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The *Saccharomyces cerevisiae MER3* gene, encoding a novel helicase-like protein, is required for crossover control in meiosis

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The *MER3* gene is identified as a novel meiosis-specific gene, whose transcript is spliced in an *MRE2/MER1*-dependent manner. The predicted Mer3 protein contains the seven motifs characteristic of the DExH-box type of helicases as well as a putative zinc finger. Double strand breaks (DSBs), the initial changes of DNA in meiotic recombination, do not disappear completely and are hyperresected late in *mer3* meiosis, indicating that *MER3* is required for the transition of DSBs to later intermediates. A *mer3* mutation reduces crossover frequencies, and the remaining crossovers show random distribution along a chromosome, resulting in a high incidence of non-disjunction of homologous chromosomes at the first meiotic division. *MER3* appears to be very important for both the DSB transition and crossover control.

Keywords: crossing over/helicase/interference/meiosis/recombination

Introduction

During meiosis, two successive rounds of chromosome segregation occur, following a single round of DNA replication, producing haploid gametes from diploid cells. The first meiotic division (meiosis I) is unique in that homologous chromosomes (homologs) are synapsed and then distributed to opposite poles. Crossovers and non-crossovers, two types of recombinants, are generated between homologs during meiotic prophase. Crossovers are associated with reciprocal exchanges of chromosome arms and are needed for faithful segregation of homologs, but non-crossovers are not (for reviews, see Carpenter, 1988; Kleckner, 1996; Roeder, 1997). Among chromosomes, crossovers are distributed non-randomly in that every homolog sustains at least one, even though the average number per homolog is very low (1–3). Along a chromosome, multiple crossovers are further apart than expected on a random basis; this phenomenon is called crossover interference. Although the distribution of crossovers among and along chromosomes is likely to represent different manifestations of the same underlying regulation (Sym and Roeder, 1994; Chua and Roeder, 1998), the mechanism of crossover control is not understood.

In *Saccharomyces cerevisiae*, meiosis-specific double strand breaks (DSBs) are resected rapidly to produce 3′ overhanging single strands and are converted to strand exchange intermediates that contain double Holliday junctions (Cao *et al*., 1990; Sun *et al*., 1991; Schwacha and Kleckner, 1994, 1995). Rad51 and Dmc1, strand exchange proteins, are required for the generation of both crossovers and non-crossovers (Bishop *et al*., 1992; Shinohara *et al*., 1992, 1997; Bishop, 1994). During or after the transition of DSBs to strand exchange intermediates, homologs are synapsed along their entire lengths forming synaptonemal complexes (SCs) (Padmore *et al*., 1991; Schwacha and Kleckner, 1994). Zip1, a component of the central region of SC, is required for crossover interference. It has been proposed that, after SC polymerization, Zip1 transmits negative signals from crossover sites to neighbors in order to prevent additional crossovers (Sym *et al*., 1993; Sym and Roeder, 1994). However, it has also been suggested that, before SC polymerization, Zip1 acts in crossover control, due to the observation that a *zip1* mutation in an SC formation-deficient background further reduces crossing over (Storlazzi *et al*., 1996). The step of recombination at which the crossover control takes place is as yet unresolved. Intact DNA duplexes containing heteroduplex regions appear shortly before or concomitant with the appearance of mature recombinants (Goyon and Lichten, 1993; Nag and Petes, 1993). This may reflect the coordination between the formation and resolution of strand exchange intermediates.

Mutations in either *MRE2*, *MER1* or *MER2* impair DSB formation (Rockmill *et al*., 1995; Storlazzi *et al*., 1995; Nakagawa and Ogawa, 1997). *MRE2* and *MER1* encode RNA-binding proteins and are required for efficient splicing of the *MER2* intron, which contains a non-canonical 5′ splice site (Engebrecht *et al*., 1991; Nakagawa and Ogawa, 1997). Elimination of the *MER2* intron in an *mre2* mutant restores the formation of DSBs and non-crossovers. However, this *mre2 cMER2* (intronless *MER2*) strain is still defective in crossing over and produces inviable spores (Nakagawa and Ogawa, 1997). Similarly, overexpression of *MER2* in a *mer1* mutant restores the formation of non-crossovers, but not crossovers (Engebrecht *et al*., 1990; Storlazzi *et al*., 1995). Thus, an unidentified target(s) of *MRE2/MER1*-dependent splicing specifically required for crossing over has been suggested.

Here, we identify the *MER3* gene, the transcript of which is a new target of *MRE2/MER1*-dependent splicing. The predicted Mer3 protein has the seven motifs conserved amongst the DExH-box type of DNA/RNA helicases as well as a putative zinc finger. Meiosis-specific DSBs do not disappear completely and are hyperresected late in *mer3* meiosis, indicating the role of *MER3* in the transition of DSBs to later intermediates. A *mer3* mutation decreases the frequency and alters the distribution of crossovers,

resulting in a high incidence of homolog non-disjunction at meiosis I. Our results indicate the requirement of *MER3*, encoding a novel helicase-like protein, for both the DSB transition and crossover control.

Results

Identification of a novel gene, MER3, which suppresses the post-initiation recombination defect of an mre2 cMER2 mutant

An *mre2 cMER2* mutant is defective in crossing over but is proficient in generating non-crossovers. To identify a gene specifically required for crossing over, we searched for multicopy suppressors of the crossover defect using the *mre2N cMER2* strain. The *mre2N* allele confers temperature-sensitive spore formation but impairs crossing over at all temperatures (Nakagawa and Ogawa, 1997). *mre2N cMER2* cells were transformed with a yeast genomic library constructed on a multicopy plasmid and induced to undergo meiosis at 23°C. Recombinants were selected based on crossing over in the *LEU2–HIS4* or *TRP5–CYH2* interval as well as on genome haploidization (see legend to Figure 1A). Among ~9000 transformants, eight displayed increased frequencies of recombinants compared with background. Restriction mapping of plasmids recovered from these eight transformants revealed that two of these contain *MRE2* and the remainder contain overlapping inserts. We named the suppressor gene *MER3*. Multicopy *MER3* suppresses the recombination deficiency in *mre2N cMER2* and *mre2*Δ *cMER2*, but not in *mre2N* or *mre2*Δ mutants (Figure 1A), indicating that *cMER2* is required for suppression. *mre2N cMER2* mutants harboring multicopy *MER3* produce higher frequencies of recombinants and viable spores than *mre2*Δ *cMER2* mutants harboring the *MER3* plasmid (Figure 1A). This difference may be due to residual activity of Mre2N.

