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Regulation of Gene Expression during Meiosis in *Saccharomyces cerevisiae*: *SPR3* Is Controlled by both ABFI and a New Sporulation Control Element

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The *SPR3* gene encodes a sporulation-specific homolog of the yeast Cdc3/10/11/12 family of bud neck filament proteins. It is expressed specifically during meiosis and sporulation in *Saccharomyces cerevisiae*. Analysis of the sporulation-specific regulation of *SPR3* has shown that it is strongly activated under sporulating conditions but shows low levels of expression under nonsporulating conditions. A palindromic sequence located near the TATA box is essential to the developmental regulation of this gene and is the only element directly activating *SPR3* at the right time during sporulation. Within the palindrome is a 9-bp sequence, gNCRCAAA(A/T) (midsporulation element [MSE]), found in the known control regions of three other sporulation genes. A previously identified ABFI element is also needed for activation. The MSE has been shown to activate a heterologous promoter (*CYC1*) in a sporulation-specific manner. Related sequences, including an association of MSE and ABFI elements, have been found upstream of other genes activated during the middle stage of *S. cerevisiae* sporulation. One group of these may be involved in spore coat formation or maturation.

Sporulation of the yeast *Saccharomyces cerevisiae* is a simple developmental process in which cells undergo premeiotic DNA replication, high-frequency recombination followed by the two divisions of meiosis, and packaging of the four haploid nuclei into spores in an ascus (9, 12). This process involves cells in a sequence of genetic, morphological, and biochemical changes (10), and it provides not only a model system for the study of the control of cellular development but also one in which to analyze the regulation of meiosis.

From a large-scale analysis of gene expression in *S. cerevisiae*, Burns et al. (4) have shown that sporulation involves 93 to 135 meiotically induced genes. While not all sporulation regulation is due to the activation of sporulation-specific genes, it is clear that novel transcripts appear at distinct times during the developmental process (27, 40). More than 30 of these genes have been isolated and characterized on the basis of the timing of their expression. They have been broadly categorized as early, middle, and late genes depending on the timing of their transcription during sporulation (29).

The mechanisms leading to the activation of early sporulation genes are been fairly well established, and overall a common theme on their regulation has emerged (1, 4, 39). These early genes may be activated in a meiosis-specific manner at their URS1 site by Ume6, which is converted from a negative regulator to a positive one by Ime1 (2, 37). Compared to those for the early meiotic genes, the mechanisms controlling the expression of middle and late sporulation genes have not been well defined. Although the early regulatory genes *IME2*, *MCK1*, and *SME2* have been shown to play a role in the regulation of some middle to late sporulation genes (24, 30, 32), their mode of action is currently unknown. Extensive analyses of the regulation of one middle (*SPS4*) and one late gene (*SGA1*) have shown that they are activated during sporulation by different elements (19, 25). The *SGA1* gene requires two elements for its expression; one element responds to nutritional control, and the other (negative response element) responds to both nutritional and mating-type control (25). The sporulation-specific expression of *SPS4* has been shown to depend on a 15-bp sequence termed UAS^{SPS4} (19).

The midsporulation gene *SPR3* was originally isolated by differential cDNA hybridization (6). This gene is expressed only in sporulating cells (20), during both divisions of meiosis. It is therefore regulated in a developmentally specific manner and provides an example of a sporulation gene suited to analysis of midsporulation control. The 5′-flanking region contains several potential regulatory motifs, including an ABFI element which was shown to be essential to the regulation of *SPR3* (33). Here we report a detailed examination of the promoter of *SPR3*, which has led to the identification of its sporulation-specific element within a 20-bp palindromic sequence which harbors a 9-bp sequence common to the regulation of two other sporulation genes.

MATERIALS AND METHODS

Strains and media. *Escherichia coli* JM101 was used as host for plasmid DNA preparation and for M13 phage. The strain BHM71-*18 mut*S215::Tn*10* with suppressed in vivo mismatch repair was used to select for mutant constructs. *S. cerevisiae* strains used were the diploid strain 3C (*MAT***a**/*MAT*α *HO*/*HO arg4-1*/*arg4-1 ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112 his4-519*/*his4-519*) (7), which was derived from several backcrosses of haploid strain BWG1-7A (*MAT***a** *leu2-3,112 his4-519 adel-100 ura3-52*) (16) to derivatives of the homothallic diploid strain S41 (*MAT***a**/*MAT*α *HO*/*HO arg4-1*/*arg4-1*). Yeast transformations were performed with lithium acetate (15).

E. coli cells were grown on 2× YT liquid medium. *S. cerevisiae* cells were propagated in liquid YEPA medium (2% peptone, 1% yeast extract, 2% potassium acetate) and sporulated in sporulation medium (2% potassium acetate, 0.004% auxotrophic requirements) according to the protocol of Fast (14).

Construction of deletion mutants. Plasmids constructed for analysis of the 5′-flanking regulatory region of *SPR3* were derived from pGK11 (20). A 1.34-kb *Bam*HI fragment consisting of the *SPR3* upstream untranslated regulatory region and the first 180 bp of coding sequence was inserted at the *Bam*HI site of pTZ18R to give pTZΔSPR3. This plasmid was linearized with *Xba*I and *Sph*I and treated with exonuclease III for various intervals to generate a set of nested deletions. Each deletion endpoint was determined by sequence analysis.

