The EMBO Journal Vol.18 No.22 pp.6448–6454, 1999

Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in *Saccharomyces cerevisiae*

Jianxin Xie, Michael Pierce, Valérie Gailus-Durner, Marisa Wagner[1], Edward Winter[1] and Andrew K.Vershon[2]

Waksman Institute and Department of Molecular Biology and Biochemistry, 190 Frelinghuysen Road, Rutgers University, Piscataway, NJ 08854-8020 and [1]Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107, USA

[2]Corresponding author
e-mail: vershon@mbcl.rutgers.edu.

Meiotic development in yeast is characterized by the sequential induction of temporally distinct classes of genes. Genes that are induced at the middle stages of the pathway share a promoter element, termed the middle sporulation element (MSE), which interacts with the Ndt80 transcriptional activator. We have found that a subclass of MSEs are strong repressor sites during mitosis. *SUM1* and *HST1*, genes previously associated with transcriptional silencing, are required for MSE-mediated repression. Sum1 binds specifically *in vitro* to MSEs that function as strong repressor sites *in vivo*. Repression by Sum1 is gene specific and does not extend to neighboring genes. These results suggest that mechanisms used to silence large regions of chromatin may also be used to regulate the expression of specific genes during development. *NDT80* is regulated during mitosis by both the Sum1 and Ume6 repressors. These results suggest that progression through sporulation may be controlled by the regulated competition between the Sum1 repressor and Ndt80 activator at key MSEs.

Keywords: *HST1*/MSE/sporulation/*SUM1*/transcription

Introduction

Meiosis and sporulation in *Saccharomyces cerevisiae* are characterized by the sequential expression of large numbers of genes (Kupiec *et al*., 1997; Chu *et al*., 1998). Sporulation-specific genes are expressed exclusively during this developmental program and can be broadly divided into early, middle or late temporal categories. Genome-wide analysis of the transcriptional program during sporulation revealed that >300 genes are induced during the middle period of sporulation around the time of meiotic chromosome segregation (Chu *et al*., 1998). Mid-sporulation genes are activated by the Ndt80 transcription factor that binds to a conserved sequence (gNCRCAAA$^{A}/_{T}$) termed the middle sporulation element (MSE) found in most middle gene promoters (Hepworth *et al*., 1995, 1998; Ozsarac *et al*., 1997; Chu and Herskowitz, 1998). *NDT80* is itself a mid-sporulation-specific gene that has been implicated as a target of checkpoint controls that operate during meiosis. MSEs are also present in several B-type cyclin promoters (*CLB1*, and *CLB3–6*) and Ndt80 has a role in the expression of these genes during meiosis (Chu and Herskowitz, 1998; Hepworth *et al*., 1998). These observations have led to the idea that Ndt80 plays a central role in the transcriptional cascade and in coordinating checkpoint signals that regulate the progression through meiosis and sporulation.

One of the genes that is activated by Ndt80 is *SMK1*, which encodes a mid-sporulation-specific MAP kinase homolog that is required for spore wall morphogenesis (Krisak *et al*., 1994; Wagner *et al*., 1997, 1999). The *SMK1* promoter contains an MSE that is required not only for transcriptional activation during mid-sporulation but also for repression of the Abf1-dependent activator site during vegetative growth and early meiosis (Pierce *et al*., 1998). Here we show that MSEs found in other mid-sporulation genes can also function to repress transcription during vegetative growth, and have identified *SUM1* and *HST1*, genes previously associated with transcriptional silencing, as being involved in this repression.

Results

A subclass of MSEs function as mitotic repression sites

The MSE in the *SMK1* promoter represses expression during vegetative growth and early sporulation (Pierce *et al*., 1998). We were interested in whether other MSEs also function as repressor sites. MSEs found in the promoters of several sporulation-specific genes were used to replace the URS1 site in a *HOP1-LacZ* reporter plasmid. These constructs were assayed for their ability to regulate transcription during vegetative growth and mid-sporulation, and under conditions in which *NDT80* is ectopically expressed (Figure 1). The MSEs from the *SMK1* and *BBP1* promoters function as strong vegetative repressor sites and reduce expression of the promoter by ⩾20-fold. The *SPR3* MSEs also repress transcription but are weaker than the *SMK1* site. In contrast, the *SPS4*, *DIT1* and *CLB6* MSEs do not repress expression detectably. The *NDT80* promoter contains two MSEs; one (at –78) is a strong repressor site, while the other (at –221) is not a repressor site in the assay. Therefore, MSEs found in a variety of promoters that are expressed during mid-sporulation are able to repress gene expression in vegetative cells.

