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A Yeast *MRE3/REC114* Gene is Essential for Normal Cell Growth and Meiotic Recombination

Sun-Hee Leem*

Department of Biology, Faculty of Natural Science, Dong-A University, Pusan 604-714, Korea

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We have analyzed the *MRE3/REC114* gene of *Saccharomyces cerevisiae*, previously detected in isolation of mutants defective in meiotic recombination. We cloned the *MRE3/REC114* gene by complementation of the meiotic recombination defect and it has been mapped to chromosome XIII. The DNA sequence analysis revealed that the *MRE3* gene is identical to the *REC114* gene. The upstream region of the *MRE3/REC114* gene contains a T_4C site, a URS (upstream repression sequence) and a TR (T-rich) box-like sequence, which reside upstream of many meiotic genes. Coincidentally, northern blot analysis indicated that the three sizes of *MRE3/REC114* transcripts, 3.4, 1.4 and 1.2 kb, are induced in meiosis. A less abundant transcript of 1.4 kb is detected in both mitotic and meiotic cells, suggesting that it is needed in mitosis as well as meiosis. To examine the role of the *MRE3/REC114* gene, we constructed *mre3* disruption mutants. Strains carrying an insertion or null deletion of the *MRE3/REC114* gene showed slow growth in nutrient medium and the doubling time of these cells increased approximately by 2-fold compared to the wild-type strain. Moreover, the deletion mutant (Δ*mre3*) displayed no meiotically induced recombination and no viable spores. The *mre3/rec114* spore lethality can be suppressed by *spo13*, a mutation that causes cells to bypass reductional division. The double-strand breaks (DSBs) which are involved in initiation of meiotic recombination were not detected in the analysis of meiotic chromosomal DNA from the *mre3/rec114* disruptant. From these results we suggest that the *MRE3/REC114* gene product is essential in normal growth and in early meiotic stages involved in meiotic recombination.

Key words: *MRE3/REC114* gene, meiotic recombination, *Saccharomyces cerevisiae*

Meiosis is distinguished from all other types of cellular development by virtue of associated DNA metabolic events, and results in reassociation of informational content and orderly segregation of a total complement of chromosomes into gametes. Major genetic events during meiosis are genetic recombination and chromosome segregation. These events play an important role in generation of new genotypes (14). Two significant events distinguish meiosis from mitosis: and (ii) morphological changes; homologous chromosome pairing and SC formation, (i) the association of high levels of recombination with reductional chromosome segregation in meiosis.

Most current studies of meiotic mechanisms in yeast entail the identification and isolation of genes that are required to carry out the process. Classical genetic studies have established general principles about the nature of meiotic recombination, thereby leading to the development of useful models for genetic exchange (24). This central role has made the isolation of mutations in meiotic recombination functions difficult; Rec⁻ mutants produce aneuploid and inviable meiotic products. Over the last several years, this problem has been overcome in the yeast, *Saccharomyces cerevisiae*. One direct method of screening for mutants involves use of a haploid strain that is disomic for chromosome III and carries genetic markers that can be exploited in screening for decreased meiotic recombination (19).

In order to analyze the mechanism of meiotic recombination, several genes required for meiotic recombination have been isolated from *S. cerevisiae* (24) and classified into two groups. One group comprises genes affecting meiotic recombination alone, namely *HOP1, SPO11, RED1, MER1, MRE2/NAM8* and *MRE4/MEK1* (8, 13, 18, 19, 21, 26). The other consist of genes that have additional mutant phenotypes during mitosis as well as meiotic recombination deficiencies. They are *RAD6, RAD50-57, MRE11, XRS2, ESR1* and *CDC40* (27). Mutants in the genes of the latter group show the following processes: cell growth deficiency, DNA repair deficiency and hypo- or hyper-mitotic recombination. *Cdc40* mutant cells show both meiotic recombination and mitotic growth deficiencies and the *ESR1* gene is invol- ved in mitotic cell growth, DNA repair and meiotic recombination (14). These observations suggest that mi-

* To whom correspondence should be addressed.
(Tel) 82-51-200-6789; (Fax) 82-51-200-7269

totic cell growth, DNA repair and meiotic recombination pathways are closely linked and the genes that are required for those processes may overlap. The relationships between them are poorly understood at present.