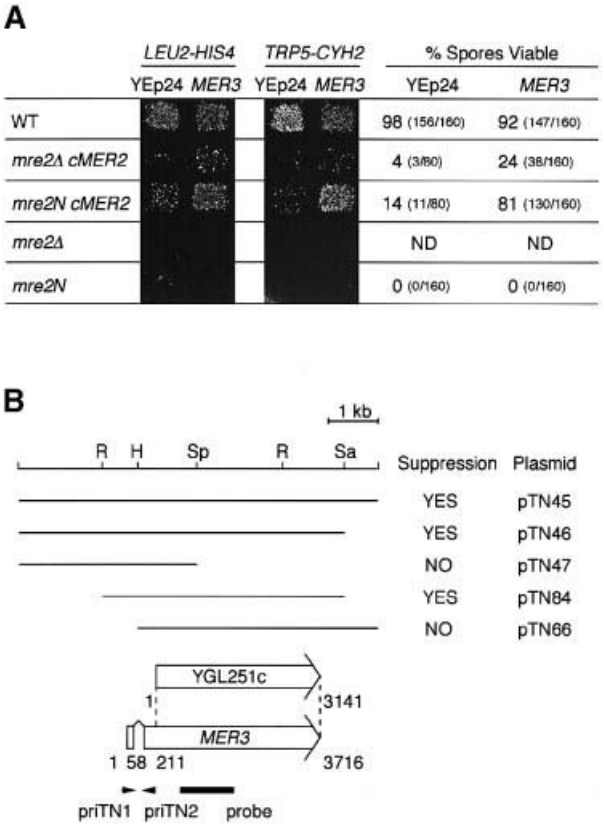

Fig. 1. Suppression of *mre2 cMER2* defects by *MER3*. (**A**) Assays for crossing over and spore viability. Wild-type (TNY171), *mre2*Δ *cMER2* (TNY240), *mre2N cMER2* (TNY169), *mre2*Δ (TNY185) and *mre2N* (TNY170) strains were transformed with a vector (YEp24) or the *MER3* plasmid (pTN45), patched on SD–Ura plates and replica plated to SPM–Ura to induce meiosis. After 4 days at 23°C, SPM–Ura plates were replicated to SD–Ura, –Arg, –Leu, –His, +CYH, +CAN and SD–Ura, –Arg, –Trp, +CYH, +CAN plates; papillae formed on these plates result from crossing over in the *LEU2–HIS4* and *TRP5–CYH2* intervals, respectively, and from haploidization. The spore viability was examined by colony formation following tetrad dissection. Numbers of viable and total spores are in parentheses. CYH, cycloheximide; CAN, canavanine; ND, not determined. (**B**) Subcloning of the *MER3* gene. A series of deletion constructs were derived from pTN45 and their suppression activities were tested by the plate assay using the *mre2N cMER2* strain. YGL251c and *MER3* ORFs are shown and their coding regions are numbered below. The positions of primers (priTN1 and 2) used in RT–PCR and a probe used for Northern blotting are illustrated. H, *Hin*dIII; R, *Eco*RI; Sa, *Sal*I; Sp, *Sph*I.

Splicing of the MER3 transcript depends on MRE2 and MER1

Subcloning and partial DNA sequencing of the *MER3* plasmid revealed that the suppression activity resides in a 4.8 kb *Eco*RI–*Sal*I fragment (pTN84, Figure 1B), includ-

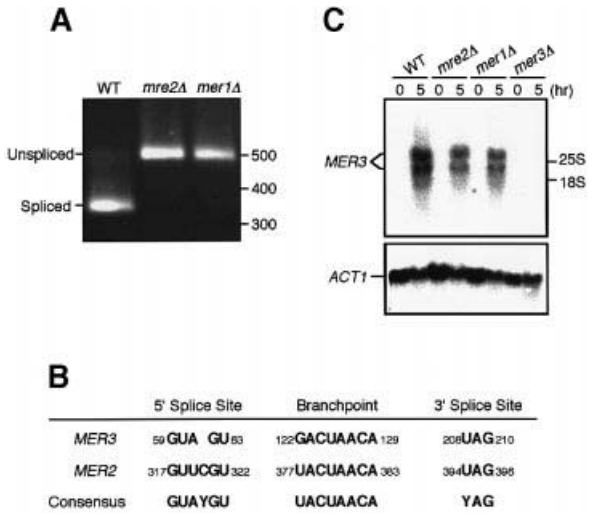

Fig. 2. Splicing and meiotic induction of the *MER3* transcript. Total RNAs were prepared from wild-type (TNY058), *mre2*Δ (TNY060), *mer1*Δ (TNY305) and *mer3*Δ (TNY286) cells immediately before the induction of meiosis and 5 h later. (**A**) RT–PCR assay for the *MER3* splicing. Meiotic RNA samples were subjected to RT followed by PCR, using priTN1 and priTN2 primers. Products were separated on a 1.2% agarose gel and visualized by staining with ethidium bromide. The positions of unspliced and spliced products are indicated on the left and the sizes of molecular weight standards are shown on the right. *ACT1* splicing was observed in *mre2*Δ and *mer1*Δ, as well as in wild type (data not shown). (**B**) The three conserved elements in introns. The 5′ splice site, branchpoint and 3′ splice site sequences are shown for *MER3*, *MER2* and the consensus (Rymond and Rosbash, 1992). The same branchpoint sequence of *MER3* has been reported in yeast (Myslinski *et al*., 1990). The positions of these elements are also indicated. (**C**) Northern blot analysis of *MER3* transcripts. *MER3* (top panel) and *ACT1* (bottom panel) transcripts were detected using the same membrane. *ACT1* was used as a standard for the amount of RNA loaded on the gel. The positions of 18S and 25S rRNAs detected by ethidium bromide staining are indicated on the right. We do not know the significance of a 3.0 kb RNA on the *MER3* blot that is shorter than the *MER3* ORF.

ing a hypothetical open reading frame (ORF), YGL251c (Coissac *et al*., 1996). However, YGL251c with an additional upstream region of 508 bp (pTN66) was not sufficient for the suppression. Given the observation that multicopy *MER2* partially suppresses the *mre2* or *mer1* defect, and that *MER2* splicing requires *MRE2* and *MER1*, an intron of *MER3* might exist in the region upstream of YGL251c, splicing of which requires *MRE2* and *MER1*. To test this possibility, we designed a pair of primers, priTN1 and priTN2, located in the region upstream (Figure 1B, see Materials and methods), and performed reverse transcription polymerase chain reaction (RT–PCR) analysis using RNAs prepared from meiotic cells. If there is no intron between the primers, a fragment of 500 bp should be amplified. However, a fragment smaller than 500 bp was amplified exclusively in the wild type (Figure 2A). DNA sequencing of the amplified fragment revealed that the *MER3* primary transcript has a 152 nucleotide intron and that the *MER3* ORF starts at –575 from YGL251c and includes it (Figure 1B). The 5′ splice site and branchpoint sequences of *MER3* differ from both the consensus and those of *MER2* (Figure 2B). Furthermore, the 5′ splice site sequence of *MER3* is unique among all introns reported in *S.cerevisiae*. In *mre2*Δ or *mer1*Δ mutants, only the unspliced fragment was amplified (Figure 2A), demonstrating that *MER3* splicing depends on *MRE2* and *MER1*.

