The *Saccharomyces cerevisiae MUM2* Gene Interacts With the DNA Replication Machinery and Is Required for Meiotic Levels of Double Strand Breaks

Luther Davis,*,[1] Maria Barbera,* Amanda McDonnell,* Katherine McIntyre,[†] Rolf Sternglanz,[†] Quan-wen Jin,[‡] Josef Loidl[‡] and JoAnne Engebrecht*

**Department of Pharmacological Sciences, Graduate Program in Genetics and [†]Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, New York 11794-8651 and [‡]Department of Cytology and Genetics, Institute of Botany, University of Vienna, Vienna, Austria A-1030*

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ABSTRACT

The *Saccharomyces cerevisiae MUM2* gene is essential for meiotic, but not mitotic, DNA replication and thus sporulation. Genetic interactions between *MUM2* and a component of the origin recognition complex and polymerase α-primase suggest that *MUM2* influences the function of the DNA replication machinery. Early meiotic gene expression is induced to a much greater extent in *mum2* cells than in meiotic cells treated with the DNA synthesis inhibitor hydroxyurea. This result indicates that the *mum2* meiotic arrest is downstream of the arrest induced by hydroxyurea and suggests that DNA synthesis is initiated in the mutant. Genetic analyses indicate that the recombination that occurs in *mum2* mutants is dependent on the normal recombination machinery and on synaptonemal complex components and therefore is not a consequence of lesions created by incompletely replicated DNA. Both meiotic ectopic and allelic recombination are similarly reduced in the *mum2* mutant, and the levels are consistent with the levels of meiosis-specific DSBs that are generated. Cytological analyses of *mum2* mutants show that chromosome pairing and synapsis occur, although at reduced levels compared to wild type. Given the near-wild-type levels of meiotic gene expression, pairing, and synapsis, we suggest that the reduction in DNA replication is directly responsible for the reduced level of DSBs and meiotic recombination.

MEIOSIS is a specialized cell cycle that enables diploid organisms to reproduce sexually by generating haploid gametes through two successive divisions. At meiosis I, also known as the reductional division, homologous chromosomes disjoin from each other. At meiosis II, as at mitosis, sister chromatids separate and move to opposite poles. The meiotic divisions, in turn, are tightly linked to gamete differentiation. Fusion of gametes at fertilization restores the diploid chromosome number and initiates zygotic development. High fidelity of meiotic chromosome segregation is essential for the propagation of all sexually reproducing organisms.

The replication of chromosomes is the first detectable cytological event in meiosis. The coordinated synthesis of genomic DNA requires multiple levels of regulation and a large number of gene products. The origin recognition complex (ORC) and the multi-subunit minichromosome maintenance (MCM) complex, among others, are essential in initiating DNA replication (reviewed in Dutta and Bell 1997). Primase and the replicative polymerases, themselves multi-protein complexes, are the machines that carry out DNA synthesis (reviewed in Baker and Bell 1998). Genetic analyses in the yeast *Saccharomyces cerevisiae* indicate that the replicative machinery used to synthesize DNA in vegetative cells is also required for the duplication of chromosomes in meiosis (Budd *et al.* 1989). In contrast, the regulatory controls for these two replication modes appear distinct (Schild and Byers 1978; Hollingsworth and Sclafani 1993; Dirick *et al.* 1998; Stuart and Wittenberg 1998).

During prophase, the duplicated homologous chromosomes align, synapse, and recombine. Alignment refers to the presynaptic association of the homologous chromosomes (Loidl *et al.* 1994; Weiner and Kleckner 1994), while chromosome synapsis is the intimate association of homologous chromosomes in the context of the synaptonemal complex (SC). The SC is a meiosis-specific structure that is elaborated along the lengths of the chromosomes (reviewed in von Wettstein *et al.* 1984). Several genes that encode structural proteins of the SC have been identified; genetic analyses indicate that the proper assembly of the SC is essential for chromosome segregation (reviewed in Kupiec *et al.* 1997).

All meiotic recombination in yeast (and presumably in other organisms as well) that has been studied to date is initiated by double strand breaks (DSBs; Sun *et al.* 1989; Cao *et al.* 1990; Cervantes *et al.* 2000). DSBs occur before the SC is fully formed, while mature recombinants do not occur until chromosome synapsis is com-

Corresponding author: JoAnne Engebrecht, Department of Pharmacological Sciences, State University of New York, Stony Brook, NY 11794-8651. E-mail: joanne@pharm.sunysb.edu

[1]*Present address:* Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024.

plete (Padmore *et al.* 1991). In most organisms reciprocal exchange is essential for proper chromosome segregation at the reductional division (Hawley 1987).

Studies in *S. cerevisiae* have been instrumental in elucidating the requirement for chromosome pairing, synapsis, and genetic recombination in ensuring proper chromosome segregation at the meiosis I division (reviewed in Kupiec *et al.* 1997). However, very little attention has been devoted to the role of DNA replication in meiosis. Recent studies indicate that DNA replication is important for meiotic recombination (Borde *et al.* 2000). Further, *SPO11* (Klapholz *et al.* 1985) and *REC8* (Klein *et al.* 1999), two yeast genes originally identified for their roles in recombination and sister chromatid cohesion, respectively, have subsequently been shown to play distinct roles in meiotic DNA replication (Cha *et al.* 2000). However, the connection between DNA replication and homologous chromosome interaction remains unknown.

The yeast *MUM2* gene was identified in a screen for meiotic mutants and is required specifically for meiotic DNA replication (Engebrecht *et al.* 1998). The Mum2 protein is not similar to other proteins; however, the C terminus is predicted to form a coiled coil. Coiled-coil domains are found in diverse classes of proteins including molecular motors and SC components. Synthetic interactions with a component of ORC and polymerase α-primase suggest that *MUM2* influences the functioning of the replication machinery. Genetic analyses of *mum2* mutants in meiosis indicate that perturbation of DNA replication modestly affects chromosome pairing and synapsis, while meiotic recombination is greatly impaired. Further, these studies reveal a direct correlation between the level of meiotic DSBs and DNA replication and suggest that DNA replication is an essential prerequisite to DSB formation.

MATERIALS AND METHODS

Yeast strains and media: Routine growth and manipulation of *S. cerevisiae* strains were performed as described (Rose *et al.* 1990). Yeast strains were transformed using the lithium acetate procedure (Ito *et al.* 1983). All integrative transformants were verified by Southern blot analysis or polymerase chain reaction (PCR). Gene disruptions were performed by one-step gene replacement; *orc2* and *pol1* alleles were introduced by two-step gene replacement (Rothstein 1991) using plasmids JR1267 (*orc2-1*) and YIp5-*pol1-17* (Budd and Campbell 1987). Yeast strains used in this study are listed in Table 1.

Diploid strains harboring the *arg4-bgl* and *arg4-nsp* alleles at different locations in the genome were created from haploids Y1244 and Y1245 after targeted integration of the appropriate plasmids as described (Goldman and Lichten 1996). The *arg4* alleles were inserted in the context of a pBR322-based plasmid that contains a 1.2-kb *URA3* *Hin*dIII fragment inserted at the *Hin*dIII site of pBR322 and a 3.3-kb *arg4* *Pst*I fragment inserted between the *Bam*HI and *Sal*I sites of pBR322 (Wu and Lichten 1995). To construct homozygous *arg4* insertions at *HIS4* and *URA3*, Y1319 was transformed with pME1141 (*MUM2 CEN4 TRP1*; Engebrecht *et al.* 1998) and induced to sporulate and resultant tetrads dissected. Haploid segregants containing pME1141 and harboring both *arg4* alleles were mated. Mitotic segregants that had lost the plasmid were isolated, thus generating isogenic *MUM2* and *mum2* strains homozygous for the *arg4* alleles. *sae2*Δ derivatives were made by transforming pME1210 (see below) into Y1244 and Y1245 derivatives. *orc* and *pol* temperature-sensitive alleles were introduced into these same strains.

hop1::LEU2, *rec104::LEU2*, *red1::LEU2*, and *spo11::TRP1* derivatives of JB128 (Bharagava *et al.* 1992) were made by transforming Y898 (JB128 *MUM2/mum2::LYS2*) with pNH37-2 (Hollingsworth and Byers 1989), pNH131-10, pNH119-8 (Hollingsworth and Johnson 1993), and pGB324 (Giroux *et al.* 1989), respectively. Transformants were induced to sporulate and the resulting tetrads were dissected to generate homozygous deletions.

