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Developmental Regulation of *SPO13*, a Gene Required for Separation of Homologous Chromosomes at Meiosis I

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Previous studies have demonstrated that the *SPO13* gene is required for chromosome separation during meiosis I in *Saccharomyces cerevisiae*. In the presence of the *spo13-1* nonsense mutation, *MAT*a/*MAT*α diploid cells complete a number of events typical of meiosis I including premeiotic DNA synthesis, genetic recombination, and spindle formation. Disjunction of homologous chromosomes, however, fails to occur. Instead, cells proceed through a single meiosis II-like division and form two diploid spores. In this paper, we report the cloning of this essential meiotic gene and an analysis of its transcription during vegetative growth and sporulation. Disruptions of *SPO13* in haploid and diploid cells show that it is dispensible for mitotic cell division. Diploids homozygous for the disruptions behave similarly to *spo13-1* mutants; they sporulate at wild-type levels and produce two-spored asci. The DNA region complementing *spo13-1* encodes two overlapping transcripts, which have the same 3′ end but different 5′ ends. The major transcript is 400 bases shorter than the larger, less abundant one. The shorter RNA is sufficient to complement the *spo13-1* mutation. While both transcripts are undetectable or just barely detectable in vegetative cultures, they each undergo a >70-fold induction early during sporulation, reaching a maximum level about the time of the first meiotic division. In synchronously sporulating populations, the transcripts nearly disappear before the completion of ascus formation. Nonsporulating cells homozygous for the mating-type locus show a small increase in abundance (<5% of the increase in sporulating cells) of both transcripts in sporulation medium. These results indicate that (i) expression of the *SPO13* gene is developmentally regulated and (ii) starvation alone, independent of the genotype at *MAT*, can trigger initial induction.

In eucaryotes, the process of meiosis plays a central role in generating genetic diversity and ensuring the proper number of chromosomes in cells that participate in sexual reproduction. This is accomplished through the coordinated induction of relatively high levels of genetic recombination and segregation of homologous chromosomes (i.e., haploidization) in two successive cell divisions. As an alternative to the mitotic cell cycle, meiosis also can be viewed as a program of cellular differentiation. In recent years, genetic analyses of the yeast *Saccharomyces cerevisiae* have provided significant insights into the role of specific genes in various stages of this developmental process (reviewed in references 3 and 6). In *S. cerevisiae*, meiosis is initiated by nitrogen starvation in the presence of a nonfermentable carbon source and is coupled to the formation of heat-resistant spores. The mating-type genes are one set of major regulatory genes determining whether meiosis and sporulation occur. Both *MAT*a and *MAT*α are required for the initiation of the meiotic divisions. They are thought to exert their effects through a regulatory cascade that involves the formation of a negative regulator from the products of the *MAT*a*1* and *MAT*α*2* loci (reviewed in references 11 and 33). This regulator has recently been shown to turn down the expression of another control gene, *RME1*, that is believed to negatively regulate specific genes required for sporulation (16, 31, 37). Other genes (*SPD1*, *SPD2*, *SPD4*, *SPO50*, *SPO51*, *SPO53*, *CDC25*, *CDC35*, *CYR1*, and *RAS2*) have been shown to be involved in the initiation of meiosis and sporulation in response to nutritional limitation (3, 26, 39, 43). Many *CDC* genes, which are required for the mitotic cell division cycle, and *RAD* genes, which are involved in DNA repair, are also required for meiosis and sporulation (reviewed in references 8 and 36).

In contrast to the genes which are indispensable for both mitosis and meiosis, the *SPO* genes are essential for normal meiosis and sporulation but are not essential for mitotic cell division. More than 30 *spo* (sporulation-defective) mutations have been isolated, and it is estimated that there may be as many as 200 *SPO* genes (5, 6, 45; R. Esposito, unpublished data). In the last few years, considerable progress has also been made in the identification of transcripts and proteins made specifically in sporulating cells (1, 9, 15, 21, 34, 48), although the functional significance of most of them is not known (see Discussion). These results, taken together, suggest that meiosis and sporulation are complex processes involving a large number of gene products, some required specifically for meiosis and others also required for the mitotic cell cycle, and that there is substantial transcriptional regulation during this process.

The *spo13-1* mutation has a particularly intriguing meiotic phenotype with respect to its effect on the meiosis I chromosome distribution. The recessive *spo13-1* ochre mutation, isolated from a naturally occurring variant, causes cells to undergo an atypical meiosis consisting of one rather than two meiotic divisions (17, 18). During this single division, *spo13-1* homozygous diploids execute some of the early landmark events of meiosis I, including premeiotic DNA synthesis as well as chromosome pairing and recombination. Proper segregation of homologs at meiosis I, which typically follows and depends upon recombination, however, is elim-

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TABLE 1. Genotypes of strains

Strain	Genotype	Origin
JK11-7B	*MATα ade2 arg4-16,36 aro7 his7 leu2-3,112 lys2 spo13-1 trp1 ura3-1*	This study
REE526	*MAT***a** *his4-713 lys2-20 SUF8-1*	M. Culbertson
K399-5C	*MAT***a** *ade2 his6 leu2 lys1 lys2 aro7 spo13-1 ura3*	S. Klapholz
K399-1D	*MATα ade2 his6 leu2 lys1 trp1 aro7 spo13-1 ura3*	S. Klapholz
K210-9C	*MAT***a** *ade2 ade5 can1*^{r} *his7 leu1 lys2-1 met13 spo13-1 trp5 tyr1 ura3-3*	S. Klapholz
JK12-2D	*MAT***a** *arg4-16,36 aro7 lys1 lys2 met4 spo13-1 ura3-1*	This study
K260-4B	*MATα ade2 cyh2*^{r} *his7 leu1 lys2 met13 spo13-1 trp5 tyr1-2 ura3-1*	S. Klapholz
K264-10D	*MAT***a** *ade2 cyh2*^{r} *his7 leu1 lys2 met13 trp5 tyr1 ura3-1*	S. Klapholz
K262-13A	*MATα ade2 ade6 cyh2*^{r} *his7 hom3 leu1 lys2 met13 trp5 tyr1 ura3-1*	S. Klapholz
W1-10C	*MATα ade2 ade6 cyh2*^{r} *his7-1 leu1-c lys2 met13 spo13::URA3(D1) trp5 tyr1 ura3-1*	This study
REE775	*MAT***a** *ade5 arg4 gal2 his5 leu1-2 lys7 pet17 thr1 trp1 ura3*	Yeast Genetics Stock Center
REE968	K262-13A with *spo13::URA3(D1)*	This study
REE969	K264-10D with *spo13::URA3(D1)*	This study
REE970	K262-13A with *spo13::URA3(D2)*	This study
REE971	K264-10D with *spo13::URA3(D2)*	This study
REE972	K262-13A with *SPO13::URA3(D3)*	This study
REE973	K264-10D with *SPO13::URA3(D3)*	This study
LM1	*MAT***a** *ade1 ade2 ADE5 CAN1 gal1 his7 LEU1 lys2 MET13 TRP5 tyr1 ura1 URA3* / *MATα ADE1 ade2 ade5 can1*^{r} *GAL1 his7 leu1 lys2 met13 trp5 tyr1 URA1 ura3*	S. Lindquist
Z270	*MAT***a** *ade2 ADE5 ade6 CAN1 cly8 cyh2*^{r} *his7 leu1 lys2 met13 trp5 tyr1 URA3* / *MATα ade2 ade5 ADE6 can1*^{r} *CLY8 CYH2 his7 leu1 lys2 met13 trp5 tyr1 ura3*	D. Plotkin
SK1	*MAT***a** *HO* / *MATα HO*	R. Roth
DL171-477	*MAT***a** *ade2 ADE5 ade6 cly8 cyh2*^{r} *his7 leu1 LYS2 met13 trp5 tyr1 URA3* / *MAT***a** *ade2 ade5 ADE6 CLY8 CYH2 his7 leu1 lys2 met13 trp5 tyr1 ura3*	D. Plotkin
DH8-303	*MATα ade2 ade6 CAN1 cly8 cyh2*^{r} *HIS7 leu1 lys2 met13 trp5 tyr1 URA3* / *MATα ade2 ADE6 can1*^{r} *CLY8 CYH2 his7 leu1 lys2 met13 trp5 tyr1 ura3*	D. Plotkin

