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Increased Copy Number of the 5′ End of the *SPS2* Gene Inhibits Sporulation of *Saccharomyces cerevisiae*

ANTHONY PERCIVAL-SMITH AND JACQUELINE SEGALL*

Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8

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We found that the introduction into a yeast cell of a high-copy-number plasmid containing the 5′ end of the *SPS2* gene, a sporulation-specific gene of *Saccharomyces cerevisiae*, led to a reduction in the efficiency of spore formation. The plasmid pAP290, which contains the sequence from −138 to +152 of the *SPS2* gene, caused a fivefold reduction in spore formation; the presence of the plasmid had no effect on transcription of the chromosomal *SPS2* gene. A plasmid containing only the sequence upstream of the TATA box of the *SPS2* gene (−350 to −68) was unable to inhibit the completion of sporulation, whereas the downstream sequence, from −70 to +404, although unable by itself to inhibit sporulation, could do so when provided with an upstream fragment containing the *CYC1* upstream activation sequence. Deletion of 22 base pairs from pAP290, which introduced a frameshift after codon 17 of the *SPS2* gene and reduced the open reading frame to 26 amino acids, generated a plasmid (pAP290Δ*Pst*) which could no longer inhibit sporulation. The *SPS2* inserts of pAP290 and pAP290Δ*Pst* were found to direct equivalent levels of sporulation-specific transcription. We conclude from these results that the presence of both the *SPS2* promoter (or a substitute promoter) and the initial coding sequence of the *SPS2* gene is required in the high-copy-number plasmid to generate the asporogenous phenotype. We speculate that the accumulation of a protein containing the amino-terminal portion of the *SPS2* gene product, synthesized from the transcripts of the truncated plasmid-borne copies of the *SPS2* gene, prevents ascus formation.

Numerous studies have been performed based on the idea that the introduction of multiple copies of a DNA regulatory sequence into a cell will result in the in vivo titration of a transcriptional regulatory factor. Such studies have contributed to the delimitation of control sequences and have demonstrated that common factors are involved in the expression of distinct genes (1, 3, 4, 22, 27, 28, 32). We tested whether the 5′ end of a sporulation-specific gene of *Saccharomyces cerevisiae*, when present at high copy number, titrates a transcriptional regulatory molecule required for the expression of a subset of sporulation-specific genes. In this report, we present the unexpected observations arising from this study.

Sporulation of *S. cerevisiae*, initiated when *MAT***a**/*MAT*α cells are transferred to medium containing an oxidative carbon source and lacking nitrogen, consists of a well-characterized series of meiotic events, including DNA replication, recombination, and chromosome segregation, followed by the formation of spore walls around the four haploid nuclei (for reviews, see references 6 and 9). Entry into the sporulation pathway is dependent on the *MAT***a**/*MAT*α genotype (for a review, see reference 14). Several different approaches have been pursued to demonstrate that progression through the subsequent developmental program is dependent on the expression of development-specific genes. Classical genetic analyses established the existence of a class of genes which are dispensable for mitotic growth but are essential for the completion of various sporulation events (8, 9, 29). More recently, differential hybridization screens have identified a large number of cloned genes expressed preferentially during sporulation (5, 11, 24). Distinct sets of sporulation-specific transcripts have been shown to accumulate at discrete times throughout sporulation (16, 17, 31; D. Law and J. Segall, manuscript in preparation). We anticipate that elucidation of the mechanisms involved in the activation of these genes will provide insights into the temporal regulation of development-specific genes.

In this study, we investigated the observation that the introduction into a yeast cell of a high-copy-number plasmid containing a DNA fragment spanning the 5′ end of the sporulation-specific gene *SPS2* (25) leads to an asporogenous phenotype. We found that the reduction in ascus formation required the presence in the plasmid not only of the transcriptional regulatory sequences of the gene but also of the initial coding sequence of the gene. We presume that the accumulation of a fusion protein containing the amino-terminal portion of the *SPS2* gene product, synthesized from the transcripts derived from the truncated *SPS2* genes present in the high-copy-number plasmid, has an adverse affect on the progression of sporulation-specific events.

MATERIALS AND METHODS

Strains and culture conditions. *S. cerevisiae* LP112 (*MAT***a**/*MAT*α *can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 ade2-1/ade2-1*) used throughout this study was constructed by S. Lindquist, University of Chicago, from the *MAT***a** and *MAT*α strains W3031A and W3031B, respectively, provided by R. Rothstein, Columbia University. Prior to sporulation, cells were grown for 36 to 48 h at 30°C in SA/SD medium (0.4% dextrose, 0.8% potassium acetate, 0.67% yeast nitrogen base) and then diluted in SA medium (1% potassium acetate, 0.67% yeast nitrogen base) and grown for an additional 24 to 48 h until a cell density of 2.5×10^6 cells per ml was reached. The cells were harvested and washed twice with sporulation medium (1% potassium acetate [pH 7.0]) and then suspended at 2×10^7 to 5×10^7 cells per ml in sporulation medium. All media contained the required supplements. The efficiency of ascus formation was monitored by microscopic examination

* Corresponding author.

