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Genetics

Nucleotide sequence and promoter analysis of *SPO13*, a meiosis-specific gene of *Saccharomyces cerevisiae*

(sporulation/transcription)

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ABSTRACT **The *SPO13* gene, required for meiosis I segregation in *Saccharomyces cerevisiae*, produces two developmentally regulated transcripts (1.0 and 1.4 kilobases) that differ in length at their 5′ ends. The shorter transcript is sufficient to complement the *spo13-1* mutation and contains a major open reading frame encoding a highly basic protein of 33.4 kilodaltons. A fragment upstream (−170 to −8) of the open reading frame confers meiosis-specific transcription on a *spo13–HIS3* fusion. Deletions at the 5′ end of *spo13–lacZ* fusions define a region between −140 and −80 that is essential for meiosis-specific expression. This region acts in an orientation-independent manner and is responsive to the *MAT–RME* regulatory cascade. It contains a 10-base-pair sequence, TAGCCGCCGA, found in a number of meiosis-specific genes, that appears to be required for *SPO13* expression. This sequence is identical to URS1, a ubiquitous mitotic repressor element.**

Initiation of meiosis and spore formation in *Saccharomyces cerevisiae* is controlled by cell type and nutritional status (1, 2). Cell-type control occurs through the products of the *MAT***a** and *MAT*α loci, which combine to negatively regulate transcription of *RME1* (3, 4). *RME1* negatively regulates *IME1*, whose expression appears to be sufficient to induce meiosis (5). *IME1* is also under negative control by the system monitoring nutritional status (5, 6).

SPO13 is required for the proper separation of homologs at meiosis I (7). *SPO13* has been cloned and encodes two overlapping transcripts with the same 3′ end (8). The 1.0-kilobase (kb) major transcript is sufficient for *SPO13* function; the role of the minor 1.4-kb transcript is unknown. The two transcripts are developmentally coregulated (8). Along with other genes such as *SPO11* (9), *SPO16* (10), *HOP1* (11), *RED1* (12), and *MER1* (13), *SPO13* is expressed early in meiosis: *SPO13* transcripts are barely detectable in vegetative cells and are induced 70- to 100-fold within the first 4.5 hr of meiosis (8). Here we report the DNA sequence of the *SPO13* gene and an analysis of its cis-acting regulatory region.[§]

MATERIALS AND METHODS

Strains. RE944 (*spo13-1/spo13-1 ura3/ura3*) is from our strain collection. LP112 (*MAT***a**/*MAT*α *ade2/ade2 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1*), obtained from S. Lindquist (University of Chicago), is a cross of W303-1A and W303-1B from R. Rothstein (Columbia University). Isogenic diploids SFY67 (*MAT***a**/*MAT***a**) and SFY68 (*MAT*α/*MAT*α) were obtained from J. Segall (University of Toronto). S104 (*MAT***a**/*MAT*α *leu1/LEU1 ura3/ura3 trp1/trp1 can1/can1 his4-519/HIS4 RME1/rme1::LEU2*), S150 (*MAT***a**/*MAT*α *leu2/leu2 ura3/ura3 trp1/trp1 his4-519/his4-712 can1/CAN1 lys1/LYS1 RME1/rme1::LEU2*), S151 (*MAT***a**/*MAT*α *leu2/leu2 ura3/ura3 trp1/trp1 his4-519/his4-712 can1/CAN1 lys1/LYS1 rme1::LEU2/rme1::LEU2*), S152 (*mat***a1-50**/*MAT*α *leu2/leu2 ura3/ura3 trp1/trp1 his4-519/his4-712 can1/CAN1 lys1/LYS1 RME1/rme1::LEU2*), and S153 (*mat***a1-50**/*MAT*α *leu2/leu2 ura3/ura3 trp1/trp1 his4-519/his4-712 can1/CAN1 lys1/LYS1 rme1::LEU2/rme1::LEU2*) were acquired from A. Mitchell (Columbia University).

Growth and Sporulation. General methods for growth and sporulation have been described (7, 8, 14). Strains were grown to midlogarithmic phase in synthetic acetate medium [1% potassium acetate plus 0.68% Difco yeast nitrogen base without amino acids, buffered to pH 6.5 with 0.05 M potassium phthalate (pH 5.5) and supplemented with auxotrophic requirements] prior to sporulation.

DNA Sequence Analysis. Dideoxy sequencing of the cloned *SPO13* gene was carried out as described (15, 16). Protein sequences were searched for homology to known sequences in the National Biomedical Research Foundation Protein Identification Resource and GenBank/EMBL sequence banks with the WORDSEARCH and TFASTA programs, respectively.

