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MEI4, a Meiosis-Specific Yeast Gene Required for Chromosome Synapsis

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The *MEI4* gene product is required for meiotic induction of recombination and viable spore production in the yeast *Saccharomyces cerevisiae*. DNA sequence analysis shows that the *MEI4* gene encodes a 450-amino-acid protein bearing no homology to any previously identified protein. The *MEI4* coding region is interrupted by a small intron located near the 5′ end of the gene. Efficient splicing of the *MEI4* transcript is not dependent on the MER1 protein, which is required for splicing the transcript of another meiotic gene, *MER2*. Expression of a *mei4*::*lacZ* fusion gene is meiosis-specific and depends on both heterozygosity at the mating-type locus and nutrient limitation. Northern (RNA) blot hybridization analysis suggests that *MEI4* gene expression is regulated at the level of transcription. A functional *MEI4* gene is not required for meiotic induction of transcription of the *MER1*, *MER2*, *MEK1*, *RED1*, *SPO11*, or *RAD50* gene. Cytological analysis of *mei4* mutant strains during meiotic prophase demonstrates that the chromosomes form long axial elements that fail to undergo synapsis. The meiosis II division is delayed in *mei4* strains.

In meiosis, a single round of DNA replication is followed by two rounds of chromosome segregation, resulting in the production of haploid gametes from diploid cells. The first meiotic division is preceded by the pairing of homologous chromosomes and high levels of genetic exchange. In the vast majority of organisms, chromosome synapsis and recombination are necessary prerequisites to proper reductional (meiosis I) chromosome segregation (3).

The pairing of homologous chromosomes during meiosis I prophase is mediated by a proteinaceous structure called the synaptonemal complex (SC) (49). Each SC is composed of two dense parallel structures called lateral elements, separated by a central element. Each lateral element represents a pair of condensed sister chromatids assembled along a protein core and is called an axial element before it becomes part of the SC. In some organisms, full-length axial elements form first and then align to make SC. In other organisms, including *Saccharomyces cerevisiae* (1), the formation of axial elements and their alignment to form tripartite SC occur contemporaneously. Most chromatin is located outside the SC and consists of a series of loops attached at their base to a lateral element.

In *S. cerevisiae*, it has been possible to isolate numerous mutants defective in meiotic recombination, chromosome synapsis, and/or meiotic chromosome segregation (35). With the exception of the *rad* mutants, which are defective in the repair of DNA damage (20), these meiotic mutants have no discernible phenotype during vegetative growth, suggesting that the genes defined by mutation function specifically in meiosis. Molecular studies indicate that these genes with meiosis-specific functions are expressed only in meiotic cells and, with the exception of the *MER2* gene, are regulated at the transcriptional level (2, 15, 25, 48, 50). The intron-containing *MER2* gene is regulated posttranscriptionally; efficient splicing of the *MER2* transcript requires the meiosis-specific MER1 protein (16).

Mutations in the yeast *MEI4* gene lead to the production of inviable meiotic progeny (32). A *mei4* null mutation completely eliminates meiotic induction of gene conversion and crossing over but does not affect mitotic recombination levels. A *spo13* mutation, which causes cells to bypass the reductional division (28), restores spore viability to *mei4* strains. The *mei4* mutation is epistatic to *rad52* in *spo13* strains, indicating that the MEI4 protein acts before the *RAD52* gene product in the meiotic recombination pathway. These results suggest that the *MEI4* gene product is required for an early step in recombination, possibly the initiation of meiotic exchange.

The DNA sequence of the *MEI4* gene has been determined in an attempt to gain insight into the function of the MEI4 protein. In addition, the dependence of *MEI4* gene expression on cell type and nutrient conditions has been determined. The effect of a *mei4* mutation on the expression of a number of other genes and on the timing of meiotic nuclear divisions has been investigated. Finally, meiotic nuclei from a *mei4* mutant have been examined in the electron microscope to determine whether the *mei4* mutation affects SC assembly.

MATERIALS AND METHODS

Yeast strains. The genotypes of yeast strains are presented in Table 1. BR2495 was obtained from Beth Rockmill (37), NKY611 was from Nancy Kleckner (1), and J319 and J252 were from JoAnne Engebrecht (13, 14). Diploid strains isogenic with BR2495 were constructed by transforming the diploid or by transforming the parental haploids, BR1919-8B and BR1373-6D (37), and then mating the transformants. Similarly, NKY611 and J319 derivatives were constructed by transforming the diploids or by transforming the haploid parents and then mating the transformants. S1593, S1594, and S1595 were generated by transformation of E100, E102, and E104 (48), respectively, with pR1199.

Genetic procedures. Yeast media and genetic methods are described by Sherman et al. (44). Yeast transformations were carried out by the lithium acetate procedure (26).

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TABLE 1. Yeast strains

Strain	Genotype
BR2495	*MAT***a** *leu2-27 his4-280 ura3-1 trp1-289 arg4-8 thr1-1 CYH10 ade2-1* *MAT*α *leu2-3,112 his4-260 ura3-1 trp1-1 ARG4 thr1-4 cyh10 ade2-1*
BR2496	BR2495 but *mer1::LEU2* *mer1::LEU2*
S1619	BR2495 but *mer2::lacZ-3'-ADE2* *MER2*
S2501	BR2495 but *ade2-1 mek1::lacZ* *ADE2 MEK1*
S2502	BR2495 but *ade2-1* with pCA161 (*spo11::lacZ*) *ADE2*
S2503	BR2495 but *ade2-1* with pR1221 (*red1::lacZ*) *ADE2*
S1608	BR2495 but *mei4::ADE2 mek1::lacZ* *mei4::ADE2 MEK1*
S1618	BR2495 but *mei4::ADE2* with pCA161 (*spo11::lacZ*) *mei4::ADE2*
S2507	BR2495 but *mei4::ADE2* with pR1221 (*red1::lacZ*) *mei4::ADE2*
S1607	BR2495 but *mei4::ADE2 mer2::lacZ-3'-ADE2* *mei4::URA3 MER2*
S2508	BR2495 but *mei4::URA3* *mei4::URA3*
S2509	BR2495 but *mei4::URA3* *MEI4*
J319	*MAT***a** *his4-260 CDC10 leu2-27 trp1-289 CYH10 lys2 ura3-1 arg4-9 THR1 ade2-1* *MAT*α *HIS4 cdc10 leu2-27 trp1-1 cyh10 LYS2 ura3-1 arg4-8 thr1 ade2-1*
S1576	J319 with pR1199 (*mei4::lacZ-5'*)
S1577	J319 with pR1200 (*mei4::lacZ-3'*)
S1578	J319 but *mer1::ADE2* with pR1199 (*mei4::lacZ-5'*) *mer1::ADE2*
S1579	J319 but *mer1::ADE2* with pR1200 (*mei4::lacZ-3'*) *mer1::ADE2*
S1593	*MAT***a** *cry1 leu2-3,112 his4-917 trp1-289 CYH10 lys2-173R2 ura3-52 ARG4 ADE2* *MAT*α *CRY1 leu2-3,112 HIS4 trp1-1 cyh10 LYS2 ura3-1 arg4-8 ade2-1* pR1199 (*mei4::lacZ-5'*)
S1594	S1593 but *MAT***a** *MAT***a**
S1595	S1593 but *MAT*α *MAT*α
NKY611	*MAT***a** *leu2::hisG ho::LYS2 lys2 ura3* *MAT*α *leu2::hisG ho::LYS2 lys2 ura3*
S2510	NKY611 with pR1216 (*mei4::lacZ-3'*)
S2511	NKY611 with pR1221 (*red1::lacZ*)
J252	NKY611 but *mer1::lacZ* *MER1*
S2512	NKY611 but *mer1::lacZ mei4::URA3* *MER1 mei4::URA3*

Deletion alleles of *MEI4* were introduced into yeast cells by one-step gene disruption (40).

