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Genetics

Isolation, DNA sequence, and regulation of a meiosis-specific eukaryotic recombination gene

(sporulation/transcription/genetic exchange/*SPO11*/*Saccharomyces cerevisiae*)

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ABSTRACT **The *SPO11* gene, required for meiotic recombination in *Saccharomyces cerevisiae*, has been cloned by direct selection for complementation of the *spo11-1* phenotype: lack of meiotic recombination and low spore viability. DNA sequencing indicates that the gene encodes a 398-amino acid protein having a predicted molecular mass of 45.3 kDa. There is no significant similarity between the SPO11 protein and other protein sequences, including those from genes known to be involved in DNA recombination or repair. Strains bearing a disruption allele are viable, indicating that *SPO11* is dispensable for mitotic growth. RNA analyses demonstrate that *SPO11* produces a 1.5-kilobase transcript that is developmentally regulated and expressed early in the sporulation process.**

Previous analysis of the *spo11-1* mutation of *Saccharomyces cerevisiae* has demonstrated (*i*) it drastically reduces meiotic recombination and ascospore viability while having no significant effect on vegetative growth or mitotic exchange (1–3); (*ii*) the low spore survival results from aneuploidy due to random segregation of homologs at meiosis I in the absence of exchange (2); (*iii*) meiotic lethality can be suppressed by *spo13-1*, a mutation that causes cells to bypass meiosis I segregation (1, 4); (*iv*) it is epistatic to other recombination (Rec) mutants, such as *rad51*, *rad52*, and *rad57*, that do not produce viable spores in the presence of *spo13-1* alone but do so when *spo11-1* is also present (ref. 5; S Klapholz, J. C. Game, and R.E.E., unpublished data); (*v*) it does not block either premeiotic DNA synthesis or synaptonemal complex formation (1); (*vi*) recombinant DNA molecules are absent in *spo11-1* strains but present in *rad52* and *rad57* mutants (6).

Based on these results, it has been proposed that the wild-type *SPO11* gene acts relatively early in the meiotic recombination process at or soon after the time of chromosome pairing. Unlike the *RAD* genes, which can affect both meiotic and mitotic recombination as well as DNA repair, the *SPO11* function appears to be meiosis specific and unrelated to repair (refs. 1 and 3; R.E.E., unpublished data; H. Roman, personal communication). Although genetic evidence clearly demonstrates that *SPO11* is essential for meiotic recombination, little is known of the nature of the gene product, its regulation, or its precise role in recombination. To gain a better understanding of the function and regulation of *SPO11*, we have cloned and undertaken a molecular analysis of this gene.‡

MATERIALS AND METHODS

Strains and General Procedures. Media and procedures for handling yeast strains used in this study have been described (1, 2, 7). Standard methods for DNA cloning and manipulation of *Escherichia coli* were used (8, 9). Procedures for RNA isolation, hybridization analysis, and quantitation of SPO11 RNA are given in ref. 7.

Cloning of *SPO11*. The *SPO11* gene was cloned by direct selection for complementation of the meiotic Rec$^-$ defect of the *spo11-1* mutation. The plasmid library used to select *SPO11* contains a partial *Sau*3A digest of strain REE526 (*MATa SPO11*) inserted into YCp19 (7). Strain BD51 (*MATa/MATα; spo11-1/spo11-1; can1/CAN1; cyh2/CYH2; HIS1/his1; hom3/HOM3; TRP5/trp5; tyr1/TYR1; ura3/ura3*) was transformed (10) with the YCp19 library, and ≈15,000 Ura$^+$ transformants were homogenized and plated as lawns on synthetic medium lacking uracil. After 2 days at 30°C, lawns were replicated to sporulation medium (SPIII). After 5 days at 34°C, the sporulation plates were replicated to synthetic medium (*i*) lacking uracil, to maintain selection for the plasmid; (*ii*) containing canavanine (can) and cycloheximide (cyh), to select for haploidization of chromosomes V and VII; and (*iii*) lacking histidine and threonine, to select for recombination on chromosome V between *his1* and *HOM3*. Can^r Cyh^r His$^+$ Hom$^+$ Ura$^+$ colonies readily occur after sporulation of a *SPO11* Rec$^+$ strain. In contrast, they are extremely rare after sporulation of a *spo11-1* Rec$^-$ strain and in vegetative cells of either background. In Rec$^+$ strains, the drug-resistance alleles segregate independently, yielding 25% spores containing both resistant alleles. In addition, recombination yields His$^+$ Hom$^+$ spores either by gene conversion at *hom3* (≈1%), or two reciprocal recombination events (1.3%), between *can1* and *hom3* (97 centimorgans) and between *HOM3* and *his1* (2.5 centimorgans). In *spo11-1* Rec$^-$ strains, viable haploid or near-haploid spores containing both drug-resistance alleles are rare (≈0.1%) and virtually always nonrecombinant. In vegetative cells, homozygosis of both *can1^r* and *cyh2^r* occurs very infrequently and requires two independent mitotic recombination events ($10^{-4} \times 10^{-4}$). This latter class is eliminated by confirming haploidization for chromosome III, containing the *MAT* locus, by testing mating competence of individual spore clones, and for chromosomes II and VII by screening for *tyr1* and *trp5* segregants, respectively.

