MOLECULAR AND CELLULAR BIOLOGY, Dec. 1986, p. 4478–4485
0270-7306/86/124478-08$02.00/0

Vol. 6, No. 12

The *SPS4* Gene of *Saccharomyces cerevisiae* Encodes a Major Sporulation-Specific mRNA

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Received 5 June 1986/Accepted 3 September 1986

The *SPS4* gene of *Saccharomyces cerevisiae*, a sporulation-specific gene identified previously in a differential hybridization screen of a genomic yeast DNA library, has been characterized further. The protein encoded by this gene was inferred from its nucleotide sequence to be 38,600 daltons with an isoelectric pH of 8.2. Consistent with this, two-dimensional polyacrylamide gel electrophoresis of the in vitro translation products of RNA purified by hybridization with the cloned *SPS4* DNA indicated that the *SPS4* gene product is a 39-kilodalton, basic protein. This protein was found to be identical in size and charge to a major, sporulation-specific protein identified in a two-dimensional polyacrylamide gel electrophoretic comparison of the in vitro translation products of total RNA from sporulating *MAT*a/*MAT*α cells and asporogenous *MAT*α/*MAT*α cells. A *MAT*a/*MAT*α strain homozygous for a partial deletion of the *SPS4* gene appeared, however, to be unaffected in its ability to form viable ascospores.

The temporal program of complex genetic and morphological events occurring during sporulation of *Saccharomyces cerevisiae* and the ease with which a variety of genetic manipulations can be performed with this organism make sporulation in yeast an appealing eucaryotic system for studying developmentally regulated gene expression. Several different approaches have been used to test the presumption that the temporally programmed events of meiosis and subsequent spore wall formation depend on the developmentally regulated expression of sporulation-specific genes (for a review, see references 7 and 9). A mutational analysis has identified several genes which are dispensable for mitotic growth but are essential for sporulation (8). Attempts to detect sporulation-specific proteins by two-dimensional polyacrylamide gel analysis of in vivo-labeled proteins have, however, been less successful (13, 14, 24, 30, 33, 34) and only one sporulation-specific enzyme, an α-glucosidase, has been described (5). In contrast, a large number of sporulation-specific genes have been identified by differential hybridization screens of yeast DNA libraries (4, 12, 22). Analyses of the in vitro translation products of RNA isolated from sporulating cells have also demonstrated the accumulation of an array of sporulation-specific mRNAs (15, 32).

In this study, we show that a sporulation-specific gene, identified previously in a differential hybridization screen of a yeast DNA library (22), encodes one of the prominent sporulation-specific proteins observed among the in vitro translation products of RNA from sporulating cells.

MATERIALS AND METHODS

Strains and culture conditions. *Escherichia coli* HB101 and CGSC 5126, a Dam$^-$ strain (provided by J. Friesen), were used for the propagation of plasmids. The *S. cerevisiae* strains used were the AP3 *MAT*α/*MAT*α, *MAT*a/*MAT*a, and *MAT*a/*MAT*α diploids previously employed (22), SR25-1A (*MAT*a *his4-912 ura3-52*) (provided by J. Friesen), CG379 (*MAT*α *ade5 his7-2 leu2-3, 112 trp1-289 ura3-52*) (provided by C. Giroux), and the diploid GR100, which was obtained by mating SR25-1A and CG379.

Transcript mapping. After a restriction enzyme map had been deduced for plasmid 27 (p27) by standard procedures (17), the approximate positions of the sequences encoding the two vegetative transcripts and the sporulation-specific transcript were determined (i) by hybridizing RNA that had been labeled in vitro (23) to a Southern blot of plasmid DNA that had been restricted with a variety of enzymes and (ii) by hybridization of purified, radioactively labeled DNA fragments of p27 to Northern blots of RNA isolated from vegetatively growing and sporulating cells. The ends of the transcript encoded by the *SPS4* gene were then positioned more precisely by an S1 nuclease analysis (1, 31). The mapping of the 5′ end of the transcript is described in Fig. 3B; a *Cla*I-*Xho*I fragment 3′ end labeled at the *Cla*I terminus was used as a probe to map the 3′ end of the gene.

In vitro translation and hybrid selection. A 10-μg sample of total yeast RNA was translated for 90 min at 37°C in a micrococcal nuclease-treated rabbit reticulocyte lysate (21) in the presence of 30 μCi of [^{35}S]methionine. Equivalent amounts of [^{35}S]methionine-labeled proteins (100,000 cpm) were then analyzed by polyacrylamide gel electrophoresis. Gene-specific RNA was obtained by hybridization of 2 μg of linearized plasmid DNA denatured in situ on a 1-cm^2 portion of Gene Screen Plus (New England Nuclear Corp.) with 150 μg of total yeast RNA in a 100-μl reaction (17, 25). The RNA recovered from the hybrids was translated as described above, and 1 μl of the reaction was analyzed. For one-dimensional analysis of the labeled proteins, the translation products were electrophoresed through a 10% polyacrylamide gel containing sodium dodecyl sulfate (SDS) (16) and detected by fluorography (2). Two-dimensional separation of the translation products was performed as described by O'Farrell et al. (20); the first dimension was nonequilibrium pH gradient electrophoresis (pH 3 to 10) for 6 h at 400 V, and the second dimension was electrophoresis in a 10% polyacrylamide gel containing SDS.