Each *SPR3* deletion mutant was cut from pTZ18U by using *Bam*HI and *Hin*dIII and fused in frame at the *Bam*HI site to the truncated *E. coli lacZ* gene

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TABLE 1. List of oligonucleotides

Oligonucleotide	Length (bp)	Sequence[a]
SPR3	28	GAAAACCATTCG**CTCGAG**CAAATAGAAG
HAP2/3	25	AGAGGTAAAC**TCGAG**TGGCCCGTTA
D331	24	TGCATCCTTC**TCGAG**TTCGTATTT
ABFIa	25	AAGTGTCTTCTCGAGTTTTGTTAGC
ABFIb	21	CGTATTTAGatcTGTTTTGTT
PAL	29	TATTTAGTGATG**CTCG**AGAGACCTCGATG
D280	25	GACACAAAAG**CTCGA**GACAGACATT
T-rich	28	GATGACAGACA**CTCGAG**CTTTACTTAAT
MSE3a	20	AATTGAGCGACACAAAAGAG
MSE3b	20	AATTCTCTTTTGTGTCGCTC

[a] Oligonucleotides are shown 5′ to 3′, from left to right. Bases which replace large deletions are in bold, and those which alter the sequence are shown in lowercase.

in either the multicopy YEP367R or integrative YIP357R reporter plasmid (31). YEP367R contains the *LEU2* gene and YIP357R contains the *URA3* gene for selection. All constructs contained identical plasmid vector sequences adjacent to test inserts; to standardize the procedure the first deletion was used as the wild-type construct (pENO as the multicopy plasmid and pINO as the integrative form).

The pINO series of constructs were linearized with *Stu*I and transformed to integrate in the genome at the *ura3* locus of the diploid strain 3C or the haploid strain BWG1-7A. Integrants were checked by Southern blot analysis as previously described (36), and only single-copy integrants were used. All plasmids constructed were monitored for *SPR3* expression by assaying for β-galactosidase activity.

Site-directed mutagenesis. The *SPR3* *Hin*dIII/*Bam*HI insert from plasmid pENO was fused to the *Hin*dIII/*Bam*HI site of M13mp18. Eight specific site-directed mutations, each of which created a unique restriction site in the *SPR3* 5′-flanking region, were generated by the double-primer method previously described (36). Duplicate mutant constructs were isolated from two different plaques and verified by sequencing and restriction digestion. The *SPR3* mutation constructs were excised from M13mp18 and ligated to YIP357R to generate the following mutant *SPR3-lacZ* fusion plasmids: YIP-SPR3, YIP-HAP2/3, YIP-Δ330, YIP-ABFIa, YIP-ABFIb, YIP-PAL, YIP-Δ280, and YIP-Trich, which correspond to the oligonucleotides shown in Table 1. The double mutant YIP-ABFIa-PAL was constructed by replacing the *Xho*I/*Bam*HI fragment of the YIP-ABFIa construct with the *Xho*I/*Bam*HI fragment from YIP-PAL. These plasmids were linearized by *Stu*I and integrated at the *ura3* locus of strains 3C and BWG1-7A to monitor *SPR3* expression as described above.

Fragment inserts in the D-deletion construct. The *Bam*HI, *Eco*RI, *Cla*I, and *Nla*III sites (located at positions −1157, −779, −461, and −328, respectively, as shown in reference 33) were used to divide the region 5′ of the ABFI and midsporulation element (MSE) sites of *SPR3* into fragments. The 378-bp *Bam*HI/*Eco*RI, 318-bp *Eco*RI/*Cla*I, and 132-bp *Cla*I/*Nla*III fragments were blunt ended with T4 DNA polymerase and ligated into the T4 DNA polymerase-treated *Hin*dIII site of the D-deletion or D-deletion ABFI constructs.

Heterologous promoter constructs. The plasmid placZi (Clontech Laboratories, Inc.) contains a basal promoter *CYC1::lacZ* fusion. Oligonucleotides MSE3a and MSE3b (Table 1) were annealed and ligated into the *Eco*RI site of the placZi polylinker region. In order to obtain constructs harboring a varied number of oligonucleotide repeats, the annealed oligonucleotides were treated with polynucleotide kinase and allowed to self-ligate prior to the addition of linearized vector.

β-Galactosidase assays. Quantitative β-galactosidase assays were performed on cells grown in appropriate medium to an optical density at 600 nm (OD_{600}) of 1 and then transferred to 0.2 vol of SPOM as described above. Extracts were prepared from 2-ml culture samples which were centrifuged, washed with homogenizing buffer (0.2 M Tris-HCl, pH 8.5; 0.1 M 2-mercaptoethanol; 1 mM phenylmethylsulfonyl fluoride [PMSF]) and resuspended in 330 μl of the same buffer. Acid-washed glass beads (2/3 volume; 40 mesh) were added and the cells were broken by agitation for 40 s in a MiniBeadbeater-8 (Biospecs). The supernatant was collected and cell debris was removed by centrifugation at 13,000 × *g* for 15 min. The assays were carried out from at least two independent transformants and for each transformant on duplicate samples with *O*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate. Protein concentration in each extract was quantitated by using the Bio-Rad assay system according to manufacturer's instructions.