Construction of the *SPO11* Disruption Allele. A 1.1-kilobase (kb) *Eco*RI fragment containing the *URA3* gene was inserted into the unique *Eco*RI site present in p(SPO11)19 (see Fig. 1). The resulting plasmid was digested with *Bgl* II and *Hin*dIII and a 3.3-kb fragment containing the disruption was purified and used to transform yeast strain REE209 to uracil

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Abbreviations: ARS, autonomously replicating sequence in yeast; ORF, open reading frame.

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‡This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02987).

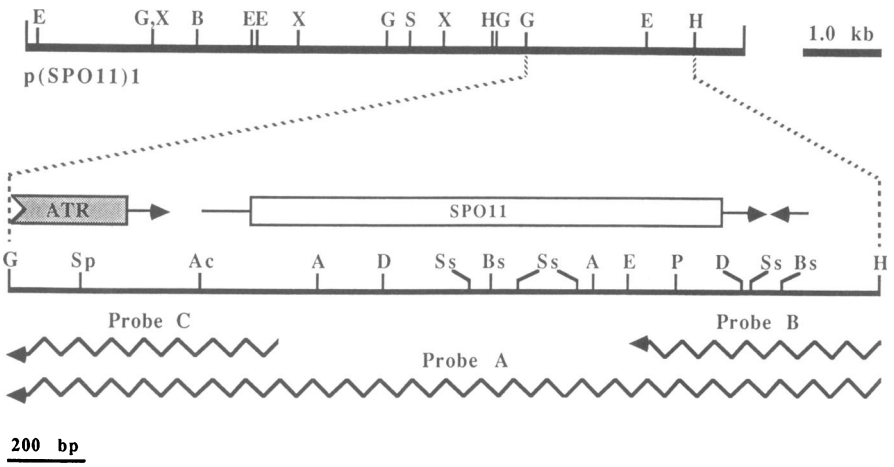

FIG. 1. Minimal subclone and transcript map of *SPO11*. The upper line gives a restriction enzyme map of the yeast DNA insert present in the original *spo11*-complementing plasmid, p(SPO11)1. The expanded map shown below represents the 2.2-kb insert contained within p(SPO11)19, the minimal *spo11*-complementing plasmid. Arrows, approximate positions of the *SPO11* and *ATR* transcripts, with ORFs indicated by open areas. Solid arrow, histidine tRNA gene (see text). Hybridization probes used in RNA mapping studies are indicated by jagged lines. A, *Ava* II; Ac, *Acc* I; B, *Bam*HI; Bs, *Bst*NI; D, *Dra* I; E, *Eco*RI; G, *Bgl* II; H, *Hin*dIII; P, *Pvu* II; S, *Sal* I; Ss, *Ssp* I; Sp, *Sph* I; X, *Xho* I.

prototrophy (11). While transformation was efficient, only 1 of 50 transformants contained the disruption allele integrated at *SPO11* (detected by DNA blot hybridization of genomic *Hin*dIII digests). Most of the remaining transformants contained the *URA3* marker on a recircularized autonomously replicating plasmid, suggesting the presence of an autonomously replicating sequence (ARS) in the *SPO11* region.

