MOLECULAR AND CELLULAR BIOLOGY, May 1990, p. 2379–2389
0270-7306/90/052379-11$02.00/0

Vol. 10, No. 5

MER1, a Yeast Gene Required for Chromosome Pairing and Genetic Recombination, Is Induced in Meiosis

JOANNE ENGEBRECHT* AND G. SHIRLEEN ROEDER
Department of Biology, Yale University, New Haven, Connecticut 06511-8112

Received 20 October 1989/Accepted 8 January 1990

The yeast *MER1* gene is required for the production of viable meiotic products and for meiotic recombination. Cytological analysis of chromosome spreads from a *mer1* mutant indicates that the *MER1* gene product is also required for normal chromosome pairing. *mer1* strains make axial elements, precursors to the synaptonemal complex; however, the chromosomes in most nuclei do not become fully synapsed. The DNA sequence of the *MER1* coding region was determined; the *MER1* open reading frame encodes a 270-amino-acid protein with a molecular mass of 31.1 kilodaltons. The *MER1* protein shows limited sequence similarity to calmodulin. Expression of the *MER1* gene was examined by RNA blot hybridization analysis and through the construction and analysis of *mer1*::*lacZ* fusion genes. Expression of the *MER1* gene is meiotically induced and required the *IME1* gene product. Thus, expression of the *MER1* gene early in meiosis is required for proper chromosome pairing and meiotic recombination.

Meiotic cell division enables diploid organisms to reproduce sexually by generating haploid gametes through two successive nuclear divisions. At meiosis I, the reductional division, homologous chromosomes disjoin from each other. At meiosis II, sister chromatids separate from each other and four haploid cells are generated. Two important events distinguish meiosis from mitotic cell division: chromosome pairing and high levels of recombination. Both of these processes are essential for the proper segregation of chromosomes at the first meiotic division (4).

Chromosome pairing is mediated by a proteinaceous structure called the synaptonemal complex (SC; reviewed in reference 56), which is composed of two lateral elements and a central core. The SC is assembled in a series of steps during prophase of meiosis I. The first observable step occurs at leptotene, in which axial elements form along condensed chromosomes. During zygotene, the axial elements corresponding to homologous chromosomes begin to align with one another. At pachytene, the central region is assembled between the axial elements (now termed lateral elements), forming the completed structure. The SC dissolves at diplotene, just before the meiosis I division. It is during pachytene that high levels of recombination occur; this observation has led to the proposal that the SC plays an important role in mediating meiotic recombination (41, 56).

Structures termed recombination nodules have been observed in association with the SC in a variety of organisms (56). Nodules are thought to be the sites of localization of recombination enzymes; this conclusion is based on the correlation between the distribution and frequency of nodules and those of meiotic exchange events as visualized by chiasmata (9). Chiasmata, the physical manifestations of reciprocal recombination events, hold the homologs together until they are oriented on the metaphase I spindle (reviewed in reference 30). At anaphase I, the chiasmata dissolve, allowing the homologs to move to opposite poles.

The *Saccharomyces cerevisiae* gene *MER1* was identified in a search for meiotic-lethal mutants and is essential for proper meiotic chromosome segregation (17). *mer1* strains produce inviable spores; however, the spore lethality can be alleviated by a *spo13* mutation. This result indicates that the *mer1* mutant is defective in a process essential for the first meiotic division, since *spo13* strains bypass this division (32).

Both reciprocal crossing over and gene conversion are reduced approximately 10-fold in *mer1* strains. The decreased level of recombination in *mer1* mutants could be due to a defect in chromosome pairing and/or recombination. Mutants believed to be defective only in homolog pairing display wild-type levels of intrachromosomal recombination (e.g., *hop1*) (27), presumably because exchange between duplicated sequences on the same chromosome is independent of SC formation. Intrachromosomal exchange is reduced in Mer$^-$ strains, suggesting that *mer1* mutants are defective in some aspect of the enzymology of recombination per se and not simply pairing.

To further clarify the role of the *MER1* gene product in recombination and pairing, we have examined meiotic chromosomes from *mer1* strains for the presence of SC. The expression and sequence of the *MER1* gene have also been examined to gain a better understanding of its function and regulation.

MATERIALS AND METHODS

Strains. Yeast strains are shown in Table 1. NKY611 was obtained from Nancy Kleckner, and 1290-1B and 1290-1C were obtained from Aaron Mitchell; all of these strains are derivatives of SK1 (20). BR2495 and BR2496 were obtained from Beth Rockmill, Y95 was obtained from Mike Snyder, and YCC75 was obtained from Connie Copeland. Bacterial strains R895 (17) and XL-1Blue (Stratagene) were used for plasmid constructions.

Plasmids. Plasmids were constructed by standard procedures (38). Plasmids pME302 containing the *spo11*::*ADE2* allele and pME303 containing the *rad50*::*ADE2* allele were described previously by Engebrecht and Roeder (17). These plasmids were used for disrupting the wild-type copies of the corresponding genes. pJT39 carrying the *cry*^{r}*1* gene on an integrating vector was obtained from John Thompson (37). The *ade2-Bgl*II allele was constructed by filling in the *Bgl*II site in the middle of the *ADE2* gene (Yp3.6 Ade, obtained from Jeff Lemontt) with Klenow fragment and inserting the

* Corresponding author.

TABLE 1. Genotypes of *S. cerevisiae* strains

Strain	Genotype
BR2495	*MATa his4-290 leu2-27 arg4-8 thr1-4 CUP1 ade2-1 ura3-1 trp1-1*
	MATα his4-260,519 leu2-3,112 ARG4 thr1-1 CUP1 ade2-1 ura3-1 trp1-289
BR2496	*MATa his4-290 leu2-27 arg4-8 thr1-4 CUP1 ade2-1 ura3-1 trp1-1 mer1::LEU2*
	MATα his4-260,519 leu2-3,112 ARG4 thr1-1 CUP1 ade2-1 ura3-1 trp1-289 mer1::LEU2
J9	*MATa arg4-8 thr1-4 CUP1 ade2-1 ura3-1 HOtrp1-1 lys2 mer1-2*
	MATα arg4-8 thr1-4 CUP1 ade2-1 ura3-1 HOtrp1-1 lys2 mer1-2
NKY611	*MATa leu2::hisG ho::LYS2 lys2 ura3*
	MATα leu2::hisG ho::LYS2 lys2 ura3
1290-1B	*MATα leu2::hisG ho::LYS2 trp1 lys2 ura3 ime1-12::TRP1*
1290-1C	*MATa leu2::hisG ho::LYS2 trp1 lys2 ura3 ime1-12::TRP1*
JE131-1A	*MATα leu2::hisG ho::LYS2 lys2 ura3 mer1*::Tn*164*
JE131-1B	*MATa leu2::hisG ho::LYS2 lys2 ura3*
JE131-1C	*MATa leu2::hisG ho::LYS2 lys2 ura3 mer1*::Tn*164*
JE131-1D	*MATα leu2::hisG ho::LYS2 lys2 ura3*
JE131-1A-1	*MATα leu2::hisG ho::LYS2 lys2 ura3 mer1*::Tn*164 ade2-Bgl*II
JE131-1B-1	*MATa leu2::hisG ho::LYS2 lys2 ura3 ade2-Bgl*II
Y95	*MATa lys2-80 ade2-101 ura3-52 Δtrp1 his3Δ200*
	MATa lys2-80 ade2-101 ura3-52 Δtrp1 his3Δ200
YCC75	*MATα LYS2 ade2-101 ura3-52 Δtrp1 his3Δ200 TYR1*
	MATα lys2-80 ade2-101 ura3-52 Δtrp1 his3Δ200 tyr1
J251	*MATa leu2::hisG ho::LYS2 lys2 ura3 mer1::LEU2*
	MATα leu2::hisG ho::LYS2 lys2 ura3 mer1::LEU2
J252	*MATa leu2::hisG ho::LYS2 lys2 ura3 MER1*
	MATα leu2::hisG ho::LYS2 lys2 ura3 mer1::Tn*164*
J253	*MATa leu2::hisG ho::LYS2 lys2 ura3 mer1::Tn222*
	MATα leu2::hisG ho::LYS2 lys2 ura3 MER1
J254	*MATa cry1 leu2::hisG ho::LYS2 lys2 ura3 MER1*
	MATa cry1 leu2::hisG ho::LYS2 lys2 ura3 mer1::Tn*164*
J256	*MATα cry1 leu2::hisG ho::LYS2 lys2 ura3 MER1*
	MATα cry1 leu2::hisG ho::LYS2 lys2 ura3 mer1::Tn*164*
J258	*MATa leu2::hisG ho::LYS2 lys2 ura3 MER1 ade2-Bgl*II
	MATα leu2::hisG ho::LYS2 lys2 ura3 mer1::Tn*164 ade2-Bgl*II
J259	*MATa leu2::hisG ho::LYS2 spo11::ADE2 lys2 ura3 MER1 ade2-Bgl*II
	MATα leu2::hisG ho::LYS2 spo11::ADE2 lys2 ura3 mer1::Tn*164 ade2-Bgl*II
J261	*MATa leu2::hisG ho::LYS2 lys2 ura3 rad50::ADE2 MER1 ade2-Bgl*II
	MATα leu2::hisG ho::LYS2 lys2 ura3 rad50::ADE2 mer1::Tn*164 ade2-Bgl*II
J263	*MATa leu2::hisG ho::LYS2 trp1 ime1-12::TRP1 lys2 ura3 MER1*
	MATα leu2::hisG ho::LYS2 trp1 ime1-12::TRP1 lys2 ura3 mer1::Tn*164*
J265	*MATa leu2::hisG ho::LYS2 lys2 ura3 mer1::LEU2*
	MATα leu2::hisG ho::LYS2 lys2 ura3 mer1::LEU2 pME3-*MER1*