inated. The cells do not terminate development at this stage but instead bypass completion of the first division and skip directly to a meiosis II-like division in which each chromosome divides equationally. Two diploid spores result from this unusual single division, which includes features of both meiosis I and meiosis II. In double-mutant studies with the *spo11-1* or *rad50-1* mutation, each of which prevents meiotic recombination, it was demonstrated that chromosome pairing and homologous recombination are not essential for the *spo13-1* single division (20, 24). The observation that the *spo13-1* mutation permits haploid cells that express both mating types to sporulate and form asci containing two haploid spores also supports the conclusion that pairing and recombination of homologs are not required for *spo13-1* meiosis (46). The *spo13-1* mutation thus allows a meiosis II-like division to occur with single, unpaired chromosomes and without prior completion of meiosis I. At least three possible roles may be postulated for the wild-type *SPO13* gene product during meiosis: (i) in meiosis I spindle development or attachment of homologous chromosomes with unduplicated centromeres to the meiosis I spindle apparatus, (ii) in regulation of centromere duplication, and (iii) in regulation of the initiation of meiosis II. No effect of the *spo13-1* mutation on mitotic cell division has thus far been detected (S. Klapholz and R. E. Esposito, unpublished data).

To gain a better understanding of the manner in which *SPO13* controls chromosome behavior during meiosis, we cloned the *SPO13* gene. Owing to its specific role in meiosis and apparent lack of function in mitosis, it seemed likely that the *SPO13* product would be regulated during meiosis and that studies with the cloned gene would provide important insights into the mechanism of differentiation operating during meiosis and sporulation. The cloned gene has been used to construct additional alleles of *SPO13*, to identify its transcripts, and to analyze its expression during meiosis and mitosis in strains of different mating type. The experiments presented below show that the *SPO13* gene is essential only for the meiosis I division. It is dispensable for mitosis and its expression is developmentally regulated.

MATERIALS AND METHODS

Procedures for *S. cerevisiae* and *Escherichia coli*. Media and standard procedures for handling the yeast strains used in this study (Table 1) have been described previously (19). Yeast transformation was carried out by the method of Hinnen et al. (12). Procedures for cloning in plasmid and M13 vectors in *E. coli* were carried out as described by Maniatis et al. (25) and Messing (30).

Yeast genomic library. Yeast DNA for the library construction was isolated from a *SPO13 ARG4* haploid yeast strain (REE526). The DNA was partially digested with *Sau*3A. Fragments greater than 4 kilobases (kb) were isolated by gel electrophoresis and ligated into the *Bam*HI site of YCp19. This vector contains yeast sequences encoding the centromere of chromosome IV (*CEN4*), *ARS1*, *TRP1*, and *URA3*, as well as the ampicillin resistance gene and plasmid replication origin of pBR322 for selection and propagation in *S. cerevisiae* and *E. coli*, respectively (40). The ligation products were transformed into *E. coli* selecting for ampicillin-resistant transformants. The pool of Amp^r transformants contained approximately 8,000 plasmids which had inserts of yeast DNA averaging about 7 kb.

Construction of the *SPO13* disruptions. Various subclones (see Fig. 2 and Results) were used to construct three disruption plasmids, p(spo13)15, p(spo13)16, and p(spo13) 21. The first two were derived from p(SPO13)12, which contains the *SPO13* fragment of p(SPO13)6 inserted into pBR322. This plasmid was modified by changing the *Hpa*I

site, previously shown to be required for complementation of the *spo13-1* mutation, into a *Hin*dIII site by the addition of linkers. A *Hin*dIII fragment containing the *URA3* gene was then inserted into this position to give p(spo13)15. The *Bst*EII site of p(SPO13)12 was also changed to a *Hin*dIII site by the addition of linkers, and the same *URA3* fragment was used to replace the original *Bst*EII-*Hpa*I region, generating p(spo13)16. Finally, the third disruption plasmid, p(spo13)21, was made by inserting the *URA3* fragment into the *Hin*dIII site of p(SPO13)11, which contains the *SPO13* fragment of p(SPO13)8 in pBR322.

Linear DNA fragments containing *SPO13* with the inserted *URA3* gene derived from each of the plasmids [by *Eco*RI and *Xba*I digestion for plasmids p(spo13)15 and p(spo13)16, and *Bam*HI and *Bst*EII digestion for p(spo13)21] were purified from agarose gels and transformed into haploid strains K262-13A and K264-10D (*SPO13 ura3*) and the diploid strain K262-13A × K264-10D (*MAT***a**/*MAT*α *SPO13/SPO13 ura3-1/ura3-1*). Ura$^+$ transformants were analyzed to confirm the presence of each of the three disruptions by blot hybridization analysis of DNA digested with appropriate restriction enzymes, *Eco*RI-*Xba*I for *D1* and *D2* and *Bam*HI-*Xba*I for *D3* (data not shown).

Preparation of RNA. For preparation of RNA from cultures under sporulation conditions, strains were grown at 30°C to a cell density of 5×10^7 cells per ml in YPA (yeast extract-peptone-acetate) medium. The cells were recovered by centrifugation and suspended at 5×10^7 cells per ml in sporulation medium which contained 2% potassium acetate and the supplements required by that strain (see reference 19 for medium recipes). The sporulating cells were incubated at 30°C and aerated by shaking at 250 rpm. The efficiency of sporulation measured at 30 h was 40% asci for Z270, 64% for LM1, and 84% for SK1. RNA was prepared from the yeast cultures by vortexing the cells with glass beads in the presence of phenol-chloroform as previously described (4).

Hybridization analysis of RNA. Denaturation of nucleic acids with glyoxal and by gel electrophoresis was carried out as described by McMaster and Carmichael (28). Transfer to nitrocellulose was done as described by Thomas (42). Hybridization probes from SP6 clones were prepared by the procedures of Melton et al. (29). Hybridization of the RNA transfer was done as described by Wahl et al. (47), except that the hybridization was done at 48°C.