DNA Sequence Analysis. The 2.2-kb *Bgl* II/*Hin*dIII fragment containing *SPO11* was subcloned into sequencing vectors M13mp8 and M13mp9 (9); deletions in the *SPO11* insert were generated by a modification of the procedure of Hong (12). Dideoxy sequencing reactions were carried out using dATP[^{35}S] and either the large fragment of DNA polymerase I (Klenow) or reverse transcriptase (13). DNA and protein sequence manipulations and data base searches were conducted using software from the University of Wisconsin Genetics Computer Group (14).§

RESULTS

Direct Selection and Subcloning of the *SPO11* Gene. The *SPO11* gene was identified on a recombinant DNA plasmid by transformation of a *spo11-1/spo11-1* diploid with a YCp19 library followed by direct selection for meiotic recombination and efficient haploidization (see *Materials and Methods* for details). One of six transformants that initially satisfied the selection criteria contained a plasmid, designated p(spo11)1, capable of conferring an Spo11$^+$ phenotype after repassage through *E. coli* (Fig. 1). The region complementing the *spo11-1* mutation was localized within a 9.4-kb insert by subcloning in YCp19 or YCp50 (15) and retesting in the original *spo11-1/spo11-1* diploid. The smallest subclone having *spo11*-complementing activity, p(SPO11)19, contains a 2.2-kb *Bgl* II/*Hin*dIII insert (Fig. 1); subclones that end at the unique *Eco*RI site of this insert fail to complement *spo11-1*. DNA blot analysis of genomic digests indicated that *SPO11* is a single copy gene (data not shown).

***SPO11* Is Dispensable During Vegetative Growth.** A haploid strain containing a genomic disruption was constructed as described in *Materials and Methods* and crossed to *spo11-1* and *SPO11* testers. Analysis of these diploids indicated that the disruption allele, *spo11-D1*, confers a Spo11$^-$ phenotype: (*i*) it fails to complement the *spo11-1* mutation, (*ii*) homozygous diploids are viable during mitosis and rescued from meiotic lethality by the *spo13-1* mutation, and (*iii*) heterozygotes are Spo11$^+$, indicating that it is recessive (Table 1). Analysis of three disruption heterozygotes yielded high spore viability (94%) and recombinant products. In all tetrads examined (18/18), the *URA3* marker and the Spo11$^-$ phenotype cosegregated, indicating that the disruption allele maps at the chromosomal location of *SPO11*. These results confirm the location of the *SPO11* gene within the *Bgl* II/*Hin*dIII fragment of p(SPO11)19 and clearly demonstrate that *SPO11* is dispensable for vegetative division.

***SPO11* Encodes a Protein of 398 Amino Acids with No Significant Sequence Similarity to Any Known Gene.** The 2.2-kb minimal fragment containing *SPO11* function was recloned and sequenced by the dideoxy-chain termination method (Fig. 2). This fragment contains a single long open reading frame (ORF) that encompasses the *Eco*RI site used to create the *spo11-D1* disruption. This ORF is 1194 base pairs (bp) long and encodes a 398-amino acid protein having

Table 1. Disruption phenotype

Strains	% asci* 25°C	30°C	34°C	Rec phenotype†	% spore viability
SPO11/*spo11-1*	82	78	68	+	84‡
SPO11/*spo11-D1*	61	83	75	+	94
spo11-1/*spo11-1*	25	22	18	−	<1
spo11-D1/*spo11-D1*	20	12	8	−	<1
spo11-1/*spo11-D1*	22	8	4	−	<1
SPO11 *spo13-1*/*spo11-1* *spo13-1*	44	62	49	+	53
SPO11 *spo13-1*/*spo11-D1* *spo13-1*	72	74	57	+	43
spo11-D1 *spo13-1*/*spo11-D1* *spo13-1*	67	70	47	−	93
spo11-1 *spo13-1*/*spo11-D1* *spo13-1*	95	84	28	−	90

*Data represent the average of 1–3 independent crosses.
†Recombination phenotype was determined for *spo11/spo11 SPO13/SPO13* strains from rare viable meiotic products after sporulation at 34°C as described (2) and by ascus dissection for all other strains. The level of recombination in *spo11*-containing strains was negligible and similar to that reported (1, 2).
‡Data from ref. 1.

§Sequence banks searched were: EMBL/Genbank Genetic Sequence Database (1986) Genbank (Bolt, Beranek, and Newman Laboratories, Cambridge, MA), Tape Release 46; EMBL/Genbank Genetic Sequence Database (1986) EMBL (Eur. Mol. Biol. Lab., Heidelberg), Tape Release 9; and Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 29.

a predicted molecular mass of 45.3 kDa. Inspection of the protein sequence indicates that *SPO11* does not contain potential Zn^{2+}-binding domains or a helix-turn-helix motif. A second partial ORF of 300 bp is present upstream of *SPO11* and represents the carboxyl terminus of an adjacent gene (see below). The presumed initiating AUG of *SPO11* is separated by 307 bp from the termination codon for this upstream ORF, and the DNA in the interval between the two ORFs is highly A+T rich (75%).