Gene Fusions. Cloning procedures were as described (17). *SPO13* DNA from −847 to +45 (+1 is the translational start) was fused in frame into the polylinker of *lacZ* in pMC1585 (18) by using *Bgl* II linkers (5′-CAGATCTG-3′). Fusions at the *Bst*EII or *Xba* I sites retained 15 and 224 amino acids, respectively, of the Spo13 protein. Sequential 5′ deletions of the fusions were made in YCp50 (19), using natural restriction enzyme sites in the *SPO13* promoter. The −80 deletion was made in plasmid p(spo13)75 by using the *Eco*RI site created as a result of an oligonucleotide-directed G → T transversion at −77. The −170 to −80 fragment in p(spo13)64R was inverted by cutting and religating at the *Eco*RI sites. The fusions were subsequently recloned into the *Bam*HI sites of YEp24 (20) or YIp5 (21). Because the *Bam*HI site was inactivated in the construction of deletions p(spo13)52 and p(spo13)53 in YCp50, *Bam*HI sites were inserted in the vector *Eco*RI site for recloning into the *Bam*HI site of YEp24. The fusions in p(spo13)65Inv, p(spo13)66Inv and p(spo13)-67Inv were inverted using *Xho* I linkers (5′-CCTCGAGG-3′) to insert the *spo13–lacZ* sequences into the *Sal* I site of YCp50 or YEp24 in the opposite orientation. *spo13–HIS3* fusions

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Abbreviation: ORF, open reading frame.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M38357).

were made in the centromeric (*CEN*) vector pRS314 (22) by first inserting an *Eco*RI–*Xho* I fragment of *HIS3* (23) into the vector polylinker and then ligating a *SPO13* fragment, from the *Eco*RI site at −170 to an *Xho* I site engineered at −10, to *HIS3* by using *Eco*RI linkers (5′-GGAATTCC-3′).

β-Galactosidase Assays. Aliquots of 1.5–5 × 10^8 cells were withdrawn at 3-hr intervals during sporulation. The cells were pelleted, resuspended in 0.3 ml of 0.1 M Tris·HCl, pH 8.0/20% glycerol/1 mM dithiothreitol, and stored at −20°C before being assayed for β-galactosidase activity (24). Peak levels of β-galactosidase activity occurred between 9 and 18 hr of sporulation, depending on the synchrony of the strain and the stability of the fusion protein. The values reported have been corrected for background by subtracting the "activity" produced by isogenic strains carrying only vector or no plasmid. Plasmid stability was measured for each experiment and ranged from 80% to 100% in selective medium.

Northern Blot Analysis. Total RNA was isolated from 5 × 10^8 cells at 3-hr intervals during sporulation. The glyoxylated RNA was loaded (20 μg per lane) and electrophoresed in 1.1% agarose gels in 10 mM sodium phosphate buffer (pH 7) with recirculation. The gels were blotted onto nitrocellulose, baked, and hybridized as described (25). The *SPO13* and *HIS3* probes were synthesized using SP6 polymerase with a 1.2-kb *Eco*RI–*Pst* I fragment of *SPO13* and a 0.4-kb *Hin*dIII–*Xho* I 3′ fragment of *HIS3* (26).

Oligonucleotide-Directed Mutagenesis. Site-directed mutagenesis was performed (27, 28) with the Muta-Gene *in vitro* mutagenesis kit from Bio-Rad. Oligomers were synthesized by P. Gardner in the laboratory of D. Steiner at the University of Chicago.

RESULTS

***SPO13* Encodes a Protein of 291 Amino Acids.** The DNA sequence of *SPO13* (Fig. 1) contains a major ORF of 291 amino acids encoded in the 1.0-kb transcript. Primer extension analysis reveals that both the major (1.0 kb) and the minor (1.4 kb) transcripts have multiple 5′ start sites. The longer transcript encodes five small ORFs, 3–26 codons long, in its long leader; the significance of these ORFs is not known. No exact matches to the TATAAA consensus sequence are found upstream of the transcriptional starts of either transcript.

Identification of the *spo13-1* ochre mutation, located at Gln-86, confirms the large ORF as the correct reading frame of *SPO13*. This ORF specifies a 33.3-kDa protein with no detectable sequence homology to other known proteins and no domains similar to any known structural or functional motifs, with the exception of a putative nuclear localization sequence (Pro-Arg-Lys-Arg; ref. 30) near the N terminus. The most striking features of the predicted protein are as follows. (*i*) It is highly hydrophilic and charged. It contains 5.5% arginine, 8.9% lysine, 6.2% glutamic acid, and 3.8% aspartic acid; 25% of the residues are hydrophobic, but these are distributed throughout the sequence so that no strong hydrophobic domain is predicted. (*ii*) The protein is very basic. The predicted isoelectric point is 10.4 with a charge of +15 at neutral pH. (*iii*) The protein lacks tryptophan and has