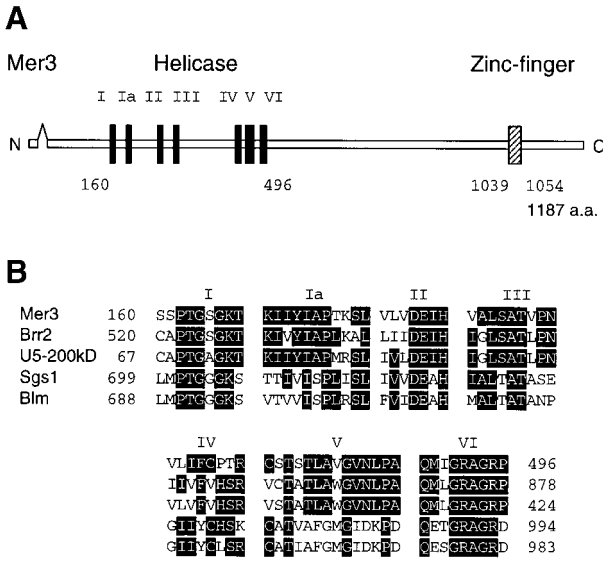

Fig. 3. The Mer3 protein contains helicase motifs and a putative zinc finger. (**A**) The seven conserved motifs (I, Ia, II, III, IV, V, VI) of DNA/RNA helicases and a putative zinc finger in Mer3. The first and last amino acid positions are shown. (**B**) The amino acid sequences of the helicase motifs. Amino acids identical in at least three cases are shaded.

Using mitotic and meiotic RNAs, Northern blotting was carried out to see the *MER3* transcript. In the wild type, the transcripts of ~4.2 and 3.0 kb were observed only in meiosis with a probe located within the pTN84 insert (Figures 2C and 1B). In *mer3*Δ (see below), those transcripts were detected in neither mitosis nor meiosis (Figure 2C). Consistent with the meiosis-specific transcripts of *MER3*, URS1 elements (Steber and Esposito, 1995) were identified around the first ATG of *MER3* (TCGGCGGGT, position –132 to –124; AGCCGCCAA, position 260–268). Even in the absence of *MRE2* or *MER1*, *MER3* transcripts were detected in meiosis, although at 60% of the wild-type level (Figure 2C). The reduction of the amount of RNA may be due to the instability of unspliced RNAs.

To confirm that *MER3* pre-mRNA is a target of *MRE2/MER1*-dependent splicing, the intron was eliminated from the genomic locus by substituting an intronless *MER3*, *cMER3*, constructed from the RT–PCR product (Figure 2A). As was seen for *cMER2*, introduction of *cMER3* did not change meiotic division, sporulation or spore viability significantly in the wild type (Table I). While only slight changes in the meiotic properties were observed when *cMER3* was introduced into *mre2*Δ and *mer1*Δ mutants, *cMER3* greatly improved the spore viability of *mre2*Δ *cMER2* and *mer1*Δ *cMER2* mutants (Table I). However, neither sporulation nor spore viability reach wild-type levels in the case of either *mre2*Δ *cMER2 cMER3* or *mer1*Δ *cMER2 cMER3*, suggesting another target(s) of *MRE2/MER1*-dependent splicing.

Table I. Meiotic properties of *mre2*Δ and *mer1*Δ derivatives

Strain	Relevant genotype	MI ± MII (%)	Sporulation (%)	Spore viability (%)
TNY058	WT	88	61	95
TNY366	c*MER3*	89	62	97
TNY101	*cMER2*	81	62	100
TNY380	*cMER2 cMER3*	82	59	100
TNY060	*mre2*Δ	85	0.9	<0.7
TNY381	*mre2*Δ *cMER3*	57	1.9	1
TNY102	*mre2*Δ *cMER2*	82	0.9	<0.7
TNY382	*mre2*Δ *cMER2 cMER3*	44	2.4	48
TNY305	*mer1*Δ	72	14	2
TNY482	*mer1*Δ *cMER3*	85	28	5
TNY481	*mer1*Δ *cMER2*	82	16	9
TNY483	*mer1*Δ *cMER2 cMER3*	83	37	86

Cultures of isogenic strains were investigated at 24 h after induction of meiosis. The occurrence of meiotic divisions was monitored by staining cells with DAPI and examining >200 cells in each aliquot by fluorescence microscopy. Cells that have completed at least one meiotic division (MI ± MII) contain more than one DAPI-staining body. Sporulation was examined by phase contrast microscopy using >400 cells and was signaled by the appearance of two to four phase-bright bodies within a cell. Spore viability was assessed by dissection of 40 tetrads produced on SPM plates using a micro manipulator, and the proportion of spores germinating to give visible colonies was determined after incubation for 3 days. All incubations were carried out at 30°C. The data presented for wild-type, *mre2*Δ, *mre2*Δ *cMER2* and *cMER2* strains were published previously (Nakagawa and Ogawa, 1997).

The Mer3 protein contains helicase motifs and a putative zinc finger

The spliced *MER3* mRNA encodes a 1187 amino acid polypeptide. The predicted Mer3 protein contains the seven motifs characteristic of the DExH-box type of DNA/RNA helicases (Gorbalenya and Koonin, 1993) and a putative zinc finger (CFHSCKDKTQCRHLCC), which may participate in protein–DNA or protein–protein interactions (Figure 3A). The BLASTP 2.0.7 search using the entire sequence of Mer3 shows that the Mer3 helicase domain has significant homology (E value $<4e^{-5}$) to yeast Brr2 and its human homolog U5-200kD, and to yeast Sgs1 and its human homolog Blm, whose helicase activity has been shown by biochemical assays (Lu *et al*., 1996; Karow *et al*., 1997; Laggerbauer *et al*., 1998; Raghunathan and Guthrie, 1998) (Figure 3B). Brr2 and U5-200kD are RNA splicing factors. On the other hand, mutations in *SGS1* or *BLM* cause a genomic instability, and individuals with Bloom's syndrome (*BLM* is mutated) show a predisposition to cancer. The sequence similarity of Mer3 to these known helicases suggests that the biochemical function of Mer3 is to unwind nucleic acid helices.