Plasmids: *SAE2* sequences were isolated by PCR amplification using primers P68, 5′-TCC AAG CTT TTG CAC GTC-3′, and P69, 5′-GTT CCC GTG GTA GAA ATG-3′. The PCR product was digested with *Sac*II and *Sal*I and the resulting 1.9-kb product was inserted into the *Sac*II and *Xho*I sites of Bluescript SK+ (Stratagene, La Jolla, CA) to generate pME1214. The 1.1-kb *Hin*dIII-*Hpa*I *URA3* fragment from Yep352 (Hill *et al.* 1986) was inserted into the *Hin*dIII-*Eco*RV sites of pME1214 to generate the *sae2*Δ*::URA3* deletion allele in pME1220. Plasmid ME1220 was digested with *Sac*I and *Hpa*I to allow substitution of the *SAE2* locus in yeast. Sequences near *THR4* (*YCR47c*), which contain a meiotic hotspot (Wu and Lichten 1994), were isolated by PCR amplification using primers P66, 5′-GAC TAC ATG GAC ACT GAG-3′, and P67, 5′-CTA GAG TTC AAC TGC AAG G-3′. The PCR product was digested with *Hin*dIII and the resulting 0.9-kb product was inserted in the *Hin*dIII site of Bluescript SK+ to generate pME1210. The 618-bp *Dra*I-*Eco*RI *SPO11* fragment from pGB320 (Giroux *et al.* 1989) was inserted into the *Eco*RV and *Eco*RI sites of Bluescript SK+ to generate pME1845.

Analysis of growth: Strains harboring conditional alleles with and without *MUM2* were grown overnight to saturation at 25°. Serial dilutions were spotted onto rich medium and incubated at the indicated temperature for 3 days. Viability was monitored by plating cells in triplicate at 25° after the indicated times at different temperatures; doubling times were calculated as described (Engebrecht *et al.* 1998).

Flow cytometric analysis: Cultures in log phase were split and propagated at 25°, 33°, and 36° for the indicated times and prepared for flow cytometric analysis as described (Engebrecht *et al.* 1998). At least 10,000 cells were analyzed per sample.

RNA isolation and analysis: Strains were cultured in YPAcetate, introduced into sporulation medium, and assessed by 4′6-diamidino-2-phenylindole (DAPI) staining as described (Engebrecht *et al.* 1998). Hydroxyurea (HU, 200 mm; Aldrich Chemicals, Milwaukee) was added after 2.5 hr in sporulation medium to a portion of the culture. RNA was prepared and Northern blot analysis was performed as described (Rose *et al.* 1995). Radioactive RNA probes were synthesized by *in vitro* transcription (Krieg and Melton 1987) of plasmids ME1845 (*SPO11*, see above) digested with *Hin*dIII using T3 polymerase (Roche Biochemicals); NH50-1 (*HOP1*; obtained from N. Hollingsworth) digested with *Bam*HI; and ME817 (*PYK1*; Rose *et al.* 1995) digested with *Xba*I using T7 polymerase (Roche Biochemicals). Quantification was done on a Molecular Dynamics (Sunnyvale, CA) 445 SI phosphoimager using Image QuaNT software.

DSBs: DSB formation was monitored by extracting DNA from cells induced to undergo meiosis (Engebrecht *et al.* 1998). The resulting DNA was digested with *Bgl*II and separated on a 0.7% agarose gel. The DNA was transferred to Gene Screen (NEN) and hybridized with sequences at *THR4* (Wu and Lichten 1994). The 0.9-kb *Hin*dIII fragment from

TABLE 1

Yeast strains

Strain	Genotype
Y1244	*MAT***a** *leu2-27 his4-260 trp1-1 ura3-1 ADE2 arg4::Kan thr1-4 lys2*
Y1245	*MAT*α *leu2-3,112 his4-280 trp1-1 ura3-1 ade2 arg4::Kan THR1 lys2*
Y1541	*MAT***a** *leu2-27 his4-260 trp1-1 ura3-1 ADE2 arg4*Δ*::Kan thr1-4 lys2* *MAT*α *leu2-3,112 his4-280 trp1-1 ura3-1 ade2 arg4*Δ*::Kan THR1 lys2*
Y1542	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2*
Y1281[a]	Y1541 *his4::URA3·arg4-nsp/his4::URA3·arg4-bgl*
Y1275	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 his4::URA3·arg4-nsp/his4::URA3·arg4-bgl*
Y1285	Y1541 *PUT2::URA3·arg4-nsp/PUT2::URA3·arg4-bgl*
Y1279	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 PUT2::URA3·arg4-nsp/PUT2::URA3·arg4-bgl*
Y1314	Y1541 *ura3::URA3·arg4-nsp/ura3::URA3·arg4-bgl*
Y1318	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 ura3::URA3·arg4-nsp/ura3::URA3·arg4-bgl*
Y1282	Y1541 *leu2-27 his4::URA3·arg4-nsp/lue2::URA·arg4-bgl his4-280*
Y1276	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 leu2-27 his4::UUA3·arg4-nsp/leu2::URA·arg4-bgl his4-280*
Y1283	Y1541 *his4::URA3·arg4-nsp/his4-280 PUT2/PUT2::URA3·arg4-bgl*
Y1277	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 his4::URA3·arg4-nsp/his4-280 PUT2/PUT2::URA3·arg4-bgl*
Y1315	Y1541 *his4-260/his4::URA3·arg4-bgl ura3::URA3·arg4-nsp/ura3-1*
Y1319	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 his4-260/his4::URA3·arg4-bgl ura3::URA3·arg4-nsp/ura3-1*
Y1284	Y1541 *leu2::URA3·arg4-nsp/leu2-3,112 PUT2/PUT2::URA3·arg4-bgl*
Y1278	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 leu2::URA3·arg4-nsp/leu2-3,112 PUT2/PUT2::URA3·arg4-bgl*
Y1316	Y1541 *leu2-27/leu2::URA3·arg4-bgl ura3::URA3·arg4pnsp/ura3-1*
Y1320	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 leu2-27/leu2::URA3·arg4-bgl ura3::URA3·arg4-nsp/ura3-1*
Y1317	Y1541 *ura3::URA3·arg4-nsp/ura3-1 PUT2/PUT2::URA3·arg4-bgl*
Y1321	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 ura3::URA3·arg4-nsp/ura3-1 PUT2/PUT2::URA3·arg4-bgl*
Y3153	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 his4::URA3·arg4-bgl/his4::URA3·arg4-bgl ura3::URA3·arg4-nsp/ura3::URA3·arg4-nsp*
Y3154	Y1541 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 his4::URA3·arg4-bgl/his4::URA3·arg4-bgl ura3::URA3·arg4-nsp/ura3::URA3·arg4-nsp + MUM2 CEN4 TRP1*
Y1679	Y1541 *orc2-1/orc2-1*
Y2032	Y1541 *orc2-1/orc2-1 mum2*Δ*::LYS2/mum2*Δ*::LYS2*
Y1243	Y1541 *pol1-17/pol1-17*
Y1287	Y1541 *pol1-17/pol1-17 1 mum2*Δ*::LYS2/mum2*Δ*::LYS2*
Y1168[b]	*MAT***a** *leu2-3,112 ura3-Stu@HIS4 HO trp1-1 ura3-1 thr1-4 ade2-1 lys2-1* *MAT*α*leu2-3,112 ura3-Stu@HIS4 HO trp1-1 ura3-1 thr1-4 ade2-1 lys2-1*
Y898[c]	Y1168 *MUM2/mum2*Δ*::LYS2*
Y1171	Y1168 *mum2*Δ*::LYS2/mum2*Δ*::LYS2*
Y1105	Y1168 *spo11::TRP1/spo11::TRP1*
Y1106	Y1168 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 spo11::TRP1/spo11::TRP1*
Y2900	Y1168 *rec104::LEU2/rec104::LEU2*
Y2901	Y1168 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 rec104::LEU2/rec104::LEU2*
Y1162	Y1168 *hop1::LEU2/hop1::LEU2*
Y1163	Y1168 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 hop1::LEU2/hop1::LEU2*
Y1169	Y1168 *red1::LEU2/red1::LEU2*
Y1170	Y1168 *mum2*Δ*::LYS2/mum2*Δ*::LYS2 red1::LEU2/red1::LEU2*

[a] Nomenclature from Wu and Lichten (1995).
[b] JB128, obtained from Bharagava *et al.* (1992).
[c] Y898 was described in Engebrecht *et al.* (1998).

pME1210 was purified and radiolabeled by random priming (New England Biolabs, Beverly, MA). Quantification was done on a Molecular Dynamics 445 SI phosphoimager using ImageQuaNT software.