The *S. cerevisiae mre3-1* mutant was isolated in a search for meiotic recombination-defective mutants (2). The diploid strain homozygous for the *mre3-1* mutant produced inviable spores. This spore lethality can be alleviated by a *spo13* mutation. While the *mre3-1* mutant has no effect on mitotic recombination and DNA repair, both inter-and intragenic recombination in meiosis are reduced approximately 500-fold than in the wild-type strain. In this paper, we report the cloning and genetic characterization of the *MRE3* gene. Δ*Mre3* disruptant cells, moreover, revealed defects in normal cell growth as well as meiotic recombination. Here we described the *MRE3* gene, a gene essential for normal cell growth, meiotic recombination and proper chromosome segregation during the first meiotic division.

Materials and Methods

Strains and plasmids

Bacterial strain DH5α was used for preparation of plasmid DNA. All yeast strains used in this study are described in Table 1. P192m3 was used to clone the *MRE3* gene and for complementation tests. The wild-type strain KJC101 was used to prepare total RNA. All strains except P192m3 were derived from the SK1 strain, but the KJ and IM strains are SK1 hybrids, not pure SK1 strains. The NKY strains were supplied by Dr. N. Kleckner.

Construction of yeast strains

Yeast manipulations and genetic methods were carried out according to Adams *et al.* (1). A mutant with disruption in the *mre3* gene was constructed from the diploid strains KJC102 and KJC103 (19) by transformation and a one-step gene disruption procedure (28). The homozygous mutant diploid strains were prepared by mating between the haploid mutant strains obtained by dissecting the asci formed after the sporulation of transformed diploids. A subclone of the 2.7 kb *Cla*I fragment of the *MRE3* gene (Fig. 1) was used to disrupt the *mre3* gene. A 1.2 kb *Hin*dIII fragment containing the *URA3* gene of YEp24 was inserted at the *Sal*I site in the *Cla*I subclone. The resultant 3.9 kb disrupted *Cla*I fragment, pSL37 (Fig. 1), was purified by gel electrophoresis and used to transform diploid strains (IMD301 and IMD302). Another null disruptant strain (IMD303) was made by transformation of the pSL38 plasmid. Gene replacement of $URA3^+$ transformants were confirmed by phenotypes and Southern blot analysis (31).

Procedure of sporulation

The diploid SK1 strains were streaked on YPG plates (1% yeast extract, 2% polypeptone, 3% glycerol, 2% agar) and incubated at 30°C for 12 h (19, 20). A single colony on a YPG plate was streaked on YPD (1% yeast extract, 2% polypeptone, 2% glucose, 2% agar) and incubated for 2-3 days. In the case of cells harboring plasmids, the cells were streaked on SD-Ura or SD-Leu (2% glucose, 0.67% yeast nitrogen base, 2% agar, supplemented with the necessary

Table 1. Genotypes of strains

Strains	Genotypes
P192m3	*MATa leu2-1 his4-4 can1^r ura3 cyh2^r ade6 TRP1 MET2 ade2 mre3-1* *MATα leu2-27 his4-290 can1^r ura3 CYH2 ADE6 trp1 met2 ade2 mre3-1*
*KJC101	*MATa leu2 his4-290 can1^r ura3 ho::LYS2 trp1 cyh2^r ade6 ade2 lys2* *MATα leu2 his4-4 CAN1 ura3 ho::LYS2 trp1 CYH2 ADE6 ade2 lys2*
*KJ1-3a	*MATα leu2 his4-290 can1^r ura3 ho::LYS2 trp1 cyh2^r ade6 ade2 lys2*
*KJ2-2a	*MATa leu2 his4-4 CAN1 ura3 ho::LYS2 trp1 CYH2 ADE6 ade2 lys2*
*KJ2-3a	*MATα leu2 his4-4 CAN1 ura3 ho::LYS2 trp1 CYH2 ADE6 ade2 lys2*
*IMH301	KJ1-3a with *mre3::URA3*
*IMH302	KJ2-2a with *mre3::URA3*
*IMD301	KJC101 with *mre3::URA3/mre3::URA3*
*IMD302	KJC101 with *mre3::URA3/MRE3*
*IMD303	KJC101 with *mre3::hisG(hisG::URA3::hisG)*
*IMD311	IMD501 with *mre3::URA3/mre3::URA3*
*IMD501	*MATa ho::LYS2 spo13::hisG ura3 lys2* *MATα ho::LYS2 spo13::hisG ura3 lys2*
NKY653	*MATa ho::LYS2 rad50::hisG spo13::hisG ura3 lys2*
NKY654	*MATα ho::LYS2 rad50::hisG spo13::hisG ura3 lys2*

All strains except P192m3 were derived from the strain SK1. *These strains are hybrids, not pure SK1 strains. NKY strains were supplied by Dr. N. Kleckner and P192m3 was supplied by Dr. M. Ajimura.