RESULTS

A 67-bp region is essential for activation of *SPR3* at the correct time during sporulation. Previous inspection of the sequence upstream of the start codon of *SPR3* for other known elements, or motifs common to other late sporulation genes, revealed five potential elements: SPR3, HAP2/3, ABFI, PAL, and a T-rich site. The site identified as SPR3 has 15 out of 19 matches to a sequence located between position −334 and −313 in *SPR6* (22). Within the palindromic sequence (PAL) there is a region highly conserved, in both sequence and position, with respect to sequences in the *SPS1*, *SPS18/19*, and *SPS100* genes. The HAP2 motif binds a heterotrimeric transcription factor, HAP2/3/4, involved in the derepression of respiratory genes in cells growing on nonfermentable carbon sources (34). The ABFI motif binds a ubiquitous transcription factor, ABFI, which is involved in the regulation of many genes, and T-rich sites have been shown to play stimulatory roles in ABFI-mediated transcription (3) and promote constitutive transcription of some yeast genes (38). Further study of the ABFI element revealed that it is essential for the regulation of *SPR3* and that the ABFI transcription factor bound to it in vitro (33).

Although ABFI is essential for *SPR3* regulation, it is an ubiquitous transcription factor which plays a role in regulating many genes with unrelated function. Hence, it is unlikely that the ABFI element is the only element involved in the sporulation-specific activation of *SPR3*. In order to identify other *cis*-acting regulatory elements responsible for sporulation-specific activation of *SPR3*, a nested series of 5′-to-3′ deletions of the upstream region was generated. These were made from position −1053 relative to the start codon. The wild type and each deletion construct were fused in frame to the reporter *lacZ* gene in the multicopy yeast shuttle vector YEp367R (31) to generate the wild-type plasmid pENO and the deletion series A to I. Each construct was transformed into cells of diploid strain 3C. Figure 1D illustrates the extent of each deletion, and Fig. 1A shows the level of *lacZ* expression from each deletion construct after 22 h in sporulation medium. These results indicated that most of the expression of *SPR3* during sporulation requires a 67-bp region within 333 bp of the start codon, since a major decrease in sporulation expression was observed on deletion from position −333 to −266 (lanes E and F in Fig. 1A).

The levels of *lacZ* expression of deletions prior to position −333 were subject to some fluctuation, possibly due to an effect of variable copy number. To test this, the extent of *lacZ* expression for each of the deletion constructs was also assayed as single-copy constructs (results not shown). Both sets of results indicated that a sporulation-specific upstream activation site (UAS) resides within the 67-bp region between positions −266 and −333, and hence both multicopy and single-copy plasmids could be used to study the sporulation-specific control of *SPR3*.

To verify that the above results were due to sporulation control and not due to a change in media conditions, these constructs were tested under conditions of vegetative growth of the diploid and starvation of the haploid strain BWG1-7A. Previous work in this laboratory used the homothallic diploid strain 3C for sporulation timing studies. There are problems making a homothallic diploid homozygous at the mating type to provide a suitable nonsporulating diploid control. We therefore used a closely related haploid strain that had been used to produce homothallic diploid strain 3C. This is not ideal, but subsequent results have shown that expression of *SPR3* in the starved haploid was very similar to that seen in the vegetatively growing diploid cells. The same series of deletion constructs (pENO series) was transformed to haploid BWG1-7A cells. *SPR3::lacZ* expression was monitored in both vegetatively grown diploid cells of strain 3C (Fig. 1B) and haploid cells starved for 22 h in SPOM (Fig. 1C). The results for both sets