Recombination frequencies: Yeast strains were grown to saturation in yeast extract, peptone, adenine, dextrose (YPAD), and aliquots of triplicate cultures were plated onto YPAD medium and synthetic media lacking the appropriate amino acids to determine the mitotic prototroph frequencies.

Meiotic (return-to-growth) recombination frequencies were determined by taking 0.8 ml from each saturated culture, washing once with dH_2O, resuspending the cells in 4 ml of 2% potassium acetate, and incubating them at 30° with aeration. Aliquots of the sporulating cultures were removed after 15 hr (this time corresponds to pachytene in this strain background under these sporulation conditions) for plating onto YPAD and synthetic media lacking the appropriate amino acids. The percentage sporulation was monitored after 2 days in all cultures to ensure that meiosis had progressed as expected.

Cytology: Chromosome spreads and fluorescence *in situ* hybridization (FISH) were performed as described (Loidl *et al.* 1994, 1998). Synaptonemal complex formation was analyzed by staining spreads with antibodies directed against Hop1 (Smith and Roeder 1997) and Zip1 (Sym *et al.* 1993).

RESULTS

Genetic interactions between *mum2* and components of the DNA replication machinery: *MUM2* was identified in a genetic screen for mutants defective in meiosis and is required specifically for meiotic DNA replication (Engebrecht *et al.* 1998). *MUM2* is expressed in vegetative cells (data not shown); however, no impairment of growth is observed in the absence of *MUM2*, suggesting that *MUM2* does not play an important role in mitotic DNA replication (Engebrecht *et al.* 1998). We tested whether a role could be uncovered by synthetic interactions with known components of the DNA replication machinery.

Orc2 is a subunit of the origin recognition complex, which is required for initiation of DNA synthesis (Bell *et al.* 1993; Ross *et al.* 1993). Strains harboring temperature-sensitive alleles of *orc2* die when shifted to the restrictive temperature due to a failure to initiate DNA synthesis (Bell *et al.* 1993; Ross *et al.* 1993; Loo *et al.* 1995). The MCM complex is also required for the initiation of DNA replication, although, unlike ORC, these proteins are transiently associated with origins of DNA replication and move with the replication fork (Aparicio *et al.* 1997). The ORC and MCM multi-protein complexes (as well as a number of other proteins, reviewed in Dutta and Bell 1997) recruit polymerases to carry out DNA synthesis. DNA polymerase α-primase (pol α) synthesizes the RNA primers and begins leading and lagging strand DNA synthesis and polymerase ε and δ are required for bulk DNA replication (review in Sugino 1995). *POL1*, *POL2*, and *POL3* encode components of pol α, ε, and δ, respectively.

To investigate the relationship between *MUM2* and the DNA replication machinery, we examined the effect of deleting *MUM2* in strains harboring temperature-sensitive alleles of genes required for initiation and synthesis of DNA. Deletion of *MUM2* in *orc2-1* strains improved growth (Figure 1A). The doubling time at 30° of the *orc2* mutant was 162 ± 20 min, while the doubling time of the *orc2 mum2* double mutant was 105 ± 12. However, deletion of *MUM2* in a *mcm5* temperature-sensitive strain (*cdc46-1*) had no effect (data not shown). In sharp contrast to the results with *orc2-1*, deletion of *MUM2* in *pol1-17* (Budd *et al.* 1989) strains lowered the restrictive temperature for growth (Figure 1A). This was confirmed by plating assays in which viability was more severely perturbed in the *pol1-17 mum2* double mutant compared to *pol1-17* at both 33° and 36° (Figure 1B). No effect on the growth of *pol2-18* and *pol3-14* mutants was observed in the absence of *MUM2* (data not shown).

To determine if deletion of *MUM2* influenced DNA replication in the mutant backgrounds, DNA content of yeast cells incubated at various temperatures was analyzed by flow cytometry. Log phase cultures have approximately equal numbers of cells in G1 and G2, as represented by the peaks of 2C and 4C content. At 33°, the restrictive temperature for *pol1-17 mum2* strains, a greater majority of the *pol1-17 mum2* cells appear to be in S phase as compared to the *pol1-17* strain (Figure 1C). At 36°, both cultures showed predominantly G1 DNA content of the cells, consistent with previous analyses (Budd *et al.* 1989). No differences were observed in the DNA content of the *orc2* and *orc2 mum2* mutants (data not shown). These results implicate *MUM2*, directly or indirectly, in DNA replication.

Meiotic early gene expression is induced in *mum2*

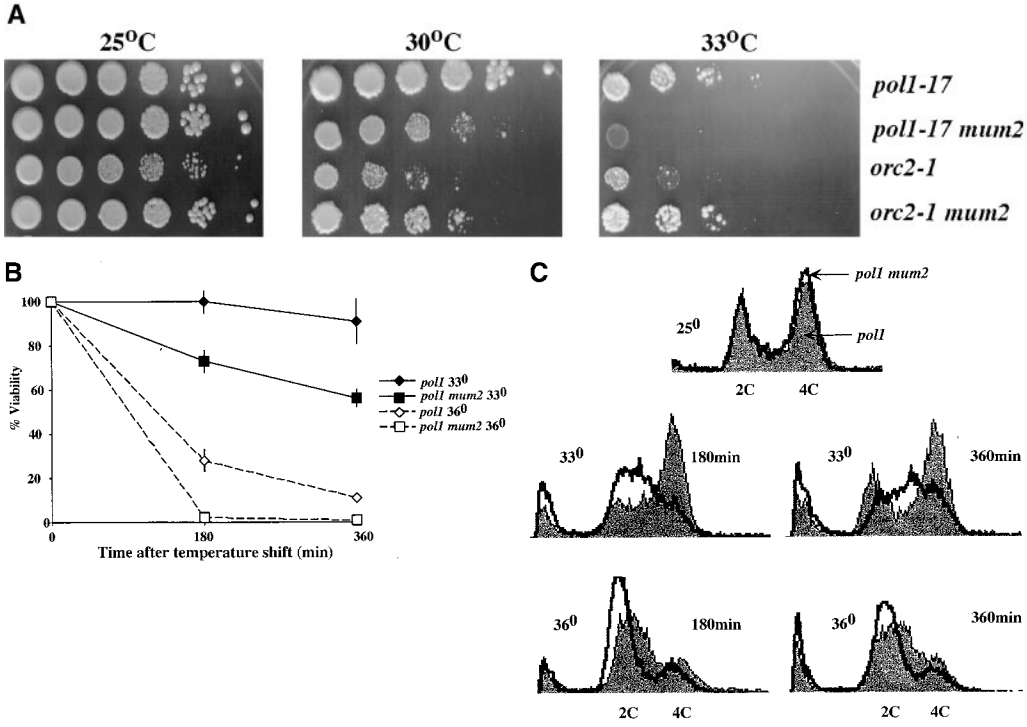

Figure 1.—*mum2* displays genetic interactions with *ORC2* and *POL1*. (A) Serial dilutions of saturated cultures were spotted on rich medium and incubated at the indicated temperature for 3 days. Strains Y1243 (*pol1-17*), Y1287 (*pol1-17 mum2*), Y1679 (*orc2-1*), and Y2032 (*orc2-1 mum2*) were used. Viability (B) and DNA content (C) of *pol1* (shaded histogram) and *pol1 mum2* (clear histogram with thick line) strains after shift to the indicated temperatures.

mutants to a greater extent than in wild-type cells treated with hydroxyurea: *mum2* mutants fail to complete meiotic DNA replication and consequently arrest prior to the first meiotic division (Engebrecht *et al.* 1998). As perturbation of DNA replication has been shown to inhibit early meiotic gene expression (Lamb and Mitchell 2001), we examined the expression level of *SPO11* and *HOP1,* prototypical meiotic early genes (Mitchell 1994), in *mum2* mutants. Northern blot analysis indicates that the steady-state level of *SPO11* and *HOP1* in meiosis in *mum2* mutants is induced similarly to wild type (Figure 2).