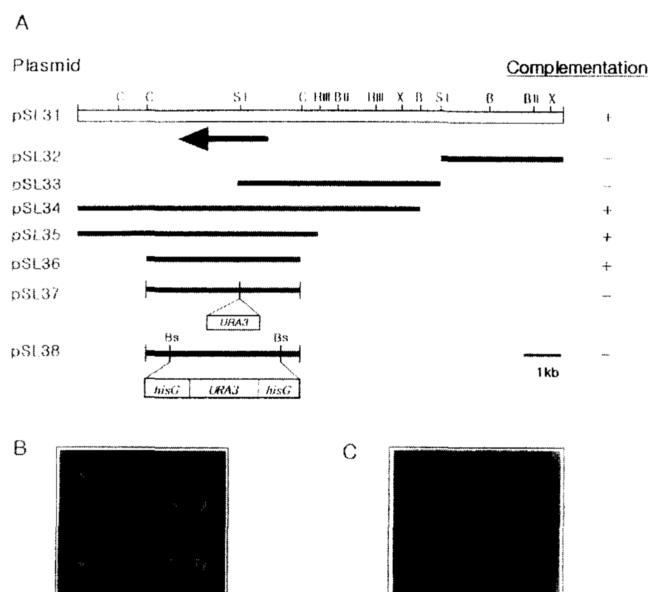

Fig. 1. Physical maps of cloned *MRE3* genes and complementation analysis. **(A)** Restriction map of 8.1 kb (pSL31) fragments containing the *MRE3* gene presents the original cloned plasmid for *mre3-1* mutation. Other lines represent the subcloned region into YCp50. Subclones were tested for their ability to complement the meiotic recombination defect in P192m3. The smallest subclone which complemented the *mre3-1* mutation is a pSL36 that contains a 2.7 kb *Cla*I fragment. The pSL37 is a disruption plasmid inserted a 1.2 kb *URA3* insert in the *Sal*I site of pSL36. The pSL38 disruption plasmid is constructed by using the *hisG::URA3::hisG* fragment to replace the region between the *Bst*X1 and *Bst*X1 sites of the *Cla*I fragment. Abbreviations for restriction enzyme sites are as follows: *Bam*HI, B; *Bgl*, BII; *Bst*X1, Bs; ClaI, C; *Eco*Rl, E; *Eco*RV, R; *Hind*, H; *Sal*I, S; and *Xho*I; X. **(B)** Tetrad analysis of heterozygous diploids at the *MRE3* gene locus. Tetrads from five asci were shown and each spore from an ascus was placed vertically. **(C)** The genetic markers of their spore clones were examined on an SD-Ura plate. All of the two small colonies appeared as Ura^+ that seemed to be *mre3::URA3*.

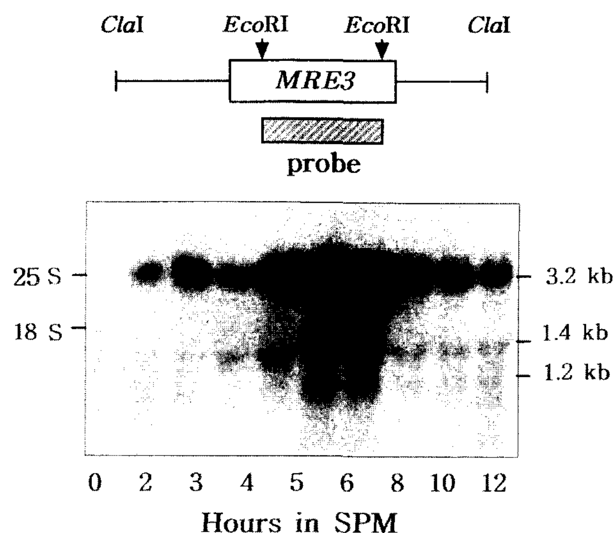

Fig. 2. Transcriptional analysis of *MRE3/REC114* gene. Northern blot analysis of *MRE3* RNA. A northern blot containing RNA prepared from a wild-type strain, KJC101, grown mitotically (lane 1) and after 2 hr to 12 hr in sporulation medium (lane 2~9). Restriction enzyme maps of the 2.7 kb *Cla*I fragment showing a closed arrow indicate the location and orientation of *MRE3* ORF. This blot was hybridized with a ^{32}P-labeled probe, a 1 kb *Eco*RI fragment. The position of size markers, 25S and 18S ribosomal RNAs are shown left of the panel, the calculated lengths of the transcripts are given in the right side of the panel.

amino acids lacking leucine or uracil) and incubated for 2-3 days. A single colony from the plate was grown in YPD or SD liquid medium for 18-24 h. Each culture was diluted 100-fold into YPA (1% yeast extract, 2% polypeptone, 2% potassium acetate) or SA (2% potassium acetate, 0.67% yeast nitrogen base, supplemented with the necessary amino acids lacking leucine or uracil) medium, for cells harboring plasmids and incubated for 12 h. Cells (1×10^7 cells/ml) were harvested, washed twice with sterile water and resuspended at the same density in SPM (0.3% potassium acetate, 0.03% raffinose). The suspension was then incubated at 30°C with vigorous shaking.