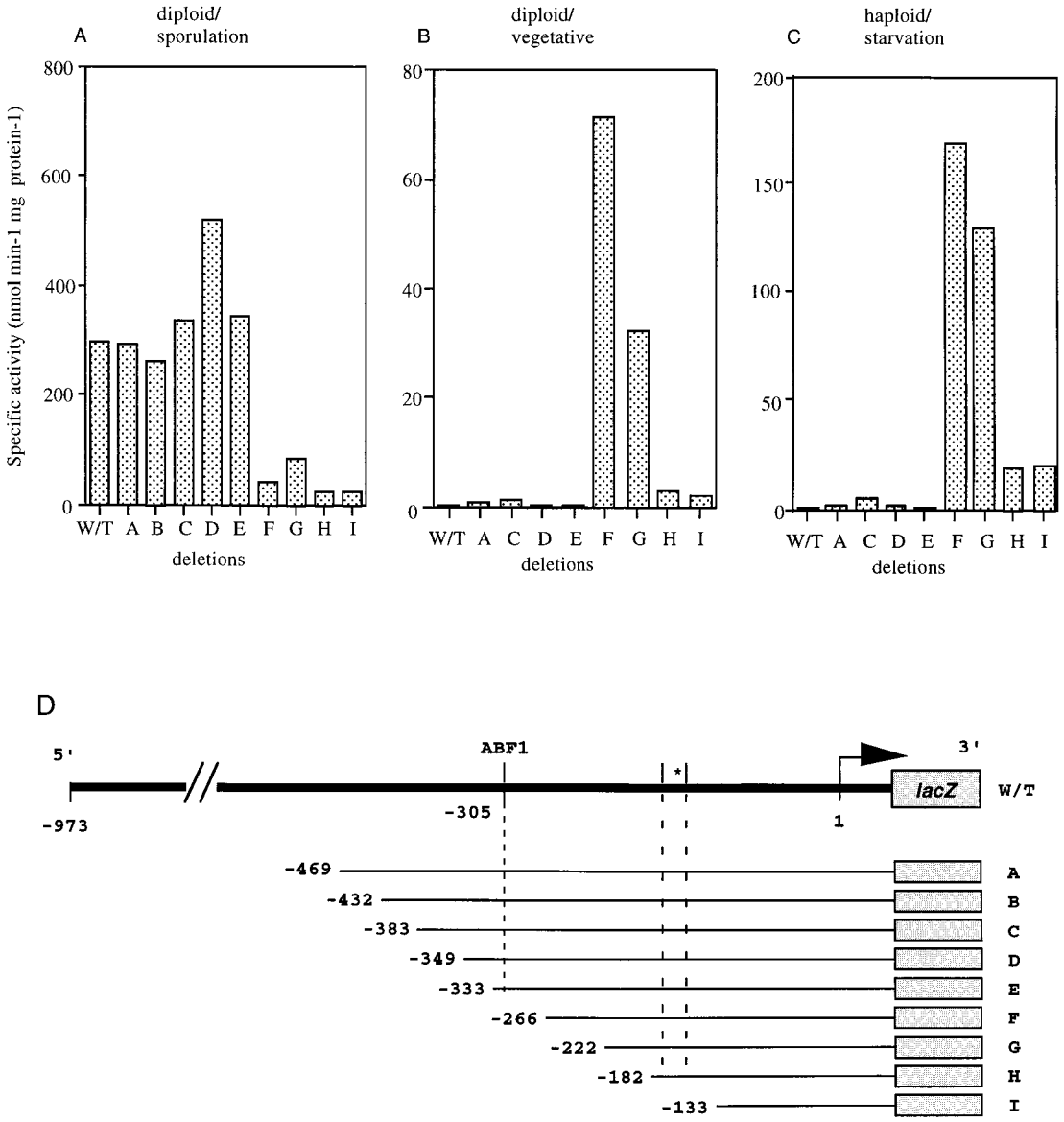

FIG. 1. β-Galactosidase activity of *SPR3* and subsequent deletion constructs during vegetative growth, starvation, and sporulation conditions. (A) Activity of β-galactosidase in transformed cells of diploid strain 3C that had been suspended in sporulation medium for 22 h. These included cells transformed with the full-length *SPR3::lacZ* multicopy (pENO) construct and the subsequent deletion constructs A to I. (B) Specific enzyme activity in vegetatively growing diploid 3C cells. (C) Enzyme activity in haploid BWG1-7A cells 22 h after suspension in sporulation medium (starvation conditions). The haploid cells were also transformed with the pENO series of multicopy constructs. (D) Extent of each deletion fused to *lacZ* in the multicopy copy vectors, pENO. The positions of the putative transcriptional start points (*) and the ABFI element are indicated.

were qualitatively similar. The full-length construct pENO was regulated normally (i.e., in a sporulation-specific way) with virtually no expression in either haploid cells suspended in sporulation medium for 22 h or vegetative diploid cells.

Deletions up to and including the 67-bp element (i.e., to position −333) did not express the *lacZ* fusion under either nonsporulating conditions (i.e., vegetative diploids or starved haploids). Further deletion led to a marked increase in expression under both nonsporulating conditions used. All of the above results indicate that sporulation-specific expression of *SPR3* requires sequences located within the 67-bp element (positions −266 to −333).

Deletions that remove the 67-bp region identified as essential for sporulation-specific expression (deletions F and G) showed a marked increase in expression under both nonsporulating conditions. However, further deletion (deletions H and I) into the potential TATA box and transcriptional start point regions (33) led to low levels of expression under all conditions tested. This increase in expression under nonsporulating conditions is discussed later.

The ABFI site and an adjacent site within an imperfect palindrome are essential for the activation of *SPR3*. From the deletion results sporulation-specific activation was expected to be mediated by sequences within the 67-bp distal to position −266. The ABFI element previously identified as being essential to the regulation of *SPR3* lies within this region. To check if there were any other essential elements within this sequence, four short window deletions were made which together completely spanned the 67-bp region of the parent construct pINO (Fig. 2B). As well, three other potential control sites (SPR3,