DNA sequence analysis. *MEI4*-complementing activity was localized to a 2.1-kbp *Sal*I-*Bam*HI fragment, as shown in Fig. 1. The *Sal*I site lies upstream of the *MEI4* gene, and the *Bam*HI site is derived from a transposon inserted just outside the 3′ end of the gene (32, 43). Deletion subclones of plasmids R1160 and R1161 were generated by using the Erase-a-Base system (Promega) (24). Prior to incubation with exonuclease III, pR1160 was digested with *Kpn*I and *Hpa*I and pR1161 was digested with *Sac*I and *Bam*HI.

The Yale Protein and Nucleic Acid Facility performed most of the sequencing, using the ABI automated DNA sequenator. Some sequencing was also done by the method of Sanger et al. (42), using [^{35}S]dATP and a T7 DNA polymerase enzyme and reagent system from Pharmacia. Sequence data were analyzed by using the Wisconsin package of computer programs (9).

RNA isolation and analysis. For isolation of RNA from meiotic cells, strains were grown at 30°C to saturation in liquid YPAD medium, diluted 50-fold into the same medium, and grown to a density of 5×10^7 to 8×10^7 cells per ml. For preparation of meiotic RNA, 2 ml of cells from the saturated cultures was pelleted and resuspended in 20 ml of 2% potassium acetate (KAc) at 30°C to induce sporulation; cells were harvested after 6 h (NKY611) or 15 h (BR2495, BR2496, and S2508) in KAc medium. RNA was extracted by the procedure of Elder et al. (11) as modified by Stewart and Roeder (46).

For Northern (RNA) blot hybridization analysis, samples containing 10 μg of total RNA were electrophoresed on a formaldehyde gel and then transferred to a Nytran membrane (Schleicher & Schuell) according to the instructions provided by the manufacturer. An 800-base riboprobe derived from the *MEI4* coding region was generated by in vitro

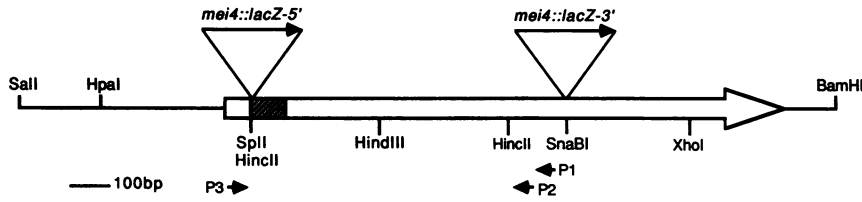

FIG. 1. Physical map of the *MEI4* gene. The large open arrow represents the limits of the *MEI4* ORF; the hatched region represents intron sequences. The locations and directions of the *lacZ* fusions are indicated by the arrows above the map. The positions and directions of the primers used for generation of cDNA copies of *MEI4* are indicated with arrows below the map. The primers have the following sequences and locations: P1, 5'-ATAGAGTAAATAAGGGT-3', bases +813 to +797; P2, 5'-GCAACGAACACAAGCGCTTGTAGTCA-3', bases +791 to +766; and P3, 5'-AGATATGGAACAAAAGGAAACATCGG-3', bases +20 to +45. P1 was used for reverse transcription of total RNA; P2 and P3 were used for PCR amplification of reverse transcription products (see Materials and Methods). The *lacZ* fusions and the primers are not drawn to scale.

transcription of pTM21 linearized with *Hin*dIII, using T7 RNA polymerase.

Amplification of *MEI4* cDNAs. The polymerase chain reaction (PCR) (41) was performed on RNA templates as described by Kawaski et al. (27) to generate cDNAs. Total RNA (10 μg) was resuspended in 100 μl of DNase I buffer (20 mM Tris [pH 7.4], 10 mM $MgCl_2$) and incubated for 15 min at 37°C with 10 U of DNase I (FPLC Pure; Pharmacia). Following DNase I digestion, the samples were phenol-chloroform extracted, ethanol precipitated, and resuspended in 20 μl of diethylpyrocarbonate-treated water. The RNA was incubated at 90°C for 1 min and then cooled on ice. The volume was brought to 100 μl with 50 mM Tris (pH 8.8), 50 mM KCl, 5 mM $MgCl_2$, 80 U of RNasin (Promega), 10 mM dithiothreitol, 50 μM deoxynucleoside triphosphates (dNTPs), 5 μg of primer 1 (Fig. 1), and 50 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Orlando, Fla.). The reaction mixture was incubated at 42°C for 60 min.

PCR was carried out by using 10 μl of the reverse transcription reaction mixture suspended in 100 μl of *Taq* polymerase buffer (50 mM KCl, 10 mM Tris [pH 9.0], 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100) containing 125 μM dNTPs, 100 ng each of primers 2 and 3 (Fig. 1), and 30 U of *Taq* polymerase (Cetus, Emeryville, Calif.). The reaction components were overlayed with Nujol mineral oil (Perkin Elmer) and placed into a DNA Thermal Cycler (Perkin Elmer Cetus) for 35 cycles of the following temperature incubations: 94°C for 2 min, 54°C for 2 min, and 72°C for 3 min. Following PCR, most of the Nujol mineral oil was removed, 10 U of RNase A was added, and the samples were incubated at 37°C for 10 min. The samples were extracted once with chloroform-isoamyl alcohol to remove the residual oil and ethanol precipitated. Samples were run on a 1.6% agarose gel after digestion with *Hin*cII to allow comparison with a *Hin*cII-digested genomic clone of *MEI4* DNA. Cloning of *MEI4* cDNAs is described below.

Plasmids. For DNA sequence analysis, the 2.1-kbp *Sal*I-*Bam*HI fragment containing *MEI4* was cloned into *Xho*I-*Bam*HI-digested SK^+ (Bluescript; Stratagene) and *Sal*I-*Bam*HI-digested SK^- (Bluescript; Stratagene), resulting in pR1160 and pR1161, respectively. For riboprobe synthesis, plasmid TM21 was constructed by inserting the 0.8-kbp *Hin*dIII-*Xho*I fragment of the *MEI4* coding region between the *Hin*dIII and *Xho*I sites of SK^+.

To make an intronless *MEI4* gene, the PCR products from RNA isolated from a wild-type strain were digested with *Hin*cII and *Hin*dIII. The resulting 257-bp *Hin*cII-*Hin*dIII fragment was cloned into SK^+ digested with *Hin*cII and *Hin*dIII to create pTM97. A full-length *MEI4* cDNA was constructed in the following way. A 1.2-kbp *Hin*dIII-*Bam*HI fragment from pR1160 was cloned into *Hin*dIII-*Bam*HI-digested pTM97 to create pTM98, a plasmid that contains all of an intronless *MEI4* gene except the first 17 codons and the 5' region. Plasmid TM62 contains the genomic *MEI4* gene on a 1.8-kbp *Hpa*I-*Bam*HI fragment cloned into *Eco*RV-*Bam*HI-digested SK^+. A 1.8-kbp fragment of pTM62 extending from the *Sal*I site in the polylinker to the *Bam*HI site at the 3' end of *MEI4* was cloned into *Xho*I-*Bam*HI-digested SK^+ to make pTM95. The 627-bp *Hin*cII fragment from pTM98 was cloned into pTM95 after digestion with *Hin*cII to remove the 715-bp *Hin*cII fragment that contains the intron. This resulted in pTM99, a full-length intronless *MEI4* gene in SK^+. A 1.8-kbp *Cla*I-*Bam*HI fragment from pTM99 was cloned into *Cla*I-*Bam*HI-digested pUN75 (12) to generate pTM100, a plasmid carrying the intronless *MEI4* gene, *CEN4*, *ARS1*, and *URA3*.