Complementation tests for *MRE3*

Homozygous strain for *mre3-1* (P192m3) was transformed with the subcloned plasmids and plated onto synthetic selective medium lacking uracil (SD-Ura). The Ura^+ transformants were sporulated and then plated onto SD-Ura, SD+Cyh, and SD-Leu. After 3-4 days, the frequencies of haploidization (*cyh*^{r}), intergenic recombination (*cyh*^{r}-*ade6*), and intragenic recombination (Leu^+) were calculated by colony counting as previously described (2).

Analysis of RNA

Total RNAs were prepared by vortexing the cells with glass beads in the presence of phenol/chloroform. Meiotic samples for isolation of RNA were prepared as previously described (19, 20).

For northern blot analysis, about 10 μg of total RNA samples were denatured and fractionated in 1% agarose gel containing 6% formaldehyde with MOPS (3-[N-Morpholino] propanesulfonic acid) buffer (29). RNA was transferred onto a nylon membrane (IMMOBILON-N, Millipore) and hybridized with the following ^{32}P-labeled DNA probes; a 1.0 kb *Eco*RI fragment of *MRE3* gene (Fig. 2). RNA sizes were estimated by comparison with 25S and 18S ribosomal RNAs.

Fluorescent microscopy

Meiotic chromosomal segregations were examined by fluorescent microscopy after staining by DAPI (4,6-diamidino-2-phenylindole, Sigma), a DNA-specific fluorescent dye. Wild-type (KJC101) and *mre3::URA3*

(IMD301) diploid strains were sporulated and then performed as described by Leem *et al.* (18). To determine the relative DNA content per cell, the intensity of fluorescence emitted from the DAPI-stained nuclei was measured by an Olympus Fluorometer equipped with 1,250×magnification.

Detection of the Double-Strand Breaks

Detection of double-stranded breaks (DSBs) at the *ARG4* locus in meiosis was carried out as described by Cao *et al.* (7) and Sun *et al.* (32) with slight modification. The DNA extraction was performed with the 10 ml of aliquots collected at hourly intervals after transfer to SPM. To detect DSBs, one-tenth of isolated DNA was digested with *Bgl*II and electrophoresed on a 0.7% agarose gel. DNAs were transferred to a NYTRAN membrane using a Vacuum transfer system (Pharmacia). After fixation by UV crosslink with 150 J/m^2, the membrane was hybridized with a ^{32}P-labeled DNA probe, a 0.9 kb *Eco*RV-*Bgl*II fragment (32).

Flow cytometry

To prepare meiotic samples for flow cytometry, 2 ml of sporulation culture were withdrawn at each time point and the cells were fixed by addition of ethanol to a final concentration of 70%. Flow cytometry was carried out as previously described by Futcher (12). Following staining with 50 μg/ml of propidium iodide and a brief sonication step, the cells were processed by FACScan analysis (Becton Dickinson).

Results and Discussion

Cloning of a DNA fragment complementing meiotic defects of an *mre3-1* mutant

The *MRE3* gene was cloned from a yeast genomic library by complementation of meiotic recombination defects. The cloning of the *MRE3* gene was performed as previously described (19). A *mre3-1* diploid strain (P192m3) carrying *his4* and *leu2* heteroalleles was transformed with a yeast genomic library carried on a YCp50 plasmid marked with the *URA3* gene. The plasmids were recovered from recombination positive transformants. Finally, we obtained one plasmid, pSL31, which carried the 8.1 kb inserted DNA fragment, among the approximately 20,000 transformants and subcloned the fragments in to a YCp50 vector (Fig. 1). Several subcloned plasmids, pSL32~pSL36, were constructed using the original plasmid (pSL31) and their complementation activities were examined by transformation to P192m3. The plasmid pSL36 contains a 2.7 kb *Cla*I fragment having the ability to complement the *mre3-1* mutation, and was used to determine the nucleotide sequence.

In an attempt to gain information regarding the structure and function of the *MRE3* gene product, the nucleotide sequence of a 2.7 kb *Cla*I fragment was determined. Then we knew that the *MRE3* gene is the same as the *REC114* gene (25).