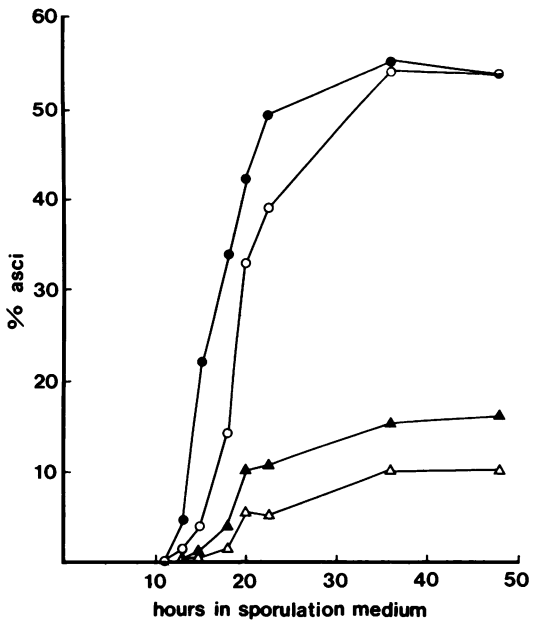

FIG. 1. Increased copy number of the 5′ region of the *SPS2* gene inhibited ascus formation. The efficiency of ascus formation was monitored for the *MAT***a**/*MAT*α LP112 strain (●) and for cells of this strain containing pYF404 (○), pAP762 (▲), or pAP290 (△) by microscopic examination of cells at various times after transfer to sporulation medium (see Materials and Methods).

of cells by using phase-contrast optics. More than 500 cells were examined for each determination.

Plasmid constructions. The *leu2d*-containing vector used in this study, pYF404, constructed and provided by E. Maicas, University of Toronto, is similar to pMP78-1 (7), except that the *Escherichia coli* portion of the vector is derived from pBR322 (*Hin*dIII-*Eco*RI fragment) rather than from pBR325. Various portions of the 5′ end of the *SPS2* gene (see Fig. 3) were purified from p18 (25) and inserted into pYF404 at its unique *Bam*HI site; pAP762 and pAP290 contain the 762-base-pair (bp) *Bgl*II fragment extending from −358 to +404 of the *SPS2* gene and the 290-bp *Sau*3A fragment extending from −138 to +152 of the *SPS2* gene, respectively. The start of transcription is denoted by +1. The plasmid pAP290Δ*Pst*, which is identical to pAP290, except that the 22-bp *Pst*I fragment extending from +94 to +116 of the *SPS2* gene has been deleted, was constructed by purification and religation of the two large *Pst*I fragments of pAP290. pAP283 contains the 283-bp *Dde*I fragment extending from −350 to −68 of the *SPS2* gene; for the construction of this plasmid, the ends of the *Dde*I fragment and the *Bam*HI-digested vector were filled in with *E. coli* DNA polymerase I prior to ligation. The orientation of the *SPS2* insert in pAP762 and pAP283, as defined by the direction of transcription of the *SPS2* gene, is opposite to the direction of transcription of the *leu2* gene in the vector; the orientation of the *SPS2* insert in pAP290 and pAP290Δ*Pst* is the same as that of the *leu2* gene. pAP474-1 and pAP474-2 contain the 474-bp *Dde*I-*Bgl*II fragment extending from −70 to +404 of the *SPS2* gene oriented in the same direction and in the opposite direction as the *leu2* gene in the vector, respectively. For the construction of these two plasmids, *Bam*HI linkers were attached to both ends of the *Dde*I-*Bgl*II fragment after the ends had been filled in.

pAP*CYC*, a pYF404 derivative with an insert containing the *CYC1* upstream activation sequence (UAS), and pAP*CYC-SPS2′*, containing an insert with the *CYC1* UAS fused to the *Dde*I-*Bgl*II fragment extending from −70 to +404 of the *SPS2* gene, were constructed as follows. A 134-bp *Xho*I-*Sma*I fragment containing the *CYC1* UAS1 and UAS2 (13) was purified from pRY65 (provided by R. Yocum, Biotechnica International, Cambridge, Mass.), and a *Bam*HI linker was attached to the *Sma*I end. This fragment was then inserted between the *Sal*I site and one of the two *Bam*HI sites of pAP474-2 (see above) by ligation with pAP474-2 DNA which had been digested with *Bam*HI in the presence of 50 μg of ethidium bromide per ml to obtain singly cut molecules (23) and then digested with *Sal*I. This generated pAP*CYC*, in which the *Sal*I-*Bam*HI fragment of pYF404 (within the *tet* gene) was replaced by the *CYC1 Xho*I-*Sma*I fragment, and pAP*CYC-SPS2′*, in which this *CYC1 Xho*I-*Sma*I fragment is fused through a *Bam*HI linker to the −70 to +404 fragment of the *SPS2* gene. The *CYC1* UAS in pAP*CYC-SPS2′* is in the reverse orientation, relative to the start of transcription, to that found in the *CYC1* gene itself. However, the *CYC1* UAS is functional in both orientations (12).

pAP.*Pst-lacZ* contains an *SPS2-lacZ* translational fusion gene consisting of the *SPS2* sequence from −358 to +96 fused in frame to the β-galactosidase gene of *E. coli* inserted into the *Hin*dIII site of pYF404.

DNA manipulations were carried out by previously described procedures (19) and constructions were verified by restriction enzyme analysis.