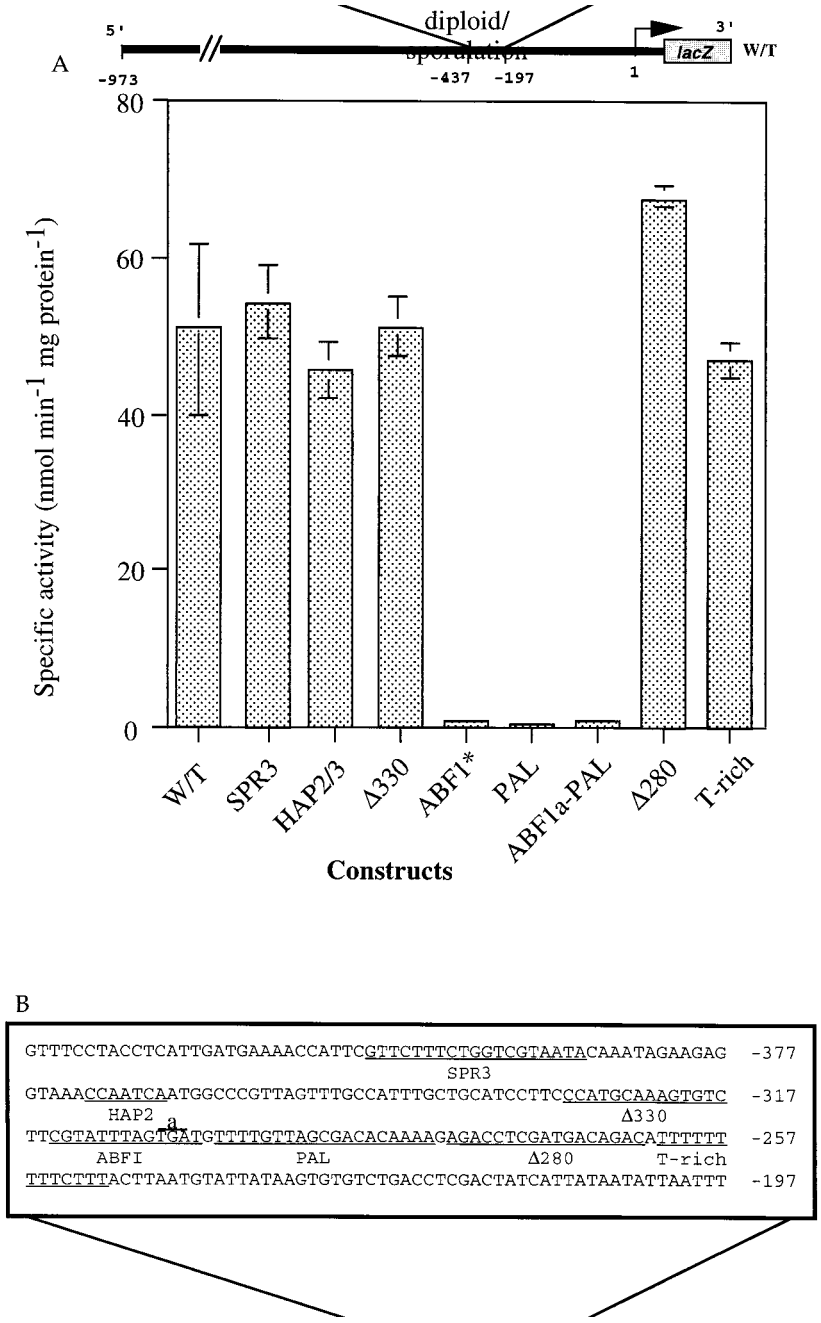

FIG. 2. Analysis of window deletions within a localized region in *SPR3*. (A) Specific activity of β-galactosidase from the parent integrative construct, pINO, and the indicated window deletions. Under vegetative and starvation conditions the specific activity of β-galactosidase in transformants with all of the above window deletions was less than 0.1 nmol min^{-1} (mg of protein)$^{-1}$. (B) Sequence of the regions spanning the window deletions are underlined. ABFI was also mutated by a 3-bp mismatch, and this is indicated (overline and a).

HAP2/3, and T-rich) identified previously (33) were also deleted by site-directed mutagenesis to see if they played a role in the regulation of the intact gene. From assays of the appropriate transformants, at least one window deletion within the 67-bp region was expected to remove sporulation-specific activation of the *SPR3* gene.

Deletions Δ331 and Δ280 (regions within the 67-bp region) and mutations of the SPR3, HAP2/3, and T-rich sites did not lead to any significant changes in sporulation expression compared to that in the wild-type construct. However, a mutation in the palindrome (PAL), another in the ABFI site, and a double mutation of both ABFI and PAL all showed 100- to 130-fold loss of expression of *SPR3* during sporulation. Previously the ABFI site was mutated by using the oligonucleotide ABFIb (Table 1), which contained a change in 3 bases essential to ABFI binding (11). Because the window deletions removed

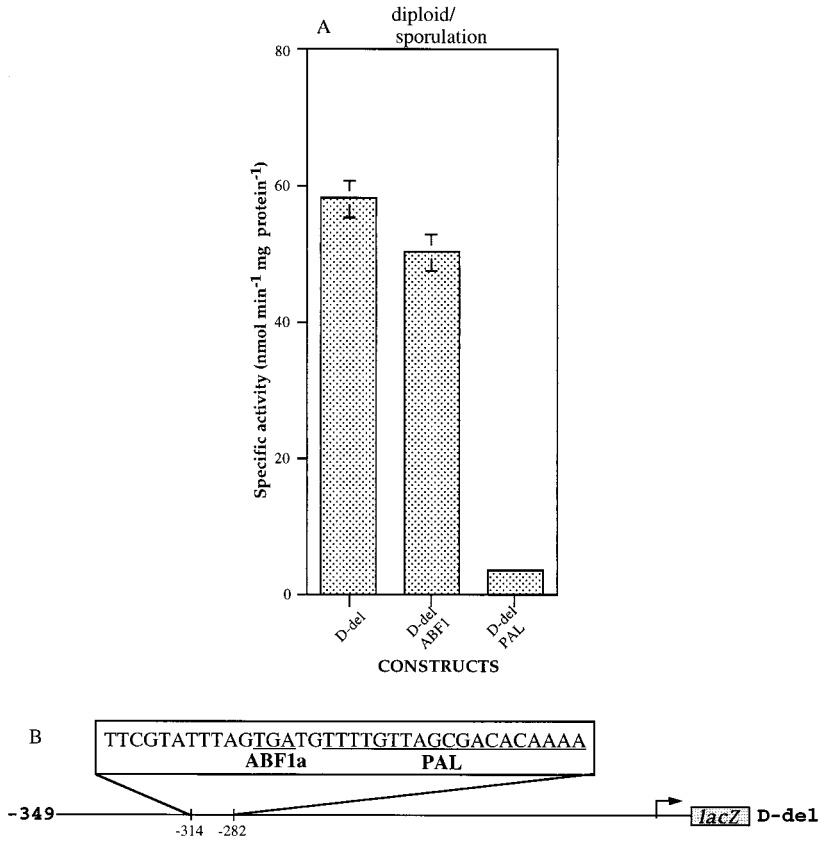

FIG. 3. Analysis of the effect of mutations of PAL and ABFI sites within a deletion construct. (A) Diploid transformants carrying the unmutated D construct or either mutated constructs were monitored for enzyme activity after suspension in SPOM for 22 h. (B) ABFI and PAL sites as indicated were mutated in the deletion construct D. ABFIa represents the oligonucleotide used for the construction of the mutation. Low levels of expression were seen for all constructs under vegetative conditions and for starved haploid cells.