The ^{32}P-labeled single-stranded DNA hybridization probes for nuclease S1 analysis were prepared by the method of Ley et al. (22) with an additional purification step to reduce the amount of complementary strand as follows. The sequencing primer for M13mp vectors obtained from New England BioLabs was used to prime synthesis of the ^{32}P-labeled strand complementary to the insert with [α-^{32}P]dCTP at about 270 Ci/mmol. This labeled single-stranded DNA was purified on an alkaline agarose gel (27). It was found that about 1% of the complementary strand was still present in the single-stranded DNA prepared from the gel. To reduce the amount of the complementary strand, the probe was incubated in 10 μl of 1 M NaCl–0.01 M sodium phosphate (pH 7)–0.001 M EDTA–50% formamide at 42°C for 1 h to anneal the probe with the contaminating complementary strand. The hybridization reaction mixture was absorbed to hydroxyapatite in 10 mM sodium phosphate (pH 7.0), and the hydroxyapatite was washed with 10 mM sodium phosphate. The single-stranded DNA was eluted from the hydroxyapatite with 0.2 M sodium phosphate (pH 7.0) and recovered by repeated isopropanol precipitations (25). Hybridizations to yeast RNA were carried out with about 30,000 dpm of probe for 18 to 36 h at 42°C in 20 μl of the same hybridization buffer used above in the self-annealing of the probe. Nuclease S1 digestions were carried out as described by Maniatis et al. (25) with 350 U of nuclease S1 per ml at 30°C for 1 h. Exonuclease VII digestions were carried out in 50 mM KCl–50 mM Tris (pH 7.5)–10 mM EDTA at 30°C for 2 h with 30 U/ml. Nuclease S1 was from P-L Biochemicals, Inc., or Bethesda Research Laboratories, Inc., and exonuclease VII was from Bethesda Research Laboratories. After nuclease digestion, samples were analyzed on 5% polyacrylamide–7 M urea gels. The gels were fixed in 7% acetic acid and dried prior to exposure.

The amount of a DNA fragment protected from nuclease S1 digestion was determined by cutting out the appropriate region of the dried gel and measuring the amount of ^{32}P by Cerenkov counting in a liquid scintillation counter. Under the hybridization conditions described above, the amount of protected DNA fragment was shown to be proportional to the amount of *SPO13* RNA in the sample. The amount of *SPO13* RNA as a fraction of total RNA was estimated from control hybridization reactions with dilutions of single-stranded DNA from M13mp18 containing the *Bam*HI-*Xba*I fragment of *SPO13* DNA. These dilutions, which contained amounts of single-stranded DNA similar to the amount of *SPO13* RNA, were used for nuclease S1 analysis in parallel with the yeast RNA samples. The counts per minute obtained from these control hybridizations with known amounts of single-stranded DNA were used to calculate the amount of *SPO13* RNA. The fraction of RNA was converted to an estimate of copies per cell by using the values for number of mRNAs per yeast cell during vegetative growth (10).

RESULTS

Cloning of *SPO13*. The wild-type *SPO13* gene was cloned indirectly by selection for the closely linked *ARG4* locus (<1 centimorgan [cM]). A yeast genomic library was constructed in YCp19, a centromere-containing vector maintained in *S. cerevisiae* at approximately 1 copy per cell (2, 40) (see Materials and Methods). This vector was used to avoid possible complications due to complementation by an independent gene at high dosage (14, 23) or to aberrant sporulation in the presence of *SPO13* at high dosage. The genomic library was transformed into strain JK11-7B (*MAT*α *arg4-16,36 spo13-1 ura3-1*). Two Arg$^+$ Ura$^+$ transformants were isolated. Each transformant was crossed to strain K399-5C (*MAT***a** *spo13-1*), and the diploids were sporulated. One of them gave four-spored asci, indicating that the parental haploid transformant contained a plasmid capable of complementing the *spo13-1* mutation. This plasmid was recovered by transforming *E. coli* to Amp^r with DNA isolated from the appropriate yeast transformant and retested by transformation into the original haploid yeast strain, JK11-7B. The plasmid again complemented both the *arg4* and *spo13-1* mutations (complementation of the latter was assayed after the appropriate cross to a *spo13-1* tester strain) and was designated p(SPO13)1 (Table 2; Fig. 1).

Further characterization of p(SPO13)1 demonstrated that it contained an insert of approximately 15 kb of yeast DNA. The minimal region necessary for *SPO13* function was localized within this insert by testing various subclones for complementation of the *spo13-1* mutation. Figure 1 summarizes these experiments and shows that the 1.2-kb *Eco*RI-*Pst*I fragment present in p(SPO13)7 is sufficient to complement the *spo13-1* mutation. Subclones that divide this region

TABLE 2. Plasmid complementation of the *spo13-1* mutation[a]

Cross	Plasmid	% Asci	One- and two-spored asci (% of total asci)
JK11-7B × K399-5C	None	33	100
JK11-7B × K399-5C	p(SPO13)1 (from pool)	53	18
JK11-7B × K399-5C	p(SPO13)1 (recovered plasmid)	51	18
JK11-7B × K399-5C	p(spo13)2	30	100
K399-1D × K210-9C	None	56	100
K399-1D × K210-9C	p(SPO13)3	68	48
JK12-2D × K260-4B	None	37	100
JK12-2D × K260-4B	p(spo13)4	43	100
JK12-2D × K260-4B	p(SPO13)5	57	37
JK12-2D × K260-4B	p(SPO13)6	57	44
JK12-2D × K260-4B	p(SPO13)7	43	32
JK12-2D × K260-4B	p(SPO13)8	81	25
JK12-2D × K260-4B	p(spo13)9	50	99
JK12-2D × K260-4B	p(spo13)10	36	99

[a] Strains JK11-7B, K399-1D, and JK12-2D were transformed with the plasmids, and the transformants were crossed to the other parent. All diploids are homozygous for *spo13-1*. For each diploid, at least 200 cells were counted to determine the values given in the table. Typically, wild-type Spo$^+$ diploids yield 10 to 50% two-spored asci (17); the spores in these asci are usually haploid and result from defective packaging of the four meiotic products.

[p(spo13)9 and p(spo13)10] do not complement the *spo13-1* mutation, indicating that the *SPO13*-complementing sequence resides within the 1.2-kb *Eco*RI-*Pst*I fragment. The close linkage of this region to the *ARG4* locus (7 kb) provides strong evidence that the complementing activity results from the presence of the *SPO13* gene.

Disruption of the *SPO13* gene. To prove further that the *SPO13* gene had been cloned and to determine whether the gene plays an essential role in vegetative cell division, genomic disruptions within the *SPO13* complementing region were constructed by the procedure of Rothstein (38). Three disruptions, designated *D1*, *D2*, and *D3* (Fig. 2) were made and crossed to *spo13-1* tester strains. Sporulation of the diploids containing *D1*/*spo13-1* and *D2*/*spo13-1* yielded the two-spored ascus phenotype typical of diploids homozygous for the *spo13-1* mutation; *D3*/*spo13-1* gave wild-type levels of four-spored asci. The sporulation phenotypes of diploids containing different combinations of each disruption are shown in Table 3. These studies demonstrate that *D1* and *D2*, which contain alterations within the *Eco*RI-*Pst*I fragment, act as recessive mutations and confer a *spo13* mutant phenotype, while *D3*, altered outside this region, has no detectable effect on sporulation. We thus conclude that the *SPO13* gene indeed resides within the *Eco*RI-*Pst*I fragment present in p(SPO13)7, originally shown to be the smallest subclone that had *SPO13*-complementing activity.