Neither the *SPO11* coding strand nor the predicted amino acid sequence shows extensive similarity to sequences available in the Genbank, NBRF, or EMBL data banks. In particular, no homology was found between *SPO11* and other genes known to be involved in DNA recombination or repair (e.g., *E. coli RecA; S. cerevisiae RAD1, RAD3, RAD6, RAD50*, and *RAD52*). A comparison of the noncoding strand to the sequence banks revealed perfect homology to a 505-base sequence surrounding and including a histidine tRNA gene 137 bp downstream from the termination codon for *SPO11* (16). In addition, a perfect match to the ARS core consensus sequence 5′ $^{A}_{T}$TTTAT$^{A}_{G}$TTT$^{A}_{T}$ 3′ (19) and a 9/11-bp match to the 3′ ARS consensus element 5′ CTTTTAG-C$^{AAA}_{TTT}$ 3′ (20) were found on the complementary strand (underlined in Fig. 2). These sequences may be responsible for the ARS plasmids formed during construction of the disruption allele (see *Materials and Methods*).

Characterization of the SPO11 Transcript. The smallest subclone complementing the *spo11-1* mutation, p(SPO11)19, hybridized to two RNAs of 1.6 and 1.5 kb (Fig. 3). The 1.6-kb RNA was most abundant when cells were first introduced into sporulation medium from logarithmic phase acetate growth ($T = 0$) and decreased during sporulation. In contrast, the 1.5-kb RNA was undetectable in vegetative cells (i.e., at $T = 0$) and increased at later times. Both the 1.5- and 1.6-kb RNAs were retained on poly(U)-Sepharose, suggesting that both are polyadenylylated (data not shown).

Nuclease S1 analysis using three single-stranded DNA probes (diagrammed in Fig. 1) demonstrated that the 1.5-kb RNA represents the *SPO11* transcript, while the 1.6-kb RNA, encoded by the same strand, is derived from an adjacent gene. When either probe A or probe C was hybridized to RNA from vegetative cells, a major fragment of 400 bases and less abundant fragments of 380 and 440 bases were protected (Fig. 4). These fragments represent multiple 3′ ends of the 1.6-kb RNA and define its position at the left end of p(SPO11)19, upstream of *SPO11*. Consistent with this conclusion is the observation that probe B failed to hybridize to this RNA (Fig. 3). The 1.6-kb RNA, designated *ATR* for *a*djacent *tr*anscript, presumably encodes the partial ORF

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-608  AGATCTAAATAATAATCTGGACTTGAATTTAATTACCACCCCAGCAAAACTAACAGAGAGATTTTTGTTTAAGTCTTTCATTAGGGGGTTGAACTTGTTCGAGAGTCATATCAATGACT

-489  TCAACAATCAATTTAGATTGCAGTTGCCCGAAAACTGTTGTGTTATCTTTAACAACAGGAGAATTTTGCATGCTAACTCTTTAACAAGCTCAAACCAGCAATGGTTAAAGGGTTGCTATT

-369  TCGATTCTGATACTTTCAAGAGTAAATTAAAGTTCTTGGAAGAGAAGTTTCCTCATGACAAATAACTTCATTTTTTCAGCATAGTTATGTGATTCTTTATATTTATTTATTTTCTCTCTG
                    >                   >                                        >     *                                   *
-249  TGTAGTGACAATTTTTTTTTTAATGTTTCATGTTAAATATATTTATTTTAAGAAGCCGTCAGTTTCCTTTTGTTTCATTGAAATCAATAACTAAAATTAAAATTATAAAAGGAATAACGT

-129  AAAAGAAAGTCTACTACCCAAGGAAAATCTACTTTATTTAGTGATTATATCCAACTAAATATCATTCAAATAACCTCACATATTTGTCTTCACCCTTAAGATTTTACGATTTACTAAGTT
               +1
  -9  CACCTTCTCATGGCTTTGGAGGGATTGCGGAAAAAATATAAAACAAGGCAGGAATTGGTCAAAGCACTCACTCCTAAAAGACGGTCCATTCACTTGAACTCCAATGGTCACTCCAACGGA
               MetAlaLeuGluGlyLeuArgLysLysTyrLysThrArgGlnGluLeuValLysAlaLeuThrProLysArgArgSerIleHisLeuAsnSerAsnGlyHisSerAsnGly   37