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                                                                -840  ACTTGATTGTCCTGCCGTCATTGGTCCCATCTAAGGTCAA
-800  TTCCAATTTGTCGTTCTCATCTTGTTCATCACTATCTTCACAGTCCAAAGTGGTGGTAGTAGCAACAAACGAAGCCTCTG
-720  GCCACGAGAGAGTATGATACATATAATATTTCATCATATAATTTTCGGGTAGGTTATGCAGGTTGGCATTTTGCATACAT
-640  ATAATGTCATTGATTGTCGCTCTGCGAATATTAATAGGCATGGTTGACGAAGTTTATTTTGGAGCTTGATCGTATGTATT
-560  TAGGTTTCCCTTTTCACTTGGCAATTACTCGTTGCTGACAAAAGGTTTCAAACTTTTCAGACGGTGCTTTTTTTTTCTGT
-480  TCAAAAAACCAATTGACGCGAGAAAGTGAGGATAGAGCCTAGAGATCAATAAAGACAAATTAGCATTGGATGCAATACTA
-400  CTAAAGCAAGCGCTTTAAGAGAAAATATTTGTCCTGGAAGTCTCATGGAGATTATTCTTAGAATTCTTAATCTCCGGGCA
-320  GAAGGCACTTCAGCGTATCATTTGTTCCATGATATTTTCTGAAGTAAACTTCAGAATTATAATACACAGTATTTACACAT
-240  CTAATTTTTGACTACAGTATAATATTCAAGCTTCCTTGATTTACCTGCAGTTATGCTTAAATTCACATTTGAATTCTTTA
-160  GTATCCGTTTAGCTAGTTAGTACCTTTGCACGGAAATGTATTAATTAGGAGTATATTGAGAAATAGCCGCCGACAAAAAG
 -80  GAAGTCTCATAAAAGTGTCTAACAGACAATTAGCGCAATAAGAAGAAAGAAAACGGATTGAAGTTGAGTCGAGAATAATT
  +1  ATGGCACCCAGAAAACGCTTTAGGCTACTCGAATTAGGGTCACCAACACATTCTAAAAGGAAAGTTCAAAAACCTCTTCAA
      MetAlaProArgLysArgPheArgLeuLeuGluLeuGlySerProThrHisSerLysArgLysValGlnLysProLeuGln   27
 +82  GAGAAGACCCCAAACTTGCGAGTTTCTCCACTTGCTTTTAAAATTGGTAAAGAAATCAAGAATAAGGAAATCAGAAAAACA
      GluLysThrProAsnLeuArgValSerProLeuAlaPheLysIleGlyLysGluIleLysAsnLysGluIleArgLysThr   54
+163  AAAAAAACAGAATCAGAAAATATATTCAATTCGAAACATGTCGATTTAAGACTAGAAAGCCCACATCCAGGATTAAATTTT
      LysLysThrGluSerGluAsnIlePheAsnSerLysHisValAspLeuArgLeuGluSerProHisProGlyLeuAsnPhe   81
+244  GTAAGTGATGCTCAACAGTACTCAAAGGCTGGAGATGTTCGATATTTGAAAAATAAATCAAGCAATACCTTGAAAAACGAA
      ValSerAspAlaGlnGlnTyrSerLysAlaGlyAspValArgTyrLeuLysAsnLysSerSerAsnThrLeuLysAsnGlu   108
+325  AGACAAACAATAGAGCGCCCCTCATTTGATAATTCCTTAAGGTTCGAGGACATTGAGCAGCCTCCAAAATCTACTTCAACG
      ArgGlnThrIleGluArgProSerPheAspAsnSerLeuArgPheGluAspIleGluGlnProProLysSerThrSerThr   135
+406  CCGGTTCTTTCTCAATCTTCTCAAATAAATGTAGAAAGAGAAGCACCCATGTTTCCAGTTCCTTACTATATTGCTCCTTCT
      ProValLeuSerGlnSerSerGlnIleAsnValGluArgGluAlaProMetPheProValProTyrTyrIleAlaProSer   162
+487  CCTATGTATAATTTTAGCCCGTATCAAAATTTCGTTGGAAACCCAACGTTCCTGACGCCAAGTCATAACCCAAACCTAAAC
      ProMetTyrAsnPheSerProTyrGlnAsnPheValGlyAsnProThrPheLeuThrProSerHisAsnProAsnLeuAsn   189
+568  TATGCCATTCCCATCCAACGGCCGGAGTTGCTTTATCCGAATGTTAACGTATACGATTCGCCATTGTTTAAGAAAACAAGA
      TyrAlaIleProIleGlnArgProGluLeuLeuTyrProAsnValAsnValTyrAspSerProLeuPheLysLysThrArg   216
+649  CTGCCCCATCAGACCAAAAGTCTAGATAAAGAAAAAAATTACCAATATCTTCCAATATATCCGGTCTCGATTTCTAATAAC
      LeuProHisGlnThrLysSerLeuAspLysGluLysAsnTyrGlnTyrLeuProIleTyrProValSerIleSerAsnAsn   243
+730  GGAGATTTTGTTGGCCAAGAGACACCAAGGGCAGCACCAAAGTTAAGTAAAAAGAGACTTTCAAATACATTGGATGTTAAT
      GlyAspPheValGlyGlnGluThrProArgAlaAlaProLysLeuSerLysLysArgLeuSerAsnThrLeuAspValAsn   270
+811  TGTTCCGATTATGAGTCTAGTGGACAAAATGCAACTTATAATGATAGTGAGTCTTCCCTTAATTAAAAAGATTAGTTTACT
      CysSerAspTyrGluSerSerGlyGlnAsnAlaThrTyrAsnAspSerGluSerSerLeuAsn   291
+892  TTTTCTGTAGAATGTTAATTAGTTTATTGCATAGAAAATCTTTAATACTGAATGACAAAATTTCCACAATTTTTTTTAAAA
+973  AAATTATCGACAACTGCAG   991
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FIG. 1. DNA and predicted protein sequence of *SPO13*. Overlapping regions of both strands were sequenced from −630 to +994. Nucleotides are numbered at left (+1 is the first base of the protein-coding region) and amino acid residues at right. Primer extension studies (17) indicate that the most prominent 5′ ends (asterisks) are between −49 and +1 (±1 base) for the 1.0-kb RNA and at −405 and −335 (±20 bases) for the 1.4-kb RNA (data not shown). The two major 3′ ends (arrowheads) were previously determined (8) and are at +931 and +954 (±14 bases). The position of the *spo13-1* ochre mutation (+256), the 11-base-pair (bp) direct repeats, and the URS1 upstream repressor sequence are underlined. The open reading frame (ORF) of the divergently transcribed *ARD1* gene begins at position −600. The sequence from −847 to −389 is identical to that previously reported (29) except for a T instead of an A at position −345 in our sequence.