In vegetative cells, the DNA synthesis inhibitor HU inhibits ribonucleotide reductase, resulting in stalled replication fork movement via a regulatory circuit that monitors nucleotide levels (Santocanale and Diffley 1998). The precise effect of HU on meiotic cells has not been delineated; however, by analogy with the mitotic block, HU-treated cells would presumably initiate meiotic DNA synthesis but arrest during fork elongation. Because the HU-induced block in meiotic DNA replication has been found to inhibit early meiotic gene expression (Lamb and Mitchell 2001), we reasoned that *mum2* mutants either bypass the HU block or arrest downstream of this block. To address this question, the level of *SPO11* and *HOP1* mRNA present in *MUM2* and *mum2* cells exposed to HU during meiosis was also examined. Treatment of wild-type and *mum2* cells with HU at a concentration that is known to elicit meiotic arrest (200 mm; Stuart and Wittenberg 1998) similarly inhibited the induction of the *SPO11* and *HOP1* genes (Figure 2). Analysis of DNA replication by cell sorting indicates that DNA synthesis does not occur in the presence of this concentration of HU (data not shown). We conclude that, in contrast to cells exposed to HU, early gene expression is not affected in the absence of *MUM2* despite the block in DNA replication. In addition, the results suggest that the *mum2* arrest occurs downstream of the arrest induced by HU after meiotic DNA synthesis is initiated.

The recombination observed in the *mum2* mutant is dependent on the meiotic recombination machinery and components of the SC: To determine whether the recombination observed in *mum2* mutants when transferred to sporulation medium is dependent on the meiotic recombination machinery or is the consequence of incompletely replicated DNA, double mutant analysis was undertaken by combining *mum2* with various mutants alleles of meiosis-specific genes required for recombination and chromosome synapsis. *SPO11* encodes a topoisomerase-like transesterase that is most likely responsible for catalyzing DSB formation, the initiating lesion in meiotic recombination (Bergerat *et al.* 1997; Keeney *et al.* 1997). Accordingly, meiotic recombination is completely eliminated in *spo11* mutants (Klapholz *et al.* 1985). *REC104* also plays an essential role in the initiation of meiotic recombination, as DSB formation does not occur in *rec104* mutants (Bullard *et al.* 1996).

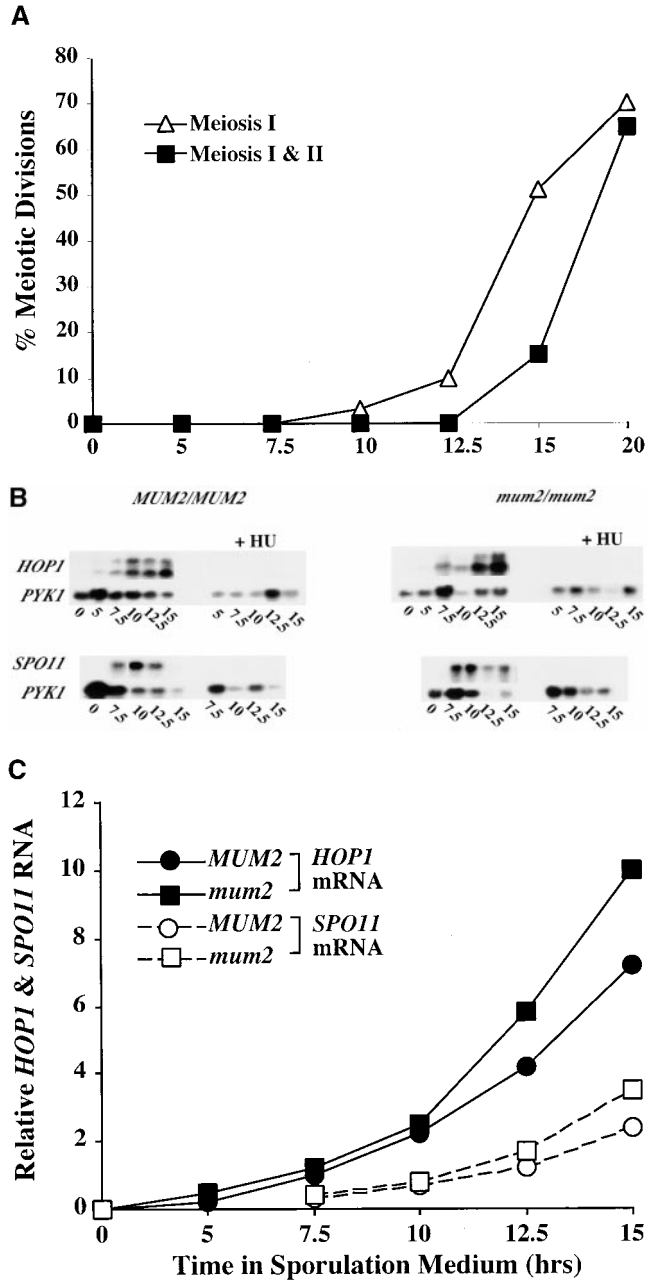

Figure 2.—Early meiotic gene expression in the *mum2* mutant. (A) Meiotic progression of *MUM2* cultures used for Northern analysis. The percentage of cells having completed the meiosis I division (△) and both the meiosis I and II divisions (■) are shown. (B) Northern blot analysis of *HOP1*, *SPO11*, and *PYK1* (as loading control) in *MUM2*, *MUM2* + HU (200 mm), *mum2*, and *mum2* + HU (200 mm). The numbers below the panels represent the hours in sporulation medium. (C) Quantification of the relative expression of *HOP1* in *MUM2* (●) and *mum2* (■) and of *SPO11* in *MUM2* (○) and *mum2* (□).

The induction of meiotic ectopic recombination between *ura3* heteroalleles on chromosomes *III* and *IV*, which occurs at a high level in *mum2* mutants (Engebrecht *et al.* 1998), was monitored. Deletion of either *SPO11* or *REC104* in *mum2* strains eliminated the induction of meiotic recombination (Table 2). These findings

TABLE 2

***mum2* recombination is dependent on the meiotic recombination machinery and SC components**

Strain	Relevant genotype	Ura prototrophs ($\times 10^7$)	
		Mitotic[a]	Meiotic[b]
Y1168	Wild type	6.7 ± 2.4 (1.0)	712.0 ± 126 (1.00)
Y1171	*mum2*	4.7 ± 2.7 (0.7)	382.0 ± 69 (0.54)
Y1105	*spo11*	9.0 ± 1.4 (1.3)	7.6 ± 2.2 (0.01)
Y1106	*spo11 mum2*	7.5 ± 0.7 (1.1)	8.0 ± 1.4 (0.01)
Y2900	*rec104*	7.3 ± 1.1 (1.1)	7.7 ± 2.3 (0.01)
Y2901	*rec104 mum2*	5.6 ± 2.1 (0.8)	8.3 ± 1.5 (0.01)
Y1162	*hop1*	7.1 ± 2.3 (1.0)	12.1 ± 1.7 (0.02)
Y1163	*hop1 mum2*	6.2 ± 3.3 (0.9)	4.1 ± 3.1 (0.01)
Y1169	*red1*	8.8 ± 3.1 (1.3)	111.0 ± 38 (0.15)
Y1170	*red1 mum2*	4.9 ± 2.9 (0.7)	9.2 ± 2.9 (0.01)

[a] Mitotic recombination frequency ± SD represents the median frequency of a mimimum of three independent cultures. Number in parentheses is the frequency normalized to the frequency of the wild-type strain, Y1168.