The mer3Δ mutant is defective in the transition of DSBs to later recombination intermediates

A *mer3*Δ strain was constructed, in which three-quarters of the *MER3* ORF including the first ATG and all the helicase motifs are deleted (Materials and methods). Under vegetative conditions, no growth defect or altered sensitivity to the DNA-damaging agent, methyl methanesulfonate, was observed in *mer3*Δ cells (data not shown). To see whether *MER3* has a role in early steps of meiotic recombination, meiosis-specific DSBs in the *HIS4::LEU2* region were examined by Southern blotting (Figure 4A). DNA was prepared from synchronous cultures, digested with *Pst*I and separated on an agarose gel. In wild type, DSBs were prominent at 4 h after the induction of meiosis (t = 4 h), and were much less prominent thereafter (Figure 4B). DSB signals were smeared downwards, indicating the processing of DSB ends. In *mer3*Δ, DSBs were first observed at t = 4 h and were still seen at t = 12 h at both 30 and 23°C (Figure 4B). A fraction of the DSBs were hyperresected at late meiosis, although less extensively than those in *rad51* and *dmc1* mutants (data not shown). These results indicate that the transition of DSBs to later recombination intermediates is partially blocked in the *mer3*Δ mutant. However, it appears that some of the late DSBs are not hyperresected, suggesting that there is an additional defect (e.g. DSB formation at late meiosis). Note that the elevation in the steady-state levels of DSBs was much more pronounced at 30 than at 23°C (Figure 4B and C). Interestingly, the *mer3*Δ mutant displays a defect in meiotic cell cycle progression, the

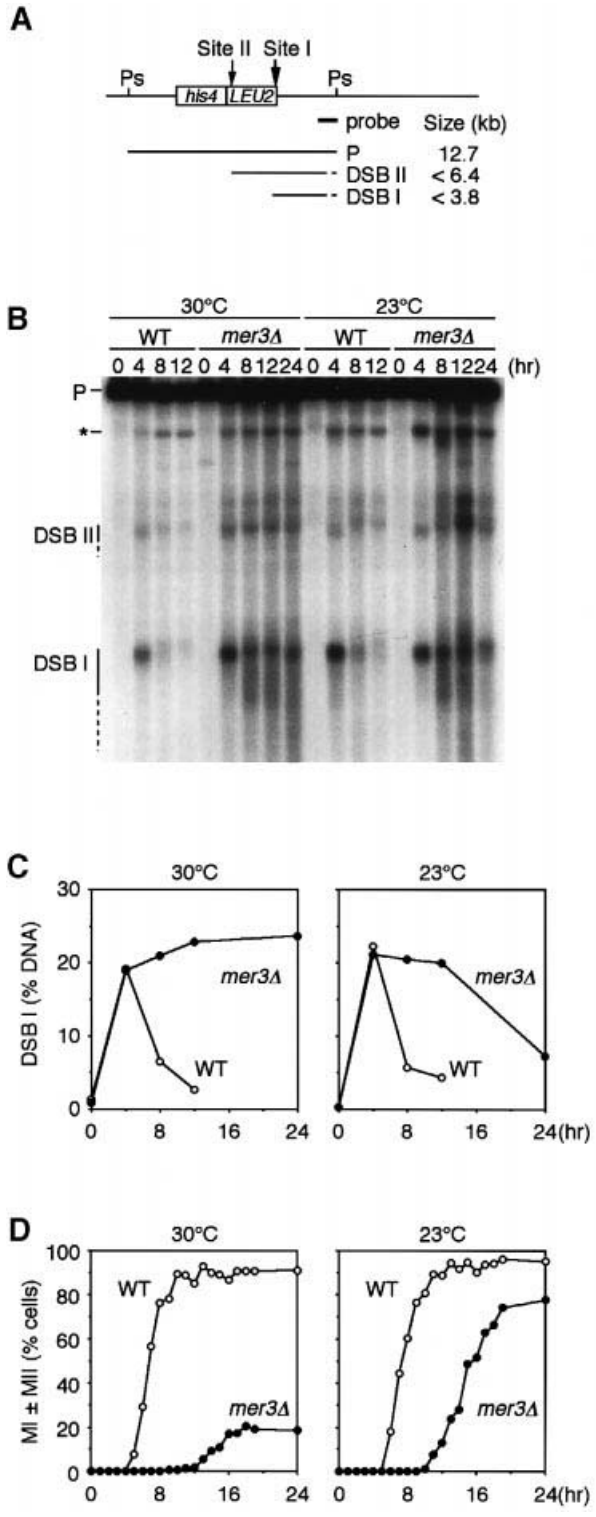

Fig. 4. DSB formation and nuclear division in meiosis. (**A**) The positions of major (site I) and minor (site II) DSB sites and *Pst*I (Ps) restriction sites in the *HIS4::LEU2* region are shown. (**B**) DNA was prepared from wild-type (NKY1551) and *mer3*Δ (TNY286) cells caused to undergo meiosis at 30 and 23°C, digested with *Pst*I, separated by agarose gel electrophoresis and transferred to a nylon membrane. A probe prepared from pNKY291 was used to detect fragments of interest by Southern hybridization. Fragments indicated by * may result from ectopic gene conversion between *his4::LEU2* and *leu2::hisG* loci. P, parental fragments; DSB I, DSB fragments at site I; DSB II, DSB fragments at site II. (**C**) The steady-state levels of DSBs at site I observed in (B) were measured by phosphoimager. The percentage of DSBs in the total DNA in each lane is shown. (**D**) Meiotic nuclear divisions were examined by 4′,6-diamidine-2-phenylindole (DAPI) staining as described in Table I at 30 and 23°C. Plotted is the percentage of cells that had undergone one or both nuclear divisions (MI ± MII) at various times throughout sporulation. Sporulation frequencies in *mer3*Δ at t = 24 h were 4 and 24% at 30 and 23°C, respectively. Spore viabilities for *mer3*Δ were 22% (44/200 spores) and 27% (53/200 spores) at 30 and 23°C, respectively; spore viability for the wild type was 97% (194/200 spores) at both temperatures.

severity of which parallels that of DSB accumulation (Figure 4D). At 30°C, only 20% of cells underwent meiotic nuclear division after a delay, while the remaining 80% arrested permanently. At 23°C, in contrast, all cells exhibited a delay in progression but 78% did eventually undergo meiosis I.