RESULTS AND DISCUSSION

Characterization of the *SPS4* gene. We previously performed a differential hybridization screen of a genomic yeast

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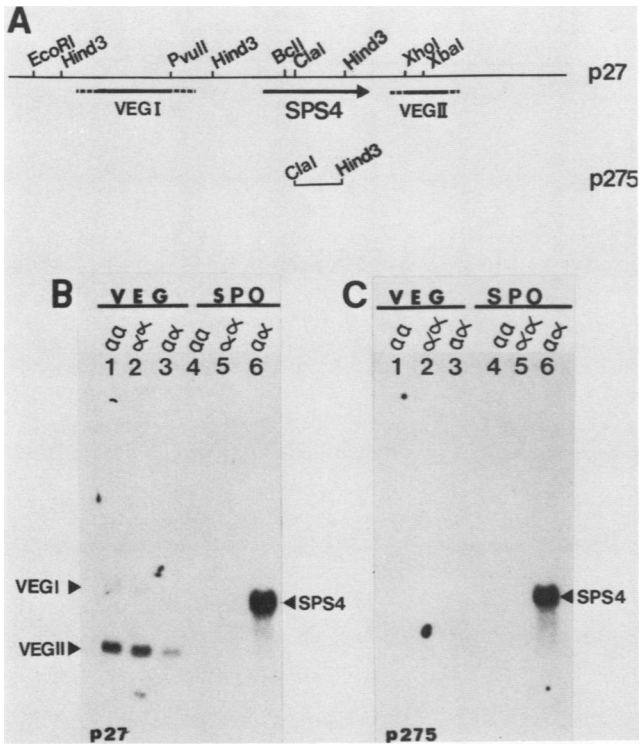

FIG. 1. Examination of the developmentally regulated expression of the *SPS4* gene. (A) The restriction map of the yeast DNA insert of p27 is shown for selected restriction endonucleases. The positions of the transcripts encoded by the *SPS4*, *VEGI*, and *VEGII* genes are indicated (see Materials and Methods). The portion of the *SPS4* gene subcloned into pDPL13 (10) to construct p275 is denoted. (B, C) Total RNA (10 μg) purified from either AP3 *MAT***a**/*MAT***a**, *MATα*/*MATα*, or *MAT***a**/*MATα* cells (as indicated at the top of each lane) growing vegetatively (VEG) or at 10 h after transfer to sporulation medium (SPO) (22) was denatured, separated by electrophoresis through a 1.5% agarose-formaldehyde gel, and then transferred to a nylon membrane. The filters were then hybridized with (B) p27 DNA or (C) p275 DNA that had been radioactively labeled by nick translation (26). The transcripts encoded by the *SPS4* and *VEG* genes are indicated.

DNA library which identified 38 plasmids representing 14 different cloned genes that are expressed preferentially during sporulation in *S. cerevisiae* (*SPS* genes) (22). Most of the *SPS* genes identified in this screen were isolated only once despite the fact that approximately 12 genome equivalents of yeast DNA were screened (22). In contrast, we had tentatively concluded that the sporulation-specific gene of p27, which we have termed *SPS4*, had been identified 15 times (22). (These 15 plasmids were assigned to groups 2 and 5 in reference 22.)

The relative ease with which plasmids containing the *SPS4* gene were identified suggested the possibility that this gene encodes a relatively abundant sporulation-specific mRNA. This would have facilitated the identification of the *SPS4* gene in the differential hybridization screen by enhancing the hybridization response of the cloned gene with the radioactive *SPS4* cDNA in the probe (22). Alternatively, the relatively high frequency with which the *SPS4* gene was identified may reflect the fact that the gene is present in several copies in the yeast genome.

As the first step in distinguishing between these possibilities, we characterized p27, one of the 15 plasmids of groups 2 and 5 (22), in more detail. The 6.5-kilobase-pair (kbp) yeast DNA insert present in p27 contains, in addition to the *SPS4* gene, two genes, termed *VEGI* and *VEGII*, which are expressed only during vegetative growth (Fig. 1B). The approximate positions of these three genes were mapped (Fig. 1A; see Materials and Methods). This analysis verified that the sporulation-specific transcript of the *SPS4* gene is encoded by a sequence distinct from the two vegetative genes; for instance, the Northern analysis depicted in Fig. 1C shows that the two transcripts present in vegetatively growing cells which hybridize with p27 DNA (Fig. 1B, lanes 1 through 3) do not hybridize with DNA from p275 (Fig. 1C, lanes 1 through 3), a subclone of p27 containing only the *SPS4* sequence (Fig. 1A; Fig. 1C, lane 6).

We next tested whether all 15 putatively related plasmids of groups 2 and 5 did indeed contain the *SPS4* gene (or portion thereof) and whether the yeast DNA insert of each plasmid had been derived from the same chromosomal gene. DNA from each plasmid and genomic yeast DNA were restricted with *Hin*dIII, *Cla*I, and *Xho*I. Southern blots (17, 28) of the restricted DNAs were then hybridized with a probe containing a portion of the coding region of the *SPS4* gene of p27, a probe spanning the 5′-flanking and initial coding region of the gene, and a probe extending from the 3′ coding region of the gene into the 3′-flanking region (Fig. 2A and 3).

All 15 plasmids hybridized with the coding region probe (Fig. 2B), confirming that each plasmid does indeed contain a portion of the *SPS4* gene. The hybridization data also indicated that the *SPS4* gene is present only once in the yeast genome; the probes corresponding to the 5′ and 3′ junctions of the *SPS4* gene of p27 hybridized with only one genomic DNA fragment each (Fig. 2F and G, respectively). Addition-