[b] The meiotic cultures were plated after 15 hr in sporulation medium and represent the mean value ± the SD from a minimum of three independent cultures. The number in parentheses is the frequency normalized to the frequency of the wild-type strain.

indicate that the recombination observed in *mum2* mutants is most likely initiated by the meiotic recombination machinery and is not the result of incompletely replicated DNA. Examination of other heteroallelic pairs indicates that all recombination observed in *mum2* mutants is dependent on *SPO11* (data not shown).

HOP1 and *RED1* encode components of meiotic chromosomes essential for the formation of the SC (Hollingsworth *et al.* 1990; Rockmill and Roeder 1990; Smith and Roeder 1997). Mutations in both genes reduce, but do not eliminate, meiotic recombination, suggesting that SC morphogenesis is required to obtain full levels of meiotic recombination. To determine if the recombination observed in *mum2* mutants is dependent on SC components, *HOP1* and *RED1* were deleted in the *mum2* mutant and recombination was measured in return-to-growth experiments. As shown in Table 2, deletion of *RED1* greatly reduced the recombination observed in the *mum2* mutant; the double mutants had a recombination level below that of the *mum2* or *red1* single mutants. These results suggest that *MUM2* functions in a pathway different from *RED1* and both pathways are required to obtain full meiotic levels of recombination. Deletion of *HOP1* resulted in a drastic reduction in the levels of recombination in this ectopic assay and introduction of the *MUM2* deletion completely eliminated the induction of meiotic recombination (Table 2).

Meiotic allelic and ectopic recombination are similarly impaired in *mum2* mutants: Examination of the *ura3* heteroalleles arranged in an ectopic configuration (see above) and *HIS4* and *LEU2* heteroalleles arranged in an allelic configuration suggested that allelic recombination was more impaired than ectopic recombination in *mum2* mutants (Engebrecht *et al.* 1998). To investigate this issue further, we introduced the *arg4* alleles described by Goldman and Lichten (1996) at multiple locations in the genome. As shown in Table 3, induction of arginine prototrophy is severely decreased in the *mum2* mutant, whether the alleles are in an ectopic or an allelic configuration (20- to 100-fold decrease). The same decrease is observed when the *arg4* alleles are placed at the same location as the *ura3* alleles (*URA3* → *HIS4*; Table 3). To determine if the level of recombination in the *mum2* mutant is influenced by the insertion being hemizygous (*i.e.*, *arg4* alleles) or homozygous (*i.e.*, *ura3* alleles), strains harboring homozygous *arg4* alleles were induced in sporulation medium and assayed for recombination. As shown in Table 3, no differences were observed whether the *arg4* alleles were hemizygous or homozygous. Therefore, the level of induction of recombination between the *ura3* alleles in *mum2* mutants cannot be accounted for by the alleles' ectopic configuration or homozygous state (see discussion).

DSB formation in mum2 mutants occurs at reduced levels: Because *mum2* mutants fail to sporulate, recombination is measured in return-to-growth experiments, which reflects both meiotic and mitotic functions within the cell. To examine the initiation of meiotic recombination, formation of DSBs was monitored physically. In wild type, DSBs are transient; therefore, to determine the total number of breaks generated during meiosis, we also analyzed DSBs in *MUM2* and *mum2* strains harboring the *sae2/com1* mutation. *sae2* mutants fail to resect DSBs, and consequently any DSBs formed are stable (McKee and Kleckner 1997; Prinz *et al.* 1997). These experiments indicate that *mum2* mutants make detectable, albeit reduced, levels of DSBs (Figure 3). The level

TABLE 3

Allelic and ectopic meiotic recombination are uniformily reduced in *mum2* mutants

Location of Arg alleles[a]	Arg prototrophs ($\times 10^4$)			
	MUM2/MUM2		*mum2/mum2*	
	Mitotic[b]	Meiotic[c]	Mitotic	Meiotic
HIS → HIS4	0.32 ± 0.06 (1.0)	220 ± 70 (1.0)	0.17 ± 0.09 (0.5)	4.5 ± 1.2 (0.02)
PUT2 → PUT2	0.26 ± 0.12 (1.0)	140 ± 30 (1.0)	0.10 ± 0.02 (0.4)	2.6 ± 0.6 (0.02)
URA3 → URA3	0.13 ± 0.03 (1.0)	97 ± 46 (1.0)	0.07 ± 0.02 (0.5)	0.71 ± 0.34 (0.01)
HIS4 → LEU2	0.28 ± 0.05 (1.0)	240 ± 20 (1.0)	0.19 ± 0.07 (0.7)	4.4 ± 1.0 (0.02)
HIS4 → PUT2	0.14 ± 0.02 (1.0)	26 ± 1 (1.0)	0.08 ± 0.03 (0.6)	1.3 ± 0.4 (0.05)
URA3 → HIS4	0.11 ± 0.01 (1.0)	76 ± 13 (1.0)	0.05 ± 0.01 (0.5)	1.1 ± 0.6 (0.01)
$\frac{URA \rightarrow HIS4}{URA3 \rightarrow HIS4}$ [d]	0.25 ± 0.04 (1.0)	84 ± 23 (1.0)	0.13 ± 0.05 (0.5)	1.8 ± 0.8 (0.02)
LEU2 → PUT2	0.16 ± 0.06 (1.0)	76 ± 11 (1.0)	0.05 ± 0.03 (0.3)	1.2 ± 0.2 (0.02)
URA3 → LEU2	0.14 ± 0.05 (1.0)	55 ± 11 (1.0)	0.07 ± 0.03 (0.5)	0.9 ± 0.4 (0.02)
URA3 → PUT2	0.20 ± 0.17 (1.0)	12 ± 2 (1.0)	0.03 ± 0.01 (0.2)	0.18 ± 0.07 (0.02)

[a] The first locus designated carries the *arg4-nsp* allele and the second locus carries the *arg4-bgl* allele in a hemizygous state.

[b] Mitotic recombination frequency ± SD represents the median frequency of a minimum of three independent cultures. Number in parentheses is the frequency normalized to the frequency of the corresponding wild-type strain.

[c] The meiotic cultures were plated after 15 hr in sporulation medium and represent the mean value ± the SD from a minimum of three independent cultures. The number in parentheses is the frequency normalized to the freqeuncy of the corresponding wild-type strain.

[d] $\frac{URA3 \rightarrow HIS4}{URA3 \rightarrow HIS4}$ represents homozygosis of the *arg4* alleles insertion as described in MATERIALS AND METHODS.

of DSBs observed in the *mum2* mutant correlates well with the level of heteroallelic recombination observed in return-to-growth experiments (>10-fold reduction).

We also monitored DSBs in *MUM2 sae2* and *mum2 sae2* cells induced for meiosis in the presence of HU. HU inhibits the formation of DSBs (BORDE *et al.* 2000) and mature meiotic recombinants (data not shown, SILVA-LOPEZ *et al.* 1975; SIMCHEN *et al.* 1976). No DSBs were detectable in the presence of HU in either culture (Figure 3). As HU also inhibits the residual DSBs in *mum2* mutants, it is unlikely that *mum2* uncouples DNA replication and events during prophase. Finally, this result is consistent with our analysis of meiotic early gene expression, in that the HU arrest is epistatic to the *mum2* arrest, and suggests a correlation between levels of DSBs and DNA replication.