*Bam*HI-*Eco*RI fragment containing *ADE2* into YIp5 to create pR943.

The *MER1* gene was mutagenized by using the shuttle mutagenesis procedure of Seifert et al. (49). The transposon used is a derivative of Tn*3* that carries the β-galactosidase-coding region and produces translational fusions when inserted in the proper orientation and reading frame of the gene of interest. pME164 and pME222 carry transposon insertions in which *lacZ* is fused in frame to the *MER1* gene (Fig. 1).

pME1 is the original *MER1*-complementing plasmid (17), and pME4 (Fig. 1) was constructed by inserting a 2.5-kilobase-pair (kbp) *Eco*RI-*Bgl*II fragment from pME1 between the *Eco*RI and *Bam*HI sites of the vector pHSS6 (49). pME8 and pME35 were made by inserting the *Mlu*I-*Xba*I fragment and the *Bgl*II-*Ssp*I fragment from pME4, respectively, into the Bluescript vector SK+ (Stratagene). A 2.0-kbp *Bgl*II-*Xba*I fragment from pME1 downstream of the *MER1*-coding region was inserted into SK+ to create pME38. The *Ssp*I-*Eco*RI fragment from pME4 was inserted into SK+ (pME21) and SK− (pME23) for the generation of subclones for sequencing. pMEΔ162, containing the *mer1*::*LEU2* allele, was used for disrupting the *MER1* gene (17).

Genetic procedures. Media were prepared and yeast manipulations were carried out as described by Sherman et al. (50). YEPAD-Cry medium was prepared as described by Meade et al. (39). The final concentration of cryptopleurine (Corkwood Enterprises, New South Wales, Australia) was 5 μM. Liquid YPA presporulation medium and KAc sporulation medium were supplemented to 75 mg/liter with uracil, leucine, lysine, and/or adenine, as required (20). Medium containing 5-fluoro-orotic acid was prepared as described by Boeke et al. (7).

Yeast transformations were carried out by the lithium acetate procedure (29). All transformants were verified by Southern blot analysis with Zetaprobe nylon membranes (Bio-Rad Laboratories, Richmond, Calif.) (46). To generate isogenic SK1 derivatives to examine the expression of *MER1*, the diploid NKY611 was transformed by one-step gene disruption (47) with pMEΔ162, pME164, or pME222 targeted with *Sma*I. The resulting transformants were sporulated, and tetrads were dissected. Cells from haploid spore colonies of the opposite mating type generated from NKY611 transformed with pMEΔ162 were mated to create the diploid J251; haploids derived from NKY611 transformed with pME222 were mated to generate J253. Haploids derived from NKY611 transformed with pME164 were designated JE131-1A, JE131-1B, JE131-1C, and JE131-1D. JE131-1A and JE131-1B were mated to create the diploid

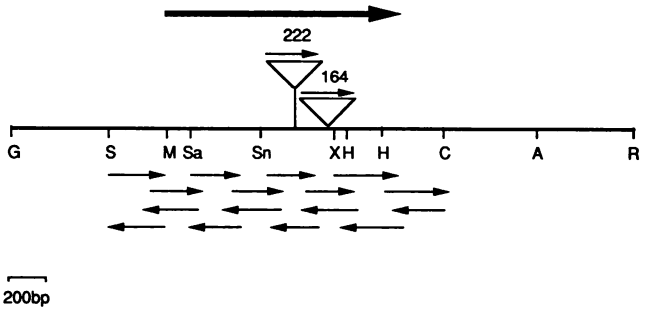

FIG. 1. Physical map of *MER1*. The map shows the *Bgl*II-*Eco*RI fragment from pME4. The minimal region containing *MER1* function resides within the *Ssp*I-*Cla*I fragment. Open triangles represent the transposon insertions that create *mer1*::*lacZ* fusions, and the arrow above the insertions indicates the orientation of the *lacZ* gene. The numbers above the triangles correspond to the insertions; plasmids and strains containing transposon insertions are identified by the same number. The large arrow indicates the direction of transcription of the *MER1* gene. The arrows below the restriction map depict the direction and extent of sequence derived from subclones generated as described in Materials and Methods. G, *Bgl*II; S, *Ssp*I; M, *Mlu*I; Sa, *Sac*I; Sn, *Sna*BI; X, *Xba*I; H, *Hin*dIII; C, *Cla*I; A, *Aat*II; R, *Eco*RI.

J252. JE131 haploids were transformed with plasmids targeted to create gene disruptions as described below.

Isogenic *MAT***a**/*MAT***a** and *MAT*α/*MAT*α derivatives of J252 were constructed in several steps. First, JE131-1B and JE131-1D were transformed with pJT39 targeted for integration at the *CRY1* locus by digestion with *Bgl*II. Ura$^+$ transformants were selected on synthetic complete medium lacking uracil; Ura$^-$ segregants which had lost the plasmid because of excision were then selected on synthetic complete medium plus 5-fluoro-orotic acid. The resulting Cry^r strains were mated to the Cry^s strains of the opposite mating type, either JE131-1A or JE131-1C. Resistance to cryptopleurine is encoded by a recessive allele (*cry1*), and *MAT* and *CRY1* are closely linked on chromosome III (39). Cryptopleurine-resistant recombinants were selected from these diploids on YEPAD-Cry medium and screened for mating ability. Frequently, both the *CRY* and *MAT* alleles become homozygous, probably because of mitotic crossing over in the interval between the centromere and *CRY1*. Alternatively, Cry^r and mating competence could be due to chromosome loss. To distinguish these two possibilities, several independent Cry^r mating-competent strains were mated to *MAT***a**/*MAT***a** (Y95) or *MAT*α/*MAT*α (YCC75) diploids. The resulting tetraploids were sporulated, and tetrads were dissected. By examining the mating phenotypes of the spore colonies, it was possible to distinguish whether the Cry^r parents were $2n - 1$ or $2n$. Two $2n$ strains, J254 and J256, were chosen for assaying β-galactosidase activity.

Ade$^-$ strains were constructed by two-step gene replacement (54) to construct strains homozygous for deletions of the *SPO11* and *RAD50* genes marked with the *ADE2* gene. JE131-1A and JE131-1B were transformed with pR943 targeted for integration at the *ADE2* locus by digestion with *Spe*I. Transformants were selected on synthetic complete medium lacking uracil, and excision of the plasmid was selected as described above. Two Ade$^-$ segregants, JE131-1A-1 and JE131-1B-1, were mated to created the diploid J258. JE131-1A-1 and JE131-1B-1 were each transformed independently with *spo11*::*ADE2* and *rad50*::*ADE2* by one-step gene disruption (47); the resultant haploids were mated to create J259 and J261.