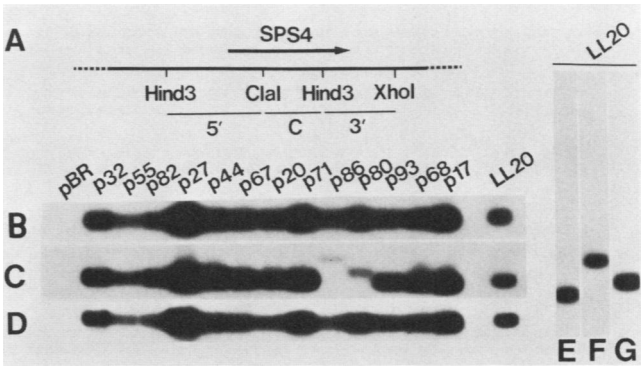

FIG. 2. The 15 *SPS4*-containing plasmids were derived from a unique genomic copy of the gene. (A) The DNA probes spanning the 5′ end (*Hin*dIII-*Cla*I), the coding region (*Cla*I-*Hin*dIII), and the 3′ end (*Hin*dIII-*Xho*I) of the *SPS4* gene of p27 are indicated as 5′, C, and 3′, respectively. The 5′-end probe was purified from p274, which contains the *Hin*dIII-*Cla*I fragment from the 5′ end of the *SPS4* gene of p27 cloned in pDPL13 (10); the coding region probe was purified from p275 (Fig. 1A); and the 3′-end probe was purified from p276, which contains the *Cla*I-*Xho*I fragment from the *SPS4* gene of p27 cloned in pDPL13. (B through G) DNA from each of the plasmids of groups 2 and 5 (22) and DNA from the yeast strain LL20 (22) were restricted with *Hin*dIII, *Cla*I, and *Xho*I. (Plasmid 26 and p19 are identical with p32 and p44, respectively [22].) Separate Southern blots (17) of the triply digested DNAs were hybridized with (B, E) the coding region probe, (C, F) the 5′ junction probe, and (D, G) the 3′ junction probe which had been radioactively labeled by nick translation. For B, C, and D only the portion of the membrane hybridizing with the probe is shown; for E, F, and G the entire lane is shown.

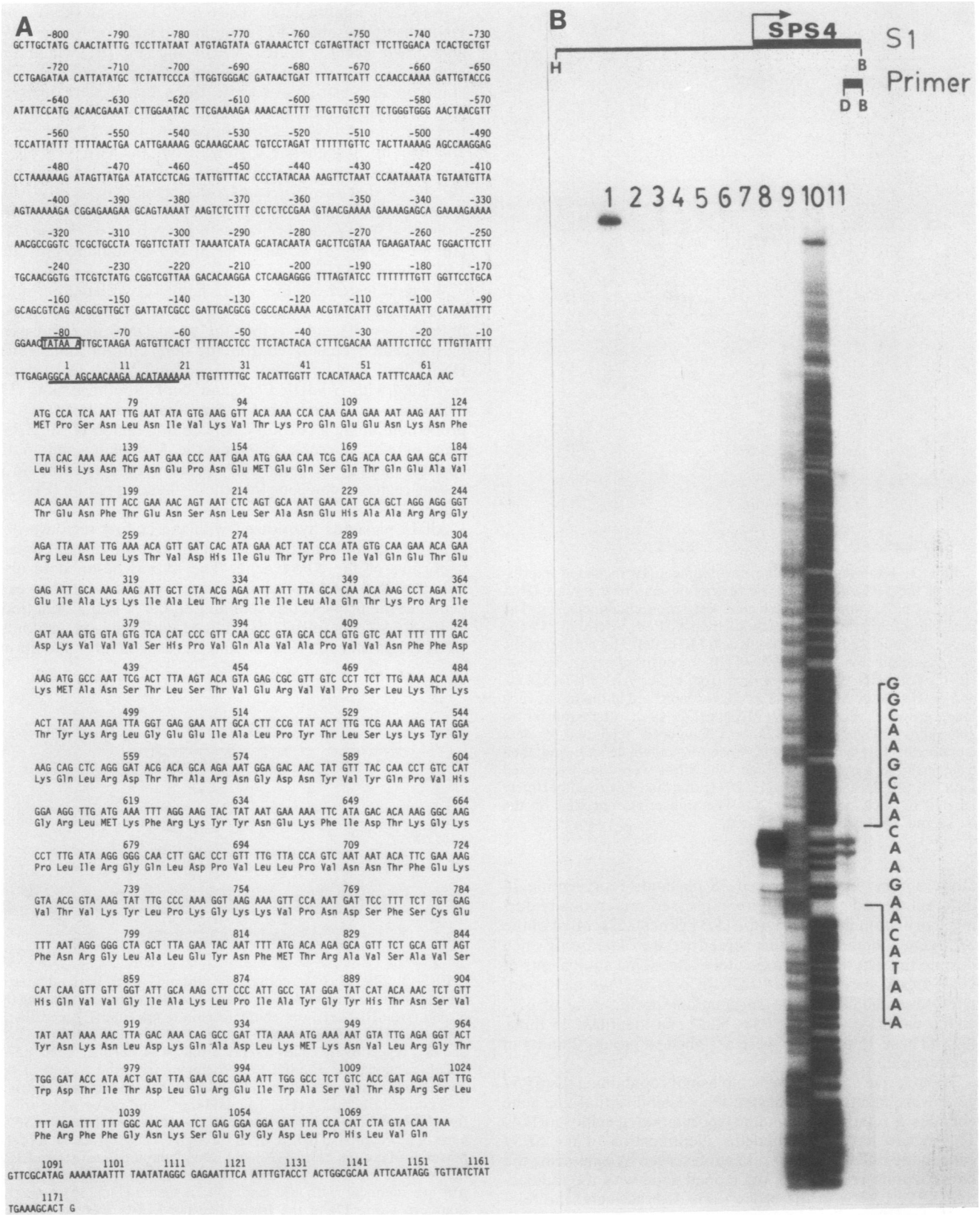

FIG. 3. Nucleotide sequence of the nontranscribed strand of the *SPS4* gene and determination of the 5′ end of the gene. (A) The nucleotide sequence of the *SPS4* gene was obtained for both strands by the method of Maxam and Gilbert (18). The sequence encompassing the multiple transcriptional initiation sites is underlined; the most prominent start site is denoted +1. The putative TATA box sequence is boxed. Translation of the predicted open reading frame of the gene is shown below the nucleic acid sequence. (B) Mapping the 5′ end of the *SPS4*

ally, this analysis indicated that 13 of the cloned copies of the *SPS4* gene extend from the *Hin*dIII restriction site in the 5'-flanking region of the gene to the *Xho*I site downstream of the gene (Fig. 2B, C, and D). In two instances, p86 and p80, the cloned fragment (a partial *Sau*3A yeast DNA fragment [22]) does not extend to the *Hin*dIII restriction site in the 5'-flanking region of the gene (Fig. 2C). The artificial 5' junction fragments appearing for these two plasmids are not detected in the yeast genome (Fig. 2C and F).

