (data not shown) when introduced on a high-copy-number plasmid. The *exo1-D173A* mutation expressed from a high-copy plasmid was previously shown to be unable to suppress the temperature sensitivity of a *msh2-L560S pol3-01* double mutant, but was shown to be stably expressed in yeast and to retain interaction with Msh2 (Sokolsky and Alani 2000).

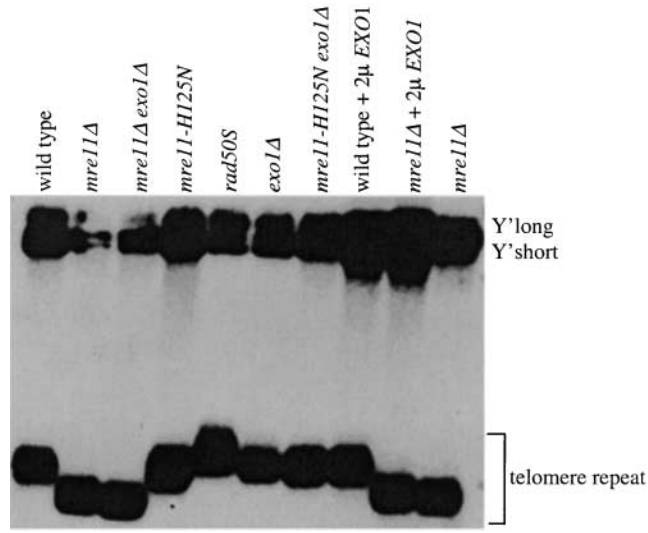


Figure 2.—*EXO1* present in high copy is unable to suppress the telomere length defect of the *mre11*Δ strain. Genomic DNA isolated from each strain was digested with *Xho*I, separated on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with a telomere-specific probe. The Y′ telomeres of the wild-type strain form a heterogeneous band of ~1.3 kb. Strains were W303-1A (wild type), LSY568 (*mre11*Δ), LSY615-1A (*mre11*Δ *exo1*), LSY716A (*mre11-H125N*), LSY739 (*rad50S*), LSY968 (*exo1*), and LSY967A (*mre11-H125N exo1*). Where indicated, the wild-type and *mre11*Δ strains were transformants containing the 2μ *EXO1* plasmid.

We have previously demonstrated normal telomere length in *mre11-H125N* strains. This finding contrasts with observations in the *rad50S* strain, in which telomeres are slightly longer than the wild-type strain (Kironmai and Muniyappa 1997; Figure 2). The *exo1* mutation by itself, or in combination with *mre11-H125N*, had no effect on telomere length (Figure 2). However, we did note a slight decrease in telomere length in the *exo1*Δ *mre11*Δ strain, which could be restored to the length characteristic of the *mre11*Δ strain by introduction of *EXO1* on a high-copy plasmid. In agreement with other published studies, *EXO1* present in high copy did not suppress the short telomere defect of the *mre11*Δ strain

TABLE 2

Efficiency of end joining a chromosomal DSB

Relevant genotype	% cell survival[a]
rad52 MRE11	0.5
rad52 MRE11 + 2μ *EXO1*	0.4
*rad52 mre11*Δ	<0.00001
rad52 mre11-H125N	0.06
*rad52 mre11*Δ + 2μ *EXO1*	<0.00001

[a] Percentage survival was measured by the number of colonies formed on galactose plates (HO endonuclease induced) divided by the number of colonies formed on glucose plates (HO repressed).

(Chamankhah *et al.* 2000; Tsubouchi and Ogawa 2000) or have any effect on telomere length of wild-type or *mre11-H125N* strains. The *rad27-6* mutation alone, or with *mre11-H125N*, caused no defect in telomere length (data not shown). The high-copy *EXO1* plasmid was also tested for suppression of the sporulation block imposed by the *mre11-H125N* mutation, but, as expected, was unable to restore sporulation to *mre11-H125N* homozygous diploids.