Estimation of plasmid copy number. The copy number of pYF404, pAP290, and pAP290Δ*Pst* was estimated by extracting DNA from cells containing these plasmids and probing a Southern blot of various dilutions of *Cla*I-digested DNA with a radioactively labeled 1-kilobase-pair (kbp) *Eco*RI-*Cla*I fragment, purified from pYF404, containing the *leu2* gene. Comparison of the hybridization responses of the chromosomal *leu2* locus and the plasmid-borne *leu2* gene indicated that the plasmids were maintained at approximately 200 copies per cell.

Transformation of yeast cells. Transformation of yeast spheroplasts generated by glusulase treatment (15) was performed as described by McNeil et al. (20).

RESULTS

Inhibition of sporulation by the 5′-end of the *SPS2* gene on a high-copy-number plasmid. We chose the *SPS2* gene of *S. cerevisiae* to test whether the presence at high copy number of the sequence from the 5′ end of a sporulation-specific gene would lead to reduced expression of a subset of coordinately activated sporulation-specific genes as a consequence of the sequestration of a transcriptional regulatory molecule. The *SPS2* gene is expressed only in *MAT***a**/*MAT*α cells after transfer to sporulation medium (24); a *Bgl*II fragment extending from −358 to +404 of the gene has been shown to direct sporulation-specific expression of an *SPS2-lacZ* translational fusion gene (25). This 762-bp *Bgl*II fragment was cloned into the high-copy-number plasmid pYF404 (7; see Materials and Methods), and the resulting plasmid pAP762 was introduced into a *MAT***a**/*MAT*α yeast strain. As an initial test for an effect of this plasmid, we monitored the efficiency with which cells containing the plasmid completed sporulation. We presumed that reduced expression of a subset of sporulation-specific genes would prevent the normal progression of developmental events. The presence of the plasmid led to a significant reduction in the efficiency of ascus formation (Fig. 1); the wild-type yeast strain and cells

containing the high-copy-number vector alone sporulated with an efficiency of 55%, whereas cells containing pAP762 sporulated with an efficiency of only 15%. Insertion into pYF404 of the 290-bp *Sau*3A fragment extending from −138 to +152 of the *SPS2* gene generated a plasmid (pAP290) which had the same inhibitory effect on sporulation as pAP762 did (Fig. 1). Curing transformed cells of either pAP762 or pAP290 generated strains which had regained the ability to sporulate as efficiently as the wild-type strain (data not shown). This confirmed that the asporogenous phenotype of cells containing pAP762 or pAP290 was due to the presence of the plasmids.

To monitor nuclear division during sporulation, we visualized the DNA of cells with the fluorescent, DNA-binding dye 33258 Hoechst (18). Cells containing pAP290 became tetranucleate after transfer to sporulation medium, although somewhat less efficiently than wild-type cells did (data not shown). The cells which did complete sporulation contained viable spores harboring the plasmid (data not shown).

Transcription of sporulation-specific in cells containing pAP290. We next monitored the effect of pAP290 on transcription of the chromosomal *SPS2* gene. This gene is normally first expressed 6 to 8 h after transfer of cells to sporulation medium, with maximal transcript accumulation occuring at 8 to 10 h (unpublished observations). We found that the presence of pAP290 did not affect the steady-state level of RNA encoded by the *SPS2* gene (Fig. 2B, cf. lanes 3 and 4 and lanes 5 and 6). Transcript accumulation from three other sporulation-specific genes, *SPS1*, *SPS3*, and *SPS4*, whose temporal pattern of expression is very similar to that of the *SPS2* gene (10, 25; unpublished observations) was also found to be unaffected by the presence of pAP290 (Fig. 2A, C, and D). Interestingly, expression of two other sporulation-specific genes, *SPS100* and *SPS101*, whose transcripts first appear approximately 4 h after the activation of the *SPS1*, *SPS2*, *SPS3*, and *SPS4* genes (Law and Segall, in preparation) was significantly reduced in cells containing pAP290 (Fig. 2E and F). The reduction in the levels of transcripts encoded by the *SPS100* and *SPS101* genes was more dramatic at 13 h (Fig. 2E and F, lane 6) than at 24 h after the transfer of cells to sporulation medium (Fig. 2E and F, lane 8), especially for the *SPS100* gene. The hybridization pattern seen for the transcript present in the lower portion of Fig. 2E verified the integrity of the RNAs used for the comparisons in this analysis.

The continued transcription of the chromosomal *SPS2* gene in cells containing pAP290 suggested that the observed inhibition of ascus formation and the reduced expression of the *SPS100* and *SPS101* genes caused by this plasmid did not result from sequestration of a transcriptional regulatory factor by the plasmid. To gain further insight into the mechanism leading to the asporogenous phenotype of cells containing pAP762 and pAP290, we subcloned additional portions of the 5′ region of the *SPS2* gene to identify the region responsible for inhibiting ascospore formation.