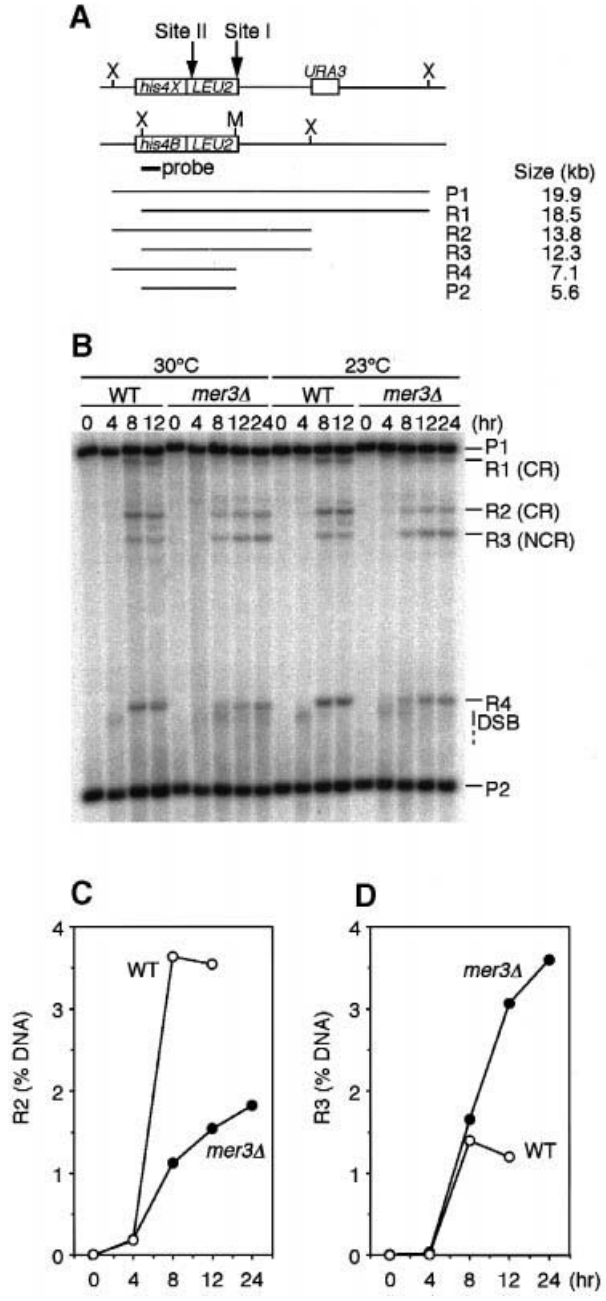

Fig. 5. Physical analysis of crossovers and non-crossovers. (**A**) The positions of polymorphic *Xho*I (X) and *Mlu*I (M) restriction sites in the *HIS4::LEU2* region. Restriction fragments (P1–2 and R1–4) produced by *Xho*I and *Mlu*I digestion are illustrated. The two DSB sites are shown by arrows. (**B**) Southern blot analysis of crossovers and non-crossovers. Wild-type (NKY1551) and *mer3*Δ (TNY286) cells were incubated at 30 and 23°C and sampled at the times indicated after induction of meiosis. Restriction fragments of interest were detected using a probe prepared from pNKY155. DSBs detected in this assay are formed at site I on the chromosome containing *his4X::LEU2-URA3*. CR, crossovers; NCR, non-crossovers. (C and D) Amounts of crossovers and non-crossovers produced at 23°C. (**C**) R2, crossovers. (**D**) R3, non-crossovers. The percentages are the mean values obtained from four blots started from two independent cultures. Similar results were obtained at 30°C.

The MER3 gene is required for normal crossing over and for faithful segregation of homologs at meiosis I

The formation of recombination products, which are either associated with crossing over or not, was examined by Southern blotting (Figure 5). DNA prepared from synchronous cultures was digested with *Xho*I and *Mlu*I, and separated on an agarose gel. Two parental (P1–2) and four recombinant fragments (R1–4) from the *HIS4::LEU2* recombination hot spot can be detected using the probe indicated in Figure 5A. In wild type, R1 and R2 are known to be correlated with events that, in tetrads, are associated with crossing over of flanking markers; R3 is not, and R4 results from both types of recombination (Storlazzi *et al*., 1995). The distribution between crossovers and non-crossovers was different in *mer3*Δ and wild-type strains (Figure 5B); crossovers (R2) were present at 50–60% of the wild-type level (Figure 5C), while non-crossovers (R3) were present at normal levels at $t = 8$ h and continued to increase to ~2.5 times the wild-type level by 24 h (Figure 5D). Although the physical analysis reveals the kinetics of recombinant formation, assignment of fragments that arise in the mutant to the crossover and non-crossover classes is based on the assumption that the relationships are the same as in wild type. Thus, we further examined the crossover frequency using *mer3*Δ tetrads formed at 23°C (Figure 4D). The tetrad analysis showed reduction of crossover frequencies in five intervals on two different chromosomes (Table II). The average decrease in crossover frequencies was 2.4-fold. In contrast, the frequency of 1:3 or 3:1 aberrant segregation of genetic markers, which can occur with or without crossing over, was increased at all three loci examined (Table III).

There is a tendency for the frequency of double crossovers in an interval to be lower than that predicted for single crossovers; this phenomenon is called interference. We tested whether *MER3* is also required for crossover interference. Non-parental ditypes (NPDs, Table II) are indicative of double crossovers in a given interval. The NPD ratio is the frequency of NPDs observed divided by that expected in the absence of interference (Materials and methods). Thus, no interference results in a ratio of 1.0. While all NPD ratios in wild type ranged from 0.20 to 0.44, those in *mer3*Δ were close to 1.0, from 0.74 to 1.32 (Figure 6). To analyze further the distribution of the crossovers along the chromosome, we examined the pattern of zero, one or two crossover events in wild-type and *mer3*Δ strains (Table IV). In the wild type, the patterns in all three intervals examined were significantly different from those predicted by a Poisson distribution. On the other hand, the patterns in *mer3*Δ were not significantly different from those predicted.

The spore viability of the *mer3*Δ mutant was 20–40%, while that of the wild type was ~97%. In *mer3*Δ, the proportion of four spore viable tetrads was decreased, and that of two or zero spore viable tetrads was increased compared with the wild-type levels (Table V). Three spore viable tetrads were not predominant among zero to three spore viable tetrads (Table V). These results suggest that non-disjunction of homologs occurs in *mer3*Δ. This

Table II. The *mer3*Δ mutation reduces crossing over

Interval	WT				*mer3*Δ				Fold decrease
	PD	TT	NPD	cM	PD	TT	NPD	cM	
MAT–CENIII	218	96	0	15	270	45	0	7	2.1
CENIII–HIS4	124	185	3	33	254	56	0	9	3.6
CAN1–URA3	365	833	21	39	973	328	11	15	2.6
URA3–HOM3	444	735	31	38	960	332	17	17	2.3
HOM3–TRP2	917	271	4	12	1052	221	4	10	1.3

Wild-type (TNY374) and *mer3*Δ (TNY375) strains were utilized to examine crossing over in the *MAT–CENIII* and *CENIII–HIS4* intervals. Wild-type (TNY367) and *mer3*Δ (TNY368) strains were employed to examine exchange in the *CAN1–URA3*, *URA3–HOM3* and *HOM3–TRP2* intervals. Only four-spore-viable tetrads that did not show aberrant segregation of the relevant markers were used to calculate map distances (cM). All the *mer3*Δ values differ significantly from those for the wild type ($P < 0.005$). PD, parental ditype; TT, tetratype; NPD, non-parental ditype.

Table III. The *mer3*Δ mutation increases aberrant segregation

Locus	WT	*mer3*Δ	Fold increase
CAN1	0.4%	1.3%	3.3
HOM3	1.1%	1.6%	1.4
TRP2	1.7%	2.6%	1.5

Absolute frequencies of aberrant segregation were scored as tetrads exhibiting 3:1 or 1:3 segregation for the indicated marker. A total of 1227 and 1344 four-spore-viable tetrads were examined for wild-type (TNY367) and *mer3*Δ (TNY368) strains, respectively. Wild-type and *mer3*Δ values are significantly different except for the *HOM3* locus.