The disruptions *D1* and *D2* further demonstrate that the *SPO13* gene plays no essential role during vegetative growth. The isolation of these disruptions in haploid cells, as well as tetrad analysis of a heterozygote for one of them (*D2*/+), generated by transformation of a diploid strain, supports this conclusion. In the latter case, 19 of 20 tetrads from sporulation of the heterozygote contained four viable ascosporal colonies; in these tetrads, the *URA3* marker and the *spo13* phenotype cosegregated 2+:2−. The *SPO13* gene also plays no essential role in spore germination, since the diploid spores formed by strains homozygous for *D1* or *D2* germinate with high efficiency.

The disruptions *D1* and *D2* behave identically to the *spo13-1* mutation except with regard to the frequency of three- or four-spored asci. Mutant *spo13-1*/*spo13-1* strains generally form >98% two-spored asci and a low but detectable level of three- or four-spored asci (17, 18). The diploid strains homozygous for *D1* or *D2* exhibit the two-spored *spo13-1* phenotype but do not form any three- or four-spored asci (e.g., 0/1,000 cells examined). We thus conclude that the *SPO13* gene is indispensable for the meiosis I division and

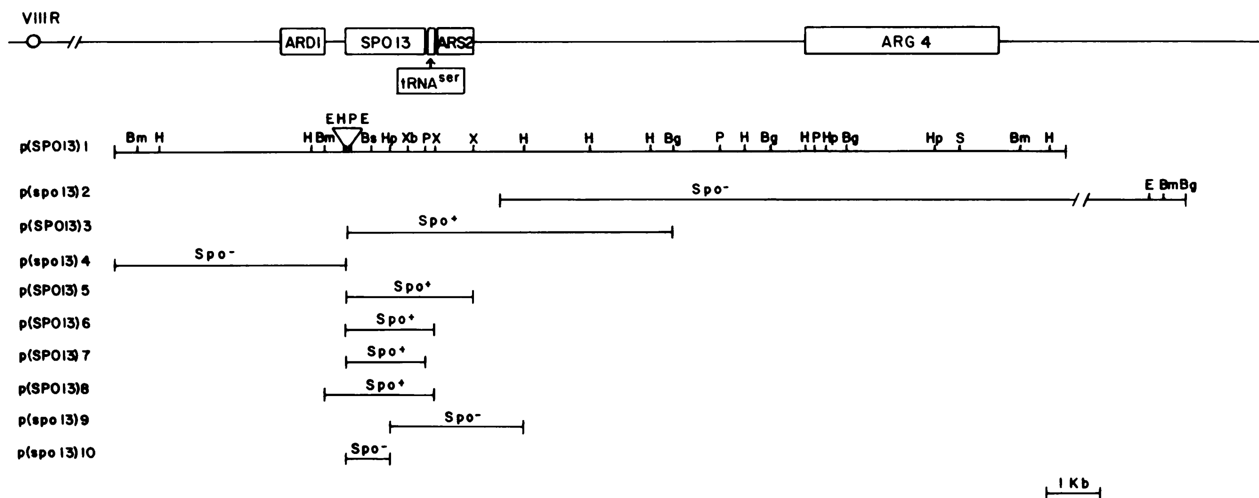

FIG. 1. Plasmids tested for complementation of the *spo13-1* mutation. The upper line gives the locations of genes in the *ARG4-SPO13* region. The locations of *ARG4* and *ARS2* are from Tschumper and Carbon (44). The location of *ARD1* is from Whiteway and Szostak (49). The location and identification of the tRNA^{Ser} are from the DNA sequence (H.-W. Tang, unpublished data). The orientation of *ARG4* and *SPO13* relative to *CEN8* was determined as described in the text. p(SPO13)1, p(spo13)2, the second Arg$^+$ plasmid isolated from the genomic library, and p(SPO13)4 have the indicated fragments in the YCp19 vector (40). All other fragments are inserted into the YCp50 vector (13). These plasmids are named according to the convention that a plasmid which complements *spo13-1* is p(SPO13), while a plasmid which does not complement is p(spo13). Restriction enzyme sites: Bg, *Bgl*II; Bm, *Bam*HI; Bs, *Bst*EII; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; S, *Sal*I; X, *Xho*I; Xb, *Xba*I.

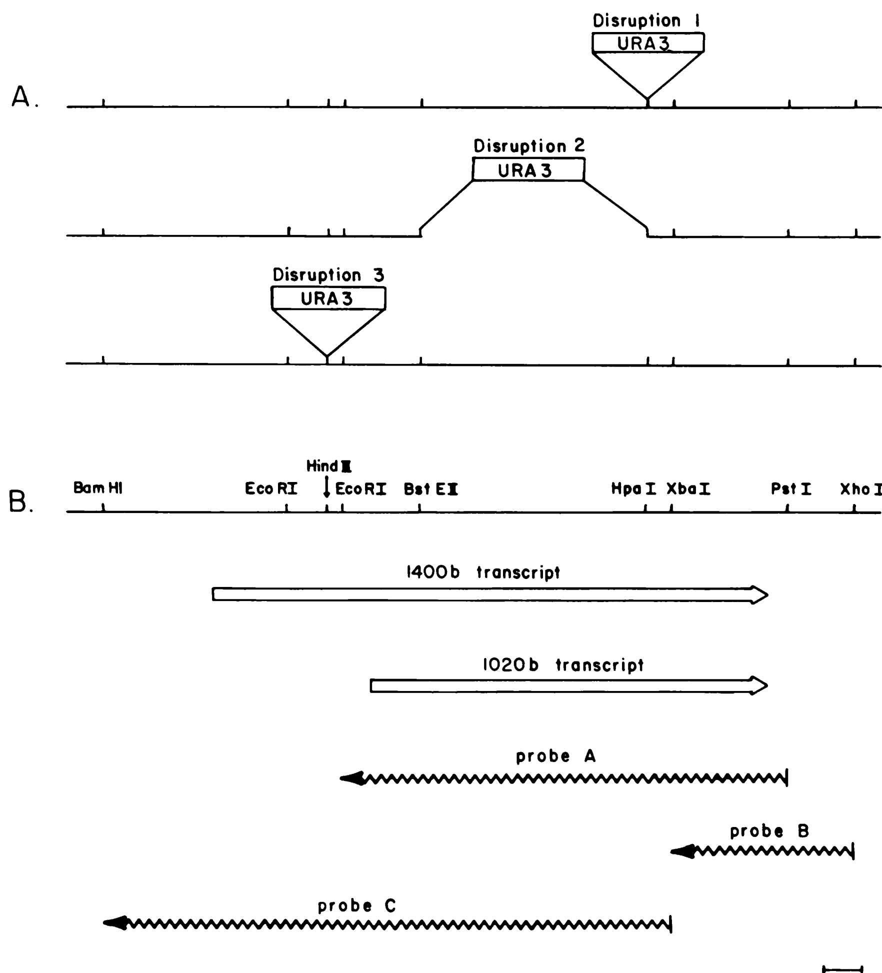

FIG. 2. Disruptions of the *SPO13* region and transcription map of the *SPO13* gene. The *D1*, *D2*, and *D3*, disruptions (panel A), whose constructions are described in Materials and Methods, are illustrated above the restriction map of the *SPO13* region (panel B). The transcription of the *SPO13* gene is illustrated below the restriction map as are the hybridization probes used to define the *SPO13* transcripts. The bar represents 0.1 kb.

that there are no other wild-type gene functions which can substitute to any significant extent for the *SPO13* function.