Having verified that the *SPS4* gene is a single-copy gene, we next determined the nucleotide sequence of the *SPS4* gene to deduce the nature of its gene product. Examination of the sequence revealed a single long open reading frame encoding a basic protein (calculated pI, 8.2) of 338 amino acids with a molecular weight of 38,600 (Fig. 3A). Neither a comparison of the predicted *SPS4* protein with the proteins in the PIR database [Protein Sequence Database of the Protein Identification Resource (PIR), National Biomedical Research Foundation, Washington, D.C., 1 March 1986] nor a comparison of the nucleic acid sequence with the Gen Bank DNA Sequence Database (1 March 1986) revealed significant homologies.

S1 nuclease analysis (1, 31) was performed to precisely map the 5' end of the transcript encoded by the *SPS4* gene. The S1 nuclease-resistant hybrids formed on hybridization of the *Hin*dIII-*Bcl*I fragment indicated in Fig. 3B with RNA extracted from *MAT***a**/*MAT*α sporulating cells revealed that there are multiple transcriptional initiation sites over a 21-nucleotide sequence 65 base pairs (bp) upstream from the predicted translational start codon of the *SPS4* gene (Fig. 3B, lanes 8 through 10; Fig. 3A). Analysis of the cDNAs obtained by using a 57-bp *Dde*I-*Bcl*I fragment as a primer for reverse transcription (11) of the *SPS4* transcripts confirmed the initiation sites defined in the S1 nuclease analysis (Fig. 3B, lane 11). A TATA box sequence (3) is located 85 bp upstream from the start of transcription (Fig. 3A).

The *SPS4* gene encodes a major sporulation-specific mRNA as assessed by analysis of the in vitro translation products of total RNA and hybrid-selected RNA. As the first step in pursuing further the suggestion that the *SPS4* gene encodes an abundant sporulation-specific mRNA, we analyzed the proteins obtained by in vitro translation of total yeast RNA. The proteins synthesized in vitro in a rabbit reticulocyte lysate (21) using as a template RNA extracted from cells growing vegetatively (Fig. 4A, lanes 2 through 4) and from cells harvested at 10 h after transfer to sporulation medium (Fig. 4A, lanes 5 through 7) (22) were compared by SDS-polyacrylamide gel electrophoresis (16). This analysis revealed a major sporulation-specific protein of 39 kilodaltons (kDa). The transcript encoding this protein is only present in *MAT***a**/*MAT*α cells during sporulation (Fig. 4A, lane 7). Kurtz and Lindquist (15) have also detected an abundant sporulation-specific mRNA encoding a protein of similar size using a wheat germ-derived in vitro translation system.

Two-dimensional separation of the in vitro-synthesized proteins by nonequilibrium isoelectric focusing in the first dimension (20) followed by SDS-polyacrylamide gel electrophoresis in the second dimension indicated that the major 39-kDa sporulation-specific protein is very basic (indicated by the arrow, Fig. 4B), migrating to a slightly more basic position than the readily identifiable abundant protein EF-1α (M_r 49,000, pI 9.2; indicated by the asterisk, Fig. 4B) (29). The 39-kDa basic protein is not observed in a two-dimensional analysis of the translation products of RNA extracted from asporogenous *MAT*α/*MAT*α cells transferred to sporulation medium (Fig. 4C) or from *MAT***a**/*MAT*α cells growing vegetatively (data not shown). The molecular weight and basic nature of this sporulation-specific protein made it a good candidate for the predicted *SPS4* gene product. Therefore, we next analyzed the translation product of the transcript encoded by the *SPS4* gene.

p27 DNA was used to hybrid select (25) the transcripts encoded by this plasmid from the RNA extracted from *MAT***a**/*MAT***a**, *MAT*α/*MAT*α, and *MAT***a***MAT*α cells growing vegetatively (Fig. 4D lanes 3, 5, and 7, respectively) or at 10 h after transfer to sporulation medium (Fig. 4D, lanes 4, 6, and 8, respectively). SDS-polyacrylamide gel analysis of the in vitro translation products of the purified RNA revealed a vegetative-specific mRNA encoding a 22-kDa protein (Fig. 4D, lanes 3, 5, and 7) and a sporulation-specific mRNA encoding a 39-kDa protein (Fig. 4D, lane 8). We assume that the 22-kDa vegetative-specific protein is encoded by the *VEGII* gene and that insufficient *VEGI*-encoded transcripts are present to detect the translation products of this latter RNA. The 39-kDa sporulation-specific protein comigrated in the SDS-polyacrylamide gel with the 39-kDa protein observed on in vitro translation of total RNA from sporulating *MAT***a**/*MAT*α cells (Fig. 4D, lanes 8 and 11), supporting the idea that the *SPS4* gene encodes this major sporulation-specific protein. The sporulation-specific mRNA encoding the 39-kDa protein was also hybrid selected by p275 DNA, which contains only a portion of the coding region of the *SPS4* gene (Fig. 1A; Fig. 4D, lane 10). As expected, this latter DNA did not hybridize with the transcripts encoding the 22-kDa vegetative protein (Fig. 4D, lane 9). Two minor sporulation-specific proteins were observed when either p27 or p275 DNA was used for transcript selection (denoted by the open arrowheads, Fig. 4D, lanes 8 and 10). We do not know whether these proteins represent proteolytic degradation products of the 39-kDa protein, the use of internal initiation codons in the *SPS4* transcript, or premature termination of translation; however, these proteins are not the translation products of transcripts which are partially homologous to the *SPS4* gene, because stringent hybridization