MRE3/REC114 transcripts are induced in early meiosis

In *S. cerevisiae*, consensus sequences have been established for RNA splicing (17). DNA sequence analysis revealed that *MRE3/REC114* ORF was composed of 1,262 bp with consensus intron splice sites close to the 3 end. To gain insight into the possible functions of the Mre3 protein, the predicted Mre3 sequence was compared with those of other proteins in NBRF-PIR and SWISS-PROT databases, but there are no significant homologies.

The genes transcripts required for meiotic recombination or SC formation are induced during meiosis,

Table 2. Comparision between the upstream region of the *MRE3* gene and the URSs of other yeast genes

Genes	5'	URS Consensus															3'
		-	T	A	G	C	C	G	C	C	G	R	R	R	R	-	
*MRE3	- 82		T	A	G	C	C	G	C	C	C	A	c	c	t		- 94
*MRE4	-127		T	A	G	C	C	G	C	C	G	A	A	A	t		-139
	-141		T	A	G	C	C	G	C	C	A	t	c	A	t		-153
*IME2	-448		T	T	G	C	C	G	C	C	G	A	A	A	t		-457
	-543		T	A	G	C	C	G	C	C	G	t	c	A	t		-552
*SPO13	- 97		T	A	G	C	C	G	C	C	G	A	c	A	A		- 85
*HOP1	-164		T	A	G	C	C	G	C	C	c	A	G	G	t		-176
*MER1	-112		T	A	G	C	C	G	C	C	G	A	c	A	G		-100
*RED1	-158		T	A	G	C	C	G	C	C	t	G	A	A	G		-170
CYC1	-240		g	A	t	C	C	G	C	C	A	G	G	c	G		-228
CYC7	-288		c	c	c	C	C	G	C	C	G	A	G	G	G		-276
CAR1	-163		T	A	G	C	C	G	C	C	G	A	G	G	G		-151
CAR2	-183		T	A	G	C	C	G	C	C	G	A	c	G	c		-171

*The expression of these genes is induced specifically during meiosis.

such as *SPO11, SPO13, HOP1, MER1, MRE4/MEK1* and *DMC1* (3, 5, 6, 9, 13, 19, 33, 34). Transcriptional induction reached a maximum level at approximately the time of meiotic recombination or chromosome segregation in meiosis I and these genes perhaps play an important role during that stage. Interestingly, the upstream region of the *MRE3/REC114* gene also contained a T_4C site, a URS (upstream repression sequence) (Table 2) and a TR (T-rich) box-like sequence, which reside upstream of many meiotic genes.

To determine the transcripts of *MRE3/REC114*, we analyzed total RNA from the wild-type strain (KJC101) by northern blot analysis using a probe of the *Eco*RI fragment involved in *MRE3/REC114* (Fig. 2). Total RNA were prepared from cultures harvested at various time points after transfer to SPM. Three transcripts 3.4 kb, 1.4 kb and 1.2 kb in size were detected with this probe, and completely separated on a northern gel approximately 20 cm in length. Northern blot analysis indicates that these transcripts are meiotically induced to accumulate maximally just prior to the first meiotic division, such as other recombination defective mutants (*hop1, rad50, spo11, red1, mer1* and *mre4/mek1*). The time of maximum accumulation of these transcripts corresponds approximately to the time in meiosis at the *MRE3/REC114* gene product is believed to act. Furthermore, less abundant transcript of 1.4 kb is present in both the mitotic and meiotic cell and its abundance induced in meiosis with kinetics similar to the other two transcripts. This result suggests that the *MRE3/REC114* gene may be required for mitosis as well as meiosis.

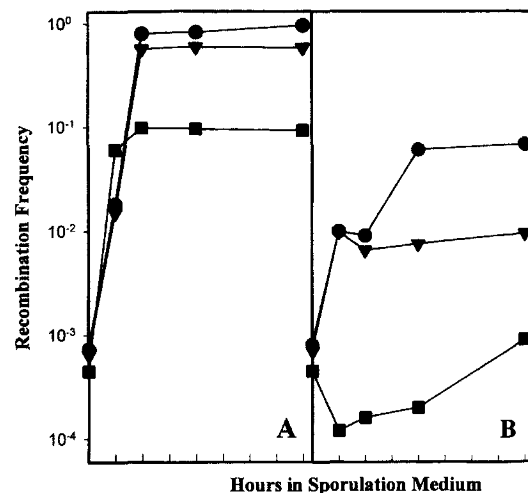

Fig. 3. Time course of haploidization and meiotic recombination by return-to-growth method. Cells grown in YPA at 2×10^7 cells/ml were collected and suspended into SPM at the same density. At approximate times after incubation in SPM, aliquots were taken and plated on SD-Ura, SD-His or SD+Cyh (1 μg/ml). Haploidization at *cyh2* locus (●), intergenic recombination at *cyh2-ade6* (▼) and intragenic recombination at *his4* (■) were examined in the wild type **(A)**, and *mre3::URA3* **(B)** strains.