In previous studies we found no defect in end-joining repair in *mre11-H125N* strains, using a plasmid ligation assay (Moreau *et al.* 1999). As the plasmid assay monitors faithful ligation of cohesive ends to regenerate the restriction enzyme site originally used to digest the plasmid, the lack of requirement for a nuclease is not surprising. Therefore, we tested the requirement for the Mre11 nuclease, and suppression by *EXO1*, in an assay that involves imprecise end joining (Moore and Haber 1996). In this method, the HO endonuclease cleaves the *MAT* locus in a *rad52* strain, which precludes repair by homologous recombination, but not by other means. Precise end joining regenerates the HO cleavage site, which will then be recut under conditions of continuous HO expression. However, if imprecise end joining occurs as a result of deletion or addition of nucleotides, the junction will be insensitive to HO cleavage and the cells will resume growth to form a colony. By placing the HO gene under the control of the *GAL1* promoter, the efficiency of end joining is a measure of the ratio of colonies formed on medium containing galactose compared to glucose. By this assay there was a >1000-fold decrease in end-joining efficiency in the *mre11*Δ strain and a 10-fold reduction in the *mre11-H125N* strain compared to the *rad52* control (Table 2). The high-copy-number *EXO1*-containing plasmid was unable to suppress the end-joining defect of the *mre11*Δ strain.

***mre11-H125N exo1* strains exhibit normal kinetics of mating-type switching:** Yeast strains with null mutations of *MRE11*, *RAD50*, or *XRS2* exhibit reduced resection of HO-induced DSBs and a delay in mating-type switching (Ivanov *et al.* 1994; Tsubouchi and Ogawa 1998).

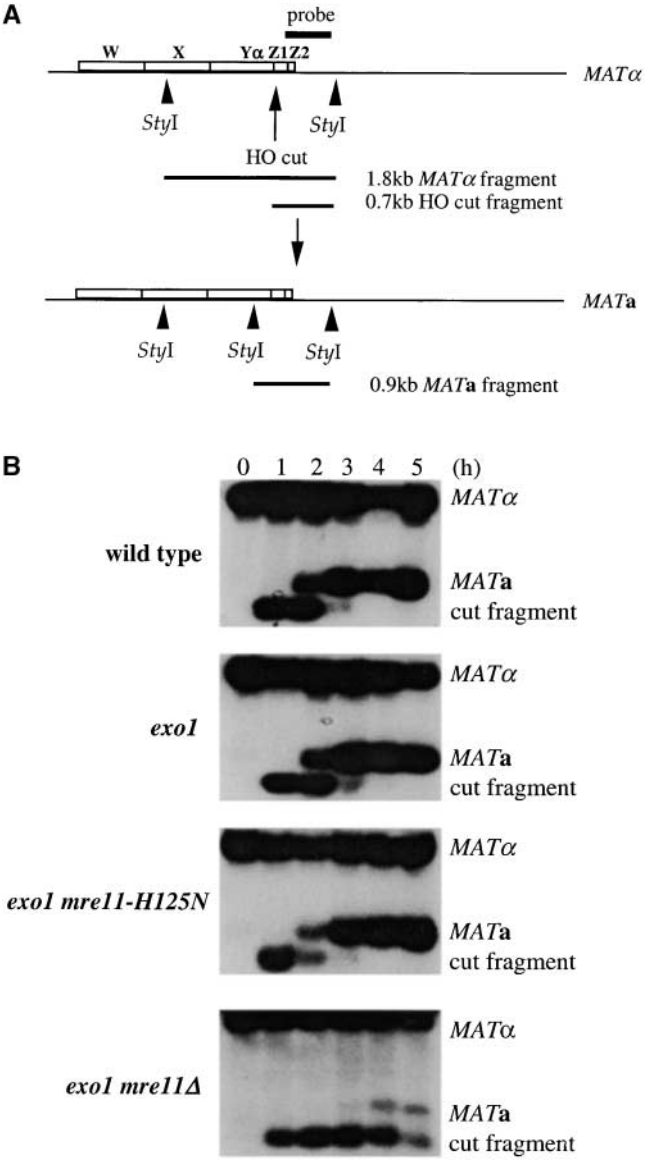


Figure 3.—Mating-type switching shows normal kinetics in the *exo1*Δ *mre11-H125N* strain. (A) Schematic representation of the *MAT*α locus. *Sty*I sites and the expected fragments before and after HO cutting are indicated. Repair of the HO-induced break by conversion from the *HMR***a** donor yields a novel 0.9-kb *Sty*I fragment. (B) Time course of repair in wild-type (W303-1B), *exo1*Δ (LSY496-20A), *exo1*Δ *mre11-H125N* (LSY967A), and *exo1*Δ *mre11*Δ (LSY615-1D) strains. All strains contain the p*GAL-HO* plasmid. Galactose was added to the cultures at time 0, for 1 hr, to induce expression of HO endonuclease and samples were removed at 1-hr intervals for DNA analysis.