a high proline content (9.9%). A 35-residue domain beginning at position 151 has an exceptionally high proline content, 25%.

Analysis of translational fusions of *SPO13* to *Escherichia coli lacZ* demonstrate that the first 224 amino acids of the protein can complement the *spo13-1* mutation. This complementation is marginal but improves greatly when the fusion gene is on a 2μ high-copy vector where more fusion protein is produced (Table 1).

A Region Between −170 and −8 Confers Meiosis-Specific Expression. A transcriptional fusion of the *SPO13* 5′ region from −170 to −8 to the *HIS3* structural gene was analyzed for *HIS3* mRNA expression during sporulation. A *CEN* plasmid bearing this fusion exhibits meiosis-specific transcription of the *HIS3* mRNA (Fig. 2 *Lower*). Accumulation of this mRNA peaks at 6–9 hr of sporulation, mimicking the accumulation of *SPO13* mRNA produced from the chromosomal *SPO13* gene. Although the *HIS3* gene alone exhibits mitotic expression, presumably initiating in vector sequences, this activity decreases substantially during meiosis and never accumulates to the levels expressed from the *spo13–HIS3* fusions (compare the 6-hr lanes in Fig. 2). Strain LP112 without plasmid exhibits a low level of hybridization to the *HIS3* probe throughout sporulation (data not shown). These data provide evidence that an element(s) capable of conferring meiosis-specific regulation on a heterologous yeast gene resides in the region from −170 to −8 of the *SPO13* gene.

A 60-bp Region from −140 to −80 Is Essential for Meiosis-Specific Expression. The 5′ boundary of the *SPO13* regulatory region was determined by deletion analysis of plasmid-borne *spo13–lacZ* fusions (Table 2). Deletions to −140 retain meiosis-specific expression whereas deletion to −80 abolishes induction. These results indicate that sequences between −140 and −80 are essential for meiosis-specific expression. The increased mitotic activity seen in the high-copy-number −140 deletion plasmid, p(spo13)61, most likely originates from vector sequences, since mitotic activity is reduced by inversion of the −47 deleted *spo13–lacZ* fusion [compare p(spo13)70 in Table 3 with p(spo13)67Inv in Table 2] and inversion of the *spo13–lacZ* fusion in *CEN* plasmids [compare p(spo13)53 with p(spo13)66Inv and p(spo13)52 with p(spo13)65Inv, Table 2].