Genetic characterization of *mre3::URA3* disruption mutants

We constructed an *mre3* disruption mutant, *mre3::URA3*, as described in Materials and Methods. Strains carrying this disruption allele failed to complement the *mre3-1* mutant, indicating that the cloned gene is the wild-type *MRE3/REC114* gene. At first, we constructed the *MRE3/mre3* strain (IMD302) and then this strain was sporulated in SPM for tetrad analysis. Tetrad analysis of heterozygous diploids at the *MRE3/REC114* locus is shown in Fig. 1 B. Dissection of 40 tetrads following sporulation of the *MRE3/mre3* diploid indicated in 38 asci, two spores proceeded with normal growth (large colony) and the other two proceded with slow growth (small colony) (Fig. 1B). Twenty of the 40 asci were chosen and their genetic markers determined. All of the large colonies were Ura^-, while all of the small colonies appeared as Ura^+ (Fig. 1C). Then we compared the doubling time of the Δ*mre3* disrupted cells (IMD301 and IMD303) and wild-type cells. As a result, the doubling time of the Δ*mre3* disrupted cells increased approximately 2-fold of the level in the wild type, approximately 2.8 and 1.5 h, respectively. The *mre3-1* mutant, however, showed normal cell growth as in the wild type. These results suggest that the original *mre3-1* mutant did not show all phenotypes of the *MRE3/REC114* gene and this gene has a mitotic role that is required for normal cell growth. This is consistent with the observation in northern blot analysis that the *MRE3/REC114* gene required mitosis as well as meiosis.

To analyze the recombination proficiency of the *mre3* disruption mutant, *mre3::URA3* (IMD301), the frequencies of intergenic recombination at the *cyh2-ade6* interval on chromosome VII, intragenic recombination between *his4* heteroalleles on chromosome III and haploidization at the recessive drug resistant marker, *cyh2*^{R}, were measured by return-to-growth experiments (10, 30). The results were compared with those of the wild-type strain at various time points during mitosis and meiosis (Fig. 3). In the *mre3::URA3* disruptant, both recombinations were less than 1% of the wild-type level, and haploidization was reduced to less than 10% of the the wild-type level during meiosis. Similar results were obtained in the analysis of *mre3-1* and IMD303 disruptant cells. These results suggest that the *MRE3* gene is essential for meiotic recombination.

The diploid strains homozygous for Δ*mre3* disruption mutants (IMD301 and IMD303) were sporulated and then the frequencies of spore formation and spore viability were measured (Table 3). The frequency of

Table 3. Viability of spores in *mre3::URA3* disruptant and *mre3 spo13* double mutant

Strains	Genotypes	viable spores/ascus					Viability of spores(%)
		0	1	2	3	4	(viable spore/total spores)
KJ101	*MATa MRE3* / *MATα MRE3*	0	0	1	3	40	97.2 (171/96)
IMD302	*MATa mre3::URA3* / *MATα MRE3*	0	0	2	7	35	93.8 (165/176)
IMD301	*MATa mre3::URA3* / *MATα mre3::URA3*	87	1	0	0	0	0.3 (62/88)
IMD501	*MATa spo13::hisG* / *MATα spo13::hisG*	6	14	24	-	-	70.5 (62/88)
IMD311	*MATa mre3::URA3 spo13::hisG* / *MATα mre3::URA3 spo13::hisG*	23	22	39	-	-	59.5 (100/168)