Plasmids carrying *lacZ* inserted upstream of the *MEI4* intron (*mei4::lacZ-5'*) were constructed as follows. Plasmid R1160 was digested with *Spl*I, and the overhangs were filled in with the Klenow fragment of DNA polymerase I in the presence of dNTPs. A *Bgl*II linker was ligated into this site to generate pR1196. A 3.1-kbp *Bam*HI-*Bgl*II fragment of pRP370 (a derivative of pLG670-Z [23] constructed by Roger Yocum and obtained from Ron Pearlman) containing the *lacZ* gene of *Escherichia coli* was cloned into *Bgl*II-digested pR1196, resulting in pR1197, a fusion of *lacZ* to the 5' exon of *MEI4* in SK^+. The 5.2-kbp *Sal*I-*Bam*HI fragment from pR1197 was cloned into *Sal*I-*Bam*HI-digested pUN100 (12) to generate pR1199, a plasmid carrying the *mei4::lacZ-5'* fusion gene, *CEN4*, *ARS1*, and *LEU2*.

Plasmids containing *lacZ* fused to the *MEI4* 3' exon (*mei4::lacZ-3'*) were constructed as follows. A *Bgl*II linker was ligated into the *Sna*BI site of pR1160 to generate pR1198. A 3.1-kbp *Bam*HI-*Bgl*II fragment from pRP366 (a derivative of pLG670-Z [23] constructed by Roger Yocum and obtained from Ron Pearlman) containing the *lacZ* gene of *E. coli* was cloned into the *Bgl*II site of pR1198, resulting in pR1202. The 5.2-kbp *Sal*I-*Bam*HI fragment from pR1202 was cloned into *Sal*I-*Bam*HI-digested pUN100 and *Sal*I-*Bam*HI-digested YCp50 to generate pR1200 and pR1216, respectively. Both pR1200 and pR1216 carry *CEN4* and *ARS1*; pR1200 is marked with *LEU2*, and pR1216 is marked with *URA3*.

Plasmid R1221 was constructed by ligating the 7-kbp *Xba*I fragment of pB69 into *Spe*I-digested pUN70 (12), which carries *CEN4*, *ARS1*, and *URA3*. Plasmid B69 (obtained from Beth Rockmill) carries a *red1::lacZ* fusion gene gener-

ated by transposon mutagenesis of *RED1*, using a Tn*3* derivative carrying *lacZ* and *LEU2* (43). The *Xba*I fragment that was cloned into pUN70 contains the entire *RED1* gene and the transposon.

The *mei4*::*ADE2* and *mei4*::*URA3* mutations were introduced into yeast cells by transformation with pTM7 and pTM6, respectively (32). BR2495 was transformed to Ade$^+$ by transformation with a *Bam*HI fragment containing the wild-type *ADE2* gene derived from plasmid Yp3.6 ADE obtained from Jeff Lemontt. Plasmids and strains carrying a *mer1*::*lacZ* fusion gene were described by Engebrecht and Roeder (15); the *mer1*::*lacZ* fusion used in this study was previously designated *mer1*::Tn*164*. Plasmid B118, carrying a *mek1*::*lacZ* fusion, was derived by insertional mutagenesis of the *MEK1* gene with a Tn*3* element marked with *lacZ* and *LEU2* (38). The *mer2*::*lacZ-3'-ADE2* gene was described by Engebrecht et al. (16). Plasmid CA161, obtained from Catherine Atcheson, contains a *spo11*::*lacZ* fusion gene, *TRP1*, and the 2μm circle origin of DNA replication.

β-Galactosidase assays. Transformants containing either the *mei4*::*lacZ-3'* fusion or *red1*::*lacZ* fusion plasmid were grown to saturation in SC medium with selection for the plasmids. Cells were then diluted 10-fold into YPA medium and grown to a density of 5×10^7 cells per ml. Two 10-ml aliquots of the culture were harvested by centrifugation, and the pellets were stored at −70°C. The remaining culture was centrifuged, resuspended in the same volume of 2% KAc, and incubated at 30°C with shaking. At the time points indicated, two 10-ml aliquots of each culture were harvested and frozen. β-Galactosidase assays were performed as described by Coney and Roeder (8), and the protein content of each sample was determined by the method of Bradford (5).

To assay expression of other meiotic genes, the appropriate strains were grown to saturation in either YPAD or SC medium lacking leucine or uracil to provide selection for plasmid maintenance. Cultures were then diluted 2-fold into YPAD and grown for a further 3 h prior to centrifugation and resuspension in a 10-fold-greater volume of 2% KAc. A 10-ml aliquot was harvested and frozen as described above. Cultures were incubated at 30°C with shaking for the times indicated; 10-ml aliquots were then removed, harvested, and frozen. β-Galactosidase assays were performed as described above.

Cytology. Cells used for cytological studies were spheroplasted prior to sporulation as described by Alani et al. (1) and modified by Rockmill and Roeder (37). Cells were prepared for examination in the electron microscope by using the general protocol of Dresser and Giroux (10) as modified by Engebrecht and Roeder (15) and Rockmill and Roeder (38).

Cells were stained with diamidino-2-phenylindole (DAPI) and examined in the fluorescence microscope as described by Thompson and Roeder (48).

Nucleotide sequence accession number. The *MEI4* gene and cDNA sequences have been submitted to GenBank and have been assigned the accession numbers M84765 and M84764, respectively.

RESULTS

The spliced *MEI4* transcript encodes a 52.9-kDa protein. The *MEI4* gene was localized to a 2.1-kbp fragment (Fig. 1) by subcloning and transposon mutagenesis. The DNA sequence of this region was determined and is presented in Fig. 2. A 1,287-bp open reading frame (ORF) was detected (from +152 and to +1439), but this ORF lacks an initiating ATG codon. Located upstream are a number of shorter ORFs. These observations raised the possibility that the *MEI4* transcript might be spliced; thus, the sequence was examined for the presence of the consensus motifs required for splicing. In yeast cells, these consist of a 5' splice junction (GTAPyGT), a 3' splice junction (PyAG), and an internal branch point sequence (TACTAAC), located 20 to 60 bp upstream of the 3' splice junction (51). A suitable 5' splice junction (GTACGT) is located beginning at +64, a TAC TAAC box is located at +102 and a 3' splice junction is located at +149 (Fig. 2). The putative intron is 88 bases long, and the region upstream contains a short ORF initiating with an ATG codon. RNA splicing using the consensus sequences identified would fuse the upstream and downstream ORFs in frame.

To investigate splicing of the *MEI4* transcript, cDNA copies of *MEI4* RNA were cloned and analyzed. Total RNA from meiotic cells was reverse transcribed by using a primer complementary to sequences near the 3' end of the *MEI4* primary transcript (Fig. 1). PCR was used to amplify an internal region of the *MEI4* cDNA, using primers that flank the putative intron (Fig. 1). The PCR products were digested with *Hin*cII and analyzed on an agarose gel. As shown in Fig. 3, the PCR product is approximately 90 bases shorter than the corresponding *Hin*cII fragment derived from the genomic clone. In addition, *MEI4* cDNAs were cloned and analyzed by DNA sequencing. The cDNA lacks the proposed 88-nucleotide intron, confirming that *MEI4* RNA is spliced (data not shown). A plasmid containing an intronless, but otherwise full-length, copy of the *MEI4* gene was constructed (pR1206; see Materials and Methods); this plasmid fully complements a *mei4* mutant (data not shown).

The spliced *MEI4* mRNA contains an ORF of 1,350 bases and specifies a protein with a molecular size of 52.9 kDa. Of the 450 amino acids in the MEI4 protein, 21 are encoded by the upstream exon and the remainder are encoded by the downstream exon. The protein is basic and unusually rich in leucine, isoleucine, and phenylalanine. The sequence of the *MEI4* protein was compared with sequences translated from the GenBank data base, but no significant similarities were detected as of August 1991.

Previous studies have identified a 10-bp consensus sequence, YCGGCGGCTA, which is required for meiosis-specific gene expression (6). Mutation of this sequence in the *SPO13* upstream region reduces meiotic expression and elevates the mitotic level of expression (6). A close match to the consensus sequence is located at −89 to −98 in the *MEI4* upstream region (TGGGCGGCTA). A sequence identical to that found in *MEI4* is found upstream of the *HOP1* gene in a region demonstrated to be required for meiotic expression (25).