Dependence of the asporogenous phenotype of cells containing pAP290 on both the 5′ regulatory region and the initial coding region of the *SPS2* gene. The 762-bp *Bgl*II fragment, which contains all the sequences necessary for directing sporulation-specific expression of the *SPS2* gene, was divided in two by taking advantage of a *Dde*I restriction site just upstream of the putative TATA box of the gene (25). The two resulting fragments, which overlap the *SPS2* sequence present in pAP290, were cloned into pYF404, generating pAP283 and pAP474-1 (Fig. 3). pAP283 presumably contains the transcriptional regulatory sequence(s) (i.e., UAS) acting

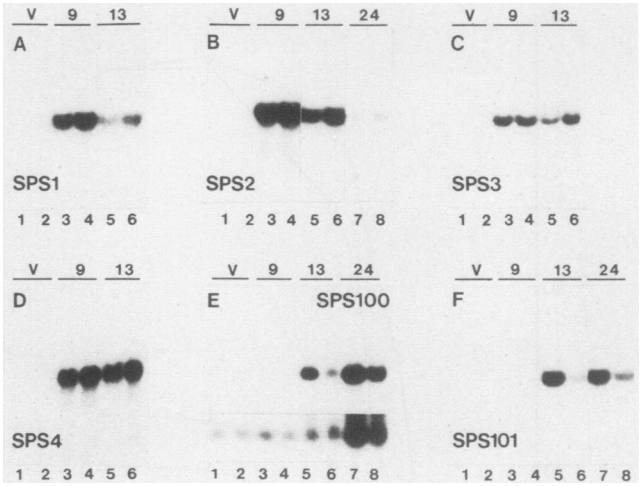

FIG. 2. Effect of pAP290 on the transcription of several sporulation-specific genes. Transcript accumulation was monitored for the *SPS1* (A), *SPS2* (B), *SPS3* (C), *SPS4* (D), *SPS100* (E), and *SPS101* (F) genes. RNA was extracted (24) from cells containing pYF404 (lanes 1, 3, 5, and 7) or pAP290 (lanes 2, 4, 6, and 8) growing vegetatively (lanes 1 and 2) or at 9 (lanes 3 and 4), 13 (lanes 5 and 6), and 24 h (lanes 7 and 8) after transfer to sporulation medium. The RNAs were denatured, fractionated through a 1.5% agarose-formaldehyde gel, and then transferred to a nitrocellulose membrane. The RNA filters were hybridized with gene-specific probes radioactively labeled by nick translation (26). The *SPS1*, *SPS2*, *SPS3*, *SPS4*, *SPS100*, and *SPS101*-gene specific DNA fragments were obtained by purification of a 1.5-kbp *Bam*HI-*Bgl*II fragment from p18 (25), a 1.5-kbp *Cla*I-*Pst*I fragment from p84, a 1.2-kbp *Eco*RI-*Cla*I fragment from p84 (25), a 1.7-kbp *Hin*dIII fragment from p277 (10), a 2.4-kbp *Eco*RI fragment from pB8 (D. Law, unpublished data), and a 1.2-kbp *Eco*RI fragment from pC2 (D. Law, unpublished data), respectively. The transcript of the *SPS100* gene is seen in the upper portion of the autoradiogram represented in panel E; the transcript present in the lower portion of the autoradiogram is an uncharacterized transcript which also hybridized with a sequence present in the yeast DNA insert of pB8. The exposure time for the lower portion of the autoradiogram was 14 times longer than that for the upper portion. In this experiment, the efficiency of sporulation for cells containing pYF404 and pAP290 was 59 and 13%, respectively.

on the *SPS2* gene; the 5′ flanking sequence of the *SPS2* gene present in this plasmid extends from −350 to −68 of the gene, ending just upstream of the putative TATA box (−64 to −59) of the gene (25). pAP474-1 contains the adjacent sequence (−70 to +404), extending from the TATA box into the coding region of the gene. Neither cells containing pAP283 nor cells containing pAP474-1 were impaired in their abilities to complete sporulation (Fig. 3). We also constructed a variant of pAP290, termed pAP290Δ*Pst*, in which the sequence from +94 to +116 of the *SPS2* gene was deleted. This deletion generated a frameshift at codon 18 of the *SPS2* gene, leading to a stop codon 9 codons after the frameshift. In contrast to cells containing the parental plasmid pAP290 which sporulated very inefficiently, cells containing pAP290Δ*Pst* were found to sporulate as efficiently as did cells containing no plasmid (Fig. 3).

These observations suggested that the entire promoter sequence as well the initial coding region of the *SPS2* gene was required to generate the asporogenous phenotype. We conjectured that accumulation of the amino-terminal portion of the *SPS2* gene product, as a result of translation of transcripts derived from the truncated plasmid-borne copies