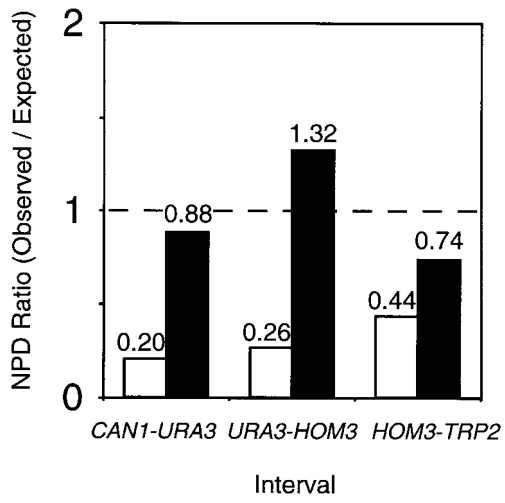

Fig. 6. Distribution of crossovers along a chromosome. NPD ratios in three intervals for wild type (open bars) and *mer3*Δ (filled bars). The number of NPDs observed was compared with that expected. In the wild type, *CAN1–URA3* and *URA3–HOM3*, $P < 0.005$; *HOM3–TRP2*, $P < 0.1$. In *mer3*Δ, *CAN1–URA3* and *HOM3–TRP2*, $P > 0.5$; *URA3–HOM3*, $P > 0.2$.

prompted us to monitor chromosome segregation during *mer3*Δ meiosis in a strain background (TNY374) where homologous *CENIII*s can be distinguished by *URA3* and *TRP1* markers. Examination of 408 two-spore-viable tetrads from *mer3*Δ diploids revealed that 388 (95%) contained pairs of sister spores (i.e. both spores were Ura^+/Trp^-, Ura^-/Trp^+ or Ura^+/Trp^+), and 68 (17%) were disomic for chromosome III (i.e. both spores were Ura^+/Trp^+). In contrast, all five two-spore-viable tetrads from wild-type diploids were pairs of non-sister spores (i.e. Ura^-/Trp^+ and Ura^+/Trp^- sets of spores). These results indicate that the *mer3*Δ mutation causes non-disjunction of homologs at meiosis I. No crossovers were seen in either the *MAT–CENIII* or the *CENIII–HIS4* intervals in the 68 pairs of chromosome III disomes, while 11 crossovers were expected based on the crossover frequency seen in four-spore-viable tetrads from *mer3*Δ (Table II; see Materials and methods). In addition, among 111 one-spore-viable tetrads from *mer3*Δ, 23 (21%) were disomic for chromosome III (i.e. Ura^+/Trp^+) and none of them were recombinant. Thus, the homolog non-disjunction is likely to be due to the *mer3*Δ defect in crossing over causing some pairs of homologs not to have any crossovers. No evidence of precocious separation of sister chromatids (i.e. Ura^+/Trp^+, Ura^-/Trp^+ and Ura^-/Trp^+ sets of spores) was observed among 81 three-spore-viable tetrads from *mer3*Δ.

Discussion

Here, we have identified the *MER3* gene as a multicopy suppressor of the crossover defect in a *mre2 cMER2* mutant. The predicted Mer3 protein contains the seven helicase motifs as well as a putative zinc finger. In a *mer3* mutant, DSBs appear at normal timing but do not completely disappear thereafter, and a fraction of DSBs are hyperresected late in meiosis. Crossovers are reduced and distributed randomly along and among chromosomes, resulting in a high incidence of homolog non-disjunction at meiosis I. Thus, a mutation in *MER3* encoding a novel helicase-like protein impairs both the transition of DSBs to later recombination intermediates and crossover control.

MRE2/MER1-dependent splicing of MER3 pre-mRNA

The *MER3* gene is transcribed only in meiosis and has an intron. *MER3* splicing depends on *MRE2* and *MER1*, which are also required for *MER2* splicing. The result that elimination of both *MER3* and *MER2* introns improves the spore viability of *mre2* and *mer1* mutants confirms that *MER3* as well as *MER2* pre-mRNA is a target of *MRE2/MER1*-dependent splicing. Non-canonical 5′ splice sites are the prominent feature shared by *MER3* and *MER2* introns (Figure 2B). It has been shown that *MER1* is no longer required when the 5′ splice site of *MER2* or U1 snRNA is mutated to increase base pairing between them (Nandabalan *et al*., 1993). In addition, Puig *et al.* (1999) have shown recently that *MRE2* (also called *NAM8*) facilitates pre-mRNA splicing if the 5′ splice site is

Table IV. Patterns of zero, one and two crossover events

Interval	Observed (%)			Expected (%)			Probability
	0-CR	1-CR	2-CR	0-CR	1-CR	2-CR	
WT							
CAN1–URA3	28	65	7	46	36	14	<<0.0001
URA3–HOM3	34	56	10	47	36	14	<<0.0001
HOM3–TRP2	77	22	1	78	19	2	0.0054
*mer3*Δ							
CAN1–URA3	73	23	3	74	22	3	0.6742
URA3–HOM3	72	23	5	72	24	4	0.0556
HOM3–TRP2	82	17	1	83	16	2	0.5476

The numbers of PD, TT and NPD for wild type and *mer3*Δ shown in Table II were used to calculate the observed patterns of zero, one and two crossover (CR) events (see Materials and methods). The patterns of 0-, 1- and 2-CR events were predicted by a Poisson distribution using observed frequencies of crossovers. The probability shows the likelihood that the difference between the observed and expected patterns is attributable to chance.

Table V. Distributions of tetrad types

Strain	Relevant genotype	Tetrad types (%)					Spore viability (%)	Total tetrads
		4-sv	3-sv	2-sv	1-sv	0-sv		
TNY374	WT	94	3	1	<0.1	1	97	335
TNY367	WT	93	6	1	<0.1	0.4	98	1331
TNY375	*mer3*Δ	14	4	19	5	58	28	2187
TNY368	*mer3*Δ	23	5	20	4	48	37	5952

sv, spore-viable.

manipulated to be non-canonical. Thus, *MER3* splicing may be regulated at the interaction of the 5′ splice site with the splicing complex by Mre2 and Mer1. However, the meiotic phenotype of *mre2* mutants is more severe than that of *mer1* mutants (Table I), suggesting different roles for *MRE2* and *MER1* in RNA splicing.

Meiotic cell cycle checkpoint

The severity of DSB accumulation correlates with the degree of cell cycle arrest; both are more pronounced at 30 than at 23°C in a *mer3* mutant. This correlation is consistent with the notion that a meiotic checkpoint monitors recombination intermediates (Bishop *et al*., 1992; Lydall *et al*., 1996; Xu *et al*., 1997). At both temperatures, however, a *mer3* mutation reduces crossovers, but increases non-crossovers. Thus, the severity of the cell cycle arrest does not correlate with that of the crossover defect. In addition, crossing over in tetrads that have completed both meiotic divisions is reduced in the mutant. Thus, it is unlikely that a *mer3* mutation simply delays the progression of meiotic events; rather, the mutation directly causes a recombination defect.