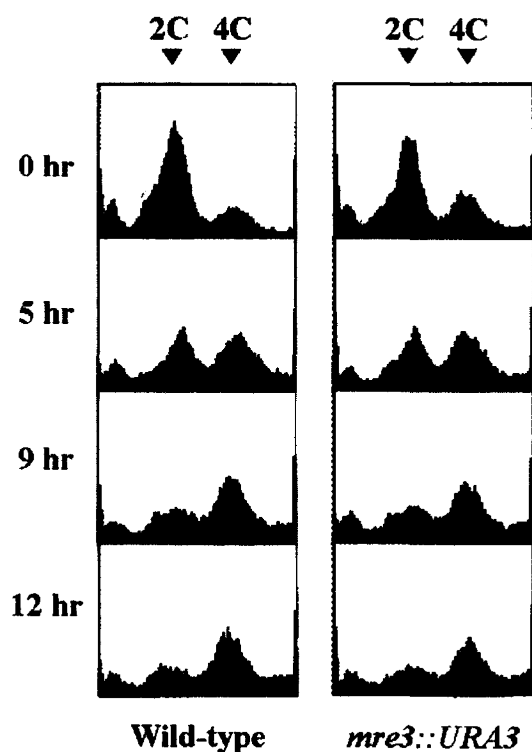

Fig. 4. Flow cytometry of Δ*mre3* cells during meiosis. KJC101 (*MRE3*) and IMD301 (Δ*mre3*) cells taken at various times after transfer to the sporulation medium were treated with RNase, stained with propidium iodide and subjected to flow cytometry. Cell number examined in FACScan is represented in the vertical axis and their DNA contents are represented horizontally.

spore formation of homozygous Δ*mre3* disruption mutants was approximately 40% of the level in the wild type. The spore viability in homozygous Δ*mre3* disruption mutants was severely decreased. In the IMD303 and *mre3-1* mutant, moreover, almost the same level was detected. The Δ*mre3* disruption mutants produced inviable spores, indicating that the *MRE3/REC114* gene is essential for spore viability.

Premeiotic DNA replication in Δ*mre3* mutant cells

We examined premeiotic DNA replication in wild-type and Δ*mre3* strains. Cells transferred to the sporulation medium were withdrawn, sonicated, stained with propidium iodide and subjected to flow cytometry. As shown Fig. 4, the DNA content in Δ*mre3* cells increased to 4C as in wild-type cells. This result suggests that premeiotic DNA replication is almost complete in Δ*mre3* strains. Thus low viability of spores in Δ*mre3* cells do not seem to be caused by the absence of premeiotic DNA replication.

MRE3/REC114 gene has a role in the early step of meiotic recombination

The *spo13* mutant has previously been shown to bypass the first reductional division and to produce two viable spores (15). The use of the *spo13* mutation allowed meiotic recombination mutations to be divided into two groups, blocked early or late in the meiotic recombination pathway (21). The *mre3* spore lethality was rescued by introduction of the *spo13* mutation. The homozygous *mre3::URA3 spo13* double mutant (IMD311) produced viable spores at a frequency of 59.5% (Table 2). Therefore, it seems likely that the defect in the *mre3* strain is in the early stage of the meiotic recombination pathway, such as *hop1*, *rad50*, *spo11*, *red1*, *mer1*, *mre2* and *mre4/mek1* (8, 13, 16, 18, 19, 21, 26).

Among the rescued spores in the *mre3 spo13* homozygous diploid strain, crossing-over between *MAT* and *CENIII* was determined by analysis of dissected diad spores. The total level of recombination in the *mre3 spo13* double mutant is reduced to approximately 4.3% of the wild type for intervals spanning. This result indicates that the defects of the meiotic recombination was shown in final meiotic products as well as return-to-growth.

In recent years, evidence show that meiotic recombination is initiated by double stranded breaks (DSBs). Meiotic DSBs were first detected at a certain

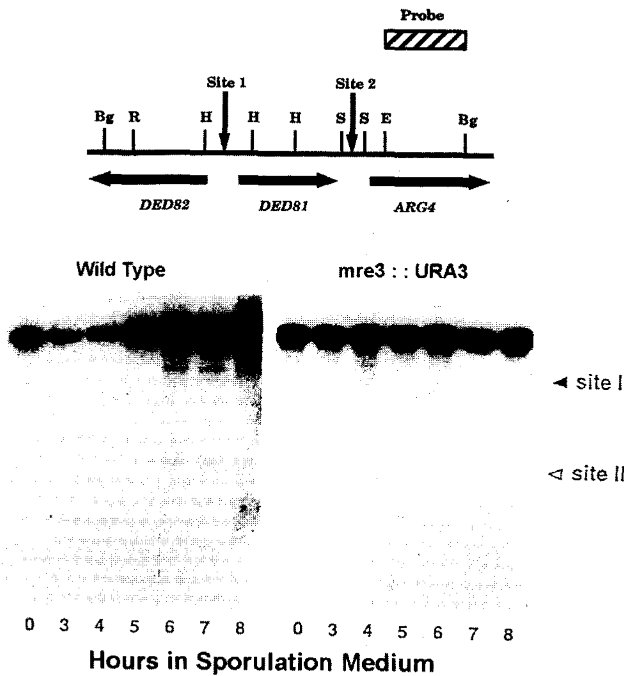

Fig. 5. Meiosis-specific double stranded breaks in the wild-type and *mre3::URA3* strains. A map of relevant restriction sites in ~5.5 kb *ARG4-DED81-DED82* region (32) and the positions of the two major DSBs sites and the probe used are shown in the top figure. Chromosomal DNA samples from either KJC101 (wild-type) or IMD301 (*mre3::URA3*) cells were digested with *Bgl*II and hybridized with a random primed *Eco*RV-*Bgl*II DNA probe.