 112  ACTCCCTGTTCAAACGCAGATGTTTTGGCTCATATTAAGCATTTCCTGTCATTGGCGGCTAATTCATTAGAGCAACATCAACAGCCTATTTCAATCGTCTTTCAAAACAAAAAAAAAAAA
      ThrProCysSerAsnAlaAspValLeuAlaHisIleLysHisPheLeuSerLeuAlaAlaAsnSerLeuGluGlnHisGlnGlnProIleSerIleValPheGlnAsnLysLysLysLys   77

 232  GGCGATACAAGCAGTCCTGACATTCACACAACATTGGACTTCCCTTTGAATGGCCCGCATCTATGCACTCATCAGTTCAAGTTGAAAAGATGCGCAATCCTTTTAAACTTATTGAAAGTC
      GlyAspThrSerSerProAspIleHisThrThrLeuAspPheProLeuAsnGlyProHisLeuCysThrHisGlnPheLysLeuLysArgCysAlaIleLeuLeuAsnLeuLeuLysVal  117

 352  GTTATGGAAAAATTACCGCTAGGTAAAAACACTACAGTGAGAGATATCTTCTACTCCAACGTGGAATTGTTTCAAAGACAAGCAAACGTAGTCCAGTGGCTGGACGTTATACGCTTTAAT
      ValMetGluLysLeuProLeuGlyLysAsnThrThrValArgAspIlePheTyrSerAsnValGluLeuPheGlnArgGlnAlaAsnValValGlnTrpLeuAspValIleArgPheAsn  157

 472  TTCAAGCTCTCTCCAAGAAAATCCTTAAACATTATACCAGCTCAAAAGGGTTTAGTTTATTCGCCTTTCCCCATTGATATTTATGACAATATTCTGACATGTGAAAATGAACCAAAGATG
      PheLysLeuSerProArgLysSerLeuAsnIleIleProAlaGlnLysGlyLeuValTyrSerProPheProIleAspIleTyrAspAsnIleLeuThrCysGluAsnGluProLysMet  197

 592  CAAAAGCAAACAATTTTCCCTGGTAAGCCCTGTCTAATTCCATTTTTCCAAGATGATGCGGTCATCAAGTTAGGGACAACAAGTATGTGTAATATTGTAATAGTGGAAAAAGAAGCTGTC
      GlnLysGlnThrIlePheProGlyLysProCysLeuIleProPhePheGlnAspAspAlaValIleLysLeuGlyThrThrSerMetCysAsnIleValIleValGluLysGluAlaVal  237

 712  TTCACCAAATTAGTAAATAATTATCACAAGTTGAGTACAAATACCATGCTCATTACAGGTAAGGGATTTCCAGATTTCTTGACAAGGTTATTCCTAAAAAAACTAGAACAATATTGCTCC
      PheThrLysLeuValAsnAsnTyrHisLysLeuSerThrAsnThrMetLeuIleThrGlyLysGlyPheProAspPheLeuThrArgLeuPheLeuLysLysLeuGluGlnTyrCysSer  277

 832  AAATTGATATCGGACTGTTCTATATTTACCGATGCGGACCCCTATGGGATTAGCATAGCCCTAAATTATACTCACTCGAATGAACGCAACGCTTATATTTGCACGATGGCAAACTATAAA
      LysLeuIleSerAspCysSerIlePheThrAspAlaAspProTyrGlyIleSerIleAlaLeuAsnTyrThrHisSerAsnGluArgAsnAlaTyrIleCysThrMetAlaAsnTyrLys  317

 952  GGAATTCGTATTACGCAAGTTTTGGCACAAAATAATGAAGTGCATAACAAATCCATTCAATTATTGAGTTTGAATCAGCGCGACTACTCCTTAGCCAAGAATTTGATAGCATCTCTGACT
      GlyIleArgIleThrGlnValLeuAlaGlnAsnAsnGluValHisAsnLysSerIleGlnLeuLeuSerLeuAsnGlnArgAspTyrSerLeuAlaLysAsnLeuIleAlaSerLeuThr  357

1072  GCCAACAGCTGGGATATTGCAACTTCACCATTAAAGAACGTCATCATAGAATGTCAGCGGGAAATTTTTTTCCAAAAGAAAGCTGAAATGAACGAGATTGATGCCAGAATTTTTGAATAC
      AlaAsnSerTrpAspIleAlaThrSerProLeuLysAsnValIleIleGluCysGlnArgGluIlePhePheGlnLysLysAlaGluMetAsnGluIleAspAlaArgIlePheGluTyr  397
                                                                                                                   >        >
1192  AAATGAATAAGAATGGGAGCTGTTGCTTTATAAAAAATGTTTTTCAAGAATTGAAAACGGTTACATTTAAATATTATATAAATTGAAATCTTTGATAATGAATACTAGTCCAAAAAAAAA
      LysEnd
         >
1312  TACAATTTCAAAAAAATAAATGCCATCTCCTAGAATCGAACCAGGGTTTCATCGGCCACAACGATGTGTACTAACCACTATACTAAGATGGCATATGATTTTACAATGATTCGATGGTGA