Chromosome pairing and SC formation in the *mum2* mutant: To assay homolog pairing, meiotic chromosomes were surface spread and painted with composite probes for chromosome *I* and *IV*. Only nuclei showing a compact appearance using DAPI were scored (LOIDL *et al.* 1994). Homologs were classified as paired if they were so close together that their FISH signals had fused into a single spot or if their signals were touching each other. In the wild-type cells harvested after 9 hr in sporulation medium, 85% of the homologous chromosome regions were paired. In the *mum2* mutant at the same time point, 40% of the homologous chromosome regions were paired (Table 4A). After 10 hr in sporulation medium, homologous pairing in the mutant approached 64% of the level seen in wild type. Thus there is a considerable capacity to pair in the mutant.

To examine the effect of the *mum2* mutation on chromosome synapsis, meiotic chromosomes were surface spread and stained with antibodies directed against Hop1, an axial element component (HOLLINGSWORTH *et al.* 1990), and Zip1, a central region component of the SC (SYM *et al.* 1993). While Hop1 staining was similar in the wild-type and mutant strains, Zip1 staining in *mum2* was much more diffuse and mesh-like, although significant staining was present (Table 4B; Figure 4). These results are consistent with the genetic epistasis studies indicating that SC components are present in the *mum2* mutant, even though full synapsis is reduced compared to wild type.

DISCUSSION

MUM2 was identified in a genetic screen for mutants defective in meiosis and is specifically required for meiotic DNA replication (ENGEBRECHT *et al.* 1998). *mum2* mutants display no growth defect as measured by doubling times (ENGEBRECHT *et al.* 1998); however, Mum2 is present in cycling cells, as both the *MUM2* transcript

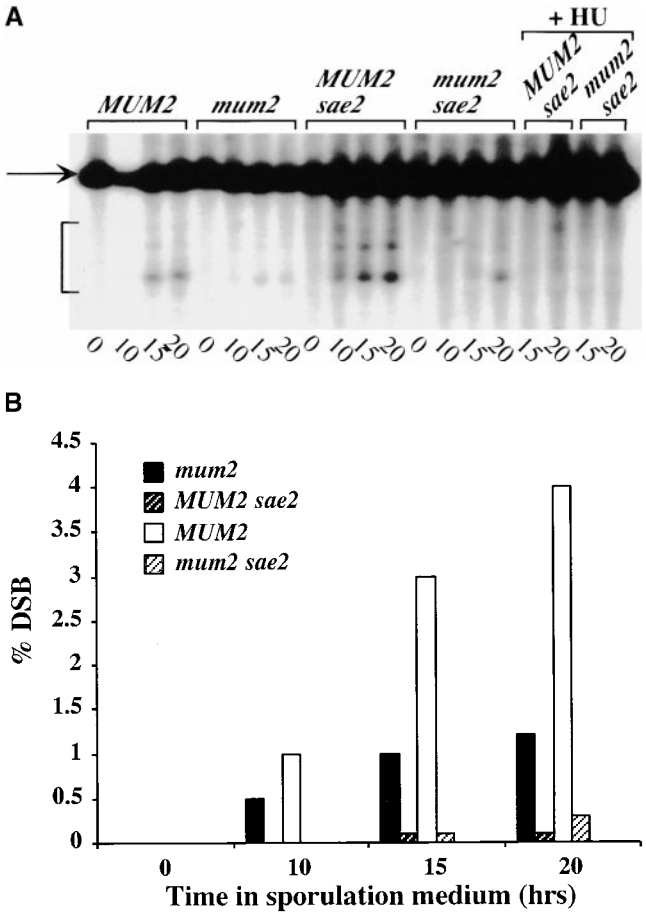

FIGURE 3.—DSBs occur at reduced levels in *mum2* mutants. (A) Southern blot anlysis of DSB at the *THR4* hotspot (WU and LICHTEN 1994). DNA was extracted from strains Y1541 (*MUM2*), Y1542 (*mum2*), Y1233 (*MUM2 sae2*), and Y1234 (*mum2 sae2*) and the latter two in the presence of HU. The numbers on the bottom refer to the hours after transfer to sporulation medium. In this experiment, ~50% of the cells had completed meiosis I by 20 hr. P indicates the parental band and the bracket indicates diagnostic DSBs. (B) Quantification of the percentage of DSB bands in comparision to the parental fragment in *MUM2* (solid bars), *mum2* (black hatched bars), *MUM2 sae2* (open bars), and *mum2 sae2* (white hatched bars).

and its protein product are detectable (data not shown). Thus, *MUM2* is essential for meiotic DNA replication, while playing a still-undefined role in mitotic DNA synthesis. In this study, we show genetic interactions between *MUM2* and components of the DNA replication machinery in vegetative cells. Interestingly, deletion of *MUM2* has opposite effects on strains harboring temperature-sensitive alleles of *ORC2* or *POL1*, improving growth of the former, but lowering the restrictive temperature of the latter. These effects may reflect a requirement for *MUM2* in facilitating the coupling of DNA synthesis with its initiation steps. Given the complex interactions and multitude of proteins required to couple initiation and DNA synthesis (DUTTA and BELL 1997), a specific requirement for *MUM2* in this process is difficult to infer from such genetic interactions. Nonetheless, these results do suggest that *MUM2* influences the functioning of the replication machinery. The differential requirements for *MUM2* in mitotic *vs.* meiotic DNA replication emphasize that these two replication modes are distinct and provide a unique tool for investigating the requirement for DNA replication in meiotic chromosome behavior. Recent studies in fission yeast also indicate that the process of replication may be fundamentally different in mitotic and meiotic cells, as some of the components essential for initiation of mitotic DNA replication are not required for meiotic S phase (FORSBURG and HODSON 2000).

Comparison of the meiotic phenotypes of yeast cells deleted for *MUM2 vs.* cells treated with HU suggests that at least some meiotic replication is initiated in *mum2* mutants. The effect on early meiotic gene expression, DSB formation, and recombination is more severe in HU-treated cells than in *mum2* mutants. These results indicate that the *mum2* arrest is downstream of the HU arrest. Inhibition of meiotic DNA replication by HU activates a Mec1p-dependent checkpoint (DIRICK *et al.* 1998; STUART and WITTENBERG 1998) and also prevents the induction of early genes (Figure 2; LAMB and MITCHELL 2001). It is not known whether it is inhibition of DNA replication itself or the activation of the replication checkpoint that is responsible for preventing the induction of early genes in HU. While this meiotic DNA replication checkpoint is functional in *mum2* mutants, it is not activated by the *mum2* replication block. In fact, meiotic prophase proceeds as demonstrated by cytological analyses, which indicate that chromosome pairing and synapsis occur, although at reduced levels in the *mum2* mutant. Thus, bulk DNA replication is not required for these prophase events. Analysis of the Coprinus *spo22* mutant also indicates that synapsis does not depend on bulk meiotic DNA replication (PUKKILA *et al.* 1995; MERINO *et al.* 2000).

SPO11 and *HOP1* represent members of a class of genes that are expressed in prophase of meiosis I (ATCHESON *et al.* 1987; HOLLINGSWORTH *et al.* 1990; MITCHELL 1994). The induction of *SPO11* and *HOP1* mRNA in *mum2* mutants most likely reflects a similar induction of the whole class of early meiotic genes, which includes genes required for meiotic recombination and chromosome synapsis (MITCHELL 1994). Thus the phenotype of *mum2* mutants in meiosis is probably not a consequence of a defect in early gene expression.