Orientation of *ARG4-SPO13* relative to *CEN8*. Previous tetrad analyses have established the map order on the right arm of chromosome VIII as *CEN8*–12 cM–*ARG4*-13 cM–*THR1* (32) and close linkage of *SPO13* to *ARG4* (<1 cM [17]). To determine the position of *SPO13* relative to the other markers, spore W1-10C, containing the *URA3* fragment inserted into the *SPO13* gene in disruption *D1*, was crossed to RE775 to give a diploid of genotype *spo13*::*URA3(D1) ARG4 THR1 ura3*/*SPO13 arg4 thr1 ura3*. Random spore rather than tetrad analysis was performed to recover enough recombinants between *URA3* and *ARG4* to permit ordering of *SPO13* relative to *THR1*. If *SPO13* is proximal to *ARG4*, then most *SPO13-ARG4* recombinants will contain the parental coupling of *ARG4-THR1*; if *SPO13* is between *ARG4* and *THR1*, then most of them will be recombined for these markers. The analysis of random spores from this cross (Table 4) indicates that the order is *CEN8-SPO13-ARG4-THR1*. This order is also in agreement with recent studies placing *ARD1*, a gene adjacent to *SPO13*, proximal to *ARG4* (49).

Mapping of the *SPO13* transcripts. Figure 3 shows the results of hybridizing the minimal fragment containing the *SPO13* gene to gel transfers of total RNA prepared from a sporulating culture. Two sporulation-specific transcripts are visible: a major one of 1.0 kb and a less abundant RNA of 1.4 kb. Since a strand-specific probe generated by the SP6 polymerase (29) was used for these hybridization experiments, both RNAs are transcribed from the same strand (Fig. 2). The other strand of this DNA fragment is not likely to be transcribed, since hybridizations with probes capable of hybridizing to both strands show only the 1.4- and 1.0-kb transcripts (data not shown). Both RNAs were retained on a poly(U)-Sepharose column during high-salt washes, indicating that the RNAs are polyadenylated (data not shown).

These two transcripts were mapped by using nuclease digestion of RNA-DNA hybrids (7). Single-stranded DNAs from the *SPO13* region labeled with ^{32}P were prepared as described in Materials and Methods. These probes were hybridized to total RNA prepared from a sporulating yeast strain. The RNA-DNA hybrids were digested with either nuclease S1 or exonuclease VII, and the protected DNA fragments were analyzed on a denaturing polyacrylamide gel. Hybridization with probe B and digestion with nuclease S1 was used to map the 3′ end of the transcripts. Only one

TABLE 3. Sporulation of strains with disrupted *SPO13* genes[a]

Genotype and strain	% Total asci	One- and two-spored asci (% of total asci)
SPO13/SPO13		
K264-10D × K262-13A	61	53
SPO13/spo13-1		
K264-10D × K399-1D	43	23
K262-13A × JK12-2D	41	30
SPO13/D1		
K264-10D × REE968	55	52
REE971 × K262-13A	60	52
SPO13/D2		
K264-10D × REE970	61	48
REE971 × K262-13A	62	48
SPO13/D3		
K264-10D × REE972	57	43
REE973 × K262-13A	43	31
spo13-1/D1		
K399-1D × REE969	92	100
JK12-2D × REE968	84	100
spo13-1/D2		
K399-1D × REE971	82	100
JK12-2D × REE970	84	100
spo13-1/D3		
K399-1D × REE973	60	36
JK12-2D × REE972	65	46
spo13-1/spo13-1		
K399-1D × JK12-2D	60	100
K399-1D × K210-9C	56	100
D1/D1		
REE969 × REE968	86	100
D2/D2		
REE971 × REE970	83	100
D3/D3		
REE973 × REE972	42	30
D1/D2		
REE969 × REE970	83	100
D1/D3		
REE969 × REE972	45	31
D2/D3		
REE971 × REE972	40	25

[a] For each diploid, at least 200 cells were counted to determine the values given in the table, except for the *D1/D1*, *D2/D2*, and *D1/D2* strains, for which at least 500 cells were counted.

major protected band of 262 bases, along with a faint band of 285 bases, was found, which indicates that the difference between the 1.0- and 1.4-kb transcripts cannot be at the 3′ end (Fig. 4).

With the 1.2-kb probe A, two protected bands could be detected, a major one of 1,070 bases and a minor one of 1,240 bases. This result indicates that the two transcripts largely overlap and cover most of fragment A. Combined with the results with probe B, this result shows that the 3′ end of the transcripts must occur less than 70 bases from the *Pst*I site at the end of fragment A and that the difference between the RNAs must be at the 5′ end of the RNAs. With probe C, two protected bands were again seen. The more abundant fragment (about 80%) was approximately 720 bases, and the less abundant fragment (about 20%) was approximately 1,150 bases. When hybridizations with probe C were digested with *E. coli* exonuclease VII (Fig. 4; lanes I and K), the protected bands were the same size as when nuclease S1 was used. This result indicates that the size difference between the two protected fragments is not due to splicing of intervening sequences but is indeed due to length differences at the 5′

TABLE 4. Map order of *SPO13*, *ARG4*, and *THR1* derived from random spore analysis[a]

Recombinant class	Spore phenotype: Ura	Arg	Thr	No. of spores[b]
I	+	+	−	42
II	−	−	+	67
III	+	−	−	5
IV	−	+	+	11
V	+	−	+	4
VI	−	+	−	1

[a] Cross: *spo13::URA3 ARG4 THR1 ura3* × *SPO13 arg4 thr1 ura3*.

[b] A total of 819 random spores were examined. The majority of spores (689) contained the parental coupling for all three markers. The most frequent classes of reciprocal recombinants shown above (I and II) result from single crossovers between *ARG4-THR1* (13 cM), in agreement with published maps of this region (32). The second most frequent classes (III and IV) result from single crossovers between *spo13::URA3-ARG4* (2 cM), as shown previously by tetrad analysis (17). The least frequent classes (V and VI) are presumed to result from double crossovers between *spo13::URA3-ARG4* and *ARG4-THR1*. Since *CEN8* is known to be proximal to *ARG4* (32) and the *ARG4* marker shows typical second-division segregation frequencies in our strains (17), the most probable map order is *CEN8-SPO13-ARG4-THR1*.

end. The lengths measured with the 3′ probe B and 5′ probe C indicate the presence of a major *SPO13* RNA of 980 bases and a minor *SPO13* RNA of 1,410 bases, in excellent agreement with the sizes (1.02 and 1.4 kb) measured on the gel transfer (Fig. 3). The 1.0- and 1.4-kb transcripts therefore differ at the 5′ end but share similar 3′ ends (Fig. 2).