1432  ATGAGTTATAAGCCATGTAAGGATCACTAGTCTTTTTCTTAAGTAACTTTTCGCTTGTGATATCTTGAAATACCATTAAACATAGATAAGCTATTGAAATGTGAAGAAAATTCAAAAA

1552  AAAAAGGGAACAGAGTCATCTTTTGGGTAGAAACTAAGCTT
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FIG. 2. DNA and predicted amino acid sequence of *SPO11*. Numbers on right, *SPO11* amino acid sequence; numbers on left, DNA sequence, with position +1 being the first base of the presumed *SPO11* initiating AUG. The next in-frame AUG occurs at +355. The 3′ end of the *SPO11* ORF occurs at position +1194. The ORF of the *ATR* transcript terminates with a TAA codon at position −307. Sequence information between positions +1023 and +1527 is in perfect agreement with that published (16); the histidine tRNA is overlined. Transcription start sites in yeast have been shown to be preferentially located at or near the consensus sequence RRYRR (where R = purine and Y = pyrimidine) (17). In agreement with this observation, the sequence AATAA is located at −137 and −164 bp (italics) from the *SPO11* AUG, and the major and minor 5′ ends of the *SPO11* transcript have been mapped to approximately −134 and −162 bp (asterisks). Transcription termination in yeast has been associated with the tripartite signal TA(T)G . . . TA(T)GT . . . TTT (18). Sequences resembling this signal are found at the 3′ end of *SPO11* at positions +1287, +1297, and +1317, and at the 3′ end of *ATR* at positions −288, −284, and −274 relative to the *SPO11* AUG. Approximate 3′ ends of both *SPO11* and *ATR* transcripts are indicated by >. Homologies to the *ARS* core and 3′ consensus sequences are underlined.

identified by DNA sequencing at the left end of subclone p(SPO11)19.

The 1.5-kb RNA, present only in sporulating cells, hybridized to all three probes. Probe A yielded a protected fragment of >1 kb in addition to the three smaller fragments protected by the *ATR* transcript (data not shown). Probe B gave two major protected fragments of 340 and 350 bases and a minor fragment of 370 bases, and probe C gave a major protected fragment of 180 bases and two minor fragments of 190 and 210 bases (Fig. 4). These data indicate that the major and two minor 5′ ends of the *SPO11* transcript (defined by probe C) lie approximately 133, 143, and 163 bases, respectively, upstream of the initiating AUG codon, and the 3′ ends (defined by probe B) lie approximately 98, 108, and 128 bases downstream of the termination codon. This analysis indicates that the full length of the predominant *SPO11* RNA is 1.44 kb, in close agreement with the 1.5 kb measured from RNA gel transfers.

Expression of *SPO11* Is Developmentally Regulated. The amount of *SPO11* RNA was quantitated in sporulating and nonsporulating cultures by nuclease S1 analysis of total RNA samples hybridized to probe B. In all cases examined, logarithmic phase cultures grown in either glucose (data not shown) or acetate (see below) showed levels of *SPO11* RNA near the limit of detection, estimated to be ≈1 molecule of *SPO11* RNA per 20 cells.

The amount of *SPO11* RNA increased significantly when *MAT***a**/*MATα* strains were transferred to sporulation medium. In strain SK1, which sporulates synchronously and efficiently (84% asci by 30 hr), an increase in transcript levels was detected early in sporulation (1.5 hr). A maximum 70-fold increase to a level of ≈5 molecules per cell occurred at 4.5 hr, approximately the time of the meiosis I division as monitored by the appearance of binucleate cells. *SPO11* RNA then decreased to nearly undetectable levels by the time of ascospore maturation (10–12 hr; Fig. 5). The pattern of *SPO11* expression was similar in two other *MAT***a**/*MATα* strains examined (Z270 and LM1), although the exact magnitude and timing of the increase varied (data not shown), probably due to differences in synchrony and sporulation efficiency (40% and 60% asci for Z270 and LM1, respectively, by 30 hr).