The Regulatory Region Imparts Meiosis-Specific Expression in an Orientation-Independent Manner. When sequences between −170 and −80 are inverted with respect to the direction of transcription in *spo13–lacZ* fusions, meiosis-specific regulation is retained [compare p(spo13)40 with p(spo13)64R and p(spo13)56 with p(spo13)63R, Table 2]. The inversion moves the −140 to −80 region, previously shown to be necessary for meiosis-specific regulation, ≈30 bases upstream of its normal position with respect to the ORF. These

Table 1. Sporulation and β-galactosidase activity in *spo13-1/spo13-1* diploids carrying *spo13–lacZ* fusions

Plasmid*	*SPO13* 3′ end	Asci, %	Tetrads/total asci, %	β-Galactosidase, unit(s)
CEN plasmids				
p(spo13)30	+45	53.9	2.8	0.8
p(SPO13)33	+671	63.2	6.8	2.2
p(SPO13)7	+873	77.3	65.5	<0.1
2μ plasmids				
p(spo13)28	+45	74.0	5.1	147.7
p(SPO13)32	+671	65.8	63.1	55.1
p(SPO13)17	+873	77.6	79.8	<0.1
pMC1585	0	51.5	2.8	0.2

*Plasmids were transformed into strain RE944 (*spo13-1/spo13-1*). All plasmids except p(SPO13)7, p(SPO13)17, and pMC1585 contain the *SPO13* gene from −847 to the indicated 3′ end, fused to +27 of the *E. coli lacZ* gene (+1 is the translational start). p(SPO13)7 and p(SPO13)17 contain only the *SPO13* gene; pMC1585 contains only the *lacZ* fragment.

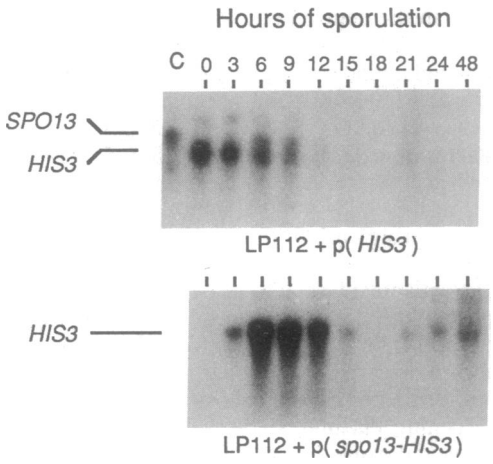

FIG. 2. Northern analysis of *SPO13* and *HIS3* expression during meiosis. Each lane contained ≈20 μg of total RNA from strain LP112 carrying the *HIS3* gene on pRS314. (*Upper*) *HIS3* lacking the upstream *SPO13* region, probed with *HIS3* and *SPO13*. The control lane (C) contained RNA from LP112 without plasmid 6 hr into sporulation. (*Lower*) *HIS3* fused 3′ to the −170 to −8 upstream fragment of *SPO13*, hybridized with the same *HIS3* probe used above.

Table 2. β-Galactosidase expression from *spo13–lacZ* fusion plasmids with 5′ deletions and point mutations

Plasmid*	*SPO13* 5′ end	Point mutation(s)†	β-Galactosidase, unit(s) Mitosis	β-Galactosidase, unit(s) Meiosis	Sporulation, %
Integrated *spo13–lacZ*					
p(spo13)42	—	—	0.1	3.6	80
CEN plasmids					
p(spo13)30	−847	—	<0.1	2.4	60
p(spo13)77	−847	−92	0.2	0.4	71
p(spo13)41	−212	—	<0.1	3.2	54
p(spo13)40	−170	—	0.1	3.4	58
p(spo13)64R‡	−170	—	0.2	3.2	57
p(spo13)72	−170	−77	<0.1	7.3	54
p(spo13)78	−170	−71 to −81	<0.1	3.6	57
p(spo13)53	−140	—	0.2	3.2	57
p(spo13)66Inv§	−140	—	0.1	5.1	46
p(spo13)75	−80	—	0.2	0.2	74
p(spo13)52	−47	—	0.3	0.6	62
p(spo13)65Inv§	−47	—	<0.1	0.2	56
2μ plasmids					
p(spo13)28	−847	—	0.1	42	63
p(spo13)55	−212	—	0.4	48	66
p(spo13)56	−170	—	2.0	86	72
p(spo13)74	−170	−77	<0.1	170	55
p(spo13)63R‡	−170	—	0.6	58	54
p(spo13)61	−140	—	18	180	65
p(spo13)67Inv§	−47	—	0.2	0.5	58

*All plasmids were transformed into strain S104. The *SPO13* sequences extend from the indicated 5′ end to +45 fused to *lacZ* at +27. The integrated *spo13–lacZ* is located at the *SPO13* locus on chromosome VIII and is preceded by all sequences normally 5′ to *SPO13*.
†The point mutation in p(spo13)77 is a G → A transition. The point mutation in p(spo13)72 is a G → T transversion. The sequence GGAAGTCTCAT is replaced with TACTCGAGATG in p(spo13)78.
‡*SPO13* sequences from −170 to −80 are in reverse orientation in p(spo13)63R and p(spo13)64R.
§The entire *spo13–lacZ* fusion is inverted within the vector in p(spo13)65Inv, p(spo13)66Inv, and p(spo13)67Inv.