A role for MER3 in crossover interference

In addition to reduced frequencies of crossovers, a *mer3* mutant shows random distribution of crossovers along and among chromosomes, resulting in a high incidence of homolog non-disjunction at meiosis I. These results indicate that *MER3* has an essential role in crossing over occurring on every pair of homologs. In contrast to crossovers, non-crossovers and aberrant segregation at some loci are increased. This raises the possibility that a *mer3* mutation impairs the crossover control that is imposed at an early step of recombination, before the differentiation of intermediates into crossovers or non-crossovers. However, it is also possible that there is a default pathway in the mutant that gives non-crossovers only, because non-crossovers are at almost the same level as in wild type at the time point when the wild-type level reaches the maximum (t = 8 h, Figure 5D), and increase further thereafter. Interestingly, immunostaining experiments using anti-Mer3 antibodies showed that the Mer3 protein localizes at discrete sites on meiotic chromosomes (T.Nakagawa and H.Ogawa, unpublished data). This result suggests that Mer3 functions at sites of recombination to impose crossover interference. It has been proposed that Zip1 transmits negative signals from crossover sites that prevent crossovers nearby (Sym and Roeder, 1994). From this point of view, Mer3 might radiate or receive the negative signal. Alternatively, Mer3 might be required for the polymerization of Zip1 along entire lengths of homologs, which is suggested to be required for Zip1 to function in interference. However, it is also proposed that there are geometrically two distinct types of double Holliday junctions, one of which is subject to the crossover control regardless of SC polymerization (Storlazzi *et al*., 1996). Thus, it is also possible that Mer3 affects the geometric conformation of Holliday junctions.

The function of Mer3

The Mer3 protein contains the seven motifs characteristic of DNA/RNA helicases and shows significant homology to several known helicases including Sgs1 and Blm (Figure 3). An alanine substitution for a highly conserved lysine in the helicase motif I, a putative nucleotide-binding region, decreases crossing over and spore viability, predominantly at a low temperature (23°C) (T.Nakagawa and H.Ogawa, unpublished data). These results indicate

Table VI. Yeast strains

Strain	Genotype
TNY171	*MAT***a** *LEU2 his4::hisG TRP5 CYH2 ura3 can1 ho::LYS2 lys2* *MATα leu2::hisG HIS4 trp5::hisG cyh2 ura3 CAN1 ho::LYS2 lys2*
TNY185	TNY171, except *mre2::hisG* *mre2::hisG*
TNY170	TNY171, except *mre2N* *mre2N*
TNY240	TNY171, except *mre2::hisG cMER2* *mre2::hisG cMER2*
TNY169	TNY171, except *mre2N cMER2* *mre2N cMER2*
TNY058	*MAT***a** *leu2::hisG his4X::LEU2-URA3 cyh2 ura3 arg4-nsp ho::LYS2 lys2* *MATα leu2::hisG his4B::LEU2 CYH2 ura3 arg4-bgl ho::LYS2 lys2*
TNY101	TNY058, except *cMER2* *cMER2*
TNY366	TNY058, except *cMER3* *cMER3*
TNY380	TNY058, except *cMER2 cMER3* *cMER2 cMER3*
TNY060	TNY058, except *mre2::hisG* *mre2::hisG*
TNY102	TNY058, except *mre2::hisG cMER2* *mre2::hisG cMER2*
TNY381	TNY058, except *mre2::hisG cMER3* *mre2::hisG cMER3*
TNY382	TNY058, except *mre2::hisG cMER2 cMER3* *mre2::hisG cMER2 cMER3*
TNY305	TNY058, except *mer1::LEU2* *mer1::LEU2*
TNY481	TNY058, except *mer1::LEU2 cMER2* *mer1::LEU2 cMER2*
TNY482	TNY058, except *mer1::LEU2 cMER3* *mer1::LEU2 cMER3*
TNY483	TNY058, except *mer1::LEU2 cMER2 cMER3* *mer1::LEU2 cMER2 cMER3*
NKY1551	*MAT***a** *leu2::hisG his4X::LEU2(Bam)-URA3 ura3 arg4-nsp ho::LYS2 lys2* *MATα leu2::hisG his4B::LEU2 ura3 arg4-bgl ho::LYS2 lys2*
TNY286	NKY1551, except *mer3::hisG* *mer3::hisG*
TNY367	*MAT***a** *leu2::hisG can1 URA3 HOM3 TRP2 ho::LYS2 lys2* *MATα leu2::hisG CAN1 ura3 hom3-10 trp2 ho::LYS2 lys2*
TNY368	TNY367, except *mer3::hisG* *mer3::hisG*
TNY374	*MAT***a** *CENIII::URA3 leu2::hisG HIS4 ura3 trp1-H3 ho::lys2 lys2* *MATα CENIII::TRP1 leu2::hisG his4B::LEU2 ura3 trp1-H3 ho::lys2 lys2*
TNY375	TNY374, except *mer3::hisG* *mer3::hisG*

TNY058, 101, 060 and 102 were described previously (Nakagawa and Ogawa, 1997). NKY1551 was described by Storlazzi *et al*. (1995).

the importance of the helicase domain for Mer3 function. Hyperresected DSBs are seen late in *mer3* meiosis. In addition, the strand exchange proteins Rad51 and Dmc1 transiently localize as foci on meiotic chromosomes in wild type, but they persist in a *mer3* mutant as shown in a *zip1* mutant (Bishop, 1994; T.Nakagawa and H.Ogawa, unpublished data). These results are consistent with the possibility that *MER3* functions in the DSB transition to later recombination intermediates. Interestingly, the RecQ protein, which is believed to be an *Escherichia coli* homolog of Sgs1 and Blm, has been shown to possess a dual role *in vitro*, promoting the formation of joined DNA

molecules catalyzed by the *E.coli* RecA and SSB protein and dissociating the joined molecules (Harmon and Kowalczykowski, 1998).

It has been proposed that crossover control is imposed during the DSB transition, from the observation that a few normally resected DSBs are detected late in meiosis in a *zip1* mutant, which also have a defect in crossover control (Sym *et al*., 1993; Storlazzi *et al*., 1996; Xu *et al*., 1997). The requirement for *MER3* for both the DSB transition and crossover control supports this hypothesis. However, we cannot rule out the possibility that *MER3* affects the expression of other genes and thus is required for different steps of recombination, as some proteins containing helicase domains are known to regulate gene expression (Eisen and Lucchesi, 1998).