Sporulation-specific expression of the *SPO13* gene. The results in Fig. 3 suggest that the *SPO13* RNAs are induced during sporulation, since no *SPO13* RNA was seen at the zero time point. To study this regulation, RNA samples were

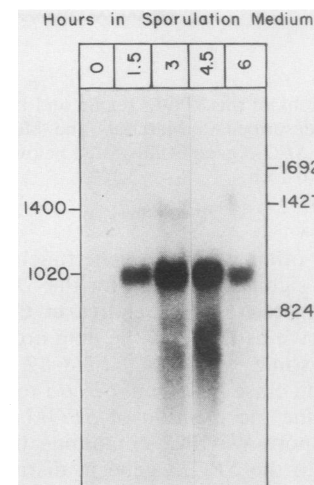

FIG. 3. Two RNAs transcribed from the *SPO13* gene. Total RNA was prepared from an SK1 yeast culture at indicated times after transfer to sporulation medium (hours in medium are given at the top of the figure), and 15 μg from each time point was fractionated on a 1.4% agarose gel. The nitrocellulose blot was hybridized with ^{32}P-labeled single-stranded RNA probe A (Fig. 2) and exposed to preflashed Kodak XAR-5 film with an intensifying screen for 12 h. Size standards, pBR322 digested with *Bam*HI and *Pvu*II and YRp7 (41) digested with *Hin*fI, were localized on the blot by hybridization with ^{32}P-labeled nick-translated YRp7. The lengths of the size standards are given to the right of the figure, and the calculated lengths of the RNAs are given to the left. The hybridizing RNAs smaller than 1 kb most probably represent degradation products of the 1.0-kb RNA and have not been further characterized.

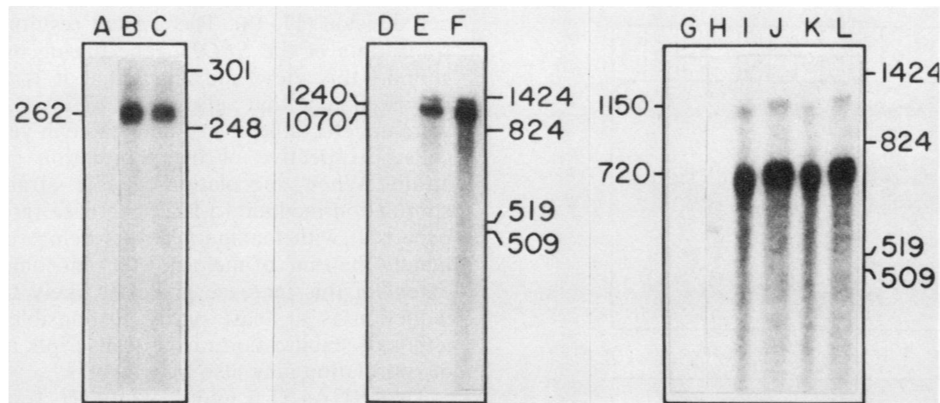

FIG. 4. Nuclease mapping of the transcripts from the *SPO13* region. Probes were ^{32}P-labeled single-stranded DNAs generated from appropriate subclones of *SPO13* DNA fragments inserted into M13mp vectors. All hybridization mixtures contained 50 μg of total RNA. Yeast RNA was prepared from strain Z270 at time points after transfer to sporulation medium. The size standards, which are ^{35}S-labeled *Hin*fI fragments of YRp7, are given to the right of the autoradiograms. The sizes of the fragments protected by hybridization to RNA are given to the left. Lanes A to C: hybridization probe B and digestion with nuclease S1 (A, *E. coli* tRNA; B, 4.5-h RNA; C, 7.5-h RNA). Lanes D to F: hybridization probe A and digestion with nuclease S1 (D, 0-h RNA; E, 1.5-h RNA; F, 3.0-h RNA). Lanes G to L: hybridization probe C (G, *E. coli* tRNA and nuclease S1; H, *E. coli* tRNA and exonuclease VII; I, 4.5-h RNA and exonuclease VII; J, 4.5-h RNA and nuclease S1; K, 7.5-h RNA and exonuclease VII; L, 7.5-h RNA and nuclease S1). Figure 2 indicates the hybridization probes and summarizes the transcription map derived from the nuclease protection experiments. Optimal exposures of lanes J and L indicate that both the 1,150- and 720-base bands are multiple fragments, which suggests that both transcripts have more than one initiation site. The upper band has a major fragment at 1,150 bases and a minor fragment at 1,100 bases. The lower band has a major fragment at 720 bases, with minor fragments at 740 and 690 bases.

isolated from *MAT***a**/*MAT*α, *MAT***a**/*MAT***a**, and *MAT*α/*MAT*α strains during vegetative growth and at time points after transfer of the cultures to sporulation medium. Hybridization with single-stranded probe C and nuclease S1 digestion were used to quantitate the level of *SPO13* transcripts in these samples. Under the hybridization conditions used for these experiments, the amount of *SPO13* RNA was measured relative to the total nucleic acid or essentially relative to the rRNAs, since the rRNAs constitute more than 80% of the total nucleic acid. For all strains tested in these experiments (Z270, DL171-477, DH8-303, and LM1), neither the 1.0- nor the 1.4-kb transcript was detectable in RNA samples prepared from log-phase cultures grown in either acetate or glucose (data not shown). Control hybridization experiments with single-stranded DNA standards indicated that less than one molecule of *SPO13* RNA was present per 20 cells in these vegetative cultures.

When *MAT***a**/*MAT*α strains were shifted to sporulation medium, a dramatic appearance of *SPO13* transcripts occurred (Fig. 5 and 6). In strain SK1, which sporulates to the highest levels with the greatest degree of synchrony (>85% asci by 24 h), the transcripts were detectable at 1.5 h, reached a peak at 3 to 4.5 h, and then tapered off. Both the 1.0- and 1.4-kb transcripts dramatically increased when the cultures were shifted to sporulation medium. For the 1.0-kb RNA, the maximum steady-state level was at least 75 times above the barely detectable level present in SK1 at the zero time point. It is important to note, however, that even at maximum accumulation, there was only a small amount of *SPO13* RNA present. The level of the 1.0-kb RNA was still only 2×10^{-6} of the total RNA, which corresponds to approximately two RNA molecules per cell.

The rate, extent, and duration of *SPO13* transcript accumulation during sporulation varied somewhat among diploids derived from different genetic backgrounds (i.e., Z270 and LM1) (Fig. 6). Not surprisingly, these parameters are strain specific and generally reflect the sporulation proficiency and kinetics of ascus formation of the particular strain examined (see also reference 21). The critical point is that in all sporulating diploids, irrespective of the sporulation kinetics, the appearance of the *SPO13* transcripts is developmentally regulated.

All three strains showed a decrease in the amount of *SPO13* RNA at later stages of sporulation. In the highly synchronous SK1 strain, the *SPO13* transcripts nearly disappeared as ascus formation was completed. Although we have not yet examined mRNA turnover, these results imply that the *SPO13* gene is not transcribed late in sporulation and that the *SPO13* RNAs are unstable during sporulation so that they do not persist in the ascospores. The higher levels of transcripts seen at the end of sporulation in the two strains that sporulate less efficiently than SK1 may be due to continued transcription or to inefficient degradation of *SPO13* RNA in cells that initiate sporulation but fail to form mature ascospores.

Figure 6 also shows the time at which some of the landmark events of the sporulation process occur relative to *SPO13* RNA synthesis in SK1. The amount of *SPO13* RNAs reached a maximum between 3 and 4.5 h. The first binucleate and tetranucleate cells, indicating the completion of the meiosis I and meiosis II divisions, respectively, were detected at 4.5 h. The amount of *SPO13* RNA thus reaches a maximum close to the time at which the meiosis I division is executed.

*MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains do not undergo premeiotic DNA synthesis or subsequent events of the meiotic process. Such asporogenous strains have been used in control studies to distinguish between events that are triggered by starvation conditions in sporulation medium (i.e., occur in **a**/**a**, α/α, and **a**/α cells) and those that are specific to meiosis and spore formation (i.e., occur only in **a**/α cells) (6). To determine whether the *SPO13* gene function is dependent on *MAT* function, we assayed for the presence of the transcripts in *MAT***a**/*MAT***a** and *MAT*α/*MAT*α cells

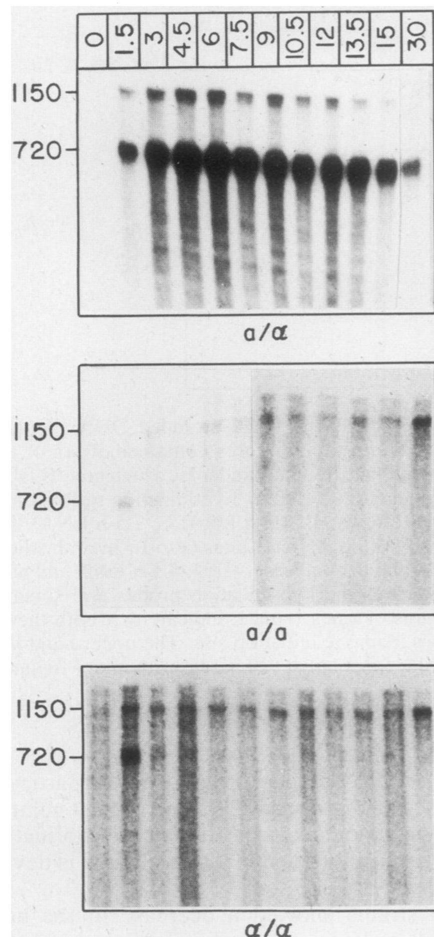

FIG. 5. The *SPO13* transcripts undergo a large increase under sporulation conditions only in **a**/α diploids. Hybridization reaction mixtures contained 50 μg of total RNA prepared at the indicated times (top of figure) after transfer of the culture to sporulation medium. Probe C was used for the hybridization reaction mixtures which were treated with nuclease S1. Top panel: Z270, a *MAT***a**/*MAT*α diploid capable of sporulation. Middle panel: DL171-477, a *MAT***a**/*MAT***a** diploid. Bottom panel: DH8-303, a *MAT*α/*MAT*α diploid. All gels were exposed to preflashed XAR-5 film with an intensifying screen for 29 h.

incubated in sporulation medium. There was a small but reproducible increase of the two *SPO13* transcripts in *MAT***a**/*MAT***a** (DL171-477) and *MAT*α/*MAT*α (DH8-303) strains, which were derived by recombination from Z270 (Fig. 5). The peak amounts of the transcripts were less than 5% of that observed in the sporulating Z270 strain and occurred at 1.5 h; the level of 1.0-kb transcript then decreased and became undetectable, while the 1.4-kb transcript remained at a low but detectable level throughout the remaining time points. These results indicate that starvation alone, independent of the genotype at *MAT*, can trigger the initial appearance of the *SPO13* transcripts, while maximal levels are dependent on heterozygosity at *MAT*.

DISCUSSION

Genetic analysis of the *spo13-1* mutation indicates that the *SPO13* function is required for meiosis I chromosome segregation and suggests that the gene is not required for mitotic cell division (17, 18). The present results characterizing the transcripts of the *SPO13* gene during mitosis and meiosis support this view and show that it is a developmentally regulated gene that acts only in meiosis. The *SPO13* RNAs were undetectable or just detectable in vegetatively growing cells, irrespective of the sporulation competence of the strain. When sporulation-capable strains are placed in sporulation medium, a large increase (greater than 75-fold) occurred, with maximum levels being reached at approximately the time of meiosis I chromosome segregation. The extent of this increase makes it likely that transcriptional induction is at least partly responsible for this change, although stabilization of the transcripts at particular stages of sporulation may also play a role.

Two RNAs (1.0 and 1.4 kb) are transcribed from the *SPO13* gene during sporulation. These two RNAs differ only at the 5′ end, where the more abundant RNA (1.0 kb) is about 400 bases shorter than the less abundant RNA. The 1.0-kb RNA is sufficient to complement the *spo13-1* mutation, since the complementing subclone p(SPO13)7 contains all the sequences for the 1.0-kb transcript but lacks about 300 bases of the 5′ end of the 1.4-kb transcript. Also, strains homozygous for the *D3* disruption, which leaves the 1.0-kb transcript intact but interrupts the 1.4-kb transcript approximately 300 bases from the 5′ end, sporulate at normal levels. An intact 1.4-kb transcript does not therefore appear to be necessary either for *SPO13* function or any other essential sporulation function.

The presence of two transcripts with different 5′ ends

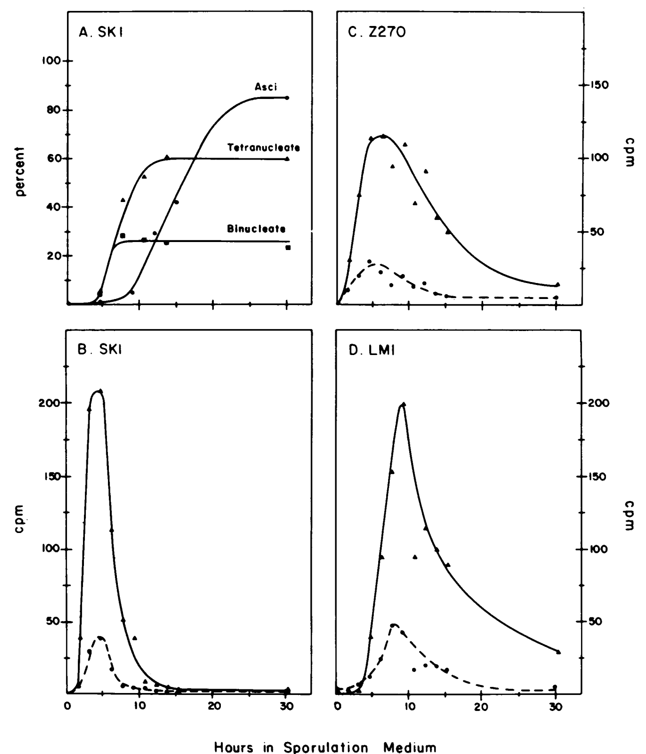

FIG. 6. Correlation of sporulation events with the levels of the *SPO13* transcripts. RNAs from three sporulating strains were analyzed as in Fig. 5. The appropriate regions of the acrylamide gel were cut out, and Cerenkov counts were determined in a liquid scintillation counter. (A) Sporulation landmarks in SK1. (B) Induction of *SPO13* RNAs in SK1. (C) Induction of *SPO13* RNAs in Z270. (D) Induction of *SPO13* RNAs in LM1.

raises the question of whether one or two regulatory regions are responsible for the sporulation-specific regulation. If one regulatory region is present, it must be capable of acting over the 400 bases separating the two 5′ ends. The properties of the *D3* disruption suggest that two regulatory regions, one for each transcript, are more likely, since *D3* homozygotes have a wild-type *SPO13* phenotype. The expression of *SPO13* occurs in this case, even when *URA3* is inserted into the 5′ end of the 1.4-kb transcript approximately 150 bp 5′ to the start of the 1.0-kb transcript. We have not yet determined, however, whether the 1.0-kb transcript is regulated correctly in this disruption.