Cytology. Spread meiotic nuclei were prepared as described by Dresser and Giroux (14) with some modifications. Ten milliliters of a culture (1×10^7 to 2×10^7 cells per ml) was transferred to 2% potassium acetate for 12 or 15 h and then centrifuged and suspended in 1% (wt/vol) potassium acetate–1 M sorbitol (pH 7.0) to an optical density at 600 nm of 4.0. To 1 ml, dithiothreitol was added to a final concentration of 10 mM, and the suspension was incubated for 10 min at 30°C. Zymolyase 100T (ICN Pharmaceuticals, Inc., Irvine, Calif.) was added to a final concentration of 37.5 μg/ml, and the cells were incubated at 30°C until 50 to 70% had spheroplasted. The cells were then washed with cold 0.1 M 2-(*N*-morpholino)ethane sulfonic acid–1 M sorbitol–1 mM EDTA–0.5 mM $MgCl_2$ (pH 6.4), and the cell pellet was kept on ice. The pellet was suspended in 70 μl of 0.1 M 2-(*N*-morpholino)ethane sulfonic acid–1 mM EDTA–0.5 mM $MgCl_2$ (pH 6.4); 490 μl of 4% (wt/vol) paraformaldehyde (pH 7.0) was added, and the mixture was immediately placed on a glass microscope slide precoated with plastic and covered with a glass cover slip. After 20 min at room temperature, the cover slip and excess liquid were removed; the surface of the slide was rinsed with 5 ml of 0.4% (vol/vol) PhotoFlo 22 (Eastman Kodak Co., Rochester, N.Y.), and the preparation was allowed to air dry. The slides were stained with silver nitrate (EM Sciences) (28) and examined under a light microscope. Areas of interest on the plastic were then transferred to 50-mesh copper grids by floating the plastic off the slide. The grids were examined with a light microscope before examination in an electron microscope.

Before coating, glass slides were washed with detergent and rinsed thoroughly in deionized water. The slides were rinsed with ethanol and wiped dry with lens paper. The slides were then dipped in plastic solution for 2 to 4 s and allowed to air dry. The plastic solution for coating slides was made by dissolving 0.5 g of Optilux petri dish plastic (Becton Dickinson Labware, Oxnard, Calif.) in 100 ml of chloroform. The quality of the coating was assessed by floating the plastic off the slide. The plastic should appear gold under fluorescent light.

The synchronously sporulating strain SK1 (20) was not used for cytological analysis because initial experiments suggested that the quality of the spreads prepared from SK1 were not as good as those obtained from our strain background (distantly related to S288C).

β-Galactosidase assays. Strains were grown to saturation in 2.5 ml of YEPAD liquid, diluted 1:100 in 100 ml of YPA, and allowed to grow at 30°C for 12 to 14 h (2×10^7 to 5×10^7 cells per ml). The cells were pelleted and suspended in 200 ml of 2% potassium acetate and incubated with shaking at 30°C to induce sporulation. Every 90 min, two 10-ml samples were removed for β-galactosidase assays, and 1 ml was removed and prepared for DAPI staining (55). The 10-ml samples were centrifuged and stored as pellets at −70°C before assaying.

β-Galactosidase assays were performed as described by Coney and Roeder (11). The total amount of protein in each sample was quantitated by the method of Bradford (8). Values for β-galactosidase activity are given as the number of nanomoles of σ-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein. The values given represent averages from two independent diploids, each assayed in duplicate.

RNA isolation and analysis. Cells were grown as described above for β-galactosidase assays. RNA was prepared by the method of Elder et al. (16), as modified by Stewart and Roeder (53). Northern blot analysis was carried out as

TABLE 2. Effect of *mer1* mutations on SC formation

Strain[a]	*MER1* genotype	Time[b] (h)	% of nuclei (no./total)				
			Uncondensed[c]	SC[d]	Axial elements[e]	Meiosis I[f]	Meiosis II[g]
BR2495	*MER1*/*MER1*	12	79 (54/68)	18 (12/68)	0 (0/68)	3 (2/68)	0 (0/68)
		15	34 (28/83)	37 (31/83)	0 (0/83)	27 (22/83)	2 (2/83)
BR2496	*mer1::LEU2*/*mer1::LEU2*	12	85 (66/78)	0 (0/78)	14 (11/78)	1 (1/78)	0 (0/78)
		15	41 (42/103)	3 (3/103)	28 (29/103)	24 (25/103)	4 (4/103)

[a] BR2495 and BR2496 are isogenic strains.
[b] Hours in sporulation medium.
[c] Percentage of nuclei with no apparent chromosome structure containing a single nucleolus and no evidence of separated spindle poles.
[d] Percentage of nuclei containing SC.
[e] Percentage of nuclei containing axial elements.
[f] Percentage of nuclei with separated spindle pole bodies and decondensed chromosomes.
[g] Percentage of nuclei with decondensed chromosomes containing two nucleoli and four separated spindle pole bodies.

described by Maniatis et al. (38). RNA riboprobes of high specific activity were synthesized by in vitro transcription of plasmids pME8, pME35, and pME38 by the procedure of Krieg and Melton (35).

DNA sequence analysis. Deletion subclones were generated from plasmids pME21 and pME23 by using the Erase-a-Base system (Promega Biotec Co., Madison, Wis.) (25). Dideoxy sequencing reactions (48) were carried out with [^{35}S]dATP and sequenase (enzyme and reagent kit from U.S. Biochemical Corp., Cleveland, Ohio). Figure 1 shows the positions, orientations, and locations of the sequences used to compile the *MER1* sequence. The BIONET computer software was used for data management and analysis (IntelliGenetics Inc., Palo Alto, Calif.).

RESULTS

***MER1* is required for complete assembly of the synaptonemal complex.** *mer1* mutants are defective in meiotic recombination (17); therefore, it was of interest to determine whether pairing occurs normally in *mer1* strains. The cytological manifestation of chromosome pairing is the formation of the SC, which can be observed in pachytene nuclei. Recently, an improved method to visualize SC in yeast chromosome spreads has been developed by Dresser and Giroux (14).

Chromosomes of isogenic Mer$^+$ and Mer$^-$ strains were examined in spreads of meiotic nuclei. Cells were harvested for cytological examination at 12 and 15 h after transfer to sporulation medium. These times were chosen on the basis of studies of the kinetics of induction of meiotic recombination in the wild-type strain, BR2495, used as a control in these experiments. At 12 h after transfer to sporulation medium, meiotic recombination is just beginning; at 15 h, meiotic recombinants are abundant (data not shown). Table 2 shows the number of nuclei observed at different stages at these two time points from the Mer$^+$ and Mer$^-$ strains.

At the 12-h time point, 18% of the nuclei observed in the wild type contained SC. This increased to 37% at 15 h (Table 2). In contrast, most nuclei with condensed chromosomes in the *mer1*Δ strain BR2496 contained axial elements (14% at 12 h, 28% at 15 h; Table 2, Fig. 2). The axial elements observed in the *mer1* strain were highly condensed; such axial elements were never observed in the wild-type strain. In *mer1* nuclei containing axial elements, small segments of paired chromosomes were occasionally observed. In addition, a few nuclei from the *mer1*Δ strain at 15 h contained fully paired chromosomes (3%; Table 2, Fig. 2), although it is unclear whether the SC was cytologically normal. Progression through meiosis in *mer1* mutants appeared to be otherwise unperturbed, as evidenced by the appearance of nuclei in meiosis I and meiosis II divisions (Table 2). Furthermore, at 15 h, the sum of the percentages of nuclei that contained axial elements and those that contained SC in *mer1* (31%) was approximately equal to the percentage of nuclei that contained SC in the wild type (37%; Table 2).

Meiotic nuclei from strains carrying the UV-induced alleles *mer1-1* and *mer1-2* were also examined (Fig. 2) (17). The proportion of nuclei containing SC and axial elements in these strains was the same as that in a strain carrying a deletion of the *MER1* gene (data not shown).

***MER1* encodes a 31-kilodalton protein.** The DNA sequence of the *MER1* gene was determined to gain information about the structure and function of the *MER1* gene product. The DNA sequence from the minimal region containing *MER1* function, as defined by analysis of subcloned fragments (Fig. 1), is presented in Fig. 3. The sequence contains a single long open reading frame that is 810 bp in length. This is capable of encoding a 270-amino-acid protein with a predicted molecular mass of 31.1 kilodaltons (Fig. 3).