gene. An S1 nuclease analysis (1, 31) was performed by using the indicated *Hin*dIII-*Bcl*I fragment spanning the 5' end of the *SPS4* gene as probe. The plasmid DNA was prepared from a Dam$^-$ strain of *E. coli*, and the probe was 5' end labeled at the *Bcl*I terminus. The probe was hybridized with 10 μg of total RNA purified from AP3 cells harvested during vegetative growth (lanes 3 through 5) or at 10 h after transfer to sporulation medium (lanes 6 through 8) (22). The reactions of lanes 3 and 6 contained RNA from *MAT***a**/*MAT***a** cells; the reactions of lanes 4 and 7 contained RNA from *MAT*α/*MAT*α cells; the reactions of lanes 5 and 8 contained RNA from *MAT***a**/*MAT*α cells; S1 nuclease was omitted from the reaction of lane 1; RNA was omitted from the reaction of lane 2. The 5' end of the gene was also mapped by a primer extension analysis (lane 11) (11) with the indicated 57-bp *Dde*I-*Bcl*I fragment 5' end labeled at the *Bcl*I terminus as a primer for reverse transcription of RNA from *MAT***a**/*MAT*α cells harvested 10 h after transfer to sporulation medium. The S1-resistant hybrids and the cDNA-RNA hybrids were heat denatured and subjected to electrophoresis on a 6% polyacrylamide–8 M urea gel adjacent to a pyrimidine (lane 9) and purine (lane 10) chemical sequencing ladder (18) of the labeled *Hin*dIII-*Bcl*I fragment. The transcribed strand of this fragment was labeled; the sequence shown on the right of the panel is for the complementary nontranscribed strand of the gene.