The *MEI4* gene is expressed only in meiosis. To examine the expression of the *MEI4* gene, two *mei4*::*lacZ* fusion genes were constructed and analyzed (Fig. 1). In one of the two fusions, *mei4*::*lacZ-5'*, *lacZ* is inserted just upstream of the intron. In the other fusion, *mei4*::*lacZ-3'*, *lacZ* is fused to the 3' exon. For both fusion genes, β-galactosidase synthesis is provided by *MEI4* transcriptional and translational signals; expression of the downstream fusion is dependent on splicing.

The kinetics of *MEI4* gene expression during meiosis were examined in an SK1 strain, which undergoes rapid and fairly synchronous sporulation (19). Expression of a *red1*::*lacZ* fusion was examined in parallel with the *mei4*::*lacZ-3'* fusion. Previous studies in an SK1 strain have shown that the *RED1* gene is transcribed only in meiosis and that *RED1*

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-528                                          CTCGACAG AGAAAGAAAA TGTCACAATT ACAACTTCAG AGGGAATTTG

-480 ACCAGAGTAA GCAGAGAAAA CACAGGTGAT GAAGAAAAAA ATTGAAAATT ACGAAAAATT GGTTCAAAAA ATGAAGAAAA

-400 TTTCGAGGTT GCACATGCAA GAATGCACAA TTAATGGTGG TAATAATAGT TATCAAAGCC TTCAGAACAA GGATAGTGAT

-320 GTTAACGGGT TTCTGAAAAT GATTGAAGAA ATGATACGCA GCTCCAGTCT ATATGATGAA TAAAAGTATT GCGCATACTT

-240 CTTATGGGAG ATCCAAATTC CCAGTACATT AGTTTGACTC TCCTTCCTTA ATGTTTTCCT CTGTACGATT ATTTATGTTT

-160 AAACGATTTA TTTTACTATG TATGTATTTC CTTTCATTTT CTTTCGGAGT CATATATATT GCTGGGCGGC TAAATGTTTC

-80  CGACTTGTAA AAGAAAACAT AACAGACATC TTGTAGTTGT GAAACAGTTT CATTAAATAA TCTTTTAGAA GAGTAAGGAC

1    ATG AGT AGA GGC AAA CTG GAA GAT ATG GAA CAA AAG GAA ACA TCG GAA GTT GAC TGG ATA ATC
     Met Ser Arg Gly Lys Leu Glu Asp Met Glu Gln Lys Glu Thr Ser Glu Val Asp Trp Ile Ile

64   GTACGTGAAATTGTCACATCCTTAACCTATTTTCTCTTTACTAACTTTTAGTAAGAGGTGTAATCTCCTCGAATATTATCTATGACAG

152  TGT TTT GCT TTA ATT CAA TCC AGG AAT CCT ACG TTG TGG AAG AGG GCA CTT TCA AGA AAG AAA GGA GAT
     Cys Phe Ala Leu Ile Gln Ser Arg Asn Pro Thr Leu Trp Lys Arg Ala Leu Ser Arg Lys Lys Gly Asp

221  GTC GAA GAT GTA GGT GCT CTC AAG AGT GAA AAA AAT TTA AAA ATA AAT CCT CGG GAA AAC TCA AAA CAT
     Val Glu Asp Val Gly Ala Leu Lys Ser Glu Lys Asn Leu Lys Ile Asn Pro Arg Glu Asn Ser Lys His

290  ATA TAC AAA TGG GTT GCG CCT TTC GAA AAT GGA TTT TTG AAT AAT AAA TCA CTG TTT GCT CAT CTG GAA
     Ile Tyr Lys Trp Val Ala Pro Phe Glu Asn Gly Phe Leu Asn Asn Lys Ser Leu Phe Ala His Leu Glu

359  CCT ATA TAC AAT TTC CTT TGC CAG AAT AAG TAC AAA AGC TTC GAA GAT GCA GTT GGT TTA AAG GAG CTG
     Pro Ile Tyr Asn Phe Leu Cys Gln Asn Lys Tyr Lys Ser Phe Glu Asp Ala Val Gly Leu Lys Glu Leu

428  CAA TCA TTT AGC AAA GAT GTA TCA ACT GCT GAT ATA AAC AAC TGG TTT TTA CCG AGA TAT AAA ATT CTG
     Gln Ser Phe Ser Lys Asp Val Ser Thr Ala Asp Ile Asn Asn Trp Phe Leu Pro Arg Tyr Lys Ile Leu

497  CTA AAA ATC CTG AGC TTA AAA ACA AAA GAA ATT GAC TTT AGA GGG TTA TCG CAA GTG TTT CAA ACA CTC
     Leu Lys Ile Leu Ser Leu Lys Thr Lys Glu Ile Asp Phe Arg Gly Leu Ser Gln Val Phe Gln Thr Leu

566  CAA ATC TTG TTA GTT TCT CAT TAT TCG CAT CGC ATA GAT TCT GAT TCT TCT TTT AAG AGG ACA CTG ATA
     Gln Ile Leu Leu Val Ser His Tyr Ser His Arg Ile Asp Ser Asp Ser Ser Phe Lys Arg Thr Leu Ile

635  GAC GTT CAT GTT TTC AAT TTC ATC GCC AAG TTT CTG TTT AAT AGA ATT TTA TTG AAG AAG AAC CAA AAC
     Asp Val His Val Phe Asn Phe Ile Ala Lys Phe Leu Phe Asn Arg Ile Leu Leu Lys Lys Asn Gln Asn

704  GAT CCA AAA TGG CTT CAA AAC TTT TAC GAC CAG GGA GAT GGC AAA CAT TTA TGT GAT AAA GTT GAC TAC
     Asp Pro Lys Trp Leu Gln Asn Phe Tyr Asp Gln Gly Asp Gly Lys His Leu Cys Asp Lys Val Asp Tyr

773  AAG CGC TTG TGT TCG TTG CAT TTT ACC CTT ATT TAC TCT ATT ATT AAC ATT CAA CTC ATC AAA ATC AAG
     Lys Arg Leu Cys Ser Leu His Phe Thr Leu Ile Tyr Ser Ile Ile Asn Ile Gln Leu Ile Lys Ile Lys

842  ACA AAC CAA ACA TTT GAA CCG CAG ATA TTA AAA TAC GTA TCT GTT CTG AAA TTA ATT GAA CAC ATA CTT
     Thr Asn Gln Thr Phe Glu Pro Gln Ile Leu Lys Tyr Val Ser Val Leu Lys Leu Ile Glu His Ile Leu

911  ATC ATC ATC GAA AGT CTG ATA CAT GTA CTT ATT AGA TTT GTA TCG AAG CAT AAA CTA ATA TGC ATC AAC
     Ile Ile Ile Glu Ser Leu Ile His Val Leu Ile Arg Phe Val Ser Lys His Lys Leu Ile Cys Ile Asn

980  CGG AAA AAG GCG TAC TGT CGA GTA TAC CTC GAA AGA GAA TTG AGT TTG AAG AAA ACC TAC TTA AAG AAC
     Arg Lys Lys Ala Tyr Cys Arg Val Tyr Leu Glu Arg Glu Leu Ser Leu Lys Lys Thr Tyr Leu Lys Asn

1049 TTT TAC AGT GTA ATA AGC GGT GTC ACA GAA AAA GAG TTA GGG GGT CTA TTG AAA ATA CTC AAG ATA GTT
     Phe Tyr Ser Val Ile Ser Gly Val Thr Glu Lys Glu Leu Gly Gly Leu Leu Lys Ile Leu Lys Ile Val

1118 ATT CTA TCA TTG CTC GAG ACA TTT GAA AGC ATT GAA TGG CAG CAC TTA AAA CCT TTC CTG GAA AAA TTT
     Ile Leu Ser Leu Leu Glu Thr Phe Glu Ser Ile Glu Trp Gln His Leu Lys Pro Phe Leu Glu Lys Phe