Recently, the gene adjacent to the 5′ region of *SPO13* has been defined (49). This gene, called *ARD1*, is required for α-factor arrest of *MAT***a** cells and for sporulation of *MAT***a**/*MAT*α diploids. It is postulated to be necessary for G1 arrest in response to α-factor or to starvation. Such arrest is in turn required for mating and for entry into sporulation. The *ARD1* and *SPO13* genes are transcribed in opposite directions with the 5′ end of the 1.4-kb *SPO13* transcript and the *ARD1* initiator AUG less than 150 bases apart. Although this proximity and the requirement of *ARD1* for sporulation suggest that transcription of the *ARD1* gene might also be induced during sporulation, as a result of a common regulatory sequence shared with the *SPO13* gene, preliminary experiments (R. Elder, unpublished observations) have shown that the level of *ARD1* RNA does not significantly increase during sporulation. Deletions in the 5′ region of the *SPO13* gene have recently been constructed (L. Buckingham, unpublished results) and are being used to define the regulatory region(s) of the *SPO13* gene. Such deletions should help clarify the relationship between the promoter and control regions of these two adjacent loci.

Genes whose transcription is induced during sporulation are most probably responding directly or indirectly to starvation and mating-type regulatory signals. Three observations support the view that starvation and mating-type signals are independently required in the regulation of sporulation. (i) Neither heterozygosity at the *MAT* locus nor starvation alone is sufficient to initiate sporulation; rather, both are typically required. For example, starvation does not induce sporulation in *MAT***a**/*MAT***a** and *MAT*α/*MAT*α diploids or those containing mutations in *MAT* such as *MAT***a**/*mat*α*2* and *mat***a***1*/*MAT*α (reviewed in references 11 and 33). Similarly, *MAT***a**/*MAT*α cells defective in responding to nutritional deprivation, e.g., in *ard1*, *cyr1-1*, or RAS_2^- mutants (26, 43, 49), also fail to undergo meiosis and sporulate. (ii) The *MAT* locus exerts its effect on sporulation through **a**/α repression of the *RME1* locus, which in turn is thought to encode a repressor of sporulation-specific gene expression (16, 31, 37). Mutant *rme1* strains, however, which no longer require both *MAT***a** and *MAT*α expression, nevertheless still must be induced by starvation to initiate sporulation. (iii) Starvation under sporulation conditions induces an early, general increase in translatable RNA in *MAT***a**/*MAT***a**, *MAT*α/*MAT*α, and *MAT***a**/*MAT*α cells; later, unique RNA species appear only in cells that are both *MAT***a**/*MAT*α and have been nutritionally deprived (21, 48).

A direct response of the *SPO13* gene to conditions of nutrient starvation may be indicated by the low induction of the *SPO13* transcripts that occur in *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains when shifted to sporulation medium. Since mating-type homozygotes fail to undergo premeiotic DNA synthesis and later events, they have been thought to be blocked in either initiation or a very early stage of sporulation (for review see references 3 and 6). It should be emphasized that the maximal induction of the 1.0-kb RNA in these strains homozygous at the *MAT* locus is only to a level of about 0.1 RNA molecule per cell, an amount that seems unlikely to be functionally important. A more probable explanation for this small, transient induction is that it reflects the dual control system that separately responds to nutritional starvation and mating-type heterozygosity in the regulation of sporulation-specific events. One possibility, for example, is that the *SPO13* gene itself may respond directly to two regulatory signals, one dependent on starvation for glucose and nitrogen and the other dependent on expression of the *MAT***a** and *MAT*α mating-type genes. These may act in sequential order such that the mating-type control system is required to stabilize the starvation response and fully induce the *SPO13* gene; alternatively, mating-type control could represent a completely independent regulatory system.

A number of sporulation-specific transcripts in *S. cerevisiae* have previously been detected by a variety of techniques. Differential hybridization studies have identified 14 or 15 transcripts that are induced during sporulation (1, 34). In vitro translation of mRNA has identified a total of 10 proteins that are induced during sporulation (21, 48). Differential hybridization and in vitro translation detect only relatively abundant RNAs. Since the 1.0-kb *SPO13* RNA even when fully induced is present at only approximately two RNA molecules per cell, it is unlikely that the *SPO13* RNA was detected in the searches for sporulation-specific transcripts. Moreover, it remains to be determined what fraction of genes encoding sporulation-specific transcripts play an essential role in meiosis and spore formation. In some cases, homozygous disruptions of the genes whose transcription is sporulation specific have resulted in diploids with no meiotic or sporulation-defective phenotype (9, 35, 50), whereas in other cases, a sporulation defect has been found (35; P. T. Magee, *Transcription during Meiosis*, in press; R. Elder and R. E. Esposito, unpublished data). Another study reported that a number of genes with no apparent function in sporulation, such as *GAL10* and *CDC10*, are induced during sporulation (15), while the *SPO15* gene, which has no detectable role in mitosis, is transcribed to give similar amounts of RNA in mitotic and meiotic cells (51). These results suggest that at least some of the sporulation-specific transcription may be incidental or unrelated to the meiotic process.

The results with *SPO13* provide a clear example of a sporulation-specific transcript from a gene known to play an important role in meiosis. We have demonstrated that in addition to *SPO13*, two other cloned *SPO* genes known to be required for proper meiotic chromosome behavior encode developmentally regulated transcripts. These are *SPO11*, which is necessary for meiotic recombination, and *SPO12*, which is also required for the meiosis I division (R. Elder and R. E. Esposito, unpublished data). *SPO11* and *SPO13* transcripts appear at the same time during sporulation, while the level of *SPO12* increases later. The sporulation-specific expression of these three *SPO* genes, coupled with estimates of 50 to 200 *SPO* genes in *S. cerevisiae*, suggests that there are likely to be many other RNAs as yet undetected that are induced to low levels during sporulation but that provide necessary functions for meiosis and sporulation.

The *D1* and *D2* gene disruptions made with the cloned *SPO13* gene provide strong evidence that *SPO13* is dispensable in mitosis and encodes a meiosis-specific function. First, these disruptions are viable in haploids and homozygous diploids. Second, they have the expected meiotic phenotype, i.e., two diploid spores per ascus, and produce

no three- or four-spored asci. It is important to note that diploids homozygous for *spo13-1* often give a low but detectable frequency (1 to 2%) of three- or four-spored asci. One interpretation of this result is that the meiosis I division can occur inefficiently in the complete absence of *SPO13* function. The failure to detect any three- or four-spored asci in a *D1* and *D2* homozygotes argues for the alternative view that the *spo13-1* nonsense allele has residual *SPO13* function (perhaps as a result of a low level of suppression) and that the *SPO13* gene plays an indispensable, direct role in the meiosis I division. The low level of *SPO13* RNA present at maximal induction indicates that only a small amount of *SPO13* gene product is necessary to carry out this essential meiotic function.

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