larger fragments, the entire ABFI site was deleted in the above experiments. Both mutations showed the same drop in expression of *SPR3* during sporulation, indicating that spacing is not a potential problem with the interpretation of these results. We have previously shown that the ABFI element is needed but is not sufficient for sporulation expression (33). The above results show that a sequence within the imperfect palindrome (PAL) is also essential.

PAL and ABFI function in different ways to regulate *SPR3*. The deletion construct D retains elements for sporulation specificity (ABFI and PAL elements) but removes all upstream sequences (beyond position −349). From the gross-deletion and window-deletion results it could be predicted that this construct should contain all sequences necessary for sporulation-specific activation, as well as any elements required for lack of expression under nonsporulating conditions.

In this deletion construct a mutation at the PAL site led to a 16-fold reduction in expression under sporulating conditions when compared to the unmutated construct (Fig. 3A). These results clearly show that a sequence within PAL is essential for sporulation-specific activation of *SPR3*. However, a mutation of the ABFI motif in this truncated construct surprisingly appeared to have no effect on expression under sporulating conditions.

The discrepancy between the results from the two ABFI mutant constructs (i.e., the truncated and the parent promoter) needed further investigation. Several explanations could account for these results. There may be an element upstream of the D-deletion boundary that represses expression under nonsporulating conditions. The role of the ABFI site would thus be to alleviate this repression under sporulating conditions (i.e., derepression). If this hypothetical repressor were removed (e.g., D-deletion), there would no longer be a requirement for ABFI function. An alternative explanation is that there is a UAS located in the vector, which when moved closer to the transcriptional start point (e.g., D-deletion) is able to substitute for the function of the ABFI motif.

The 629-bp region removed when deleting from the parent pINO to the D-deletion boundary was divided into three fragments and separately placed upstream of the D-deletion boundary in both the wild-type and ABFI mutant constructs (see Materials and Methods). The results shown in Fig. 4A reveal that all three fragments restored the requirement for a functional ABFI site for sporulation-specific expression. This result is more readily explained by the existence of an activator sequence in the vector upstream of the *SPR3* promoter region. Such a sequence, when brought closer to the transcriptional start point, would substitute and/or replace the ABFI function, thus removing the requirement for an ABFI site in some deletion constructs. However, in constructs containing more upstream *SPR3* sequence, a functional ABFI site is required for sporulation-specific activation.

Together, the above results show that the MSE element is absolutely essential for sporulation-specific expression. The adjacent ABFI site is also required but may be substituted by another element in the vector sequence. Therefore, the MSE and ABFI elements must be acting in different ways.