In *S. cerevisiae*, consensus sequences have been established for RNA splicing, transcription initiation, and termination. The *MER1* transcript does not appear to be spliced, since the highly conserved yeast splice signal (36, 44) was not found. Transcriptional initiation requires a TATA box that is located 50 to 120 bp upstream of the transcription initiation site (22, 42). The sequence TATATAT, displaying similarity to the consensus sequence TATATAA, is located at positions −161 to −155 in the *MER1* sequence; an additional TATA-like sequence is located beginning at position −48.

Three sequences have been shown to be important for polyadenylation and termination of mitotic genes in *S. cerevisiae*. The sequence TAG-X1-14-TA(T)GT-(A+T-rich)-TTT, usually located 1 to 140 bp downstream of the translation stop codon, may serve as a signal for both polyadenylation and termination (61). The sequence TTTTTATA has also been demonstrated to be important for termination of some yeast genes (26). The sequence AAUAAA is necessary for polyadenylation in higher eucaryotes, and this sequence is found downstream of a few yeast genes in what could be the region of polyadenylation (61). None of these

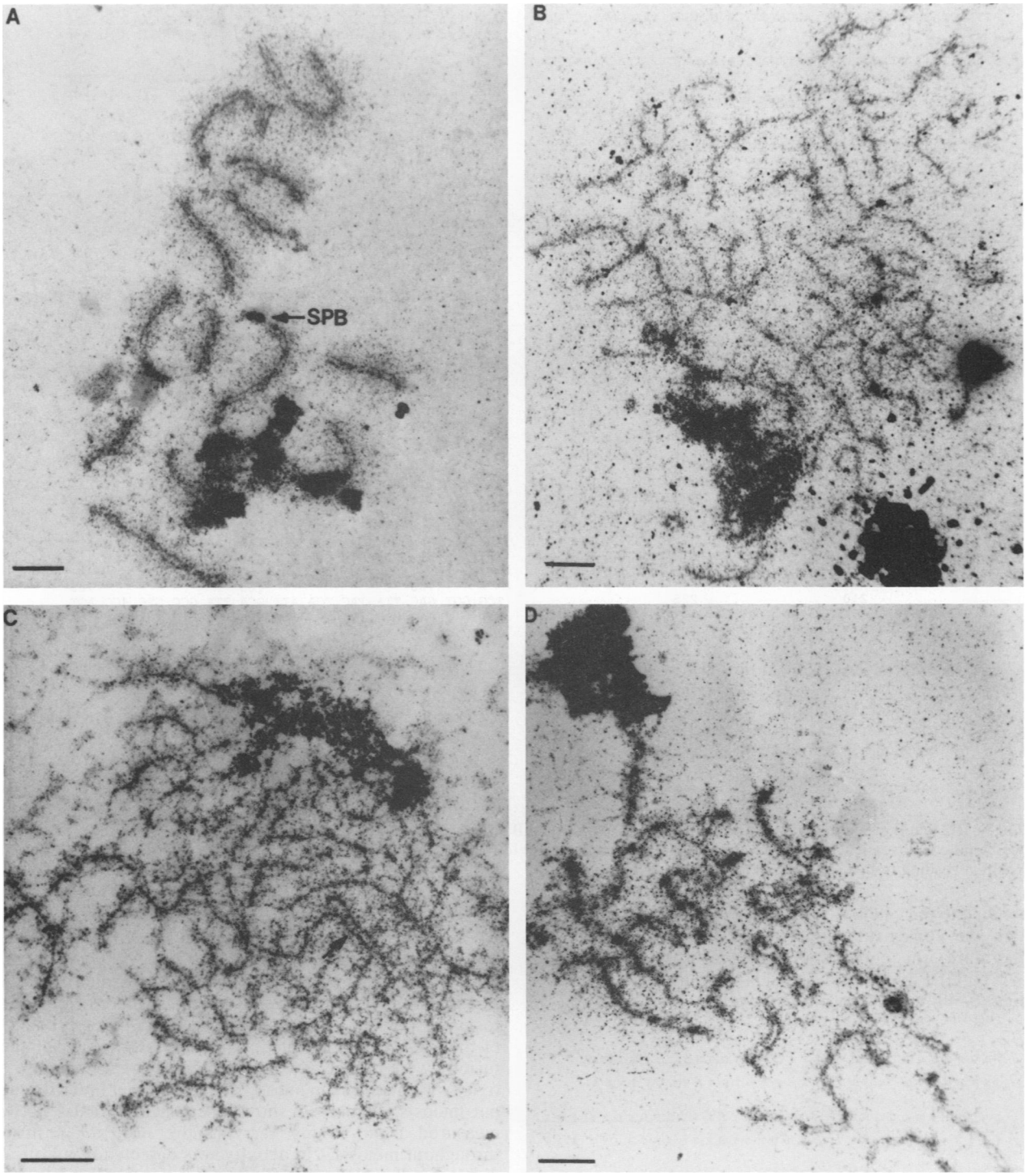

FIG. 2. Micrographs of spread nuclei from Mer$^+$ and Mer$^-$ strains. The darkly stained structures in each micrograph are nucleoli. (A) Nucleus in pachytene displaying SC from a wild-type strain, BR2495. Spindle pole bodies (SPB) are indicated. (B and C) Nuclei containing condensed axial elements from BR2496, a *mer1*Δ strain, and J9, a strain with the UV-induced *mer1-2* mutation, respectively. In panel C, the arrow indicates a short paired segment. (D) Nucleus from a Mer$^-$ strain (BR2496) in which chromosomes appear fully paired. Bars, 1 μm.

sequences was found downstream of the *MER1*-coding region.

Comparison of *MER1* with known DNA and protein sequences. The *MER1* DNA and predicted amino acid sequences were compared with sequences available in the GenBank, Protein Identification Resource, and SWISS-PROT data banks. A large region (beginning at amino acid 122 and extending to amino acid 238) of the *MER1* protein was found to be related to the Ca^{2+}-binding protein calmodulin. The best match was found with bovine brain calmodulin (60); there is 22% identity between the *MER1* protein and this calmodulin (Fig. 4). However, the *MER1* regions, cor-