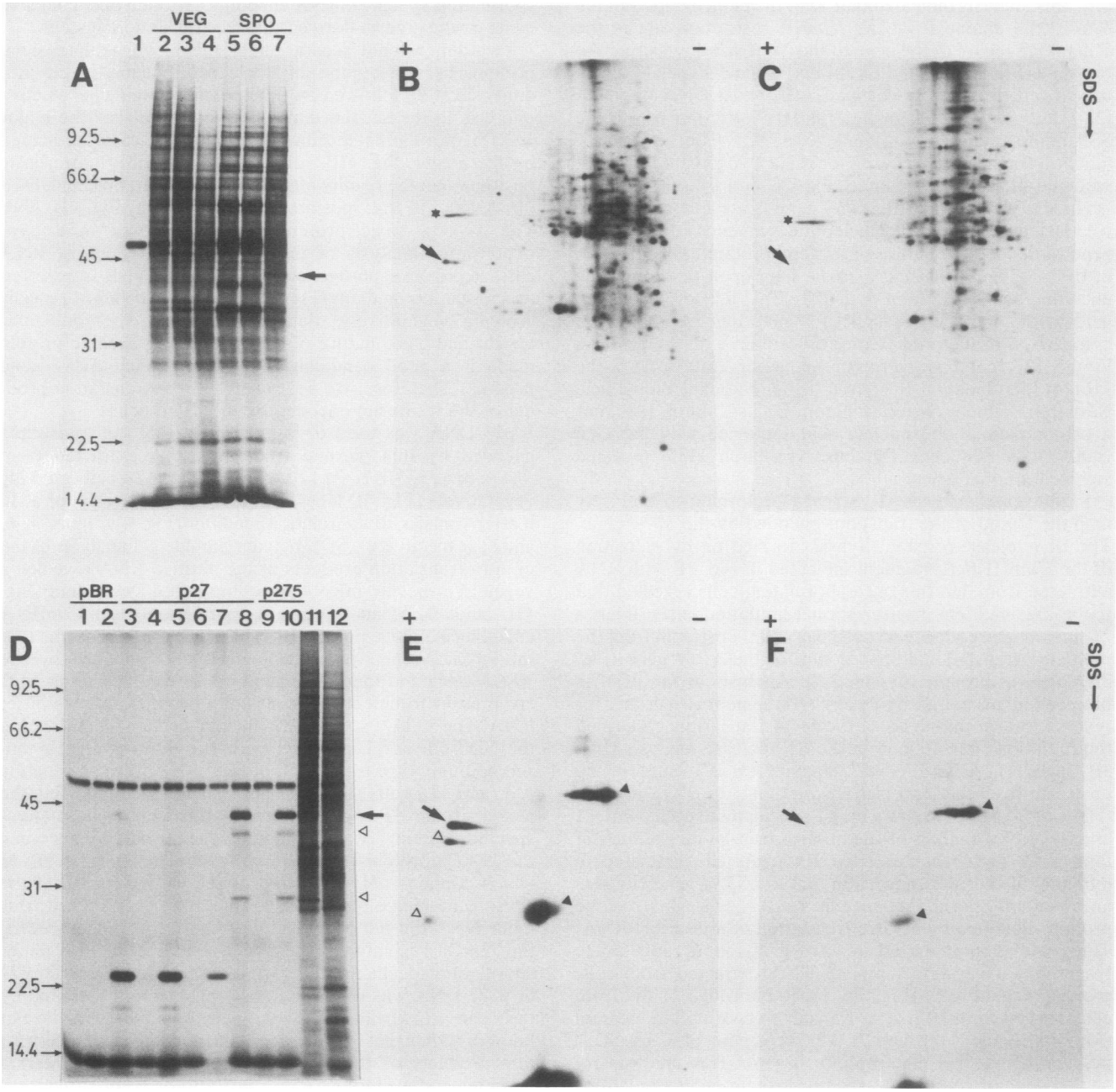

FIG. 4. The *SPS4* gene encodes a major sporulation-specific mRNA as assessed by in vitro translation. (A) One-dimensional SDS-polyacrylamide gel analysis of the in vitro translation products of total RNA. RNA extracted from AP3 *MAT***a**/*MAT***a** cells (lanes 2 and 5), *MAT*α/*MAT*α cells (lanes 3 and 6), and *MAT***a**/*MAT*α cells (lanes 4 and 7) growing vegetatively (lanes 2 through 4) or at 10 h after transfer to sporulation medium (lanes 5 through 7) (22) were translated in a rabbit reticulocyte lysate. Lane 1 contains the in vitro translation products obtained in the absence of exogenous RNA. The molecular masses of marker proteins are indicated in kDa on the left. The arrow on the right indicates the major 39-kDa sporulation-specific protein referred to in the text. (B, C) The in vitro translation products of RNA from (B) *MAT***a**/*MAT*α cells or (C) *MAT*α/*MAT*α cells at 10 h after transfer to sporulation medium were separated by two-dimensional gel electrophoresis (see Materials and Methods). The arrow denotes the presence (B) or absence (C) of the 39-kDa sporulation-specific protein, and the asterisk denotes EF-1α (29). (D) One-dimensional SDS-polyacrylamide gel analysis of the in vitro translation products of RNA recovered from the hybrids formed between pBR322 DNA (lanes 1 and 2), p27 DNA (lanes 3 through 8), or p275 DNA (lanes 9 and 10) and total yeast RNA from *MAT***a**/*MAT***a** cells (lanes 3 and 4), *MAT*α/*MAT*α cells (lanes 5 and 6), and *MAT***a**/*MAT*α cells (lanes 7 through 10) growing vegetatively (lanes 1, 3, 5, 7, and 9) or at 10 h after transfer to sporulation medium (lanes 2, 4, 6, 8, and 10). Lanes 11 and 12 contain the in vitro translation products of total RNA purified from *MAT***a**/*MAT*α cells harvested 10 h after transfer to sporulation medium and from vegetatively growing cells, respectively. The arrow denotes the 39-kDa sporulation-specific protein. The proteins denoted by the open arrowheads are discussed in the text. (E, F) Two-dimensional polyacrylamide gel analysis of the in vitro translation products of RNA recovered from the hybrids formed betwen (E) p27 DNA or (F) pBR322 DNA and RNA extracted from *MAT***a**/*MAT*α cells at 10 h after transfer to sporulation medium. The arrow denotes the position of the 39-kDa sporulation-specific protein. The proteins denoted by the arrowheads are discussed in the text.

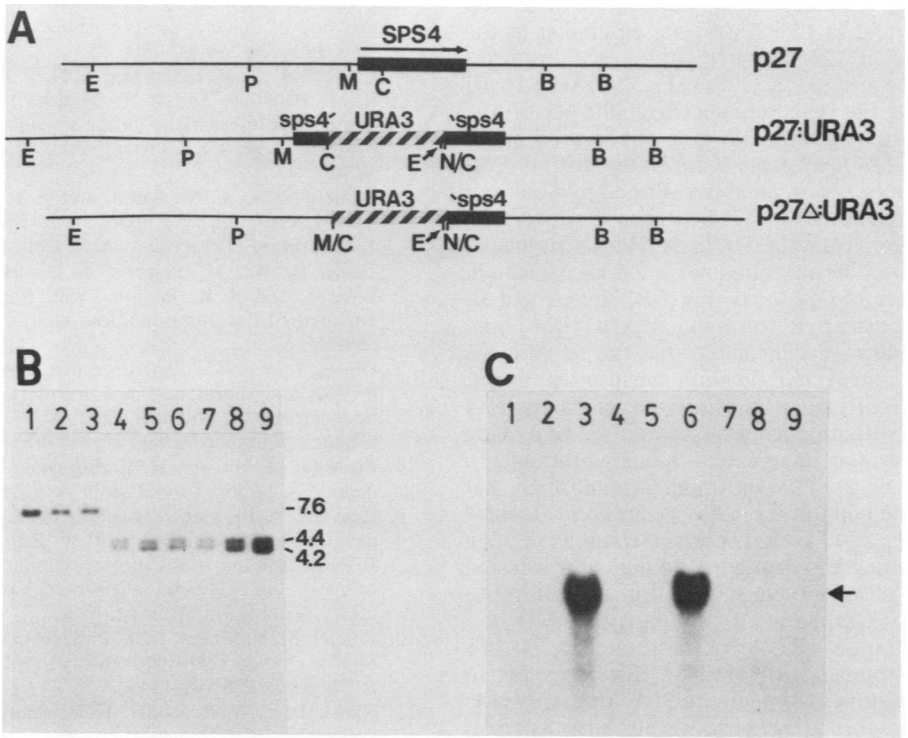

FIG. 5. Southern and Northern analysis of the nucleic acids isolated from mutant *sps4* strains. (A) p27:URA3 contains an insertion of a fragment containing the *URA3* gene at the *Cla*I site of the *SPS4* gene of p27; p27Δ:URA3 is derived from p27:URA3 by deletion of the 5′ end of the *SPS4* gene (see the text). Abbreviations: B, *Bgl*II; C, *Cla*I; E, *Eco*RI; M, *Mlu*I; N, *Nar*I; P, *Pvu*II. The arrow denotes the *Eco*RI site introduced adjacent to the mutated *sps4* gene. (B) Southern analysis of DNA extracted by the rapid isolation procedure of Davis et al. (6) from a *MAT***a** *ura3 SPS4* strain (SR25-1A) (lane 2), a *MAT*α *ura3 SPS4* strain (CG379) (lane 3), the diploid obtained by mating these two strains (lane 1), from two independent *MAT***a** *URA3 sps4* transformants (lanes 4 and 5), two independent *MAT*α *URA3 sps4* transformants (lanes 6 and 7), and the *MAT***a** *sps4*/*MAT*α *sps4* diploids obtained by mating the *sps4* haploid strains (lanes 8 and 9). The DNA was digested with *Eco*RI, and a Southern blot was probed with p277 DNA that had been radioactively labeled by nick translation (17, 26). p277 is a subclone of the *Hin*dIII fragment of p27 spanning the *Cla*I site of the *SPS4* gene (Fig. 1A). The sizes of the *Eco*RI fragments are denoted in kbp on the right of the autoradiogram. (C) Northern analysis of the RNA extracted from two *MAT***a** *sps4*/*MAT*α *sps4* strains (lanes 1, 4, and 7; and 2, 5, and 8 respectively) and from the wild-type *MAT***a** *SPS4*/*MAT*α *SPS4* strain (lanes 3, 6, and 9) growing vegetatively (lanes 7 through 9) and at 10 or 20 h after transfer to sporulation medium (lanes 1 through 3 and 4 through 6, respectively). The Northern blot was probed with p277 DNA that had been radioactively labeled by nick translation (26). The position of the transcript encoded by the *SPS4* gene is indicated by the arrow on the right.