Tsubouchi and Ogawa (2000) demonstrated greater stability of the HO-cut fragment and an even greater delay in switching in the *exo1*Δ *mre11*Δ strain, assumed to occur as a result of even slower resection of HO-induced breaks. If Exo1 were the nuclease redundant with Mre11 we would have expected the *exo1*Δ *mre11-H125N* double mutant to also show delayed kinetics of

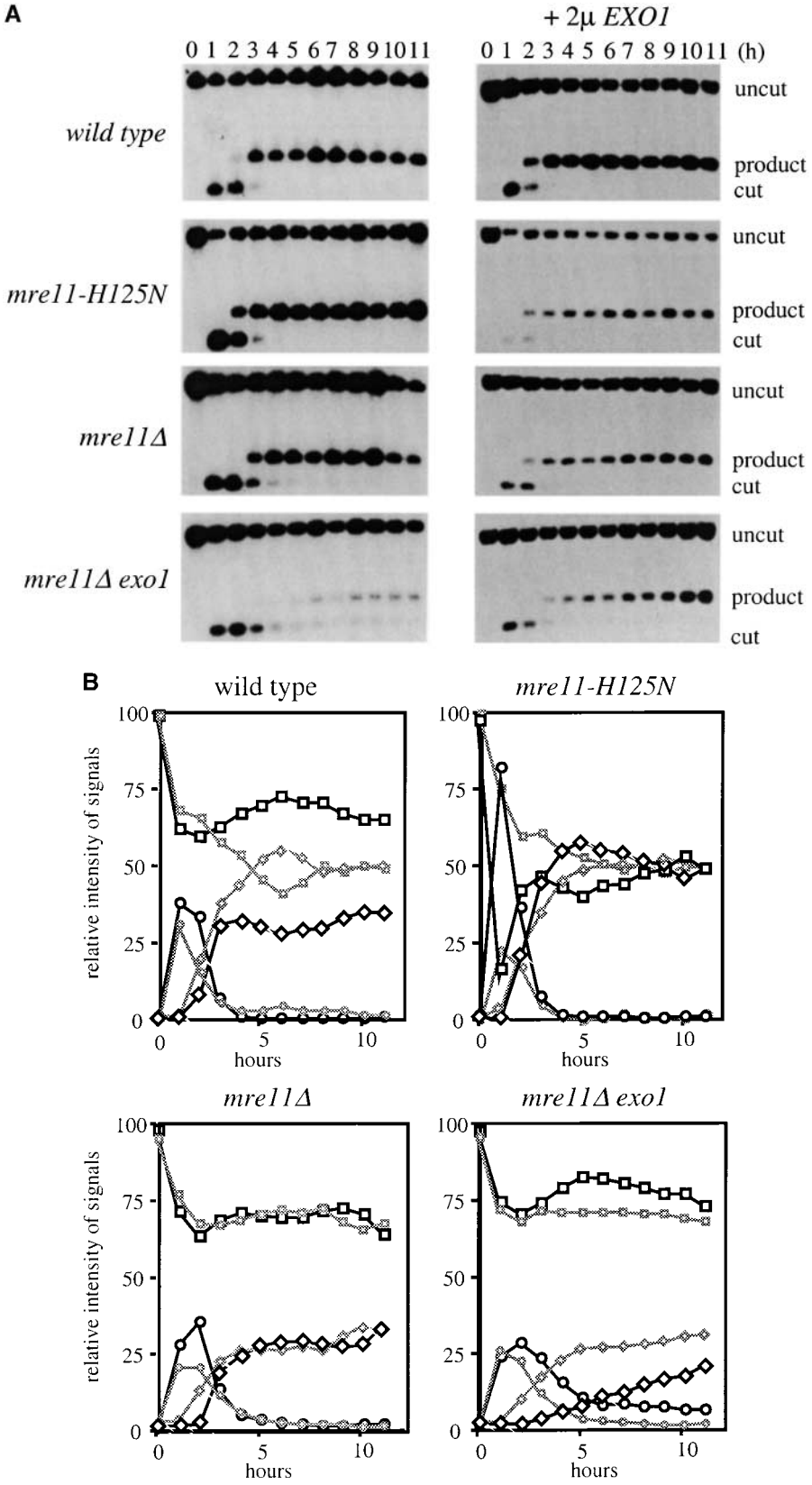


FIGURE 4.—*EXO1* in high copy partially suppresses the delay in mating-type switching of the *mre11*Δ strain. (A) On the left are strains without the *EXO1* high-copy-number plasmid and on the right are the same strains containing the *EXO1* high-copy-number plasmid. Strains are wild type (W303-1B), *mre11-H125N* (LSY716A), *mre11*Δ (LSY568), and *exo1*Δ *mre11*Δ (LSY615-1D), and all contain the p*GAL-HO* plasmid. Galactose was added to the cultures at time 0, for 1 hr, to induce expression of HO endonuclease and samples were removed at 1-hr intervals for DNA analysis. (B) Densitometric analyses of the data presented in A. For each strain the relative intensities of the (□) uncut (*MAT*α), (○) cut, and (◇) product bands (*MAT***a**) are shown with (shaded lines) and without (solid lines) the 2μ *EXO1* plasmid.