While incompletely replicated DNA is probably present in *mum2* mutants, the recombination that occurs is dependent on the meiotic recombination machinery. As *SPO11* has been shown to have separable roles in the control of meiotic S-phase length and in recombination (CHA *et al.* 2000), the loss of recombination in *mum2* mutants deleted for *SPO11* may be a consequence of altered S-phase length, absence of DSBs, or both. We favor the hypothesis that deletion of *SPO11* eliminates

TABLE 4

Chromosome pairing and synapsis in *mum2* mutants

Strain/relevant genotype	Time in meiosis (hr)	No. of nuclei with paired chromosome(s)			Nuclei with paired FISH signals (%)	
		IV	I	IV + I	Nonhomologous	Homologous[a]
		A. Homologous pairing				
Y1541 *MUM2*	0	2	8	0	5	10 (50)
	5	7	13	8	2	35 (52)
	9	2	7	39	4	85 (51)
	10	8	7	37	2	81 (55)
Y1542 *mum2*	0	6	9	7	3	14 (104)
	5	10	19	5	4	19 (101)
	9	12	12	30	2	40 (104)
	10	20	6	36	0	52 (94)
Stain/relevant genotype		No signal	Weak signal/dots		Oblong signals/ short linear	SC
		B. Zip1 staining at 10 hr				
Y1541 *MUM2*		25	16		30	29 (100)
Y1542 *mum2*		35	29		30	6 (100)

[a] Numbers in parentheses represent the total number of nuclei examined.

recombination by preventing DSB formation in *mum2* mutants, because deletion of *REC104*, another meiosis-specific gene essential for the initiation of meiotic DSBs (Bullard *et al.* 1996), also eliminates the recombination that occurs in *mum2* mutants. Taken together with the finding that the pattern of DSBs is similar in *MUM2* and *mum2* cells, these results indicate that the recombination observed in *mum2* mutants is initiated by meiosis-specific DSBs.

The recombination observed in the *mum2* mutant is also dependent on SC components. Deleting both *RED1* and *HOP1* in *mum2* mutants results in levels of recombination lower than those observed in any of the single mutants, indicating that these gene products function in different pathways, both of which are required for meiotic levels of recombination. In the case of *RED1*, there is clearly a synergistic effect of deletion of *MUM2,* indicating that these pathways cooperate to ensure meiotic levels of recombination. The low levels of meiotic recombination observed in the *hop1* mutant in our assay make it difficult to distinguish between a synergistic or additive relationship between these gene products.

MUM2 was identified in a screen for meiotic mutants proficient for the induction of ectopic recombination (Engebrecht *et al.* 1998). High levels of meiotic recombination were observed in return-to-growth experiments with the ectopic heteroallele pair used, but not allelic recombination (Table 2 and 3; Engebrecht *et al.* 1998). In this study, we show that the reduction of recombination in return-to-growth assays in the *mum2* mutant is not influenced by the location of the particular heteroallele pair being measured or by whether the introduced alleles are hemizygous or homozygous (Table 3). Further, the differences are not due to strain backgrounds (J. Engebrecht, unpublished results). There are three possible explanations for these findings. First, the level of recombination between the *ura3* heteroalleles is low and hence deletion of *MUM2* does not have much effect. Second, different heteroalleles are repaired differently in *mum2* mutants. Third, insertion of different pairs of heteroalleles at a particular locus alters an important sequence or chromatin structure such that recombination is affected differently in the *mum2* mutant.

Meiosis-specific DSBs are greatly reduced in *mum2* mutants, although not eliminated as they are in the presence of HU. The failure to form DSBs in HU-treated cells is probably due to the absence of Spo11p (as well as other meiosis-specific gene products required for DSB formation). Because Spo11 is present and functional in *mum2* mutants (Figure 2, Table 2), the reduction in DSBs may be a consequence of the defect in DNA replication. The direct coupling of DSB formation with DNA synthesis has been suggested on the basis of the observation that a delay in replication results in a similar delay in DSB formation (Borde *et al.* 2000). In addition, the finding that *SPO11* plays a role in the regulation of S phase in addition to its role in DSB formation (Cha *et al.* 2000) suggests that DNA replication and DSB formation are interrelated events. Determining the molecular requirement for DNA replication in DSB formation will be facilitated by analysis of Mum2p and elucidation of its unique role in meiotic DNA replication.

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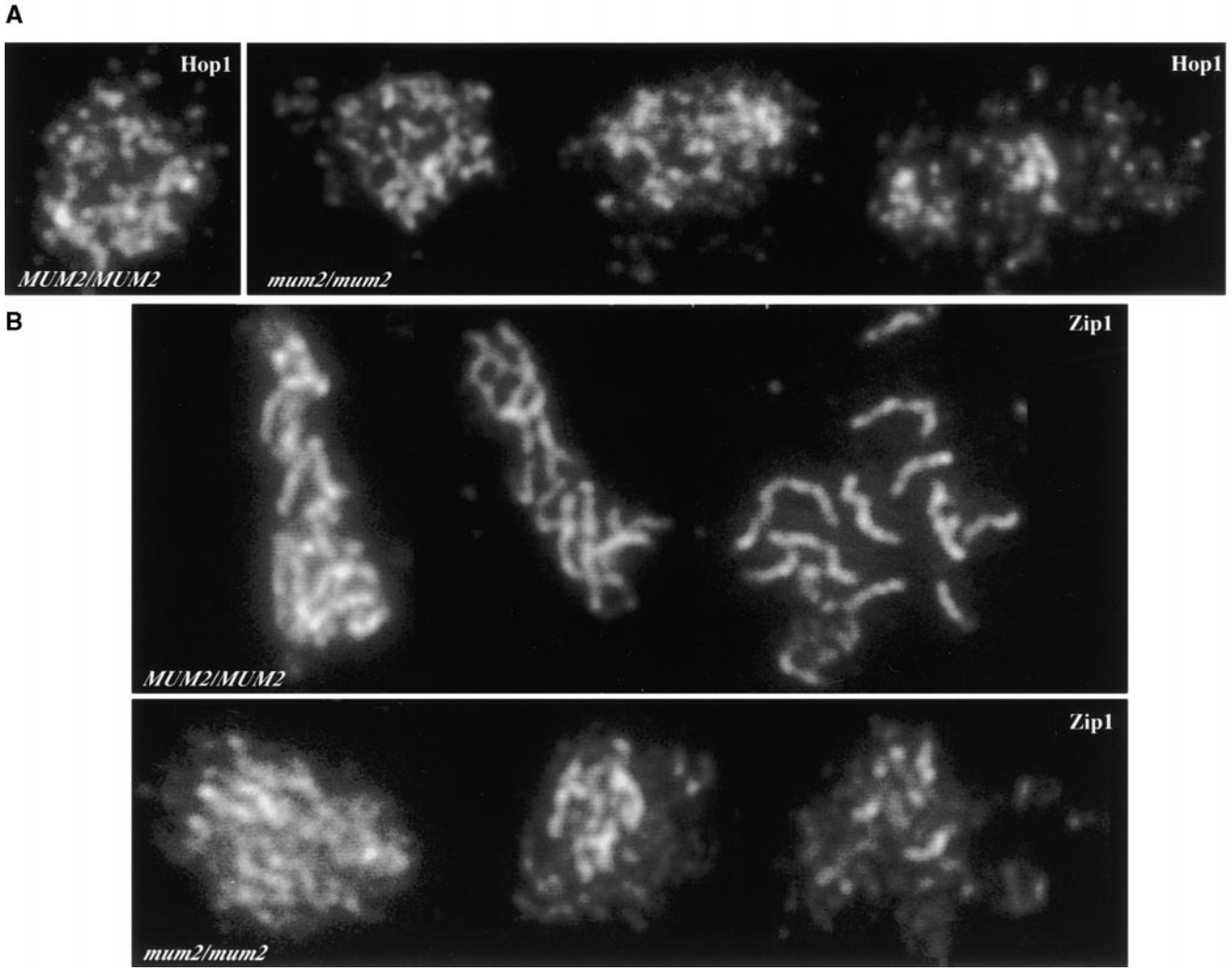

FIGURE 4.—SC formation in *mum2* mutants. Surface spread chromosomes from either wild type (*MUM2/MUM2*) or mutant (*mum2/mum2*) were labeled with antibodies directed against (A) Hop1 (SMITH AND ROEDER 1997) or (B) Zip1 (SYM *et al.* 1993).

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LITERATURE CITED

APARICIO, O. M., D. M. WEINSTEIN and S. P. BELL, 1997 Components and dynamics of DNA replication complexes in *S. cerevisiae*: redistribution of MCM proteins and Cdc45p during S phase. Cell **91:** 59–69.

ATCHESON, C. L., B. DIDOMENICO, S. FRACKMAN, R. E. ESPOSITO and R. T. ELDER, 1987 Isolation, DNA sequence, and regulation of a meiosis-specific eukaryotic recombination gene. Proc. Natl. Acad. Sci. USA **84:** 8035–8039.