conditions were used for the hybrid selection (65% formamide, 0.4 M NaCl, 50°C).

A two-dimensional polyacrylamide gel electrophoretic analysis of the protein synthesized in vitro from the RNA hybrid selected with p27 DNA confirmed that the *SPS4* gene encodes the major 39-kDa protein observed on in vitro translation of total RNA from sporulating cells; both proteins have the same mobility in nonequilibrium pH gradient gel electrophoresis (denoted by the arrows, Fig. 4B and 4E). In both instances the protein appears as a doublet, probably due to in vitro modifications (20). (The proteins in Fig. 4E denoted by the open arrowheads are the *SPS4*-related proteins referred to above [Fig. 4D, lanes 8 and 10]; the proteins denoted by the closed triangles are the proteins synthesized in the reticulocyte lysate in the absence of any added RNA [data not shown] and are present in the control translation of RNA obtained by using pBR322 DNA for the selection [Fig. 4F].) The reason the 39-kDa protein appears as a prominent sporulation-specific protein when the in vitro coding capacity of sporulation RNA is assessed but is not detected on analysis of in vivo labeled proteins (13, 14, 24, 30, 33, 34; our unpublished observations) is perplexing. We cannot exclude the possibility that the *SPS4* transcripts are not translated in vivo. Alternatively, it is possible that the *SPS4* gene product is present in a very insoluble or unextractable form, for instance, as a component of the spore walls. In support of this latter idea, the *SPS4* gene is first expressed in AP3 at 6 to 8 h of sporulation with maximal transcript accumulation occurring at 8 to 12 h (data not shown), a time at which the meiotic events of sporulation have been completed and the deposition of spore wall components is beginning (13).

The *SPS4* gene is not essential for sporulation. The in vitro demonstration that the *SPS4* gene encodes a major sporulation-specific protein suggested that the *SPS4* gene product may have an important role in the sporulation process. To investigate this, a *MAT***a** *sps4*/*MAT*α *sps4* strain was constructed by the one-step gene disruption procedure of Rothstein (27) to replace the wild-type *SPS4* gene with a mutated copy of the gene. As the first step in creating a mutation in the *SPS4* gene, a *Cla*I-*Nar*I fragment containing the *URA3* gene was isolated from pDPL13-URA3 (23) and inserted into the unique *Cla*I site of p27 located within the *SPS4* gene. The *Cla*I site of pBR322 had been previously removed. The resulting plasmid DNA (p27:URA3; Fig. 5A) was then digested with *Mlu*I and *Cla*I and religated after the termini had been treated with the Klenow fragment of DNA

polymerase I. This resulted in the deletion of a 521-bp fragment at the 5′ end of the *SPS4* gene extending to the codon for amino acid 100 of the putative protein. After digestion of this plasmid (p27Δ:URA3, Fig. 5A) with *Pvu*II and *Bgl*II to separate the yeast sequence from the vector, the plasmid DNA was used to transform a *MAT***a** *ura3* strain (SR25-1A) and a *MAT*α *ura3* strain (CG379) to Ura$^+$ (19, 27). The genotype of the transformants was verified by Southern analysis (28) of genomic DNA that had been digested with *Eco*RI. DNA extracted from the *MAT***a** *SPS4 ura3* strain and the *MAT*α *SPS4 ura3* strain contained a 7.6-kbp fragment spanning the wild-type *SPS4* locus (Fig. 5B, lanes 2 and 3). Analysis of DNA extracted from the *MAT***a** *URA3* and *MAT*α *URA3* transformants indicated that the *SPS4* locus had undergone the expected deletion-substitution event. Introduction of an *Eco*RI site adjacent to the truncated *SPS4* gene resulted in *Eco*RI fragments of 4.4 and 4.2 kbp being generated from the mutant locus (Fig. 5B, lanes 4 through 7). The disappearance of the 7.6-kbp fragment confirmed that there is only a single copy of the *SPS4* gene. A *MAT***a** *sps4 URA3* strain and a *MAT*α *sps4 URA3* strain were then mated, and the resulting homozygous *sps4* diploid was tested for the presence of *SPS4* RNA and for its ability to sporulate.

Northern analysis of RNA extracted from *MAT***a** *sps4*/*MAT*α *sps4* strains at 10 or 20 h after transfer to sporulation medium, times at which the *SPS4* transcript was present in wild-type sporulating cells (Fig. 5C, lanes 3 and 6), indicated that no detectable *SPS4* RNA accumulated in the mutant cells (Fig. 5C, lanes 1, 2, 4, and 5). However, as assessed by light microscopic examination, the homozygous mutant cells formed asci as efficiently as the wild-type strain (60%), and the viability of both wild-type and mutant spores was found to be similar (80%). Thus, rather surprisingly, the *SPS4* gene, although it encodes a major sporulation-specific mRNA, is not essential for the formation of viable spores.