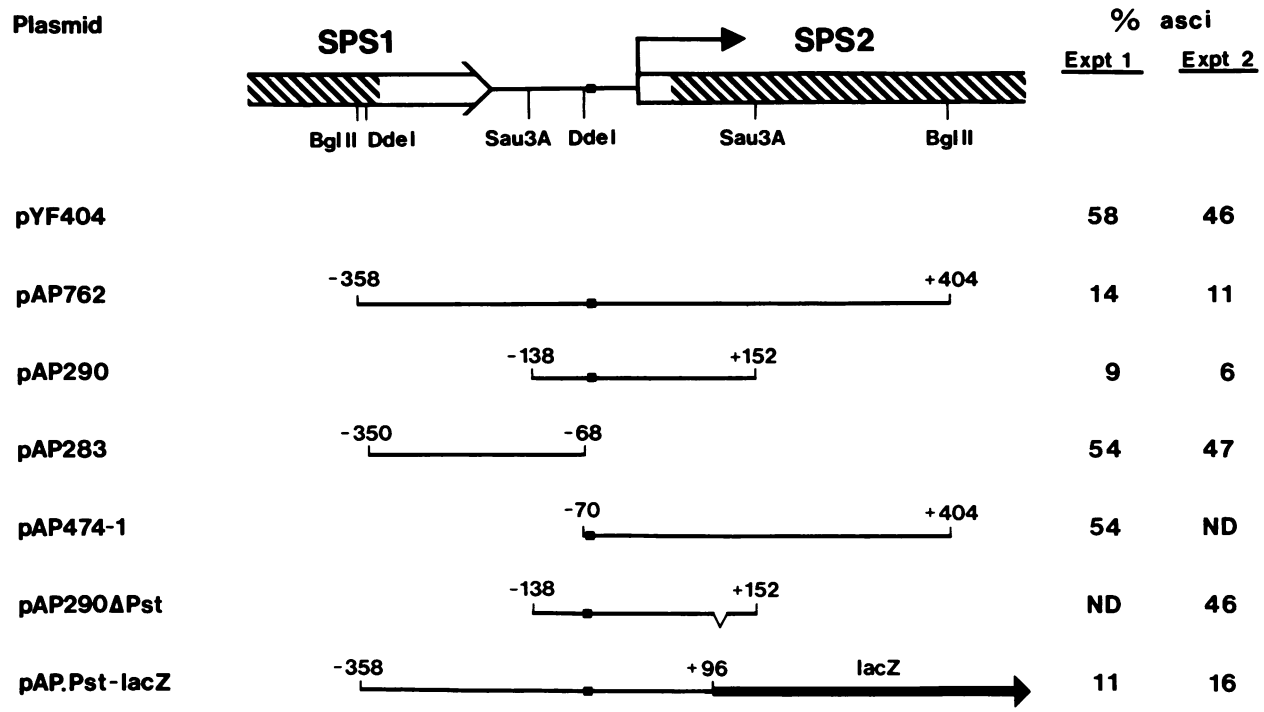

FIG. 3. Assessment of the ability of sequences from the 5′ region of the *SPS2* gene, when present at high copy number, to inhibit ascus formation. The fragments from the 5′ region of the *SPS2* gene that were cloned into the high-copy-number vector pYF404 are depicted below the map extending from the 3′ region of the *SPS1* gene into the 5′ region of the *SPS2* gene (25). The putative TATA sequence of the *SPS2* gene is denoted by the small box. The hatched and open regions of the genes represent coding and noncoding sequence, respectively. The names of the plasmids containing these inserts are given on the left, and their construction is described in Materials and Methods. The efficiency of ascus formation for cells containing these plasmids is shown on the right for two typical experiments. ND, Not determined.

of the *SPS2* gene, has a deleterious affect on the progression of sporulation-specific events. This proposal assumes that the *SPS2* insert of pAP290 contains a functional *SPS2* promoter. To test this assumption, a probe specific for plasmid-derived *SPS2* transcripts was used to perform an S1 nuclease analysis of RNA extracted from cells containing pAP290. No plasmid-derived transcripts could be detected in RNA isolated from vegetatively growing cells (Fig. 4, lane 2). However, 10 h after the transfer of cells to sporulation medium, we could detect significant accumulation of plasmid-derived *SPS2* transcripts (Fig. 4, lane 4). The estimated size of the S1 nuclease-resistant hybrids (470 bp) was in good agreement with the size expected (427 bp) for transcription initiating at the wild-type start site. The sequence downstream of −138 in the *SPS2* gene is therefore sufficient to direct sporulation-specific gene expression.

A similar analysis of RNA isolated from cells containing pAP290Δ*Pst* indicated that the *SPS2* sequence present in this plasmid contained as active an *SPS2* promoter as the *SPS2* insert of pAP290 did (Fig. 4, cf. lanes 4 and 5). The inability of pAP290Δ*Pst* to inhibit sporulation, despite the observation that it contains a functional promoter, is consistent with the idea that at least a portion of the coding sequence of the *SPS2* inserts of pAP762 and pAP290 is necessary to generate the sporulation defect.

The *SPS2*-derived transcripts of pAP762 and pAP290 encode 136- and 330-amino-acid hybrid proteins, respectively, for which the first 119 and 35 codons, respectively, are contributed by the *SPS2* sequence and the final 17 and 298 codons, respectively, are derived from the adjacent pBR322 sequence. As indicated above, only the first 17 amino acids of the predicted 26-amino-acid peptide generated from the *SPS2* insert of pAP290Δ*Pst* are from the same frame as the *SPS2* gene product itself. Since the presence of pAP762 and pAP290 inhibited sporulation but the presence

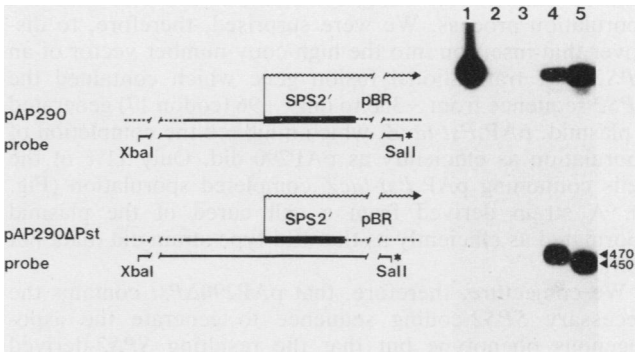

FIG. 4. *SPS2* inserts of both pAP290 and pAP290Δ*Pst* promoted sporulation-specific transcription. An S1 nuclease analysis was performed (2, 30) to detect plasmid-derived, *SPS2*-generated transcripts in cells containing pAP290 (lanes 2 and 4) or pAP290Δ*Pst* (lanes 3 and 5). The indicated *XbaI*-*SalI* fragment, which was purified from either pAP290 (lanes 2 and 4) or pAP290Δ*Pst* (lanes 3 and 5), was 5′ end labeled at the *SalI* site. The labeled DNA was hybridized with 10 μg of total RNA extracted from cells growing vegetatively (lanes 2 and 3) or at 10 h after transfer to sporulation medium (lanes 4 and 5). The reaction of lane 1 was similar to the reaction of lane 4, expect for the omission of S1 nuclease. The S1 nuclease-resistant RNA-DNA hybrids were analyzed by electrophoresis through a nondenaturing 2% agarose gel.