BAKER, T. A., and S. P. BELL, 1998 Polymerases and the replisome: machines within machines. Cell **92:** 295–305.

BELL, S. P., R. KOBAYASHI and B. STILLMAN, 1993 Yeast origin recognition complex functions in transcription silencing and DNA replication. Science **262:** 1844–1849.

BERGERAT, A., B. DE MASSY, D. GADELLE, P. C. VAROUTAS, A. NICOLAS *et al.*, 1997 An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature **386:** 414–417.

BHARAGAVA, J., J. ENGEBRECHT and G. S. ROEDER, 1992 The *rec102* mutant of yeast is defective in meiotic recombination and chromosome synapsis. Genetics **130:** 59–69.

BORDE, V., A. S. H. GOLDMAN and M. LICHTEN, 2000 Direct coupling between meiotic DNA replication and recombination initiation. Science **290:** 806–809.

BUDD, M., and J. L. CAMPBELL, 1987 Temperature-sensitive mutations in the yeast DNA polymerase I gene. Proc. Natl. Acad. Sci. USA **84:** 2838–2842.

BUDD, M. E., K. D. WITTRUP, J. E. BAILEY and J. L. CAMPBELL, 1989 DNA polymerase I is required for premeiotic DNA replication and sporulation but not for X-ray repair in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:** 365–376.

BULLARD, S. A., S. KIM, A. M. GALBRAITH and R. E. MALONE, 1996 Double strand breaks at the *HIS2* recombination hot spot in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **93:** 13054–13059.

CAO, L., E. ALANI and N. KLECKNER, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

CERVANTES, M. D., J. A. FARAH and G. R. SMITH, 2000 Meiotic DNA breaks associated with recombination in *S. pombe*. Mol. Cell **5:** 883–893.

CHA, R. S., B. M. WEINER, S. KEENEY, J. DEKKER and N. KLECKNER, 2000 Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev. **14:** 493–503.

DIRICK, L., L. GOETSCH, G. AMMERER and B. BYERS, 1998 Regulation

of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science **281:** 1854–1857.

Dutta, A., and S. P. Bell, 1997 Initiation of DNA replication in eukaryotic cells. Annu. Rev. Cell. Dev. Biol. **13:** 293–332.

Engebrecht, J., S. Masse, L. Davis, K. Rose and T. Kessel, 1998 Yeast meiotic mutants proficient for the induction of ectopic recombination. Genetics **148:** 581–598.

Forsburg, S. L., and J. A. Hodson, 2000 Mitotic replication initiation proteins are not required for pre-meiotic S phase. Nat. Genet. **25:** 263–268.

Giroux, C. N., M. E. Dresser and H. F. Tiano, 1989 Genetic control of chromosome synapsis in yeast meiosis. Genome **31:** 88–94.

Goldman, A. S., and M. Lichten, 1996 The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. Genetics **144:** 43–55.

Hawley, R. S., 1987 Exchange and chromosomal segregation in eukaryotes, pp. 497–527 in *Meiosis*, edited by P. B. Moens. Academic Press, New York.

Hill, J. E., A. M. Myers, T. J. Koerner and A. Tzagoloff, 1986 Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2:** 163–167.

Hollingsworth, N. M., and B. Byers, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics **121:** 445–462.

Hollingsworth, N. M., L. Goetsch and B. Byers, 1990 The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61:** 73–84.

Ito, H., Y. Fukada, K. Murata and A. Kimura, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Klapholz, S., C. S. Waddell and R. E. Esposito, 1985 The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:** 187–216.

Klein, F., P. Mahr, M. Galova, S. Bunonmo, C. Michaelis, *et al.*, 1999 A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell **98:** 91–103.

Krieg, P. A., and D. A. Melton, 1987 In vitro RNA synthesis with SP6 RNA polymerase. Methods Enzymol. **155:** 397–415.

Kupiec, M., B. Byers, R. E. Esposito and A. P. Mitchell, 1997 Meiosis and sporulation in *Saccharomyces cerevisiae*, pp. 889–1036 in *The Molecular and Cellular Biology of the Yeast Saccharomyce: Cell Cycle and Cell Biology*, edited by J. R. Pringle, J. R. Broach and E. W. Jones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Lamb, T. M., and A. P. Mitchell, 2001 Coupling of *Saccharomyces cerevisiae* early meiotic gene expression to DNA replication depends upon *RPD3* and *SIN3*. Genetics **157:** 545–556.

Loidl, J., F. Klein and H. Scherthan, 1994 Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. J. Cell Biol. **125:** 1191–1200.

Loidl, J., F. Klein and J. Engebrecht, 1998 Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast, pp. 257–285 in *Nuclear Structure and Function*, edited by M. Berrios. Academic Press, San Diego.

Loo, S., C. A. Fox, J. Rine, R. Kobayashi, B. Stillman *et al.*, 1995 The origin recognition complex in silencing, cell cycle progression, and DNA replication. Mol. Biol. Cell **6:** 741–756.

McKee, A. H., and N. Kleckner, 1997 A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene *SAE2*. Genetics **146:** 797–816.

Merino, S. T., W. J. Cummings, S. N. Acharya and M. E. Zolan, 2000 Replication-dependent early meiotic requirement for Spo11 and Rad50. Proc. Natl. Acad. Sci. USA **97:** 10477–10482.

Mitchell, A. P., 1994 Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. **58:** 56–70.

Padmore, R, L. Cao and N. Kleckner, 1991 Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell **66:** 1239–1256.

Prinz, S., A. Amon and F. Klein, 1997 Isolation of *COM1*, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. Genetics **146:** 781–795.

Pukkila, P. J., K. B. Shannon and C. Skrzynia, 1995 Independent synaptic behavior of sister chromatids in *Coprinus cinereus*. Can. J. Bot. **73**(Suppl. 1): S215–S220.

Rockmill, B., and G. S. Roeder, 1990 Meiosis in asynaptic yeast. Genetics **126:** 563–574.

Rose, K., S. A. Rudge, M. A. Frohman, A. J. Morris and J. Engebrecht, 1995 Phospholipase D signaling is essential for meiosis. Proc. Natl. Acad. Sci. USA **92:** 12151–12155.

Rose, M. D., F. Winston and P. Hieter, 1990 *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ross, M., F. J. McNally, P. Laurenson and J. Rine, 1993 Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae*. Science **262:** 1838–1843.

Rothstein, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast, pp. 281–301 in *Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego.

Santocanale, C., and J. F. Diffley, 1998 A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. Nature **395:** 615–618.

Schild, D., and B. Byers, 1978 Meiotic effects of DNA-defective cell cycle mutations of *Saccharomyces cerevisiae*. Chromosoma **70:** 109–130.

Silva-Lopez, E., T. J. Zamb and R. Roth, 1975 Role of premeiotic replication in gene conversion. Nature **253:** 212–214.

Simchen, G., D. Idar and Y. Kassir, 1976 Recombination and hydroxyurea inhibition of DNA synthesis in yeast meiosis. Mol. Gen. Genet. **144:** 21–27.

Smith, A. V., and G. S. Roeder, 1997 The yeast Red1 protein localizes to the cores of meiotic chromosomes. J. Cell Biol. **136:** 957–967.

Stuart, D., and C. Wittenberg, 1998 *CLB5* and *CLB6* are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. **12:** 2698–2710.

Sugino, A., 1995 Yeast DNA polymerases and their role at the replication fork. Trends Biochem. Sci. **20:** 319–323.

Sun, H., D. Treco, N. P. Schultes and J. W. Szostak, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

Sym, M., J. Engebrecht and G. S. Roeder, 1993 ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell **72:** 365–378.

von Wettstein, D., S. W. Rasmussen and P. B. Holm, 1984 The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18:** 331–413.

Weiner, B. M., and N. Kleckner, 1994 Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell **77:** 977–991.

Wu, T., and M. Lichten, 1994 Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science **263:** 515–518.

Wu, T. C., and M. Lichten, 1995 Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics **140:** 55–66.

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