Table 3. Effect of the *MAT* locus on β-galactosidase expression from *spo13–lacZ* fusions

MAT genotype	2μ plasmid*	*SPO13* 5′ end	β-Galactosidase, unit(s) Mitosis	Meiosis	Sporulation, %
a/α	p(spo13)28	−847	0.1	58	47
	p(spo13)56	−170	0.2	110	77
	p(spo13)63R†	−170	2.7	170	77
	p(spo13)74‡	−170	<0.1	61	79
	p(spo13)61	−140	16	190	63
	p(spo13)70	−47	17	9.6	80
a/**a**	p(spo13)28	−847	<0.1	0.1	<0.1
	p(spo13)56	−170	0.1	0.3	<0.1
	p(spo13)63R†	−170	1.7	13	<0.1
	p(spo13)74‡	−170	<0.1	3.8	<0.1
	p(spo13)61	−140	6.1	16	<0.1
	p(spo13)70	−47	14	14	<0.1
α/α	p(spo13)28	−847	<0.1	0.1	<0.1
	p(spo13)56	−170	0.1	0.3	<0.1
	p(spo13)63R†	−170	0.7	3.7	<0.1
	p(spo13)74‡	−170	<0.1	2.0	<0.1
	p(spo13)61	−140	6.6	19	<0.1
	p(spo13)70	−47	15	7.8	<0.1

*All plasmids were transformed into the isogenic strains LP112 (**a**/α) SFY 67 (**a**/**a**), and SFY 68 (α/α).
†The −170 to −80 region is in reverse orientation in p(spo13)63R.
‡p(spo13)74 has a G·C → T·A point mutation at −77.

results indicate that this region can function in both orientations and that its precise location with respect to the main ORF is not critical for proper expression.

The −140 to +45 Region Responds to *MAT* Control and the *RME* Pathway. To determine whether meiosis-specific regulation from the region between −140 and −80 responds to the *MAT* regulatory cascade, the behavior of *spo13–lacZ* fusions was examined in isogenic strains heterozygous and homozygous at the *MAT* locus. The fusions were introduced on multicopy plasmids to facilitate detection of their basal mitotic activity in the asporogenous strains SFY67 and SFY68. The results in Table 3 show that β-galactosidase activity mediated by the −140 to −80 region is under cell-type control—i.e., it is repressed in *MAT* homozygotes in sporulation medium. A small starvation-induced increase in β-galactosidase activity, consistent with a previously reported 5-fold increase in *SPO13* mRNA (8), also occurs. Regulation by *MAT* is independent of the orientation of the regulatory sequences (Table 3).

The −140 and −80 fusions, carried on *CEN* plasmids, were further tested in *mat* and/or *rme1* mutant strains (Table 4). Normal induction of *spo13–lacZ* occurs in *MAT***a**/*MAT*α strains, regardless of the state of *RME1*, but is not observed in the *mat***a1**/*MAT*α *RME1*/*RME1* mutant. Since meiosis-specific expression is restored in the absence of *RME1* (in the *mat***a1**/*MAT*α *rme1*::*LEU2*/*rme1*::*LEU2* double mutant), we conclude that an Rme1-responsive site(s) resides between −140 and +45.

Table 4. β-Galactosidase expression from fusion plasmids in strains carrying mutations at *MAT* and *RME1*

Plasmid*		Host genotype *MAT*	*RME1*	β-Galactosidase, unit(s) Mitosis	Meiosis	Sporulation, %
p(spo13)53	(−140)	**a**/α	+	0.3	11.7	49
		a/α	−	0.1	3.1	48
		a1/α	+	0.1	<0.1	<0.1
		a1/α	−	0.1	6.1	8
p(spo13)75	(−80)	**a**/α	+	0.2	0.2	34
		a/α	−	0.2	0.2	41
		a1/α	+	0.1	0.3	<0.1
		a1/α	−	<0.1	<0.1	4

*Plasmids were transformed into strains S150, S151, S152, and S153. The 5′ end of the *SPO13* sequence is indicated in parentheses. The 3′ end of the *SPO13* sequences in both plasmids is +45.

Table 5. Comparison of the 10-bp sequences in meiotically induced genes

Gene	Position	Sequence	Reference
SPO13	−88 to −97	TCGGCGGCTA	This study
SPO11	+163 to +172	TTGGCGGCAA	9
SPO16	−92 to −83	TGGGCGGCTA	10
HOP1	−173 to −164	TGGGCGGCTA	11
RED1	−166 to −157	TCAGCGGCTA	12
MER1	−103 to −112	TCGGCGGCTA	13
(URS1 consensus)		YCGGCGGCTA	31