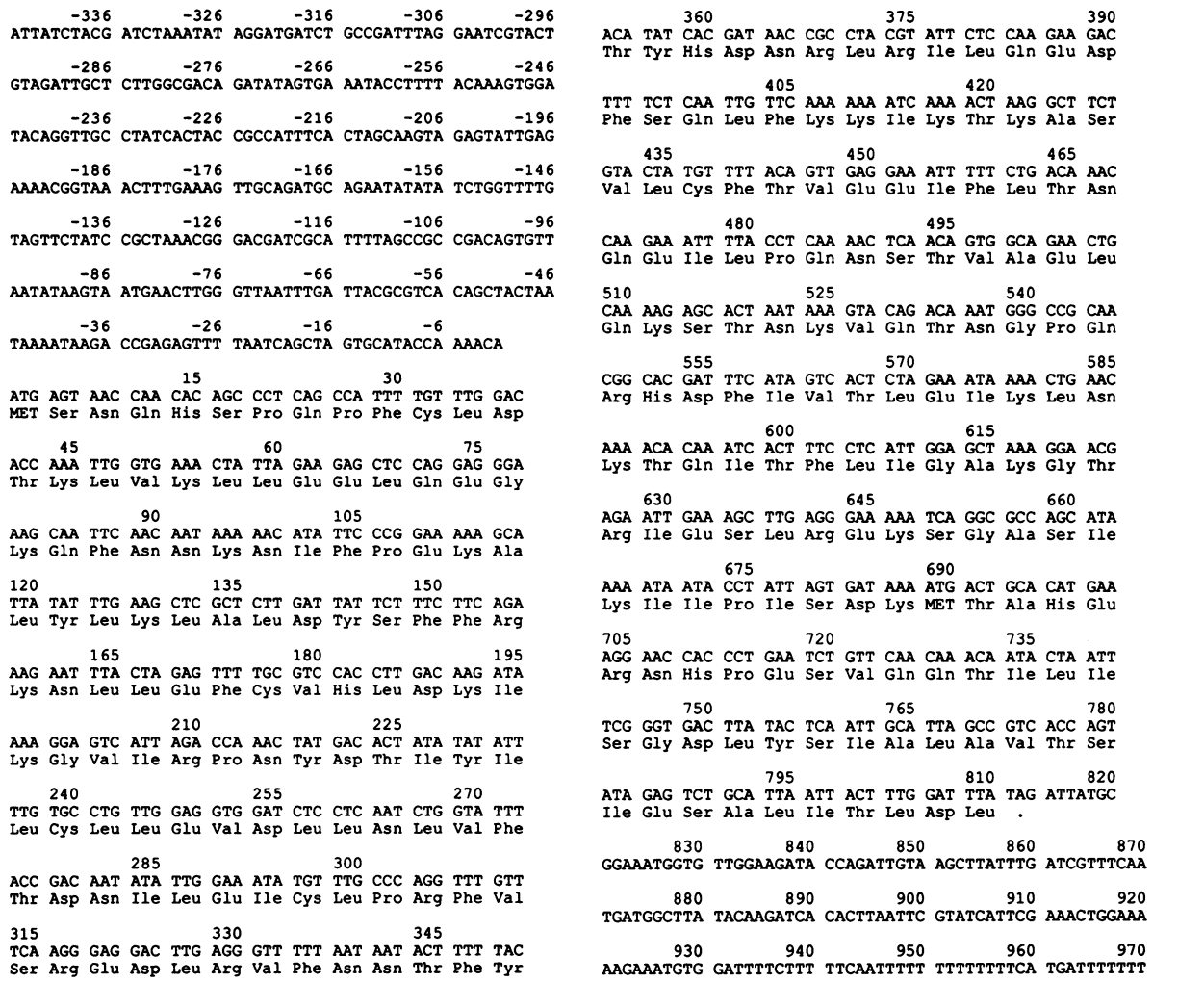

FIG. 3. DNA and predicted amino acid sequences of *MER1* (GenBank/EMBL accession number M31304). The DNA sequence and the deduced amino acid sequence of *MER1* are presented. Numbers refer to the DNA sequence, with position +1 indicating the first base of the *MER1* open reading frame.

responding to the calcium-binding sites in calmodulin, do not contain the consensus sequence for calcium binding (5). Furthermore, the consensus sequences for DNA-binding (43), GTP-GDP-binding (13), ATP-binding (58), and zinc-binding (6, 40) sites were not detected.

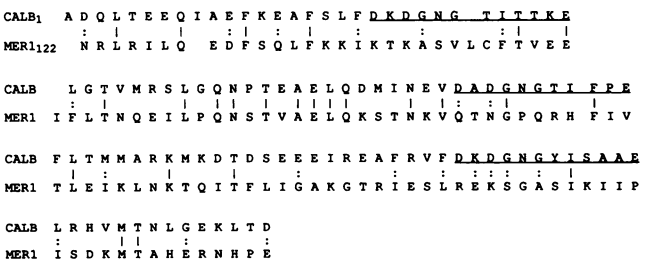

FIG. 4. Amino acid sequence comparison of bovine brain calmodulin (CALB) and the *MER1* protein (MER1). The proteins are aligned to show maximal similarity. The numbers indicate the first amino acid where the similarity begins for each protein. The single-letter designations for amino acids are used. Connecting lines indicate identical amino acids; colons indicate similar amino acids. The underlined amino acids in calmodulin represent the regions of Ca^{2+} binding.

***MER1* protein is synthesized only during meiosis.** To examine the expression of *MER1*, protein fusions with β-galactosidase were created (see Materials and Methods). In such a fusion, β-galactosidase synthesis is controlled by *MER1* transcriptional and translational signals; therefore, β-galactosidase activity provides a direct measure of the production of the *MER1* gene product. Strains heterozygous for these fusions were induced to sporulate, and β-galactosidase activity was measured in mitosis and at different times throughout meiosis. Figure 5A shows the β-galactosidase activity measured from strains carrying the *mer1*::*lacZ* fusions throughout meiosis. No activity was detected in mitotically dividing cells. After transfer to starvation medium, β-galactosidase activity steadily increased until approximately 7.5 h and then decreased.

To correlate the timing of *MER1* synthesis with other events during meiosis, cells from each time point were examined for chromatin segregation by diamidino-2-phenylindole (DAPI) staining and for sporulation by light microscopy. At about the same time that maximal β-galactosidase activity was detected, the cell population underwent the meiosis I division, as determined by a peak in the percentage of binucleate cells (Fig. 5B). Cells undergoing meiosis II division (tetranucleates) were also present at this time;

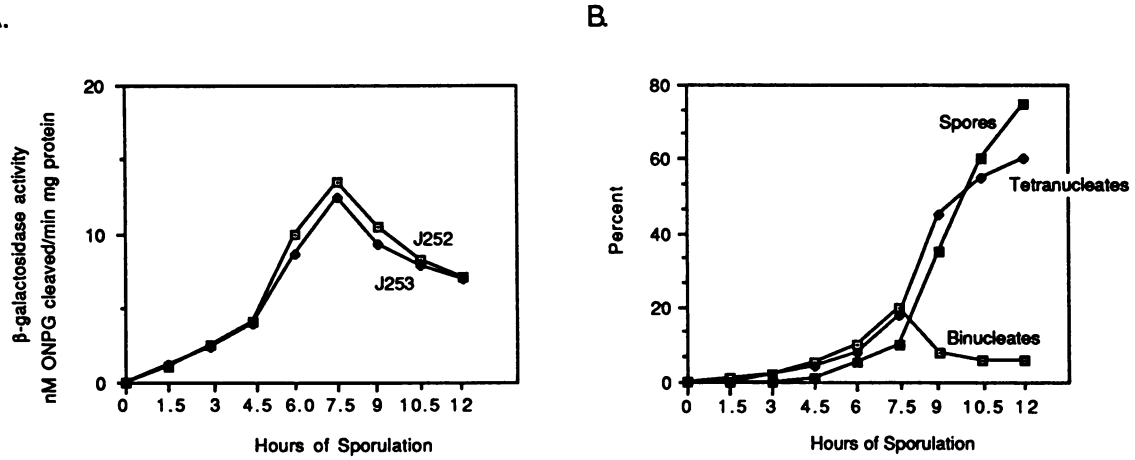

FIG. 5. Meiotic time course of *MER1* expression. (A) β-Galactosidase activity from strains J252 (Tn*164*) and J253 (Tn*222*), carrying *mer1*::*lacZ* fusions assayed at the indicated times after transfer to sporulation medium. β-Galactosidase units are defined as nanomoles of σ-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein. (B) Landmark events in meiosis. Samples from the same time points used in β-galactosidase assays were prepared for visualization by DAPI fluorescence. At least 100 cells from each strain were examined at each time point and then averaged to determine the percentage of binucleate and tetranucleate cells. Cells were also examined for sporulation with a light microscope.

therefore, the cell population was not entirely synchronous. (The kinetics of meiosis in the SK1 cells used for β-galactosidase assays is significantly different from the kinetics of meiosis in the strains used for cytology.)

S. cerevisiae enters meiosis when two conditions are met: starvation for carbon and nitrogen and expression of both *MAT***a** and *MAT*α mating-type information (reviewed in reference 18). Expression of meiosis-specific genes requires both conditions; however, many genes can be induced by starvation alone. To determine whether the expression of *MER1* is meiosis specific, β-galactosidase activity was measured in *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains under starvation conditions. β-galactosidase activity in these strains was considerably less than that in the *MAT***a**/*MAT*α diploids; a low level of β-galactosidase activity was detected, probably because of the low level of constitutive sporulation found in this strain background (Table 3). Thus, the *MER1* gene product is expressed only under conditions that induce meiosis.

MER1* expression requires *IME1* but not *RAD50* or *SPO11*.** The product of the *IME1* gene is required for entry into meiosis (31). The signals that induce sporulation (starvation and expression of *MATa** and *MAT*α information) are believed to act through *IME1* to stimulate the expression of other meiosis-specific genes (51). To examine the expression of *MER1* in the absence of the *IME1* gene product, strains homozygous for an *IME1* deletion and heterozygous for a *mer1*::*lacZ* fusion were assayed for β-galactosidase activity throughout meiosis. Very little activity was detected (Table 3), indicating that *IME1* is required for the proper expression of *MER1*.