To determine whether the increase of *SPO11* RNA in *MAT***a**/*MATα* strains is sporulation specific or simply a starvation response, RNA samples were analyzed from strains homozygous at the *MAT* locus, which fail to undergo premeiotic DNA synthesis and subsequent events in sporulation medium (21, 22). Both *MAT***a**/*MAT***a** and *MATα*/*MATα* strains showed a small transient increase in *SPO11* transcript

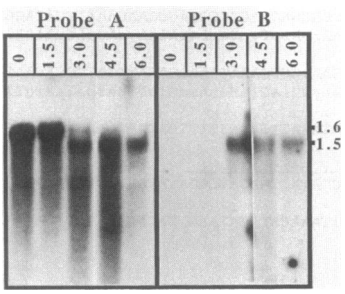

FIG. 3. RNA blot hybridization analysis of the *SPO11* region. RNA was prepared from strain SK1 at the indicated times (hr) after transfer to sporulation medium. After denaturation with glyoxal, 15 μg of total RNA from each time point was fractionated on a 1.4% agarose gel and the gel was blotted to nitrocellulose. The blot was hybridized to ^{32}P-labeled RNA probes generated by SP6 polymerase (Fig. 1). The size standards used were *Pvu* II fragments of pBR322 or *Hin*fI fragments of YRp7. Calculated lengths of the RNAs are given to the right.

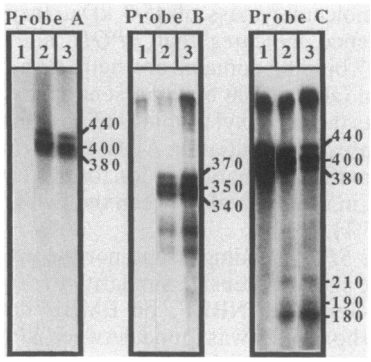

FIG. 4. Nuclease S1 mapping of the 1.5- and 1.6-kb transcripts. ^{32}P-labeled single-stranded DNA probes (Fig. 1) were hybridized to total RNA samples, digested with nuclease S1, and fractionated on 5% acrylamide/urea gels. Probe A contains the entire p(SPO11)19 insert, probe B contains the right end of p(SPO11)19 including the last 242 bases of the *SPO11* ORF, and probe C contains the left end of p(SPO11)19, encompassing the 3′ end of the adjacent gene and the first 47 bases of the *SPO11* ORF. The size standards used were ^{35}S-labeled *Hin*fI fragments of YRp7. Approximate sizes of the protected fragments are given on the right, rounded to the nearest 10 bases. Size measurements are accurate to ±5%. Probe A, 100-μg samples: lane 1, *E. coli* tRNA; lanes 2 and 3, Z270 logarithmic phase in glucose. Probes B and C, 25-μg samples, SK1 at various times of sporulation: lane 1, 0 hr; lane 2, 3 hr; lane 3, 4.5 hr. Bands obtained with probe B that are smaller than 300 bases may represent additional 3′ ends of *SPO11* or may result from artifactual nuclease S1 cleavages within A+T-rich regions of the RNA·DNA hybrids.

levels at 1.5 hr, reaching only 5–10% of the peak level in a near isogenic *MAT***a**/*MATα* strain before returning to vegetative levels (compare Fig. 6*A* with Fig. 6 *B* and *C*). Maximal expression of *SPO11* RNA thus requires heterozygosity at the mating-type locus as well as nutrient deprivation. In contrast to *SPO11*, levels of RNA from the 5′ adjacent gene (*ATR*) decreased during sporulation of SK1 (Fig. 5) as well as in *MAT***a**/*MAT***a** and *MATα*/*MATα* diploids (data not shown). Since this occurred irrespective of *MAT* genotype, it most likely represents a starvation response.

DISCUSSION

Molecular analysis of the cloned *SPO11* gene is consistent with the previous genetic data demonstrating that *SPO11* encodes a meiosis-specific Rec function. First, the *spo11-D1*

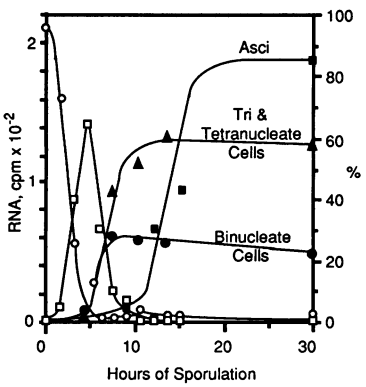

FIG. 5. Analysis of *SPO11* RNA during sporulation of *MAT***a**/*MATα* diploids. Total RNA samples (25 μg) prepared from strain SK1 at the indicated times after transfer to sporulation medium were analyzed with probe B as in Fig. 4. The amount of each protected fragment was quantitated by cutting out the appropriate region of the gel and measuring Cerenkov counts per minute. Open symbols, quantitation of RNA (○, *ATR*; □, *SPO11*); solid symbols, sporulation landmarks of SK1.