The Essential Regulatory Region Contains a 10-bp Sequence That Appears to be Important for Meiosis-Specific Control. Two intriguing candidates for specific short sequences within the 60-bp region that might govern meiosis-specific expression were examined further. One, an 11-bp sequence, GGAAGTCTCAT, repeated twice upstream of the *SPO13* ORF at −365 to −355 and at −81 to −71, apparently is not involved in meiosis-specific regulation, since (*i*) deletion analysis has eliminated the distal repeat as being important for fusion regulation, (*ii*) deletion to −170 along with an alteration at −77 in the proximal sequence does not affect nutritional or mating type control of *spo13–lacZ* [compare p(spo13)40 with p(spo13)72 in Table 2 and compare p(spo13)56 with p(spo13)78 in Tables 2 and 3], and (*iii*) deletion to −170 and replacement of the proximal 11 bases with an unrelated sequence of the same G+C content (effectively eliminating both repeats) retains regulation [compare p(spo13)40 with p(spo13)72 in Table 2]. In contrast, the second candidate, a 10-bp sequence, TAGCCGCCGA (−97 to −88), appears to be required for regulation. Evidence for this role is based on the effect of an ethyl methanesulfonate-induced C·G → T·A point mutation at −92 (mutant obtained from R. Surosky of this laboratory). This alteration strongly decreases meiotic expression [compare p(spo13)77 with p(spo13)30, Table 2]. The −92 point mutation also exhibits a reproducible elevation in the mitotic level of expression, which has been confirmed in more recent studies of *spo13–URA3* fusions (L.E.B., C. Steber, and R.E.E., unpublished work; also see *Discussion*). Examination of five other meiosis-specific genes, *SPO11*, *SPO16*, *RED1*, *MER1*, and *HOP1*, induced at the same time as *SPO13* during meiosis, indicates they all contain close matches to this 10-bp sequence (Table 5). For *HOP1*, this sequence is within a region required for meiotic expression and, as noted by Hollingsworth *et al.* (11), contains a 6-bp homology to the binding site for the mammalian transcriptional activator Sp1. Surprisingly, this sequence element, which appears to have a role in meiosis-specific expression, is identical to URS1, an upstream repressor sequence found adjacent to a number of yeast genes (31).

DISCUSSION

Dramatic changes occur in gene expression as vegetative cells shift from mitosis to meiosis and sporulation (32). The appearance of meiosis-specific mRNAs can be divided into early, middle, and late classes, depending on the time of their peak accumulation (33, 34). This program of expression appears to be controlled at the level of transcription, rather than RNA turnover (R. Surosky and R.E.E., unpublished data). *SPO13*, required for successful completion of meiosis I, is one of the genes transcribed early in meiosis; peak

transcript accumulation occurs approximately at the time of the first meiotic division (8). We have demonstrated that the region from −170 to −8 is sufficient for meiosis-specific regulation, since it can impart meiosis-specific expression to a heterologous gene. Deletion analysis has further shown that sequences within the 60-bp region from −140 to −80 are essential for meiosis-specific regulation. The regulatory element(s) within this region operates like other upstream regulatory sequences in that the region is functional in both orientations (35). The position of the control region relative to the site that promotes transcriptional initiation is not known. *SPO13* lacks a TATAAA consensus (35, 36) at the expected position and may be one of an increasing number of "TATA-less" genes (37, 39).

What sequences within the 60-bp region between −140 to −80 impart regulation? The best candidate for a critical sequence is the 10-bp sequence TAGCCGCCGA, found at −97 to −88. Three lines of evidence support the importance of this element in regulation. (*i*) This sequence is found in the 5′ region of the coregulated meiosis-specific genes *SPO16*, *RED1*, *MER1*, and *HOP1* (Table 5). For *HOP1*, it is located within a 105-bp region necessary for meiosis-specific expression (11). (*ii*) A good match to this sequence is found in the translated portion of *SPO11* gene. Although such a location for a regulatory element is unusual, deletion analysis has shown that a regulatory element(s) is present in this region (C. Atcheson, personal communication). (*iii*) A mutation at −92 results in loss of meiosis-specific induction of *spo13–lacZ*. Based on this evidence, this sequence is likely to be required for meiosis-specific transcriptional activation.

Surprisingly, a sequence very similar and possibly identical to this one has been identified as a repressor of transcription during the mitotic cycle. This upstream repressor sequence, URS1, is present in a number of diverse yeast genes (31, 39) and has been shown to repress transcription of *CAR1*, *SSA1*, and *ENO1* during growth (31, 39, 40). The point mutation at −92 within this sequence also results in increased mitotic expression of *spo13–lacZ* (Table 2). In addition, point mutations at −91, −92, −93, and −94 allow mitotic expression of a *spo13–URA3* fusion (C. Steber, L.E.B., and R.E.E., unpublished results). Therefore, in the context of *SPO13* sequences this element also functions in mitotic repression.

In yeast it has been reported that complex regulation involving both repression and activation can be facilitated by common components. For example, the *MCM1* gene product, an activator, is required for binding of the α2 repressor protein to adjacent sites (41, 42). Overlapping cis-acting sites also occur (43, 44), e.g., in the yeast *HSP70* cognate gene *SSA1*, where a cis-acting heat shock element required for activation overlaps URS1, required for repression (39). It remains to be determined whether *URS1* participates in meiosis-specific regulation in a similar manner.