1187 CCG GCT CAT GAA ATA TCG CTT CAG AAG AAA AGG AAA TAT ATA CAG GCG GCC TTA TTA ATT ACT GCC GAA
     Pro Ala His Glu Ile Ser Leu Gln Lys Lys Arg Lys Tyr Ile Gln Ala Ala Leu Leu Ile Thr Ala Glu

1256 AGA AAT TTG ATA GCG CGC TTT CGA TTG TCA AGA TGG TTC AAT GAG ACA GAA ACA TTT ATT TTC TTT TGC
     Arg Asn Leu Ile Ala Arg Phe Arg Leu Ser Arg Trp Phe Asn Glu Thr Glu Thr Phe Ile Phe Phe Cys

1325 AGT AGG GGG CGC ATT TTG GAA AAC ACA AAA ATA TCG AAA GCT CTT TCA TTT CGG GGA CAA CAA CTT CAG
     Ser Arg Gly Arg Ile Leu Glu Asn Thr Lys Ile Ser Lys Ala Leu Ser Phe Arg Gly Gln Gln Leu Gln

1394 TTG AAA ATT ACA GTG AAC ACA ACA TCT TCC CCA ACA GAC CTA CAT TAA
     Leu Lys Ile Thr Val Asn Thr Thr Ser Ser Pro Thr Asp Leu His Stop

1442 AACGCTTCTT CCGGACTTGC CCATGATTAA CCTAATCTTA TACGAACTGA ATTAAACTTT ACGGTATTAC CGATAGGAAA

1520 CTTCTATTTT ATGATTTTTT GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
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FIG. 2. DNA sequence of the *MEI4* gene and deduced amino acid sequence of the encoded gene product. Numbers refer to the DNA sequence, with position +1 indicating the first base of the 5′ *MEI4* exon. The upstream regulatory sequence and the splicing consensus sequences are underlined.

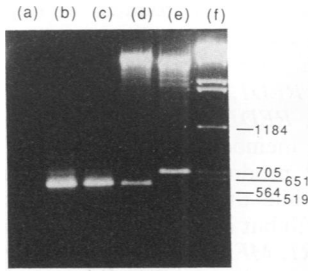

FIG. 3. Agarose gel analysis of *MEI4* cDNA. To generate *MEI4* cDNA copies, reverse transcription and PCR were carried out on total RNA isolated from wild-type (BR2495) mitotic cells (lane a), wild-type meiotic cells (lane b), and *mer1* (BR2496) meiotic cells (lane c). The reaction products were digested with *Hin*cII to remove the primers prior to electrophoresis. Also included on the gel were a *Hin*cII digest of a plasmid containing a cloned *MEI4* cDNA (lane d), a *Hin*cII digest of plasmid containing the genomic *MEI4* gene (lane e), and λ phage DNA digested with *Hin*dIII plus *Bgl*II (lane f).

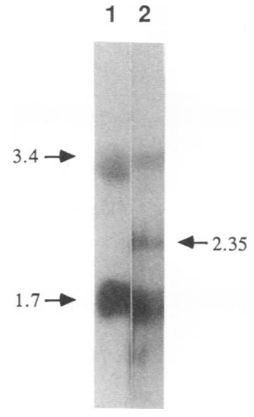

FIG. 5. Northern blot hybridization analysis of *MEI4* RNA. RNA was isolated from the SK1 strain NKY611 before transfer to sporulation medium (lane 1) and 6 h after transfer to sporulation medium (lane 2). The 2.35-kb *MEI4*-specific transcript is indicated on the right. Cross-hybridizing 35S (3.4-kb) and 18S (1.7-kb) rRNAs are indicated on the left. *MEI4* RNA was also observed at 3 h after transfer to sporulation medium (data not shown).

RNA accumulates maximally at 7.5 h after introduction into sporulation medium (48). Figure 4 shows the amount of β-galactosidase activity detected in an SK1 diploid carrying the *mei4*::*lacZ-3'* or *red1*::*lacZ* fusion gene on a centromere-containing plasmid. Neither fusion gene produced any detectable β-galactosidase activity in vegetative cells. The activity of both fusions increased between 2.5 and 7.5 h after the transfer to sporulation medium and remained fairly constant between 7.5 and 12.5 h. To correlate the timing of *MEI4* gene expression with other events in meiosis, cells from each time point were examined for chromatin segregation by staining with DAPI and for sporulation by light microscopy (Fig. 4). β-Galactosidase activity from the *MEI4* fusion gene was detected before binucleate cells were observed, indicating that *MEI4* gene expression precedes the meiosis I division.

The results of β-galactosidase assays demonstrate that the MEI4 protein is synthesized only during meiosis. Northern blot hybridization analysis indicates that *MEI4* gene expression is regulated at the level of transcription. A 2.35-kb *MEI4*-specific transcript is observed only in sporulating cells (Fig. 5).

MEI4* gene expression is not induced by starvation alone.** For meiosis to occur, a yeast cell must be heterozygous at the mating type locus (*MATa**/*MAT*α) and starved for nitrogen in the presence of a nonfermentable carbon source (reviewed by Esposito and Klapholz [17]). Expression of meiosis-specific genes requires all of these conditions; however, some genes can be induced by starvation alone. To determine whether *MEI4* gene expression can be induced by starvation, isogenic *MAT***a**/*MAT*α (S1593), *MAT***a**/*MAT***a** (S1594), and *MAT*α/*MAT*α (S1595) strains carrying the *mei4*::*lacZ-5'* fusion gene on a centromere-containing plasmid were constructed and analyzed. As shown in Table 2, β-galactosidase activity was not detected in *MAT***a**/*MAT***a** or *MAT*α/*MAT*α strains under starvation conditions, indicating that *MEI4* gene expression is meiosis specific.

***MEI4* splicing is MER1 independent.** The *MER2* gene, required for meiotic recombination, specifies an RNA that contains an intron. Furthermore, splicing of *MER2* mRNA is dependent on the product of the *MER1* gene, which is expressed only in meiosis (16). To determine whether splicing of the *MEI4* mRNA is dependent on the *MER1* gene

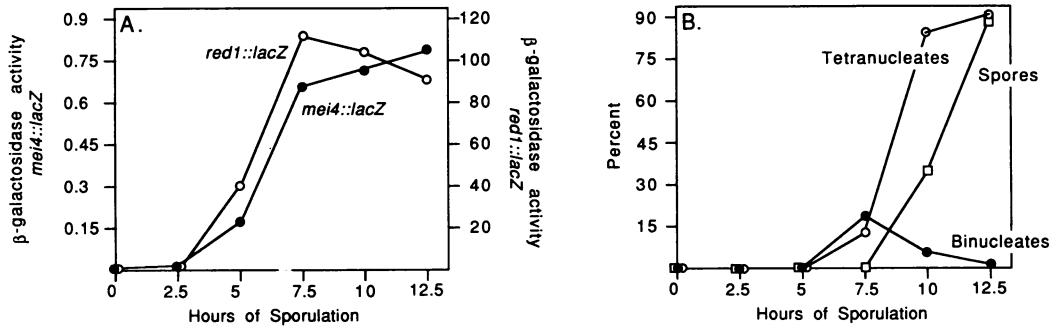

FIG. 4. Meiotic time course of *MEI4* expression. (A) β-Galactosidase activity from strains S2510 (*mei4*::*lacZ-3'*) and S2511 (*red1*::*lacZ*) assayed at various times after transfer to sporulation medium. β-Galactosidase activity is measured as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein, and values presented represent the averages of the values obtained with two independent transformants. (B) Landmark events in meiosis. Samples from each points were stained with DAPI and examined in the fluorescence microscope to determine the percentage of binucleate and tetranucleate cells (48). Cells from each time point were also examined in the light microscope to assess sporulation. At least 300 cells were examined at each time point. In previous return-to-growth studies using SK1 strains (1, 36), commitment to a low level of meiotic gene conversion was detected at 2.5 to 3 h and commitment was complete by 4 to 5 h.