mating-type switching. As shown previously, the *exo1*Δ and *mre11-H125N* strains exhibited normal kinetics of switching to *MAT***a** (MOREAU *et al.* 1999; TSUBOUCHI and OGAWA 2000), but the same kinetics were also found for the *exo1*Δ *mre11-H125N* strain (Figure 3). *EXO1* present in high copy increased the rate of switching to *MAT***a** of the *mre11*Δ strain (Figure 4), but had no discernible effect on the kinetics of mating-type switching in the

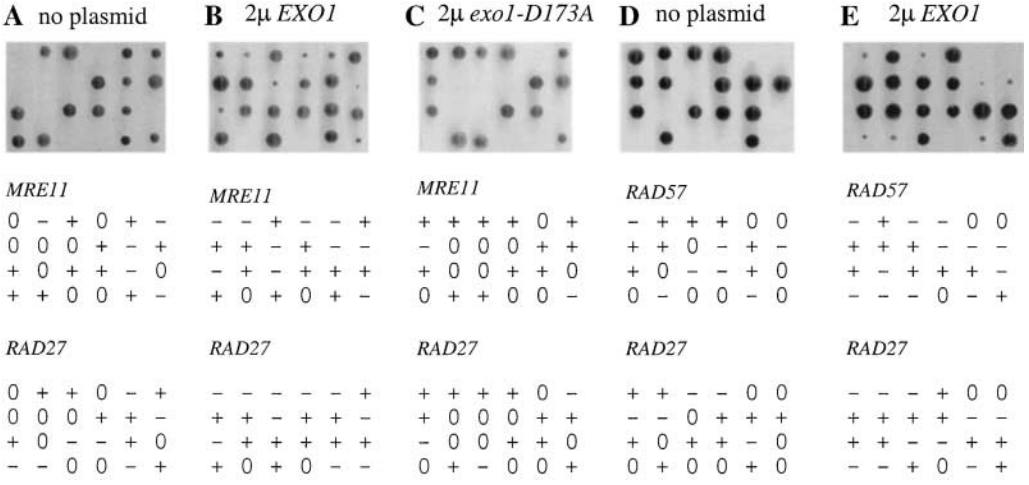


FIGURE 5.—*EXO1* in high copy suppresses the inviability of *rad27*Δ *mre11*Δ and *rad27*Δ *rad57*Δ double mutants. (A) Spores derived from a diploid heterozygous for *RAD27* and *MRE11* (LSY568 × LSY702-3B). (B) Spores from a diploid heterozygous for *RAD27* and *MRE11* (LSY568 × LSY702-3B) containing the 2μ plasmid expressing *EXO1* (pSM502). (C) Spores derived from a diploid heterozygous for *RAD27* and *MRE11* (LSY568 × LSY702-3B) containing the 2μ plasmid expressing the *exo1-D173A* allele (pSM638). (D) Spores derived from a diploid heterozygous for *RAD27* and *RAD57* (LSY702-3B × LSY543). (E) Spores derived from a diploid heterozygous for *RAD27* and *RAD57* (LSY702-3B × LSY543) containing the 2μ plasmid expressing *EXO1* (pSM502). In each case, the grid below the tetrad dissection indicates the genotype of each spore: +, wild type; −, mutant locus; 0, dead spore.

wild-type or *mre11-H125N* strains. The *EXO1* high-copy plasmid partially complemented the delay in mating-type switching of the *exo1*Δ *mre11*Δ strain, but switching efficiency was not restored to wild-type levels.