We have previously performed a mutational analysis of two other *SPS* genes, the *SPS1* and *SPS2* genes (23). Mutation of the *SPS1* gene resulted in a readily detectable asporogenous phenotype; a *MAT***a** *sps1*/*MAT*α *sps1* strain failed to form asci when transferred to sporulation medium. The *SPS2* gene (23), however, and the sporulation-specific *SGA* gene of *S. cerevisiae* (35) have been found to be dispensable for the formation of viable spores. More detailed analyses are required to determine whether the products of such genes as *SPS2*, *SPS4*, and *SGA* contribute in a subtle manner to the sporulation process; whether the absence of these gene products can be compensated for by alternate gene products; or whether these genes, although activated in a sporulation-specific manner, have no role in the developmental process.

ACKNOWLEDGMENTS

We thank Larry Moran and Valerie Watt for valuable comments on this manuscript.

This work was supported by the Medical Research Council (Canada) (grant MA-6826). A.T.G. is a Medical Research Council Fellow, and J.S. is a Medical Research Council Scholar.

LITERATURE CITED

1. **Berk, A., and P. Sharp.** 1977. Sizing and mapping of early adenovirus mRNA's by gel electrophoresis of S1 endonuclease digested hybrids. Cell **12**:721–732.
2. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
3. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
4. **Clancy, M. J., B. Buten-Magee, D. J. Straight, A. L. Kennedy, R. M. Patridge, and P. T. Magee.** 1983. Isolation of genes expressed preferentially during sporulation in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:3000–3004.
5. **Clancy, M. J., L. M. Smith, and P. T. Magee.** 1982. Developmental regulation of a sporulation-specific enzyme activity in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2**:171–178.
6. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padget.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**:404–411.
7. **Dawes, I. W.** 1983. Genetic control and gene expression during meiosis and sporulation in *S. cerevisiae*, p. 29–64. *In* J. F. T. Spencer, D. M. Spencer, and A. W. R. Smith (ed.), Yeast genetics. Springer-Verlag, New York.
8. **Esposito, M. S., and R. E. Esposito.** 1974. Genes controlling meiosis and spore formation in yeast. Genetics **78**:215–225.
9. **Esposito, R. E., and S. Klaphotz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. W. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Gendel, S., N. Straus, D. E. Pulleyblank, and J. Williams.** 1981. Shuttle cloning vectors for the cyanophyte *Anacystis nidulans*, J. Bacteriol. **256**:148–154.
11. **Ghosh, P. K., V. B. Reddy, J. Swingcoe, P. Lebowitz, and S. M. Weissman.** 1978. Heterogeneity and 5′ terminal structures of the late RNAs of Simian virus 40. J. Mol. Biol. **126**:813–846.
12. **Holaway, B. L., D. J. Lehman, D. A. Primerano, P. T. Magee, and M. J. Clancy.** 1985. Sporulation-regulated genes of *Saccharomyces cerevisiae*. Curr. Genet. **10**:163–169.
13. **Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall.** 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619–628.
14. **Kraig, E., and J. E. Haber.** 1980. Messenger ribonucleic acid and protein metabolism during sporulation of *Saccharomyces cerevisiae*. J. Bacteriol. **144**:1098–1112.
15. **Kurtz, S., and S. Lindquist.** 1984. Changing patterns of gene expression during sporulation in yeast. Proc. Natl. Acad. Sci. USA **81**:7323-7327.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the bacteriophage T4. Nature (London) **227**: 680–685.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–559.
19. **McNeil, J. B., R. K. Storms, and J. D. Friesen.** 1980. High frequency recombination and the expression of genes cloned on a chimeric yeast plasmid: identification of a fragment of 2-μm circle essential for transformation. Curr. Genet. **2**:17–25.
20. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**:1133–1142.
21. **Pelham, R. B., and J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
22. **Percival-Smith, A., and J. Segall.** 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Sacharomyces cerevisiae*. Mol. Cell. Biol. **4**:142–150.
23. **Percival-Smith, A., and J. Segall.** 1986. Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation in *Sacharomyces cerevisiae*. Mol. Cell. Biol. **6**:2443–2457.
24. **Petersen, J. G. L., M. C. Keilland-Brandt, and T. Nillson-**

Tillgren. 1979. Protein patterns of yeast during sporulation. Carlsberg Res. Commun. **44**:149–162.

25. **Ricciardi, R. P., J. S. Miller, and B. E. Roberts.** 1979. Purification and mapping of specific mRNAs by hybridization selection and cell free translation. Proc. Natl. Acad. Sci. USA **76**: 4927–4931.
26. **Rigby, P. W. J., M. Diekmann, C. Rhodes and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
27. **Rothstein, R.** 1983. One-step gene replacement in yeast. Methods Enzymol. **101**:202–211.
28. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–516.
29. **Thiele, D., P. Cottrelle, F. Iborra, J.-M. Buhler, A. Sentenac, and P. Fromageot.** 1985. Elongation factor 1α from *Saccharomyces cerevisiae*. Rapid large-scale purification and molecular characterization. J. Biol. Chem. **260**:3084–3089.
30. **Trew, B. J., J. D. Friesen, and P. B. Moens.** 1979. Two dimensional protein patterns during growth and sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **138**:60–69.
31. **Weaver, R. F., and C. Weissman.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure. The 5′ termini of 15S-globin mRNA precursor and mature 10S-globin mRNA have identical coordinates. Nucleic Acids Res. **7**:1175–1193.
32. **Weir-Thompson, E. M., and I. W. Dawes.** 1984. Developmental changes in translatable RNA species associated with meiosis and spore formation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:695–702.
33. **Wright, J. F., N. Ajam, and I. W. Dawes.** 1981. Nature and timing of some sporulation-specific protein changes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:910–918.
34. **Wright, J. F., and I. W. Dawes.** 1979. Sporulation-specific protein changes in yeast. FEBS Lett. **104**:183–186.
35. **Yamashita, I., and S. Fukui.** 1985. Transcriptional control of the sporulation-specific glucoamylase gene in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:3069–3073.