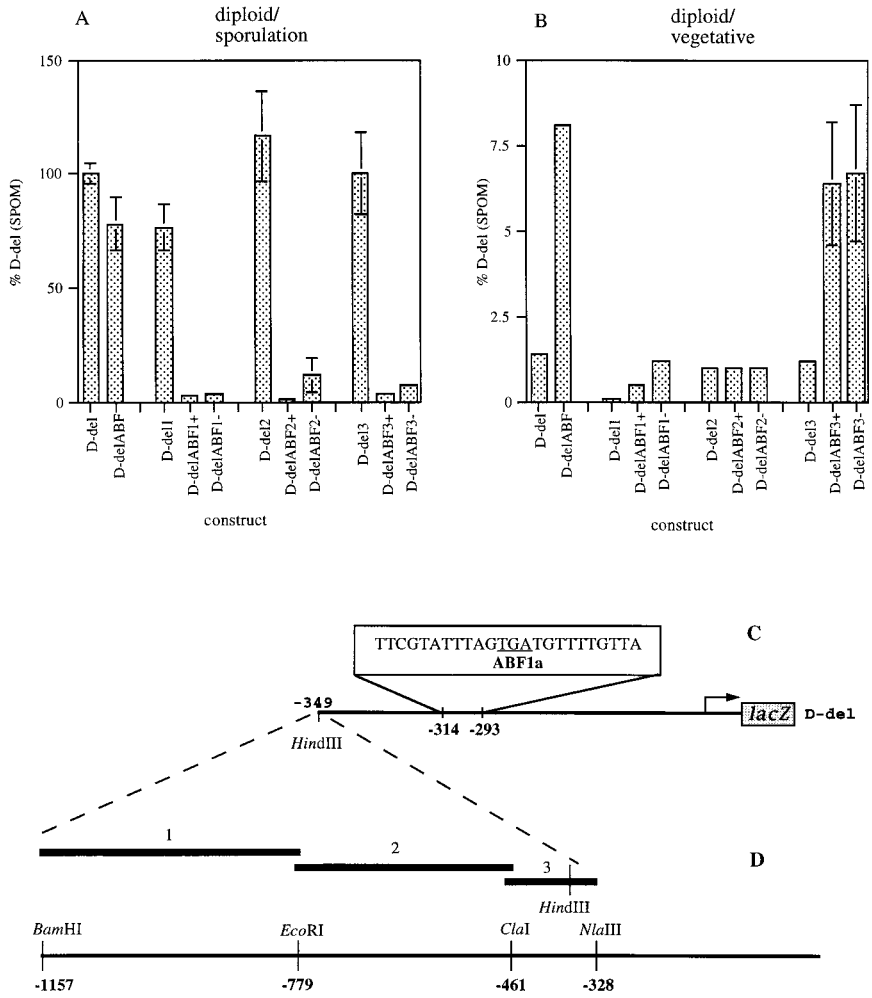

FIG. 4. Effect of inserting fragments from the 5′ region of *SPR3* into D-deletion (D-del) constructs. (A and B) Relative activities of the various constructs. All activities are standardized to the wild-type deletion construct D under sporulation conditions, which represents 100% activity (first bar in panel A). Activity was examined after the transformants were suspended in SPOM for 22 h (A) or under vegetative conditions (B). (C) D-deletion construct showing the region altered in the ABFI mutation and the *Hin*dIII site at which fragments were inserted. (D) Restriction sites of the genomic *SPR3* region, with the three fragments indicated above. Fragments (1, 2, and 3) from the 5′ region of *SPR3* were inserted in the correct orientation (+) or incorrect orientation (−) at the *Hin*dIII site of the D-deletion construct carrying either the wild-type (D-del) or mutated (D-delABF) ABFI site.

An element within the palindrome of *SPR3* can activate a heterologous promoter in a sporulation-specific way. A region in the PAL sequence of *SPR3* has an 8-of-9-bp match with the one that Hepworth et al. showed is involved in the sporulation-specific activation of *SPS4* (19). This 9-bp sequence, which in *SPR3* we have termed the MSE, forms one of the inverted repeats of the PAL. An oligonucleotide spanning the MSE was inserted into a basal *CYC1::lacZ* fusion construct (see Materials and Methods) and tested for its ability to activate expression from this heterologous promoter.

From Fig. 5 it can be seen that under vegetative conditions there was no significant activation of the heterologous promoter. However, the MSE was capable of stimulating transcription from the minimal *CYC1* promoter by 25-fold under sporulation conditions, in an orientation-independent way. The level of activation increased with increasing number of copies of the MSE element in the *CYC1* promoter region.

DISCUSSION

The results from this study indicate that *SPR3*, a midsporulation gene, is regulated in a complex manner by at least two essential promoter elements: a palindromic sequence, containing the MSE identified in this study, and an ABFI element previously identified (33). The *SPR3* gene contains four potential TATA boxes, with the most proximal one adjacent to the transcription start sites identified previously (33). The MSE is located 43 to 102 bp upstream of these TATA boxes, locating the sporulation-specific element very close to the site for assembly of the general transcription machinery.

The MSE alone has been shown to confer sporulation-specific regulation on a heterologous promoter, suggesting that this element is directly involved in the regulation of timing of expression of the *SPR3* gene. However, these experiments also indicate that there are differences between the *CYC1* basal promoter element and the intact *SPR3* promoter, as the requirement for a functional ABFI site is not seen in the heterologous promoter. This may indicate that ABFI is needed in *SPR3* to modulate chromatin structure (28) or to promote the accessibility of an MSE-binding protein in the native promoter. This difference between the activity of the ABFI and MSE motifs is highlighted by the results obtained with the D-deletion construct (see Fig. 3), since mutation of the ABFI motif no

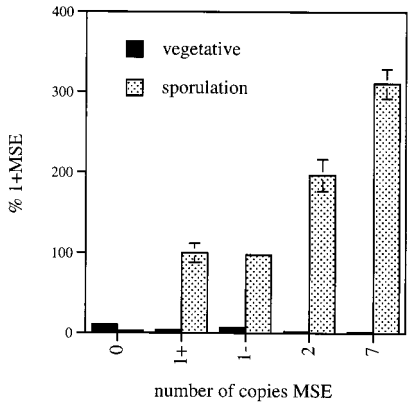

FIG. 5. Induction of a heterologous promoter by the MSE. The MSE oligonucleotide was inserted in the correct (+) or incorrect (−) orientation or in multiple copies at the multicloning site of placZi. Diploid transformants carrying these constructs were assayed under vegetative conditions or after suspension in SPOM for 22 h. The relative activities are standardized to a single copy of the MSE in the correct orientation under sporulating conditions.

longer affected sporulation expression but the requirement for the MSE motif remained.

A 9-bp sequence element and an ABFI or ABFI-like element are involved in the regulation of at least three sporulation-specific genes. Our experiments have shown that a 15-bp element from *SPR3* can confer sporulation-specific expression on a *CYC1::lacZ* promoter. Hepworth et al. (19) showed that a similar sequence with an 8-bp match was involved in the sporulation-specific regulation of *SPS4* and identified this element in the *DIT2* and *SPR2* genes. Figure 6A illustrates the comparison between these sequences and additionally the *SPS18*/*SPS19* and *SMK1* sporulation genes and *CDC10*, which is subject to sporulation-specific upregulation (8, 21, 26). The consensus sequence is gNCRCAAA(A/T). A detailed analysis of the divergently transcribed *SPS18*/*SPS19* gene pair has localized sporulation-specific activation to an 87-bp region within the 273-bp intergenic promoter (8). A number of small window deletions within this region of *SPS19* revealed that the A_4 track in the 9-bp motif is essential to the sporulation-specific regulation of *SPS19*, but a deletion which removed the gN within the consensus only decreased activity by 25% and did not affect sporulation specificity (43). These results indicate that a minimal element, CRCAAA(A/T), is sufficient for sporulation specificity.