High-copy suppression of the synthetic lethality of *rad27* with *RAD52* group mutations by *EXO1*: Deletion of *RAD27* is lethal in combination with mutation of any one the *RAD52* group genes, including *mre11*Δ and *mre11-H125N* (SYMINGTON 1998; MOREAU *et al.* 1999; DEBRAUWERE *et al.* 2001). A diploid heterozygous for *MRE11* and *RAD27* was transformed with the high-copy-number *EXO1* plasmid and sporulated, and the resulting tetrads were dissected to determine whether viable *mre11*Δ *rad27*Δ spores could be obtained. *EXO1* did suppress the lethality, but the spore colonies were very small (Figure 5). This suppression is most likely due to suppression of the *rad27*Δ defect because the *EXO1* plasmid also suppressed the lethality of *rad27*Δ *rad57*Δ and *rad27*Δ *rad59*Δ double mutants (Figure 5 and data not shown). The suppression was not observed using the plasmid containing the *exo1-D173A* mutation, indicating a requirement for the nuclease activity of Exo1.

DISCUSSION

The Mre11 complex of *S. cerevisiae*, consisting of Mre11, Rad50, and Xrs2, functions in several aspects of DNA metabolism, including meiotic recombination, DNA repair, telomere maintenance, and nonhomologous end joining (PAQUES and HABER 1999). Although the Mre11 protein exhibits 3′ to 5′ exonuclease and endonuclease activities *in vitro* (FURUSE *et al.* 1998; PAULL and GELLERT 1998; USUI *et al.* 1998; MOREAU *et al.* 1999), the Mre11 complex is implicated in resection of HO-induced and meiosis-specific DSBs to produce 3′ single-stranded tails (IVANOV *et al.* 1994; TSUBOUCHI and OGAWA 1998). To explain this paradox, it has previously been suggested that the MRX complex unwinds ends to provide a single-stranded substrate for the Mre11 endonuclease activity. Alternatively, there may be an as yet unknown cofactor or associated protein that reverses the polarity of the Mre11 exonuclease. The polarity of the *Escherichia coli* RecBCD nuclease is reversed after interaction with a Chi site (ANDERSON and KOWALCZYKOWSKI 1997). The human Mre11/Rad50/Nbs1 complex also shows 3′ to 5′ polarity, indicating that the other components of the complex are insufficient to reverse the polarity (TRUJILLO *et al.* 1998; PAULL and GELLERT 1999). Although point mutations eliminating the Mre11 nuclease activity result in the accumulation of meiosis-specific DSBs, the processing of HO-induced DSBs is unaffected by the *mre11-H125N* mutation (FURUSE *et al.* 1998; USUI *et al.* 1998; MOREAU *et al.* 1999). Because meiosis-specific DSBs retain Spo11 covalently bound to the 5′ termini, but HO-induced breaks have free ends, it seems likely that other nucleases are redundant with Mre11 in mitotic cells. To explain the inability of these redundant nucleases to process meiosis-specific DSBs, we suggest these activities are exonucleases that require a free 5′ end or possibly endonucleases that are excluded from the meiotic DSB forming/processing complex.

Exo1 appeared to be a likely candidate for the redundant activity because the polarity of degradation is 5′ to 3′ and *EXO1* has been identified as a high-copy suppressor of the MMS sensitivity of *mre11*Δ mutants (TSUBOUCHI and OGAWA 2000). Furthermore, *exo1*Δ *mre11*Δ double mutants grow very poorly (30% plating efficiency) and show higher sensitivity to MMS and ionizing radiation than *mre11*Δ single mutants (TSUBOUCHI and OGAWA 2000; Figure 1). The greater synergism observed between *mre11*Δ and *exo1*Δ for MMS sensitivity than we