TABLE 2. *MEI4* expression[a]

Strain	Relevant genotype		β-Galactosidase (U)
S1593	*MATa MER1* *MATα MER1*	*mei4::lacZ-5'*	2.2
S1594	*MATa MER1* *MATa MER1*	*mei4::lacZ-5'*	0.0
S1595	*MATα MER1* *MATα MER1*	*mei4::lacZ-5'*	0.0
S1576	*MATa MER1* *MATα MER1*	*mei4::lacZ-5'*	1.5
S1578	*MATa mer1* *MATα mer1*	*mei4::lacZ-5'*	1.2
S1577	*MATa MER1* *MATα MER1*	*mei4::lacZ-3'*	0.4
S1579	*MATa mer1* *MATα mer1*	*mei4::lacZ-3'*	0.4

[a] β-Galactosidase units are given as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein. Values are averages obtained from two independent transformants. Activity was assayed at 20 h after introduction into sporulation medium, which corresponds approximately to the time of maximum induction in this strain background. β-Galactosidase activity was measured in mitotic cells prior to the transfer to sporulation medium for all strains, and no activity was detected. Strains S1593-S1595 are isogenic; strains S1576-S1579 are isogenic.

product, expression of the *mei4::lacZ-5'* and *mei4::lacZ-3'* fusion genes was examined in wild-type and *mer1* strains. If the MER1 protein is required for *MEI4* RNA splicing, then the level of expression of the *mei4::lacZ-3'* fusion gene (but not the *mei4::lacZ-5'* fusion gene) should be reduced in a *mer1* mutant. As shown in Table 2, expression levels of both fusion genes are unaffected by the genotype at *MER1*. Thus, the *MER1* gene product is not required for splicing the *MEI4* transcript.

Additional evidence that *MEI4* splicing is *MER1* independent was obtained by analysis of *MEI4* cDNAs derived from a *mer1* mutant. PCR was used to amplify such *MEI4* cDNAs, and the products were analyzed on an agarose gel (Fig. 3). The major product was the same size as the *MEI4* cDNA derived from wild-type meiotic cells (Fig. 3). A cloned *MEI4* cDNA derived from the *mer1* mutant was analyzed by DNA sequencing and found to be intronless.

Effect of *mei4* on meiosis-specific gene expression. One possible function of the *MEI4* gene is to regulate the expression of other genes whose products are required for meiotic recombination. To explore this possibility, the effect of a *mei4::ADE2* mutation on expression of the *SPO11*, *MER1*, *MER2*, *MEK1*, *RED1*, and *RAD50* genes was investigated. *SPO11*, *MER1*, *RED1*, and *MEK1* are expressed only in meiosis and are members of the early class of genes (2, 15, 38, 48). *MER2* produces a functional transcript only in meiosis (16), while the *RAD50* gene is expressed during vegetative growth but is induced in meiosis (18). Expression of *SPO11*, *MER1*, *MER2*, *MEK1*, and *RED1* was examined by using the appropriate *lacZ* fusion genes, either borne on plasmids or integrated into the genome in a single copy. Mitotic and meiotic levels of β-galactosidase activity were determined in isogenic *MEI4* and *mei4::ADE2* diploids carrying such fusions. In no case did the *mei4* mutation significantly affect the level of β-galactosidase activity generated by the fusion protein (Table 3). Expression of the *RAD50* gene was examined by Northern blot analysis; no difference in mitotic or meiotic levels of *RAD50* mRNA was detected when RNAs extracted from the wild type and a *mei4* mutant were compared (data not shown).

These results demonstrate that *MEI4* is not required to induce the expression of several meiotic genes examined, suggesting that the MEI4 protein is not a general transcription factor for the early class of meiotic genes. In the *mer2::lacZ* fusion used in this analysis (Table 3), *lacZ* was inserted downstream of the *MER2* intron; thus, these results also indicate that the *MEI4* gene product is not required for meiosis-specific splicing of *MER2* RNA.

***mei4* mutants are defective in SC assembly.** To examine the effect of a *mei4* mutation on chromosome synapsis, spreads of meiotic nuclei from wild-type and *mei4* strains were prepared and examined in the electron microscope as described in Materials and Methods.

Wild-type cells in meiosis I prophase could be classified into three groups based on whether they contained SC or SC-related structures. One class of nuclei, referred to as homogeneous in Table 4, contain diffusely stained chromatin and duplicated, but not yet separated, spindle pole bodies (SPBs). A second class, referred to as precursors in Table 4, contain a number of short single structures, assumed to be segments of axial elements, with occasional limited pairing to form short stretches of tripartite SC. Such morphology has been shown to precede temporally the formation of SC (1). Nuclei in the third category (called SC) are in pachytene

TABLE 3. Meiotic gene expression in *mei4* strains[a]

Strain	Relevant genotype	β-Gal (U)	Strain	Relevant genotype	β-Gal (U)
S1619	*MEI4 mer2::lacZ-3'* *MEI4 MER2*	1.2	S1607	*mei4 mer2::lacZ-3'* *mei4 MER2*	1.7
S2501	*MEI4 mek1::lacZ* *MEI4 MEK1*	42.5	S1608	*mei4 mek1::lacZ* *mei4 MEK1*	32.4
J252	*MEI4 mer1::lacZ* *MEI4 MER1*	2.8	S2512	*mei4 mer1::lacZ* *mei4 MER1*	2.6
S2502	*MEI4* p(*spo11::lacZ*) *MEI4*	2.8	S1618	*mei4* p(*spo11::lacZ*) *mei4*	3.8
S2503	*MEI4* p(*red1::lacZ*) *MEI4*	6.2	S2507	*mei4* p(*red1::lacZ*) *mei4*	7.5

[a] β-Galactosidase (β-Gal) units are expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein. Values are averages obtained from three or four independent transformants. The units represent the activity observed at 6.5 h after introduction into sporulation medium for J252 and S2512 and at 20 h for all other strains. Time courses of *mei4::lacZ* and *red1::lacZ* activity have shown that levels of expression are at or near maximum at these time points (data not shown). Strains J252 and S2501 are isogenic; all other strains are isogenic with each other but not with J252 and S2501. *MEI4* derivatives of BR2495 strains were transformed to *ADE2*, because *ade2* auxotrophy altered the timing of meiotic events (data not shown) and all *mei4* strains were *ADE2* due to the *mei4::ADE2* disruption.

TABLE 4. Effect of *mei4* on SC assembly[a]

Strain	Relevant genotype	% of nuclei (no./total)					
		Homogeneous	Precursors	Axials	SC	Meiosis I	Meiosis II
BR2495	*MEI4*/*MEI4*	34 (48/142)	25 (35/142)		18 (26/142)	17 (24/142)	6 (9/142)
S2508	*mei4*::*URA3*/*mei4*::*URA3*	34 (54/159)	27 (43/159)	21 (33/159)		18 (29/159)	

[a] Nuclei classified as homogeneous show uniform staining and contain no evidence of SC or axial elements. Nuclei classified as precursors contain multiple short stretches of axial elements; in BR2495, precursor nuclei may show some pairing of axial elements. Nuclei in the axial category contained medium to long stretches of axial elements with no apparent pairing, while those nuclei in the SC category contain fully synapsed chromosomes. In most of the prophase nuclei, duplicated by unseparated SPBs are apparent. Meiosis I nuclei contain two separated SPBs and no SC; meiosis II nuclei contain four separated SPBs and no SC. In addition to the nuclei indicated in the table, 50 nuclei from the *MEI4* strain and 25 nuclei from the *mei4* strain were examined but could not be classified because of fixation and staining artifacts and/or because of the adhesion of membrane fragments. BR2495 and S2508 are isogenic. Prespheroplasted cells harvested 20 h after introduction into starvation medium were used to prepare spreads. Because the cells were spheroplasted prior to the transfer to sporulation medium, they proceeded through meiosis more slowly than did the intact cells indicated in Fig. 7.

and contain SC, as evidenced by two lateral elements lying parallel to each other (Fig. 6A). Under the conditions used, the percentage of wild-type nuclei in pachytene reaches a maximum at about 20 h and then decreases (Table 4) (37).