The data of Hepworth et al. (19) show that in *SPS4* a region adjacent to the MSE-like motif is responsible for more than a 10-fold increase in activation of a heterologous *CYC1::lacZ* promoter. A similar requirement for an adjacent element has been observed by us in the *SPS18*/*SPS19* divergent promoter (8). Curiously, closely associated with the minimal element in both *SPS4* and *SPS19* is a 14-bp sequence which resembles the ABFI element found in *SPR3*; however, these contain an extra base pair between the essential flanking sequences (Fig. 6B). For ABFI it is well established that the insertion of one base pair leads to a motif with low affinity for the binding of ABFI protein in vitro (17, 18). Nonetheless, the ABFI-like sequences have been shown to be important for the correct level of activation of both genes but not for the sporulation specificity of *SPS4* (that for *SPS19* has not been determined). In all of this set of genes, except *SPS19* (which is part of a divergent system with *SPS18*), ABFI, or a similar sequence, is directly upstream of the MSE site. Clearly, further experiments are required to determine how these elements play a role and whether ABFI or a related protein is acting at this site(s).

***CDC10* and *SPR3* may be regulated by the same mechanism during sporulation.** The *SPR3* gene was previously shown to encode a sporulation-specific homolog of the yeast *CDC3*/*10*/*11*/*12* family of bud neck filament genes (33). Of these, *CDC10* expression is known to increase during sporulation (21). The upstream regulatory region of *CDC10* also contains a putative strong ABFI element and directly downstream an MSE element. The existence of both of these elements in *CDC10* may indicate that the sporulation-specific activation of *CDC10* is controlled in a way similar to that of *SPR3*. However, the lack of expression under vegetative conditions may be unique to *SPR3*, since *CDC10* is expressed and is necessary for optimal growth under vegetative conditions (21).

In some genetic backgrounds, homozygous diploid null *spr3* mutants sporulate at lower efficiency (23). *SPR3* may have a role similar to that of the homologous *CDC3*/*10*/*11*/*12* genes involved in bud formation and cytokinesis in haploid cells (33), especially since it has been shown that the *CDC10* and *SPR3* gene products localize to the membrane that invaginates the haploid nuclei during the initial stages of spore formation (13).

Changes may occur in the basal transcription machinery during sporulation. In the gross deletion experiments, removal of both the MSE and the ABFI element (F-deletion) led to a significant increase, under nonsporulation conditions, of expression of the *lacZ* construct. This may be explained by the approach of a vector-derived UAS element. However, a similar change did not occur under sporulation conditions. In fact a decrease in activity was observed during sporulation conditions

A

SPR3	->	-314	CGTATTTAGTGATGTTTTGTTAGCGACACAAAA
SPS4	->	-132	GCGCCACAAAA
SPS19	->	-204	ACGTCACAAAA
SPR2	<-	-218	TAGCCACAAAA
SPR2	<-	-229	GGGACACAAAA
DIT2	->	-378	GCGTCGCAAAA
SMK1	<-	-69	GTGTCACAAAT
CDC10	->	-136	AGACCACAAAT
Consensus for MSE			gNCRCAAA(A/T)

B

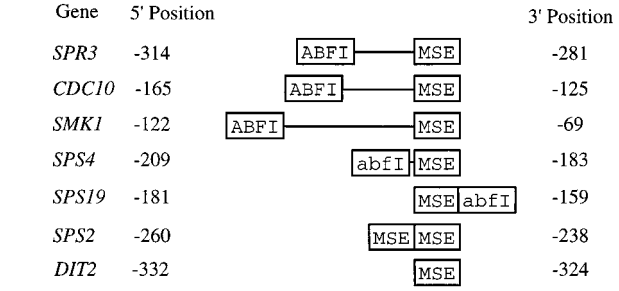

FIG. 6. Consensus sequence for an MSE and its relation to ABFI or an ABFI-like element. (A) The consensus sequence gNCRCAAA(A/T) is found in the upstream regulatory region of five other genes; in *SPR2* it appears twice (35) as shown here. This motif is also found in *CDC10*, which is expressed both during vegetative growth and during sporulation. The underlined region in *SPR3* represents the ABFI element, and the arrow represents the direction of MSE with respect to the start codon. (B) Relative position of ABFI or ABFI-like (abfI) sequences with respect to the MSE. The ABFI-like sequences (abfI) have one extra base pair.

in the F-deletion after 10 h (data not shown). A similar effect was observed with the basal *CYC1::lacZ* promoter construct. These results may indicate that there is a change (or changes) during sporulation in the general transcription machinery and that this may be involved in the regulation of sporulation-specific genes. Analysis of the yeast RNA polymerase II has revealed that subunits RPB4 and RPB9 are dispensable for normal growth but are essential for transcription during heat shock and postexponential growth phases (5, 41, 42). Given that some subunits are only essential for cells to survive stress, it is possible that some of the RNAP II subunits are dispensable during sporulation and vice versa or that RNA polymerase II subunits may change during sporulation.

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