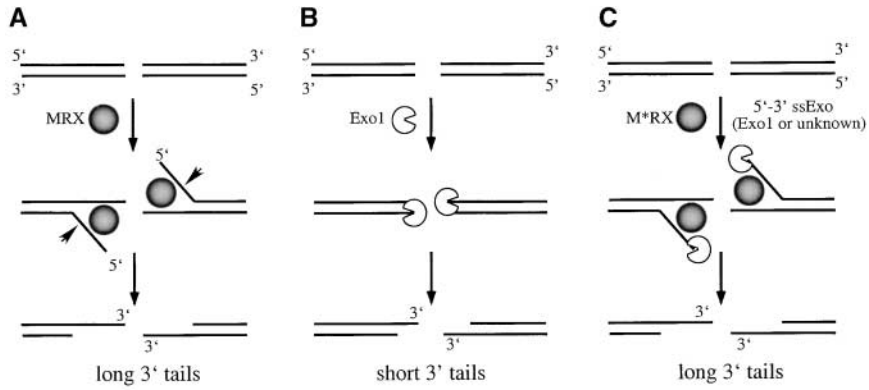


FIGURE 6.—Model for end processing in *mre11*Δ and *mre11-H125N* strains. (A) *MRE11*, (B) *mre11*Δ, (C) *mre11-H125N*. The MRX complex unwinds ends to produce single-stranded tails for cleavage by the Mre11 endonuclease. In *mre11*Δ strains the MRX complex is absent and ends can be resected only by a 5′ to 3′ double-stranded exonuclease, such as Exo1. This is normally inefficient, but can occur if *EXO1* is present in high copy. In *mre11-H125N* cells, the ends are still unwound by the M*RX complex (M* refers to the Mre11-H125N subunit) to produce single-stranded tails that can be removed by other nucleases in the absence of the Mre11 nuclease activity. This can occur through the activity of Exo1 or by another single-strand exonuclease.

observe for γ-ray sensitivity could be due to the different lesions generated by these DNA damaging agents. *exo1* mutants show no sensitivity to ionizing radiation, but are sensitive to high concentrations of MMS. Because *EXO1* in high copy also reduces the MMS sensitivity conferred by *rad50* and *xrs2* mutations (TSUBOUCHI and OGAWA 2000), it appears to bypass the requirement for the MRX complex rather than simply substitute for the nuclease function of Mre11. We suggest that, in the absence of the MRX complex, the 5′ to 3′ exonuclease activity of Exo1 inefficiently resects DSBs (Figure 6). This can occur with greater efficiency, and hence the partial suppression, if *EXO1* is present in high copy. Consistent with this hypothesis, the putative nuclease-defective alleles of *EXO1*, *exo1-D78A* and *exo1-D173A*, are unable to suppress the *mre11*Δ DNA repair defect when introduced in high copy.

The *exo1*Δ *mre11-H125N* strain was modestly more γ-ray sensitive than the *mre11-H125N* strain and considerably more resistant than the *mre11*Δ strain. If Exo1 were the only activity redundant with Mre11, then we would have expected the *exo1*Δ *mre11*Δ and *exo1*Δ *mre11-H125N* strains to exhibit similar phenotypes. The Mre11-H125N protein interacts normally with Rad50 and Xrs2 by the two-hybrid system (data not shown), suggesting the complex is still present in this strain. If the MRX complex processes DSBs by coupled unwinding and endonuclease activities then the formation of single-stranded DNA in *mre11-H125N* strains would be expected, whereas duplex ends should be present in *mre11*Δ strains (Figure 6). The observation that Exo1 has activity on both single- and double-stranded DNA could account for the weak synergism observed for γ-ray sensitivity of the *exo1*Δ *mre11*Δ and *exo1*Δ *mre11-H125N* double mutants (FIORENTINI *et al.* 1997). The *mre11-H125N* and *exo1*Δ *mre11-H125N* strains are sensitive to high doses of ionizing radiation, but have no apparent defect in the repair of a single HO-induced DSB. We consider two possible interpretations of these results. First, a nuclease redundant with Mre11 may be present in limiting amounts and able to process one DSB made by HO endonuclease, but not multiple breaks in the same cell. Second, the high doses of irradiation that are required to sensitize *mre11-H125N* mutants may cause severe base and sugar damage in addition to strand breaks and the Mre11 nuclease may be required to endonucleolytically remove damaged nucleotides or to remove phosphate or phosphoglycolate groups from damaged termini. Thus, the major function of the Mre11 nuclease could be to remove end-blocking lesions to provide a substrate for the resection nuclease. The weak synergism between the *exo1* and *mre11-H125N* mutations suggests the existence of at least one other redundant 5′ to 3′ exonuclease for the resection of DSBs.

We have previously shown no defect in telomere length or end joining in the *mre11-H125N* strain and concluded that the Mre11 nuclease activity of the MRX complex is either unimportant for these functions or fully redundant. *EXO1* present in high copy is unable to suppress the telomere maintenance and end-joining defect of *mre11*Δ strains, indicating that Exo1 is not the redundant activity for these functions of the complex or that 5′ to 3′ processing is not required. Alternatively, the specialized protein/DNA structures present at telomeres and as intermediates in end joining may be inaccessible to Exo1, but accessible to the hypothetical single-strand 5′ to 3′ exonuclease.

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