Materials and methods

Strains and media

Yeast strains are listed in Table VI. All are of the SK1 strain background (Kane and Roth, 1974), except for the TNY367 and TNY368 strains which are SK1 congenic and derived from MY263 (Sym and Roeder, 1994). *his4::hisG* and *trp5::hisG* strains were constructed by replacement of 1.3 kb *Sna*BI–*Bgl*II and 0.7 kb *Spe*I–*Bgl*II regions, respectively, with a 1.2 kb *hisG* fragment. The *mer1::LEU2* strain was derived from NKY2204 (Storlazzi *et al*., 1995). To make *mer3::hisG* and *cMER3* strains, a 4.6 kb *Sac*I fragment from pTN105 and a 5.6 kb *Eco*RI fragment from pTN149, respectively, were introduced into a yeast diploid strain. In *his4::hisG*, *trp5::hisG*, *mer3::hisG* and *cMER3* constructions, uracil auxotrophs were selected by plating cells on SD plates supplemented with 5-fluoro-orotic acid. DNA integration was carried out by lithium acetate transformation (Ito *et al*., 1983) and verified by Southern blot analysis.

Yeast media were prepared according to Treco and Lundblad (1992). MYPD, MYPL, YPA, SPM and synthetic medium were prepared as described earlier (Nakagawa and Ogawa, 1997). Cycloheximide and canavanine were added to the media at final concentrations of 3 and 60 μg/ml, respectively.

Induction of meiosis

For induction of meiosis at 23°C, cultures in both pre-sporulation medium (YPA) and sporulation medium (SPM) were incubated at 23°C. Synchronous meiotic cultures were obtained as described previously (Nakagawa and Ogawa, 1997). Tetrad dissection was carried out using spores produced on SPM plates.

Plasmids

Plasmids were constructed by standard methods (Sambrook *et al*., 1989). The original *MER3*-containing plasmid, pTN45, has an ~9 kb fragment of yeast genomic DNA in YEp24 (New England Biolabs). To create the *mer3::hisG-URA3-hisG* plasmid, pTN105, a 4.8 kb *Nco*I–*Sal*I fragment from pTN45 was introduced into the *Sac*II–*Sal*I sites of pBluescriptII KS$^+$ (Stratagene) to give pTN97, and then a 3.8 kb *hisG-URA3-hisG* fragment (Alani *et al*., 1987) was substituted for a 3.5 kb *Afl*II–*Bst*XI *MER3* region (–15 to 3466) of pTN97. To create the *cMER3-URA3-MER3* plasmid, pTN149, a 2.5 kb *Eco*RI–*Cla*I fragment containing the *cMER3* N-terminal region and a 3.0 kb *Bam*HI–*Sal*I fragment containing the *MER3* C-terminal *Sph*I–*Sal*I region, in which the *Sph*I site had been destroyed, were introduced into the *Eco*RI–*Cla*I and *Bam*HI–*Sal*I sites of YEp24, respectively. In pTN149, a 0.7 kb *Sph*I–*Cla*I *MER3* region is directly duplicated and flanking *URA3*. A 0.3 kb *Afl*II–*Spe*I *cMER3* fragment prepared from the RT–PCR product was used for DNA sequencing and construction of the *cMER3* gene.

Calculation of interference and statistical analysis

The frequency of NPDs expected was calculated from the Papazian equation (Papazian, 1952), $NPD = 1/2[1 - T - (1 - 1.5T)^{2/3}]$, where T is the frequency of tetratypes shown in Table II. Because the T value for the *CAN1–URA3* interval in wild type was >3/2, the expected frequency of NPDs in that interval only was determined as follows: $NPD = T^2/8(1 + 2T/3)$ (Papazian, 1952).

Since only one class gives NPDs among four types of two-crossover (CR) tetrads, the numbers of zero, one and two CR events shown in Table IV were calculated as follows, assuming no chromatid interference: 0-CR = PD – NPD; 1-CR = TT – 2NPD; 2-CR = 4NPD.

Data sets were analyzed using the χ^2 test. Values of $P < 0.05$ were considered significant.

Calculation of recombination frequencies among disomes

From the crossover frequency seen in four-spore-viable tetrads of *mer3*Δ (Table II), 9.7 and 12.3 crossovers among 68 disomes are expected to occur in the *MAT–CENIII* and *CENIII–HIS4* intervals, respectively. However, crossovers in the *MAT–CENIII* or *CENIII–HIS4* interval followed by homologous non-disjunction will generate *MAT***a***/MAT***a** and *MAT*α*/MAT*α sets or His$^-$/His$^-$ and His$^+$/His$^+$ sets of spores in half of all meioses, owing to random segregation of meiosis II. Thus, the expected number of recombinants in 68 disomes is (9.7 + 12.3)/2 = 11.

Northern blotting and RT–PCR analysis

RNA of yeast cells was prepared by glass bead and phenol extraction (Treco, 1989a). For Northern blotting, total RNAs were separated on a 0.7% agarose gel in MOPS/formaldehyde buffer (Sambrook *et al*., 1989), soaked in a 0.05 M NaOH buffer for 20 min for partial digestion of RNA and transferred to NYTRAN nylon membranes (Schleicher & Schuell) in a 10× SSC buffer. For the detection of *MER3* and *ACT1* RNAs, a 1.0 kb *Bst*BI–*Cla*I fragment from pTN45 and a 0.6 kb *Cla*I fragment from pTN7 (Nakagawa and Ogawa, 1997), respectively, were ^{32}P-labeled by the random primer method (Sambrook *et al*., 1989) and used as hybridization probes.

A 2.5 μg aliquot of total RNA was treated with RNase-free DNase I FPLCpure™ (Pharmacia) to eliminate contaminating DNA and subjected to reverse transcription with 16 U of M-MuLV reverse transcriptase (New England Biolabs) using 3 pmol of priTN2 (5′-CGCCTCTTCATC-AGGTGTCTGCTCTAAATCG-3′; position 437–467). PCR (Saiki *et al*., 1988) was performed using 20 pmol each of priTN1 (5′-GGTGGATTTG-ACAACTTAAGAGGCGTCG-3′; position –33 to –6) and priTN2 under the following conditions: 1 min at 94°C and then 30 s at 94°C, 10 s at 54°C and 30 s at 74°C for 35 cycles. A total of 2.5 U of KOD dash DNA polymerase (Toyobo) was used for each PCR.

Physical detection of meiotic recombination events

DNA was prepared as described by Treco (1989b). Detection of restriction fragments of interest was performed as described earlier (Storlazzi *et al*., 1995). Digested DNA samples were separated by electrophoresis on a 0.7% agarose gel and transferred to NYTRAN nylon membrane (Schleicher & Schuell). A 1.5 kb *Pst*I–*Eco*RI fragment from pNKY291 (Cao *et al*., 1990) or a 1.6 kb *Pst*I–*Sac*I fragment from pNKY155 (Cao *et al*., 1990) labeled with ^{32}P by the random primer method were used as probes for Southern hybridization. Southern and Northern blot signals were quantified with a Fuji BAS2000 phosphoimager.

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