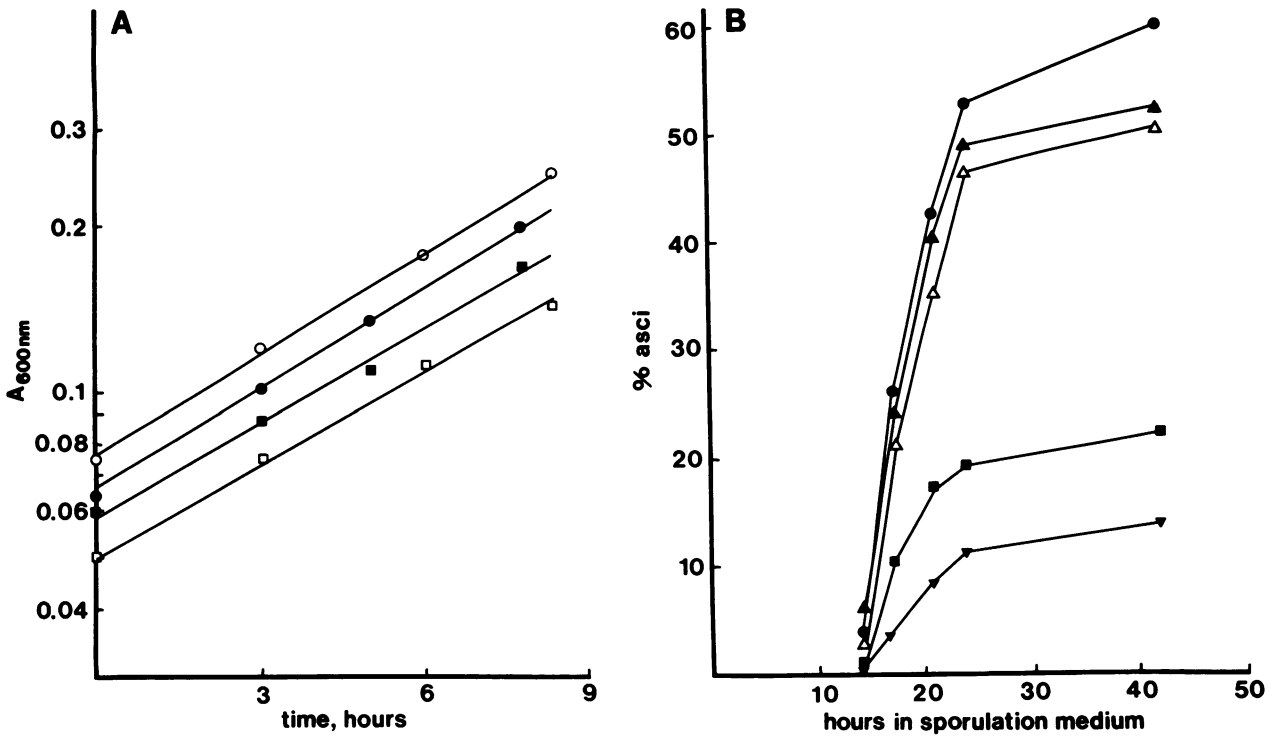

FIG. 5. Presence of a high-copy-number vector containing a *CYC1-SPS2'* transcriptional fusion gene did not affect vegetative growth but did inhibit ascus formation. (A) Growth in presporulation medium (see Materials and Methods), as monitored by A_{600} for cells containing pYF404 (● and ○) and for cells containing pAP*CYC-SPS2'* (■ and □). The data for two independent experiments (open and closed symbols) are shown. (B) Efficiency of ascus formation for cells containing pYF404 (●), pAP*CYC* (△), pAP474-2 (▲), pAP*CYC-SPS2'* (■), and pAP290 (▼). pAP*CYC* and pAP474-2 contain the *CYC1* UAS and the sequence from −70 to +404 of the SPS2 gene, respectively, inserted into pYF404, while pAP*CYC-SPS2'* contains the *CYC1* UAS fused to the *SPS2* insert of pAP474-2 to create a transcriptional fusion gene (see Materials and Methods). The cells containing pYF404 and pAP*CYC-SPS2'* represented by the closed symbols in panel A were used for the experiment represented in panel B.

of pAP290Δ*Pst* did not, we initially concluded that the putative overproduced hybrid proteins required more than 17 amino-terminal *SPS2*-specified amino acids, with less than 35 amino acids being sufficient, to interfere with the sporulation process. We were surprised, therefore, to discover that insertion into the high-copy-number vector of an *SPS2-lacZ* translational fusion gene which contained the *SPS2* sequence from −358 to only +96 (codon 17) generated a plasmid, pAP.*Pst-lacZ*, which inhibited the completion of sporulation as efficiently as pAP290 did. Only 11% of the cells containing pAP.*Pst-lacZ* completed sporulation (Fig. 3). A strain derived from a cell cured of the plasmid sporulated as efficiently as the wild-type strain did (data not shown).