The *IME1* gene product could be acting directly to turn on the expression of *MER1* or indirectly through or in combination with another gene(s). *rad50* (21, 57) and *spo11* (33) mutants are completely defective in the initiation of meiotic recombination. Mutations at *RAD50* and *SPO11* are epistatic to mutations at the *MER1* gene (17), suggesting that the *SPO11* and *RAD50* gene products act before the *MER1* gene product. Therefore, it was of interest to determine whether *MER1* expression is altered in *spo11* or *rad50* mutants. Although sporulation was slightly decreased in the *spo11* and *rad50* strains, *MER1*-directed β-galactosidase activity was close to wild-type levels (Table 3). Therefore, neither the *SPO11* nor *RAD50* gene product is required for *MER1* expression.

The *MER1* transcript is meiotically induced. To determine whether the pattern of protein synthesis observed correlates with the pattern of transcriptional regulation, RNA blot analysis was performed on total RNA prepared from mitotic and meiotic cells. When the RNA was hybridized to an antisense RNA probe derived from sequences internal to the *MER1* coding region, a meiosis-specific transcript 3.2 kilo-

TABLE 3. *MER1* expression

Strain	Relevant genotype	β-Galactosidase[a] (U)	% of spores at 12 h[b]
J252	*MAT***a** *MER1* *MAT*α *mer1*::Tn*164*	13.5	72
J254	*MAT***a** *MER1* *MAT***a** *mer1*::Tn*164*	0.68	5
J256	*MAT*α *MER1* *MAT*α *mer1*::Tn*164*	0.53	4
J263	*MAT***a** *ime1-12*::*TRP1* *MER1* *MAT*α *ime1-12*::*TRP1* *mer1*::Tn*164*	0.26	0
J259	*MAT***a** *spo11*::*ADE2* *MER1* *MAT*α *spo11*::*ADE2* *mer1*::Tn*164*	12.3	65
J261	*MAT***a** *rad50*::*ADE2* *MER1* *MAT*α *rad50*::*ADE2* *mer1*::Tn*164*	11.8	53

[a] β-Galactosidase units are given as nanomoles of σ-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein. The units given represent the activity observed at the time of maximal induction, which occurred at 7.5 h for all strains. β-Galactosidase activity was measured from mitotic cells before transfer to sporulation medium in all strains. These values were not above background.

[b] A minimum of 200 cells were examined in the light microscope for the presence of spores. Sporulation was almost complete at 12 h. All of the strains used for this analysis were isogenic derivatives of SK1. SK1 sporulates efficiently and synchronously. The conditions necessary for sporulation (starvation and heterozygosity at the mating type locus) are probably not as stringently controlled in this strain background, as evidenced by the small fraction of cells that were able to sporulate in the *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains.

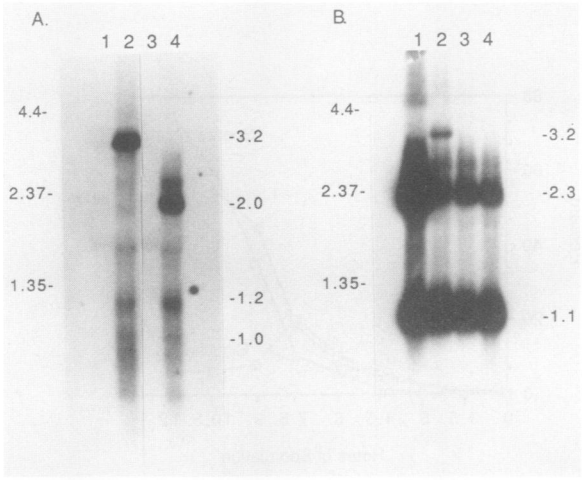

FIG. 6. Transcription of *MER1*. Northern blots containing RNA prepared from the wild-type strain, NKY611, before transfer to sporulation medium (lanes 1), RNA from NKY611 6 h after transfer to sporulation medium (lanes 2), RNA from strain J251, carrying a *mer1*Δ at 6 h after transfer to sporulation medium (lanes 3), and RNA from a *mer1*Δ strain carrying a centromere plasmid containing the *MER1* coding region and about 1 kb of downstream sequences at 6 h after transfer to sporulation medium (lanes 4). (A) The blot was probed with a riboprobe synthesized from pME8, containing sequences internal to the *MER1* gene. (B) The blot was probed with sequences 1 kb downstream of the *MER1* gene (pME38 used as template for riboprobe synthesis). The position and sizes of RNA markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) are indicated to the left of the blot; the positions and sizes of the transcripts detected are shown to the right.

bases (kb) in length was detected (Fig. 6A). The size of the transcript was surprising, because the coding region of the *MER1* gene is only 810 bp in length. Two lines of evidence indicate that the 3.2-kb mRNA is the *MER1* transcript. First, RNA from a strain containing a deletion of the *MER1* gene lacks this transcript (Fig. 6A). Second, transposon insertions within the *MER1* gene alter the size of the transcript (data not shown). In addition to the 3.2-kb transcript, two smaller and less abundant transcripts (approximately 1.0 and 1.2 kb) were observed; these are also specific to the *MER1* gene (Fig. 6A).

The *MER1* transcript proceeds 2 kb 3′ of the coding region. To determine the source of the extra sequences in the *MER1* transcript, antisense probes from sequences 5′ and 3′ of the *MER1* coding region were hybridized to total RNA from mitotic and meiotic cells. A probe for sequences upstream of the *MER1* coding region did not detect any message (data not shown), whereas a probe containing sequences beginning 1 kb downstream of the *MER1* coding region detected the 3.2 kb transcript (Fig. 6B), suggesting that the additional sequences are transcribed from the 3′ end. Consistent with this conclusion, the *MER1* transcript size is altered in strains carrying a *CEN* plasmid containing the *MER1* coding region and approximately 1 kbp of downstream sequences (Fig. 6A).

The probe containing sequences downstream of the *MER1* coding region detected 2.3- and 1.1-kb transcripts in addition to the 3.2-kb *MER1* transcript. These messages are present in both mitotic and meiotic cells (Fig. 6B).

DISCUSSION

The *mer1* mutant is defective in chromosome pairing. Recombination and homologous pairing are essential for proper chromosome segregation at the meiosis I reductional division (4). *mer1* strains are defective in both of these processes. *mer1* mutants recombine at 10% of wild-type levels, and the frequency of nuclei containing SC is approximately 10% of the wild-type frequency.

The pairing defect in *mer1* mutants is manifested by the accumulation of axial elements at the expense of SC. Axial elements are precursors to the SC and represent unpaired chromosomes that have partially condensed and assembled with some of the proteinaceous components of the complex. In *S. cerevisiae*, axial elements are observed in leptotene as short discontinuous threads (14); consequently, these are difficult to detect and were scored as uncondensed chromosomes in both the wild type and the *mer1* strain. This is in contrast to the highly condensed continuous axial elements observed in the *mer1* strain; these structures were never observed in the wild-type strain.

The number of *mer1* nuclei containing axial elements was about 10 times the number of *mer1* nuclei containing fully synapsed chromosomes. Small regions of pairing were occasionally observed in *mer1* nuclei; these paired segments could be due to the way the chromosomes happened to lie in the spreads or could represent pairing between either homologous or nonhomologous chromosomes. No nuclei that contained intermediate levels of synapsis were found. This all-or-none effect of pairing suggests that a critical threshold of some *MER1*-dependent process may be necessary for synapsis. The recombination events that occur in *mer1* strains (10% of wild-type events) do not occur preferentially in a subset of cells (data not shown); this lack of coincidence of recombination events suggests that recombination does not occur predominantly in nuclei that pair normally. Furthermore, the SC formed in *mer1* nuclei does not ensure proper disjunction, since the fraction of cells undergoing complete synapsis (approximately 10% of the nuclei containing condensed chromosomes) is greater than the spore viability (approximately 1%; 17).