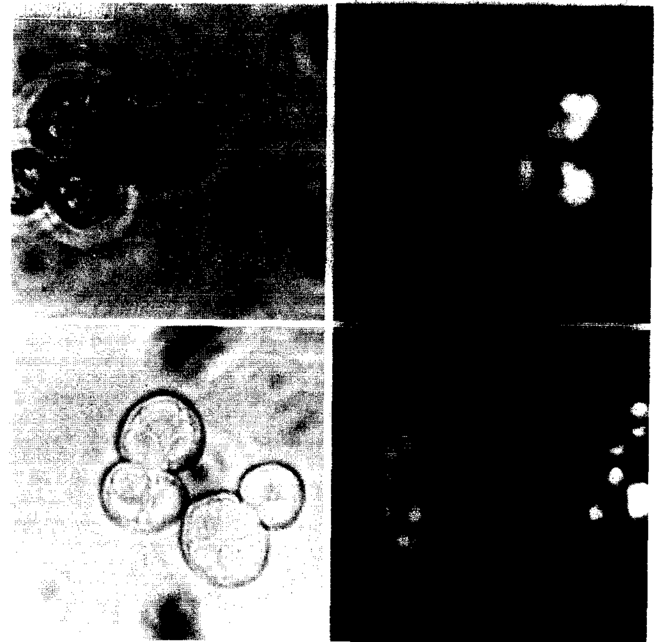

Fig. 6. Meiotic chromosome segregation in the tetrads of the *mre3::URA3* strain. IMD301 cells were presporulated in YPA and transferred to SPM at 30°C. At 12 h after sporulation, aliquots were fixed and stained with DAPI solution and photographed by phase-contrast microscopy **(A, C)** or fluorescence microscopy **(B, D)** at 1,250 × magnification.

loci which revealed elevated levels of meiotic recombination (hot spots), such as *ARG4* (23), *HIS4::LEU2* insertion allele on chromosome III (7) and *HIS4* (22). Therefore, the occurrence of DSBs is critical in the early step of meiotic recombination. We examined the fate of the DSBs at the *ARG4* locus in the *mre3::URA3* strain, IMD301. In a wild-type strain, KJC101, the DSBs are introduced at this locus after 5 h in SPM and reached a maximum level after 6-7 h in SPM. However, in the *mre3::URA3* mutant, introduction of DSBs was completely eliminated (Fig. 5).

The same results were obtained in *rad50* and *spo11* mutants (7) which completely abolished both meiotic recombination and chromosome synapsis. Therefore, these results suggest that the *MRE3/REC114* gene has a role at or/and before the introduction of DSBs, early in the meiotic recombination pathway.

Unequal chromosome segregation with *mre3::URA3* mutants in meiosis

Morphological development during sporulation of yeast has been followed by light and fluorescent microscopy. The segregation of chromatin during two meiotic divisions is readily visualized by light microscopy and by fluorescence using DAPI (in Materials and Methods). These procedures permit analysis of cells into several stages; mononucleate, binucleate (first division) and tetranucleate (second division).

In meiosis, the *mre3* mutant produces spores, but the spores are inviable, as observed in other recombination mutants (*hop1*, *rad50*, *spo11*, *red1*, *mer1*, *mre2* and *mre4/mek1*) (8, 13, 16, 18, 19, 21, 26). Most meiotic recombination-defective mutants produced inviable spores which were aneuploid, as expected if meiotic recombination is required for normal segregation at meiosis I (11). To examine chromosome segregation in *mre3* mutants (IMD301), cells were sporulated and stained with DAPI. The *mre3::URA3* diploid cells showed several spots unequal in size in the tetrad in SPM, but most wild-type cells showed four spots of equal size concurrently (Fig. 6). Thus, this result suggests that the *mre3* spore lethality is caused by chromosomal nondisjunction during meiotic division.

In this study, genetic characterization has demonstrated that the *MRE3/REC114* gene product is essential for normal mitotic cell growth and proper chromosome segregation during the first meiotic division. Northern blot analysis also indicates that the *MRE3/REC114* gene is required for mitosis as well as meiosis. Therefore, the Mre3/Rec114 protein appears to

be functionally separable into two parts (cell growth and meiotic recombination).

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