Although specific binding activities have been detected in yeast whole-cell extracts for the URS1 elements found in *SSA1* (39) and *CAR1* (38), the genes encoding these activities have not been defined. Recently, six genes have been identified in our laboratory, *UME1–UME6*, that repress the mitotic expression of several early meiosis-specific genes including *SPO13* (ref. 34; R. Surosky, unpublished work). It is not known whether some of the *UME* gene products affect gene expression through the URS1 site and are responsible for the binding activities described above.

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1. Esposito, R. E. & Klapholz, S. (1980) in *The Molecular Biology of The Yeast Saccharomyces: Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 211–287.
2. Mitchell, A. P., Driscoll, S. E. & Smith, H. E. (1990) *Mol. Cell. Biol.* **10**, 2104–2110.
3. Kassir, Y. & Simchen, G. (1976) *Genetics*, **82**, 187–206.
4. Mitchell, A. & Herskowitz, I. (1986) *Nature (London)* **319**, 738–742.
5. Kassir, Y., Granot, D. & Simchen, G. (1988) *Cell* **52**, 853–862.
6. Smith, H. E. & Mitchell, A. P. (1989) *Mol. Cell. Biol.* **9**, 2141–2152.
7. Klapholz, S. & Esposito, R. E. (1980) *Genetics* **96**, 589–611.
8. Wang, H.-T., Frackman, S., Kowalysin, J., Esposito, R. E. & Elder, R. (1987) *Mol. Cell. Biol.* **7**, 1425–1435.
9. Atcheson, C. L., DiDomenico, B., Frackman, S., Esposito, R. E. & Elder, R. T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8035–8039.
10. Malavasic, M. J. & Elder, R. T. (1990) *Mol. Cell. Biol.* **10**, 2809–2819.
11. Hollingsworth, N. M., Goetsch, L. & Byers, B. (1990) *Cell* **61**, 73–84.
12. Thompson, E. A. & Roeder, G. S. (1989) *Mol. Gen. Genet.* **218**, 293–301.
13. Engebrecht, J. & Roeder, G. S. (1990) *Mol. Cell. Biol.* **10**, 2379–2389.
14. Klapholz, S. & Esposito, R. E. (1982) *Genetics* **100**, 387–412.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
16. Innis, M. A., Myambo, K. B., Gelfard, D. H. & Brow, M. A. D. (1988) *Proc. Natl. Acad. Sci. USA* **58**, 9436–9440.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Casadaban, M., Martinez-Arias, A., Shapira, S. K. & Chou, J. (1983) *Methods Enzymol.* **100**, 293–308.
19. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1987) *Gene* **60**, 237–243.
20. Botstein, D., Falco, S. C., Stewart, S. E., Brennan, M., Scherer, S., Strichcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Gene* **8**, 17–24.
21. Struhl, K., Stinchcomb, D. T., Scherer, S. & Davis, R. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1035–1039.
22. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.
23. Struhl, K. (1985) *Nature (London)* **317**, 822–824.
24. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
25. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
26. Struhl, K. & Davis, R. (1980) *J. Mol. Biol.* **136**, 309–322.
27. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
28. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154**, 367–382.
29. Whiteway, M. & Szostak, J. W. (1985) *Cell* **43**, 483–492.
30. Moreland, R. B., Nam, H. G., Hereford, L. M. & Fried, H. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6561–6565.
31. Sumrada, R. A. & Cooper, T. G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3997–4001.
32. Kurtz, S. & Lindquist, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7323–7327.
33. Percival-Smith, A. & Segall, J. (1984) *Mol. Cell. Biol.* **4**, 142–150.
34. Strich, R., Slater, M. R. & Esposito, R. E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 10018–10022.
35. Struhl, K. (1987) *Cell* **49**, 295–297.
36. Hahn, S., Hoar, C. T. & Guarente, L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8562–8566.
37. Hahn, S., Buratowski, S., Sharp, P. A. & Guarente, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5718–5722.
38. Luche, R. M., Sumrada, R. & Cooper, T. G. (1990) *Mol. Cell. Biol.* **10**, 3884–3895.
39. Park, H.-O. & Craig, E. (1989) *Mol. Cell. Biol.* **9**, 2025–2033.
40. Cohen, T., Yokoi, T., Holland, J. P., Pepper, A. & Holland, M. J. (1987) *Mol. Cell. Biol.* **7**, 2753–2761.
41. Herskowitz, I. (1989) *Nature (London)* **342**, 749–757.
42. Keleher, C. A., Goutte, C. & Johnson, A. D. (1988) *Cell* **42**, 237–247.
43. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
44. Levine, M. & Manley, J. (1989) *Cell* **59**, 405–408.