The appearance of axial elements and the absence of SC in *mer1* mutants suggests that *mer1* mutants usually fail to synapse. The proposition that the axial elements observed in *mer1* strains represent precursors to the SC is based on the similarity in the kinetics of axial element assembly in *mer1* and SC assembly in the wild type. However, alternative interpretations are that *mer1* mutants synapse transiently and that they form unstable complexes that are sensitive to the spreading procedure. Nevertheless, it is clear that *mer1* mutants are defective in some aspect of SC structure or assembly.

Relationship between pairing and recombination. A number of lines of evidence suggest that pairing is required for meiotic levels of recombination. In a variety of organisms, there is a strong correlation between the presence of SC and recombination. For example, SC formation and crossing over are absent in *Drosophila melanogaster* males (12), whereas both events occur in females. In organisms in which there is incomplete SC formation, recombination is limited to the regions of synapsis (56). The phenotypes of meiotic mutants also suggest that the SC is required for recombination. *Drosophila c(3)G* mutants do not assemble SC (52); these mutants also have reduced fertility and display reduced levels of exchange between homologs (23). In *S. cerevisiae*, the *HOP1* gene product is believed to encode a component of the SC (27; N. Hollingsworth and B. Byers, personal communication); *hop1* mutants do not make SC and display reduced levels of interchromosomal recombination. The yeast gene *RED1* is also believed to play a role in

synapsis; *red1* mutants do not make SC, and interchromosomal recombination is reduced (B. Rockmill and S. Roeder, unpublished results). However, *c(3)G* (24), hop1 (27), and *red1* (B. Rockmill and S. Roeder, unpublished results) mutants are proficient in intrachromosomal recombination, indicating that the meiotic recombination machinery is intact. The phenotypes of such "pairing" mutants support the hypothesis that the SC is required for meiotic levels of recombination.

The SC is probably required for recombination, but is recombination required for synapsis? Carpenter (10) has proposed that synapsis initiates by the joining of two chromosomes via the formation of a short stretch of SC. Strand exchange, which may sometimes result in gene conversion, checks for sequence homology; if extended homology is found, SC formation continues, bringing the homologs together by zippering. This model predicts that a recombination event must occur for synapsis to take place. Consistent with this hypothesis, recombination nodules have been observed in association with axial elements at points of convergence between homologs at zygotene (1, 2).

The dependence of pairing on recombination is further supported by the phenotypes of yeast mutants believed to be defective in the initiation of meiotic recombination (*mer1* [17], *rad50* [21], *spo11* [33]). These mutants are defective in intrachromosomal as well as interchromosomal recombination, indicating that the recombination machinery is defective. SC is not observed in spreads of meiotic nuclei from any of these recombination mutants (*mer1* [this study], *rad50* [19], *spo11*Δ [15]), suggesting that either pairing is required for recombination or the SC is destabilized in the absence of recombination. If recombination is required for synapsis, then the *MER1* gene product may function to bring the axial elements corresponding to homologous chromosomes together by initiating recombination.

One possible exception to the rule that all mutants defective in recombination are also defective in pairing is the *spo11-1* mutant. SC was observed in thin sections of *spo11-1* meiotic nuclei (33), although no SC was observed in spreads from a *spo11*Δ mutant (15). This difference may be due to the different methods of analysis, the nature of the different mutations, or both. It is possible that the sections of SC in *spo11-1* nuclei represent segments of incompletely paired chromosomes or pairing between nonhomologous chromosomes. Even if *spo11-1* mutants do synapse completely, this does not disprove the hypothesis that recombination is required for pairing. *spo11-1* mutants may be able to initiate events that lead to heteroduplex formation but unable to process or resolve these to generate recombinant products. For example, a heteroduplex formed in a *spo11-1* mutant might be unzippered by a helicase analogous to the *E. coli* helicase II. This enzyme catalyzes the removal of the incoming donor strand from a parental recipient duplex, resulting in the abortion of the recombination event (45).

MER1 shows similarity to calmodulin. *MER1* encodes a protein with a predicted molecular weight of 31,100. A large region of the *MER1* protein is similar to the regulatory protein calmodulin, suggesting that these proteins may be derived from a common ancestor. Calmodulin is a ubiquitous protein that binds calcium ions and is thereby activated to modulate various proteins (34). Because the *MER1* protein does not contain the consensus calcium-binding site, it is tempting to speculate that the *MER1* protein may regulate a protein or proteins via some other messenger. If the *MER1* gene product is a modulator, then it may act through another protein(s) to ensure that recombination and pairing occur properly.

Expression of the *MER1* gene is meiotically induced. Both *MER1* RNA and fusion protein are synthesized only during meiosis and are maximally accumulated during prophase I just before the first meiotic division. The kinetics of expression of *MER1* is similar to those of other yeast genes essential for meiosis I (*RED1* [55], *SPO13* [59], *SPO11* [3], *HOP1* [N. Hollingsworth, L. Goetsch, and B. Byers, Cell, in press]). Comparison of *MER1* and *RED1* transcripts indicates that maximal induction occurs simultaneously for both transcripts (data not shown). The timing of expression of *mer1*::*lacZ* and *red1*::*lacZ* fusion genes is also identical (B. Rockmill and S. Roeder, unpublished results).

Transcriptional activation of genes necessary for meiosis is probably coordinately controlled. *IME1* and *IME2* (51) are likely candidates for the genes responsible for this transcriptional regulation; mutations in both genes have been shown to affect the expression of a number of meiosis-specific genes (this study; A. Mitchell and H. Smith, personal communication).

The major 3.2-kb *MER1* transcript is considerably longer than predicted based on the coding region of the *MER1* gene (17). The additional sequences are derived from sequences downstream of the *MER1* coding region. The length of the transcript may be due to lack of efficient termination signals downstream of the *MER1* coding region. In fact, transcription termination may occur at the end of one of the adjacent genes, since the probe containing sequences downstream of *MER1* detected additional overlapping transcripts. In addition to the 3.2-kb transcript, two smaller and less abundant transcripts were detected for *MER1*. These small transcripts could be breakdown products of the 3.2-kb transcript, or they could result from termination or processing sites closer to the end of the *MER1* coding region. It is unclear whether these *MER1*-specific transcripts share the same 5′ end. Multiple transcripts have been observed in other meiosis-specific genes (i.e., *SPO13* [3], *RAD50* [19], *RED1* [55]). In the case of *SPO13*, two transcripts differing at the 5′ termini were identified (3).

Several lines of evidence indicate that the additional 3′ sequences on the *MER1* transcript are not required for *MER1* function. First, strains with transposon insertions in this region have no discernible defect (17). Second, *MER1*-complementing activity can be provided in *trans* on a plasmid containing the *MER1* coding region and only about 400 bases of downstream sequence (Fig. 1). It is possible that a truncated protein is functioning in both of these cases; however, this is unlikely, since there are multiple stop codons in all three reading frames in the sequence downstream of the proposed *MER1* coding region (Fig. 3 and an additional 162 bp not shown). Last, *trpE*::*mer1* fusion proteins containing approximately 400 bases downstream of the putative *MER1* stop codon produce a protein in *Escherichia coli* of the predicted molecular weight (unpublished results).

Summary. *mer1* mutants are defective in both pairing and recombination; the absence of SC may be a direct consequence of the failure to recombine. The *MER1* gene product could act directly or indirectly as a modulator of other proteins to effect these processes. A search for suppressors of *mer1* may identify genes whose products interact with the *MER1* protein and provide further insight into the role of recombination and pairing in proper meiotic chromosome segregation.

ACKNOWLEDGMENTS

We thank C. Bonin for excellent technical assistance, B. Piekos and B. Rockmill for help with the electron microscopic analysis, and M. Dresser and C. Giroux for advice on the chromosome spreading technique. We also thank R. Doolittle for helpful discussions and B. Rockmill, T. Menees, J. Trimmer, and M. Synder for critical reading of the manuscript.

This work was supported by Public Health Service grant GM28904 from the National Institutes of Health and by National Science Foundation grant PCM-8351607. J.E. is a Fellow of The Jane Coffin Childs Memorial Fund for Medical Research. This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research. BIONET is funded by Public Health Service grant 5 U41 RR01685-03 from the National Institutes of Health.