We conjecture, therefore, that pAP290Δ*Pst* contains the necessary *SPS2*-coding sequence to generate the asporogenous phenotype but that the resulting *SPS2*-derived peptide is simply too small to impair sporulation; that is, a minimum protein size is required for the effect, even if a portion is derived from a non-*SPS2*-coding sequence. Alternatively, it is possible that the very small pAP290Δ*Pst*-derived *SPS2* peptide is innocuous because it is rapidly degraded. It should be noted that various fusion proteins containing the amino-terminal portion of the *SPS2* gene product but different non-*SPS2* carboxy-terminal sequences resulted in decreased ascus formation. This rules out the possibility that the non-*SPS2* sequence is directly responsible for the effect.

To investigate further the hypothesis that the inhibition of sporulation is a posttranscriptional event, we constructed a transcriptional fusion gene which would allow expression of the amino-terminal portion of the *SPS2* gene independently of the putative *SPS2* UAS. This was done by fusing a 134-bp fragment containing the *CYC1* UAS to the sequence extending from −70 to +404 of the *SPS2* gene and inserting this hybrid sequence into pYF404. Cells containing the resulting plasmid, named pAP*CYC-SPS2'*, showed a reduced frequency of ascus formation (22%) relative to cells containing either the vector plasmid alone (60%) or the control plasmid pAP*CYC* (50%), which contains only the *CYC1* UAS inserted into pYF404 (Fig. 5B). Loss of the plasmid pAP*CYC-SPS2'* generated a strain which had recovered the ability to sporulate as efficiently as the wild-type strain did (data not shown). The sequence from −70 to +404 could not by itself lead to an asporogenous phenotype (Fig. 3, pAP474-1 and Fig. 5B, pAP474-2). These results support the idea that the asporogenous phenotype caused by the high-copy-number plasmids is dependent on the production of the *SPS2*-derived transcripts, with the 5' flanking sequence being required solely to promote transcription. Thus, the region upstream of −70 can be substituted for by the *CYC1* UAS. The inhibition of sporulation caused by pAP*CYC-SPS2'*, however, was neither as dramatic nor as reproducible as the effect obtained with pAP290 (Fig. 5B and data not shown).

The observation that cells containing the *CYC1-SPS2'* fusion gene could grow in a presporulation medium containing acetate as the carbon source, which would lead to derepression of the *CYC1* gene, indicated that the synthesis

of the amino-terminal portion of the *SPS2* gene product was not lethal to vegetatively growing cells. Indeed, cells containing pAP*CYC-SPS2'* grew with approximately the same generation time as did cells containing the vector plasmid pYF404 (Fig. 5A). An S1 nuclease analysis indicated that the *CYC1-SPS2'* fusion gene was expressed more efficiently in vegetatively growing cells than in cells at 10 h after transfer to sporulation medium (data not shown). These results indicate that the asporogenous phenotype of cells containing the 5' end of the *SPS2* gene at high copy number was not due to a general toxic affect arising from the accumulation of the putative hybrid proteins but resulted from the interruption of a sporulation-specific event.

DISCUSSION

We found that a high-copy-number plasmid containing the 5' end of the *SPS2* gene inhibited the completion of spore formation. The presence of the sequence that extends from −138 to +152 of the *SPS2* gene at high copy number (pAP290) led to reduced efficiency of spore formation, but neither the sequence from −350 to −68 (pAP283) nor the sequence from −70 to +404 (pAP474) of the *SPS2* gene affected sporulation. Deletion of 22 bp from pAP290, from +94 to +116 within the coding region of the *SPS2* gene, generated a plasmid (pAP290Δ*Pst*) which could no longer inhibit sporulation, although transcription from the *SPS2* promoter was not affected by the deletion.

We consider that the most plausible interpretation of these observations is as follows. First, the asporogenous phenotype is dependent on the synthesis of plasmid-derived *SPS2* transcripts and hence requires that a promoter be present. There is not, however, a stringent requirement for the *SPS2* promoter itself; we found that the *SPS2* sequence upstream of −70 could be replaced by the *CYC1* UAS. Second, the phenotype results from the overproduction of a protein containing the amino-terminal portion of the *SPS2* gene product. It is possible that this either leads to the accumulation of an aberrant sporulation-specific structure or prevents the assembly of a sporulation-specific structure, which in the former case inhibits and in the latter case is required for the continuation of the temporal progression of sporulation-specific events, including the activation of the *SPS100* and *SPS101* genes and spore wall formation. The first 17 amino acids of the *SPS2* gene product are sufficient for this predicted effect, since the presence in a high-copy-number plasmid of an *SPS2-lacZ* translational fusion gene containing only the first 17 codons of the *SPS2* gene (pAP.*Pst-lacZ*) led to a reduced efficiency of sporulation. It should be noted that truncation of the *SPS2* gene does not by itself lead to an asporogenous phenotype; the presence of the *SPS2-lacZ* fusion gene of pAP.*Pst-lacZ* in a centromere-containing vector does not affect the completion of sporulation (unpublished data).