In the *mei4* strain, nuclei in the precursor class did not show pairing of axial elements (Fig. 6B). Moreover, SC was never observed in preparations of nuclei from *mei4* strains despite their examination at several meiotic time points (data not shown). Instead, *mei4* strains contain a fourth class of nuclei, referred to as axials in Table 4. These nuclei have long (perhaps full-length) but unpaired axial elements (Fig. 6C and D). This morphology was not observed in preparations of wild-type cells, consistent with a previous report that synapsis is concurrent with axial element formation (1).

The proportion of *mei4* nuclei containing long unpaired axial elements is equal to the proportion of wild-type nuclei with SC, suggesting a correspondence between these phases. The timing of progression into the first meiotic division appears to be otherwise unperturbed in the *mei4* mutant (Table 4). However, the absence of nuclei undergoing the meiosis II division at 20 h prompted us to investigate more carefully the timing of the meiosis I and meiosis II divisions.

Meiosis II is delayed in *mei4* mutants. To examine the timing of the meiotic divisions, the increase in number of nuclei per cell was monitored throughout meiosis in *MEI4* and *mei4* diploids. Cells removed from sporulation medium at successive time points were fixed and stained with DAPI and then examined by fluorescence microscopy. The results of one such time course are presented in Fig. 7. While binucleate cells appear coincidentally in both wild-type and *mei4* cultures, completion of the meiosis II division is delayed by approximately 1 h in the mutant. This is evidenced by the accumulation of cells that have completed the meiosis I division (binucleates) and a delay in the appearance of both tetranucleate cells and spores. Although some *mei4* cells appeared to arrest at the binucleate stage (Fig. 7A), their level in the culture dropped to approximately 1% by 36 h (data not shown). Therefore, all cells that complete meiosis I ultimately undergo the meiosis II division in the absence of MEI4 protein. Spore formation in *mei4* strains occurs at wild-type levels.

DISCUSSION

***MEI4* expression is meiosis specific.** The results presented above demonstrate that the *MEI4* gene is expressed only in meiotic cells. A *mei4*::*lacZ* fusion gene produces detectable β-galactosidase activity only when cells heterozygous at the mating-type locus are starved for nitrogen in the presence of a nonfermentable carbon source. Northern blot hybridization analysis suggests that *MEI4* gene expression is regulated at the transcriptional level.

Previous studies have identified three classes of meiosis-specific genes, classified as early, middle, or late according to the time in meiosis at which maximum transcript accumulation is observed (2, 34, 47, 50). Studies of the *mei4*::*lacZ* fusion gene do not permit a precise analysis of *MEI4* gene regulation because the amount of activity is influenced not only by transcript accumulation but also by protein stability. However, some information can be gained by comparison of the *mei4*::*lacZ* and *red1*::*lacZ* fusions. For both fusion genes, β-galactosidase activity increases dramatically between 2.5 and 7.5 h and then decreases (*red1*::*lacZ*) or increases (*mei4*::*lacZ*) only slightly. Previous studies have demonstrated that *RED1* RNA accumulates maximally at 7.5 h after SK1 strains are introduced into sporulation medium and that the kinetics of *RED1* transcript accumulation (48) parallels that of *SPO13*, one of the early genes (50). These results suggest that *MEI4*, like *RED1* and *SPO13*, is a member of the early class of meiotic genes.

***MEI4* splicing is MER1 independent.** In *S. cerevisiae*, intron-containing genes are quite rare. The identification of introns in two genes with meiosis-specific functions, *MER2* and *MEI4*, was therefore unexpected. Previous studies demonstrated that *MER2* splicing requires the product of the *MER1* gene and suggested that there is at least one additional gene whose expression is *MER1* dependent (16). These observations raised the possibility that splicing of the *MEI4* transcript might also require MER1. However, a *mei4*::*lacZ* fusion in which *lacZ* is inserted downstream of the intron is efficiently expressed in a *mer1* mutant, demonstrating that *MEI4* splicing is MER1 independent.

The lack of a requirement for the MER1 protein in *MEI4* splicing is consistent with two other observations. First, unlike *MER2*, the *MEI4* gene is not transcribed in vegetative cells; there is therefore no apparent need for regulation at the level of splicing. Second, the splicing signal sequences in the *MEI4* intron (including the 5′ and 3′ splice junctions and the TACTAAC box) are perfect matches to the consensus sequences; it is therefore expected that the normal splicing apparatus (in the absence of the MER1 protein) should be able to process the *MEI4* primary transcript. In contrast, the *MER2* 5′ splice junction differs from the consensus sequence at one position; it is possible that the meiosis-specific *MER1*