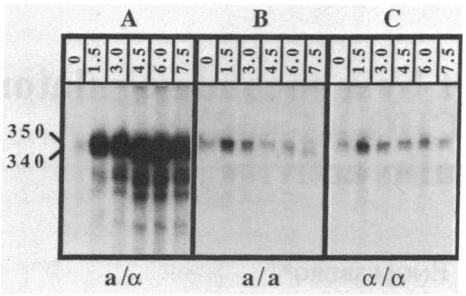

FIG. 6. *SPO11* RNA in *MATa/MATa* and *MATα/MATα* diploids under sporulation conditions. RNA samples (50 μg total) were analyzed with probe B as in Fig 4. The time (hr) after transfer to sporulation medium is shown at the top. *MATa/MATa* (DL171-477) and *MATα/MATα* (DH8-303) strains were derived by recombination from the *MATa/MATα* (Z270) strain. Gel exposures were for 78 hr on preflashed Kodak XAR-5 film using an intensifying screen.

disruption mutation, which presumably eliminates *SPO11* function, behaves similarly to *spo11-1*: (*i*) it is recessive, (*ii*) only ≈1% of the spores are viable and these are nonrecombinant, and (*iii*) a decrease by a factor of >100 in recombination is observed in the *spo13-1* meiosis I bypass system where spore viability is high. Second, both haploids and homozygous diploids carrying the *spo11-D1* mutation are viable during vegetative growth, indicating that the gene is dispensable for mitotic division. Third, the developmental regulation of *SPO11*—i.e., the extremely low level of *SPO11* RNA in vegetative cultures and substantial increase during sporulation—is compatible with the observation that it is not required for mitotic recombination (1, 3) and is suggestive of a meiosis-specific function.

In addition to *SPO11*, enhanced expression during sporulation has also been observed for several other genes required for meiosis—i.e., *SPS1*, *SPO12*, and *SPO13* (refs. 7 and 23; see below), implicating transcriptional control as a major regulatory mechanism during meiosis. The magnitude of the increase generally suggests that a higher transcription rate is at least partly responsible, although stabilization of RNA during sporulation may be a contributing factor. At present, genes whose expression is induced during sporulation may be grouped into at least three induction classes: early, middle, and late, based on the times at which increases in RNA are first seen (24–29). Analysis of *SPO11* and *SPO13* transcripts in the same RNA samples indicates that their patterns of regulation are indistinguishable and that they are members of the early class. Since *SPO11* and *SPO13* are required for meiotic events that occur at different times—i.e., recombination and meiosis I segregation—this indicates that the time of induction does not necessarily correlate directly with the time that the mutant defect is first detected. Additional support for this conclusion comes from analysis of the *SPO12* gene, which like *SPO13* is required for completion of the meiosis I division but is induced and exhibits maximum transcript levels 2–3 hr later (ref. 7; R.E.E. and R.T.E., unpublished data).

In addition to their similar pattern of expression in sporulating *MATa/MATα* cells, *SPO11* and *SPO13* also respond identically to nonsporulating conditions, raising the possibility that they are coregulated genes. In strains homozygous at the *MAT* locus, both exhibit a small transient increase in transcript levels. This increase in *SPO11* RNA (to 0.3 molecule per cell) probably has no functional significance since no increase in recombination is seen under these conditions (21, 22). As discussed more fully elsewhere (7), it most likely represents a direct response to the starvation conditions of sporulation medium. Thus far, sequence comparisons of the 5′ regions of *SPO11* and *SPO13* have revealed no striking similarities that might represent common regulatory elements responding to either a starvation signal or to *MAT* control.

One final point may be made about the regulation of *SPO11*. The major 5′ end of the *SPO11* transcript seems surprisingly close to the 3′ end of the *ATR* transcript, being separated by only ≈70 bp. Although regulatory sequences could potentially be located within the transcribed region of the *SPO11* gene, upstream sequences specifying regulation of *SPO11* must either lie within the small intergenic region or be located within the DNA transcribed by the *ATR* gene. Preliminary evidence suggests that *ATR* transcription probably plays no role in *SPO11* regulation, since strains containing a chromosomal disruption of this gene are viable and exhibit wild-type growth and sporulation (C.L.A., unpublished data).

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