Our previous mutational analysis of the *SPS2* gene indicated that the product of this gene is not required for the formation of viable spores (25). Our present results suggest that although the *SPS2* gene product may be dispensable, it nonetheless contributes to a sporulation-specific event. The cell can compensate for the absence of the gene product, but the putative overproduction of its amino-terminal portion is deleterious and prevents the completion of sporulation. We do not know whether overproduction of the entire protein has the same effect. It is interesting that two genes required for chromosome transmission were identified by Hartwell and co-workers (21) by screening a yeast DNA library in a high-copy-number vector for plasmids that caused a high frequency of chromosome loss or recombination. This approach was based on the idea that abnormal stoichiometries of proteins involved in multicomponent mitotic structures might lead to aberrant chromosome transmission, although the absence of a single gene product may not itself lead to a defect in cell division (21).

A definitive proof of our speculation that the asporogenous phenotype of cells containing sequences from the 5' end of the *SPS2* gene results from overproduction of the amino-terminal portion of the *SPS2* gene product awaits a direct demonstration that translation of the *SPS2*-derived transcripts is essential for generation of the phenotype. However, the inability of pAP290Δ*Pst* to inhibit sporulation, despite the fact that this plasmid contains a functional *SPS2* promoter, as well as the observation that the sequence upstream of −70 can be replaced by the *CYC1* UAS argues persuasively against the phenotype being a consequence of sequestration of a transcription factor by a regulatory sequence.

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LITERATURE CITED

1. **Banner, C. D. B., C. P. Moran, Jr., and R. Losick.** 1983. Deletion analysis of a complex promoter for a developmentally regulated gene from *Bacillus subtilis*. J. Mol. Biol. **168**:351–365.
2. **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.
3. **Brady, J., M. R. Loeken, and G. Khoury.** 1985. Interaction between two transcriptional control sequences required for tumor-antigen-mediated simian virus 40 related gene expression. Proc. Natl. Acad. Sci. USA **82**:7299–7303.
4. **Buchanan-Wollaston, V., M. C. Cannon, and F. C. Cannon.** 1981. The use of cloned *nif* (nitrogen fixation) DNA to investigate transcriptional regulation of *nif* gene in *Klebsellia pneumonia*. Mol. Gen. Genet. **184**:102–106
5. **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.
6. **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.
7. **Erhart, E., and C. P. Hollenberg.** 1983. The presence of a defective *LEU2* gene on 2μ DNA recombinant plasmids of *Saccharomyces cerevisiae* is responsible for curing and high copy number. J. Bacteriol. **156**:625–635.
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. **Garber, T., and J. Segall.** 1986. The *SPS4* gene of *Saccharyomyces cerevisiae* encodes a major sporulation-specific mRNA. Mol. Cell. Biol. **6**:4478–4485.

11. **Gottlin-Ninfa, E., and D. B. Kaback.** 1986. Isolation and functional analysis of sporulation-induced transcribed sequences from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:2185–2197.
12. **Guarente, L., and E. Hoar.** 1984. Upstream activation sites of the *CYC1* gene of *Saccharomyces cerevisiae* are active when inverted but not when placed downstream of the "TATA box." Proc. Natl. Acad. Sci. USA **81**:7860–7864.
13. **Guarente, L., B. Lalonde, P. Gifford, and E. Alani.** 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. Cell **36**:503–511.
14. **Herskowitz, I., and Y. Oshima.** 1981. Control of cell type in *Saccharomyces cerevisiae*: mating type and mating type interconversion, p. 181–210. *In* J. N. 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.
15. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
16. **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.
17. **Kurtz, S., and S. Lindquist.** 1984. Changing patterns of gene expression during sporulation in yeast. Proc. Natl. Acad. Sci. USA **81**:7323–7327.
18. **Lemke, P. A., B. Kugelman, H. Morimoto, E. C. Jacobs, and J. R. Ellison.** 1978. Fluorescent staining of fungal nuclei with a benzimidazol derivative. J. Cell Sci. **29**:77–84.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **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.
21. **Meeks-Wagner, D., J. S. Wood, B. Garvik, and L. H. Hartwell.** 1986. Isolation of two genes that affect mitotic chromosome transmission in *S. cerevisiae*. Cell **44**:53–63.
22. **Mercola, M., J. Goverman, C. Mirell, and K. Calame.** 1985. Immunoglobulin heavy-chain enhancer requires one or more tissue-specific factors. Science **227**:266–270.
23. **Parke, R. C., R. M. Watson, and J. Vinograd.** 1977. Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis. Proc. Natl. Acad. Sci. USA **74**:851–855.
24. **Percival-Smith, A., and J. Segall.** 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:142–150.
25. **Percival-Smith, A., and J. Segall.** 1986. Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation of *Saccharomyces cerevisiae*. Mol. Cell. Biol.**6**:2443–2451.
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. **Scholer, H. R., and P. Gruss.** 1984. Specific interaction between enhancer-containing molecules and cellular components. Cell **36**:403–411.
28. **Seguin, C., B. K. Felber, A. D. Carter, and D. H. Hamer.** 1984. Competition for cellular factors that activate metallothionein gene transcription. Nature (London) **312**:781–785.
29. **Tsuboi, M.** 1983. The isolation and genetic analysis of sporulation-deficient mutants in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **191**:17–21.
30. **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.
31. **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.
32. **Xiao, H., and J. T. Lis.** 1986. A consensus sequence polymer inhibits in vivo expression of heat shock genes. Mol. Cell. Biol. **6**:3200–3206.