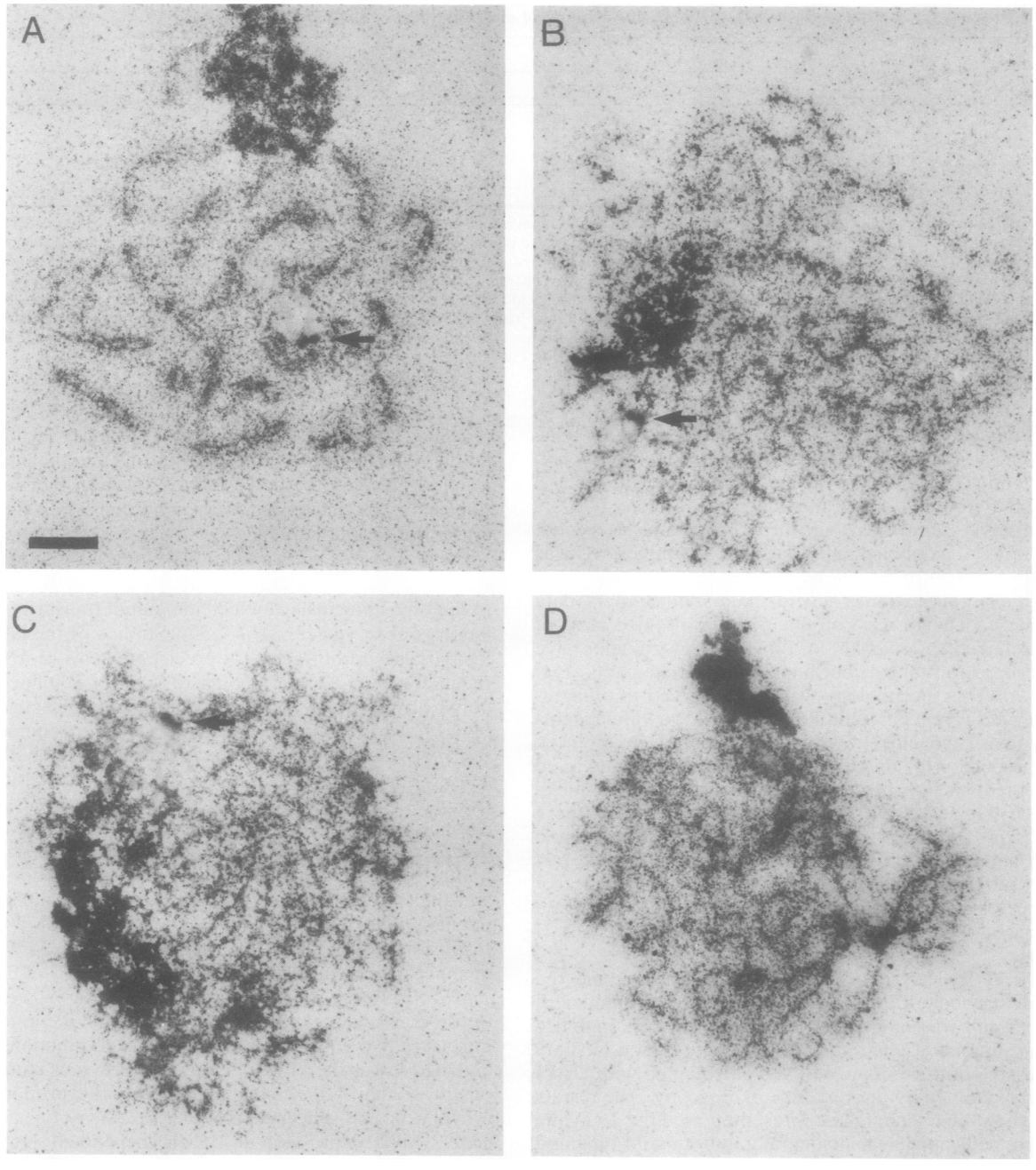

FIG. 6. Electron micrographs of meiotic nuclei from a wild-type strain (BR2495) and a homozygous *mei4*::*URA3* (S2508) diploid. (A) Wild-type nucleus in pachytene (SC class); (B) *mei4* nuclei containing segments of axial elements (precursor class); (C and D) *mei4* nuclei containing long axial elements (axial class). The darkly staining structure in each micrograph is the nucleolus. Duplicated but unseparated SPBs are indicated by arrows in panels A to C. Bar = 1 μm.

gene product is required to overcome the effect of this substitution (16).

***mei4* mutants fail to assemble SC.** Examination of spread meiotic nuclei from *mei4* strains demonstrates a defect in SC assembly. Although long axial elements develop in mutant cells, they do not align with each other to produce tripartite SC. While axial element formation and synapsis occur simultaneously in wild-type cells (1), uncoupling of these processes has now been noted in several meiotic mutants. Like *mei4*, the *mer1* mutation causes most nuclei to accumulate full-length but unpaired axial elements; however, full synapsis is observed in about 10% of *mer1* nuclei (15). The *spo11*, *rad50*, and *rec102* mutations cause a meiotic phenotype very similar to that of *mei4*, in that meiotic recombination is not induced and spore viability is restored by the *spo13* mutation (4, 21, 29, 30, 31). However, *spo11* null mutants show little or no axial element development (22), *rad50* strains develop only short to medium-length axial elements (1), and a *rec102* null mutant, like the *mei4* mutant, develops long unpaired axial elements (4). The *rad50S*

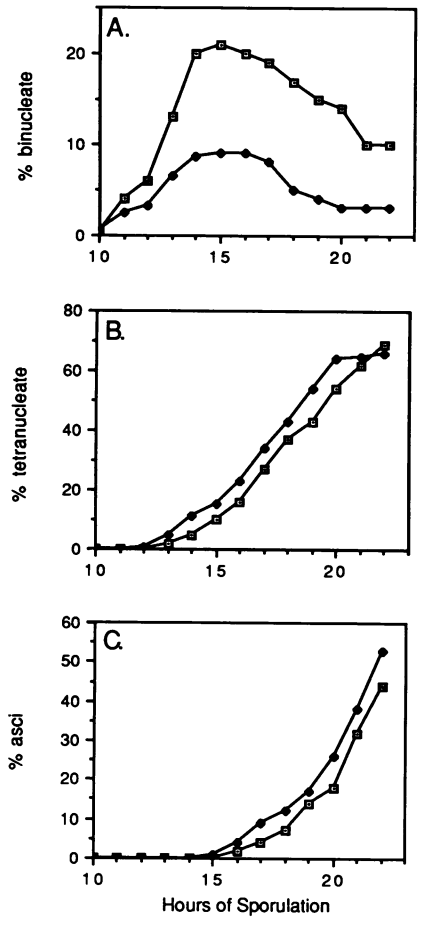

FIG. 7. Timing of the meiotic nuclear divisions. Cultures of isogenic *mei4::URA3/MEI4* (S2509) and *mei4::URA3/mei4::URA3* (S2508) diploids were sporulated in 2% KAc. At the time points indicated, cells were removed, fixed, and stained with DAPI as described by Thompson and Roeder (48). Samples were then examined by fluorescence microscopy and scored for completion of meiosis I (binucleate cells) and meiosis II (tetranucleate cells). Formation of asci was also monitored by using Nomarski interference microscopy on the same samples. A total of 500 cells were counted at each time point. Identical kinetics have been observed in duplicate cultures on two other occasions. The open and filled boxes indicate the behaviors of mutant and wild-type strains, respectively.

mutant progresses further into meiotic prophase than does the corresponding null mutant, and its spore viability is not restored by the *spo13* mutation; *rad50S* strains also produce long axial elements with rare tripartite structure (1). In this and other respects (see below), the phenotype of *mei4* is similar to that of *rec102* and intermediate to that of *rad50* and *rad50S*.

Null mutations in all of the genes just mentioned not only result in a failure to assemble SC but also cause a defect in meiotic recombination (1, 4, 14, 21, 29–31). The only mutant in which these processes are differentially affected is the *spo11-1* mutant, which is defective in recombination but does assemble SC (29). The simultaneous elimination of both synapsis and recombination by a single mutation in each of several different genes can be accounted for economically by the proposal that these processes have common prerequisites. For example, both recombination and synapsis may depend on the success of a chromosomal homology search (1, 7, 13, 39, 45).

The *mei4* mutation delays meiosis II. While *mei4* strains complete the meiosis I division at the same time as do control strains, binucleate cells accumulate and the appearance of tetranucleate cells and spores is delayed by about 1 h. Mutants with phenotypes otherwise similar to that of *mei4* show different kinetics, indicating complex regulation of the meiotic divisions. A *rec102* null mutant shows a 1- to 2-h delay in completing both the meiosis I and II divisions, coupled with a slight accumulation of binucleate cells (4). While a null mutant of *SPO11* has been reported to complete both meiotic divisions on or ahead of schedule (22), the *spo11-1* mutation seems to cause aberrations in the timing of both meiotic divisions (29). Null mutants of *rad50* experience no delay or a slight delay in meiosis II (21, 33), but like *mei4*, the *rad50S* mutant undergoes a delay in meiosis II (1). Binucleate cells accumulate to high levels in *rad50S* strains, and meiosis II SPB duplication and separation occur much later than in wild-type nuclei, leading to a defect in spore formation. Separation of duplicated meiosis I SPBs is also delayed and aberrant in several respects in *rad50S* strains (1), even though binucleate cells appear with kinetics equivalent to those of control strains. It is possible that the *mei4* mutation also affects events in meiosis I. The delay in meiosis II in *mei4*, *rec102*, and *rad50S* strains may be a secondary consequence of perturbations in chromosome metabolism during prophase I.

Role of MEI4. We have previously suggested four possible roles for the *MEI4* gene product. The *MEI4* gene might encode (i) a structural component of the SC, (ii) a regulator of other genes, (iii) a recombination enzyme, or (iv) a protein required for the homology search. Unfortunately, the *MEI4* DNA sequence has provided little insight into the function of the MEI4 protein. The observation that *mei4* mutants fail to assemble SC is consistent with all four possible functions.

The low abundance of the *MEI4* gene product makes it unlikely that the MEI4 protein is a major structural component of the SC. A *mei4::lacZ* fusion gene produces 100-fold less β-galactosidase activity than does a *red1::lacZ* fusion (Fig. 4A). A previous study indicated that the *RED1* transcript is present at low copy number (48).

It also seems unlikely that the MEI4 protein serves as a meiotic transcriptional regulator for a large group of genes, since no change in the expression of six meiotically induced genes was detected in a *mei4* background. However, MEI4 might regulate the expression of one or a few other genes not tested; it could also regulate genes posttranscriptionally.

It is possible that the *MEI4* gene product plays a catalytic role in recombination and/or synapsis. The last two hypotheses may not be mutually exclusive if recombination enzymes play a role in the homology search process, as suggested previously (7, 39, 45). The observation that the MEI4 protein is very basic raises the possibility that it has DNA-binding activity.

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