LITERATURE CITED

1. **Albini, S. M., and G. H. Jones.** 1987. Synaptonemal complex spreading in *Allium cepa* and *A. fistulosum*. Chromosoma **95**:324–338.
2. **Anderson, L. K., and M. A. Stack.** 1988. Nodules associated with axial cores and synaptonemal complexes during zygotene in *Psilotum nudum*. Chromosoma **97**:96–100.
3. **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.
4. **Baker, B. S., A. T. C. Carpenter, M. S. Esposito, R. E. Esposito, and L. Sandler.** 1976. The genetic control of meiosis. Annu. Rev. Genet. **10**:53–134.
5. **Baum, P., C. Furlong, and B. Byers.** 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca^{2+}-binding proteins. Proc. Natl. Acad. Sci. USA **83**:5512–5516.
6. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232**:485–487.
7. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
8. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–252.
9. **Carpenter, A. T. C.** 1975. Elerctron microscopy of meiosis in *Drosophila melanogaster* females. II. The recombination nodule—a recombination-associated structure at pachytene? Proc. Natl. Acad. Sci. USA **72**:3186–3189.
10. **Carpenter, A. T. C.** 1987. Gene conversion, recombination nodules, and the initiation of meiotic synapsis. BioEssays **6**:232–236.
11. **Coney, L. R., and G. S. Roeder.** 1988. Control of yeast gene expression by transposable elements: maximum expression requires a functional Ty activator sequence and a defective Ty promoter. Mol. Cell. Biol. **8**:4009–4017.
12. **Cooper, K. W.** 1950. Normal spermatogenesis in *Drosophila*, p. 1–61. *In* M. Demerec (ed.), Biology of *Drosophila*. Hafner Press, New York.
13. **Dever, T. E., M. J. Glynias, and W. C. Merrick.** 1987. GTP-binding domain: three consensus sequence elements with distinct spacing. Proc. Natl. Acad. Sci. USA **84**:1814–1818.
14. **Dresser, M. E., and C. N. Giroux.** 1988. Meiotic chromosome behavior in spread preparations of yeast. J. Cell Biol. **106**:567–573.
15. **Dresser, M. E., C. N. Giroux, and M. J. Moses.** 1986. Cytological analysis of meiosis using synaptonemal complexes in spread preparations of yeast nuclei. Yeast **2**:S96.
16. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80**:2432–2436.
17. **Engebrecht, J., and G. S. Roeder.** 1989. Yeast *mer1* mutants display reduced levels of meiotic recombination. Genetics **121**:237–247.
18. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Farnet, C., R. Padmore, L. Cao, E. Alani, and N. Kleckner.** 1988. The *RAD50* gene of *S. cerevisiae*. UCLA Symp. Mol. Cell. Biol. New Ser. **83**:201–215.
20. **Fast, D.** 1973. Sporulation synchrony of *Saccharomyces cerevisiae* grown in various carbon sources. J. Bacteriol. **116**:925–930.
21. **Game, J. C., T. J. Zamb, R. J. Braun, M. Resnick, and R. M. Roth.** 1980. The role of radiation (rad) genes in meiotic recombination in yeast. Genetics **94**:51–68.
22. **Hahn, S., D. T. Hoar, and L. Guarente.** 1986. Each of three "TATA elements" specifies a subset of the transcription initiation sites at the *CYC-1* promoter of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8562–8566.
23. **Hall, J.** 1972. Chromosome segregation influenced by two alleles of the meiotic mutant *c(3)G* in *Drosophila melanogaster*. Genetics **71**:367–400.
24. **Hall, J.** 1977. Recombination influenced by two alleles of the meiotic mutant *c(3)G*. Drosophila Inform. Serv. **52**:143–144.
25. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
26. **Henikoff, S., J. D. Kelly, and E. H. Cohen.** 1983. Transcription terminates in yeast distal to a control sequence. Cell **33**:607–614.
27. **Hollingsworth, N. M., and B. Byers.** 1989. *HOP1*: a yeast meiotic pairing gene. Genetics **121**:445–462.
28. **Howell, W. M., and D. A. Black.** 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36**:1014–1015.
29. **Ito, H., Y. Fukada, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
30. **Jones, G. H.** 1987. Chiasmata, p. 213–244. *In* P. B. Moens (ed.), Meiosis. Academic Press, Inc., Orlando, Fla.
31. **Kassir, Y., D. Granot, and G. Simchen.** 1988. *IME1*, a positive regulator gene of meiosis in *S. cerevisiae*. Cell **52**:853–862.
32. **Klapholz, S., and R. E. Esposito.** 1980. Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics **96**:589–611.
33. **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.
34. **Klee, C. B., T. H. Crouch, and P. G. Richman.** 1980. Calmodulin. Annu. Rev. Biochem. **49**:489–515.
35. **Krieg, P. A., and D. A. Melton.** 1987. In vitro RNA synthesis with SP6 RNA polymerase. Methods Enzymol. **155**:397–415.
36. **Langford, C. J., F. Klinz, C. Donath, and D. Gallwitz.** 1984. Point mutations identify the conserved intron-contained TACTAAC box as an essential splicing signal sequence in yeast. Cell **36**:645–653.
37. **Larkin, J. C., and J. F. Woolford.** 1983. Molecular cloning and analysis of *CRY1* gene: a yeast ribosomal protein gene. Nucleic Acids Res. **11**:403–420.
38. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Meade, J. H., M. I. Riley, and T. R. Manney.** 1977. Expression of cryptopleurine resistance in *Saccharomyces cerevisiae*. J. Bacteriol. **129**:1428–1434.
40. **Miller, J., A. D. McLaughlin, and A. Klug.** 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. EMBO J. **4**:1609–1614.
41. **Moses, M. J.** 1968. Synaptonemal complex. Annu. Rev. Genet. **2**:363–412.
42. **Nagawa, F., and G. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8557–8561.
43. **Pabo, C. O.** 1984. Protein-DNA recognition. Annu. Rev. Bio-

chem. **53**:293–321.
44. **Pikielny, C. W., J. L. Teem, and M. Rosbash.** 1983. Evidence for the biochemical role of an internal sequence in yeast nuclear mRNA introns: implications for U1 RNA and metazoan mRNA splicing. Cell **34**:395–403.
45. **Radman, M.** 1988. Mismatch repair and genetic recombination, p. 169–192. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
46. **Reed, K. C., and D. A. Mann.** 1985. Rapid transfer of DNA from agarose gel to nylon membranes. Nucleic Acids Res. **13**:7207–7221.
47. **Rothstein, R.** 1983. One step gene disruption in yeast. Methods Enzymol. **101**:202–211.
48. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
49. **Seifert, H. S., E. Y. Chen, M. So, and F. Heffron.** 1986. Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83**:735–739.
50. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics: laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Smith, H. E., and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**:2142–2152.
52. **Smith, P. A., and R. C. King.** 1968. Genetic control of synaptonemal complexes in *Drosophila melanogaster*. Genetics **60**: 335–351.
53. **Stewart, S. E., and G. S. Roeder.** 1989. Transcription by RNA polymerase I stimulates mitotic recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**:3464–3472.
54. **Struhl, K.** 1983. The new yeast genetics. Nature (London) **305**:391–397.
55. **Thompson, E. A., and G. S. Roeder.** 1989. Expression and DNA sequence of *RED1*, a gene required for meiosis I chromosome segregation in yeast. Mol. Gen. Genet. **218**:293–301.
56. **von Wettstein, D., S. W. Rasmussen, and P. B. Holm.** 1984. The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18**:331–413.
57. **Wagstaff, J. E., S. Gottlieb, and R. E. Esposito.** 1986. The role of *RAD50* in meiotic intrachromosomal recombination, p. 75–83. *In* A. Klar and J. N. Strathern (ed.), Mechanisms of yeast recombination, current communications in molecular biology. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
58. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1**:945–951.
59. **Wang, H., S. Frackman, J. Kowalisyn, R. E. Esposito, and R. Elder.** 1987. Developmental regulation of *SPO13*, a gene required for separation of homologous chromosomes at meiosis I. Mol. Cell. Biol. **7**:1425–1435.
60. **Watterson, D. M., F. Sharief, and T. C. Vanaman.** 1980. The complete amino acid sequence of the Ca^{2+}-dependent modulator protein (calmodulin) of bovine brain. J. Biol. Chem. **255**: 962–975.
61. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.