We also measured the ability of the sites to induce expression during the middle stages of sporulation (Figure 1). All of the MSEs tested showed an increase in the level of *LacZ* expression during meiosis relative to the level of expression during vegetative growth. This result suggests that during meiosis, not only are the MSEs derepressed, but they also serve as activator sites, presumably by an Ndt80-dependent mechanism. To test

Site	Position	Sequence	Mitotic repression units	Mitotic repression fold	Meiotic activation units	Meiotic activation fold	Gal-Ndt80 induction units	Gal-Ndt80 induction fold
Vector		————————	23		27		15.4	
SMK1	-69	ccactaATTTGTGACactt	0.3	77	75	2.7	19	1.2
NDT80	-78	cctccaTTTTGTGTCacct	0.7	33	272	10	315	20
NDT80	-221	ctactcTTTTGTGTCatac	14	1.6	170	6.3	357	23
SPS4	-191	atacgtTTTTGTGGCgcgc	21	1.1	306	11	493	32
SPR3	-14	ggtctcTTTTGCGTCgcta	5.0	4.6	203	7.5	127	8.2
SPR3	-289	ggtctcTTTTGTGTCgcta	3.6	6.4	313	12	342	22
DIT1	-342	acccttATTTGTGAGgagt	39	0.6	57	2.1	84	5.5
DIT1	-555	accttttTTTTGCGACgcgc	35	0.7	157	5.8	92	6.0
BBP1	-131	acccgtTTTTGTGTCgctc	0.2	115	280	10	464	30
CLB6	-345	ttttttTTTTGCGACggta	27	0.9	182	6.7	75	4.9

Fig. 1. Repression and activation by MSEs in a heterologous promoter. Oligonucleotides containing MSEs from the indicated promoters were cloned into the *Xho*I site of the *HOP1-LacZ* reporter vector, pAV124 (Pierce *et al*., 1998). The mitotic repression activity of each construct was measured in strain W303A. Meiotic activation was measured in strain RSX2-7B 10 h after transfer to sporulation media. Ndt80-dependent activation of the reporters was measured in transformants of strain MPY2 grown in galactose. Values are β-galactosidase units and are the average of three independent transformants. The fold repression and activation were calculated by comparison of the level of β-galactosidase activity with a vector lacking an MSE.

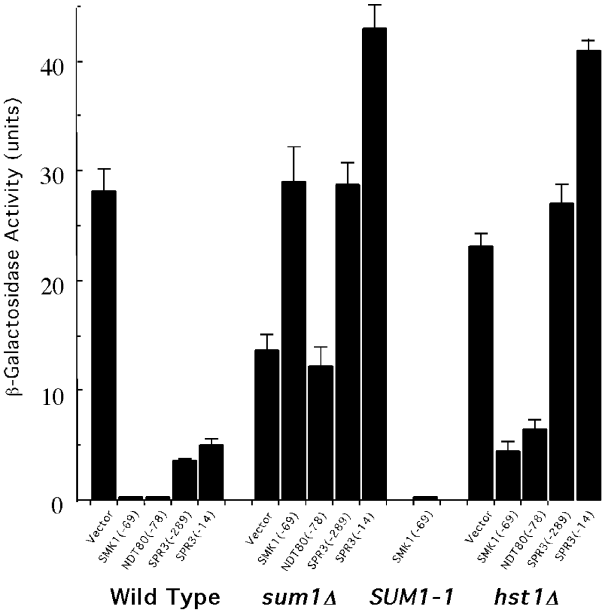

Fig. 2. Mutations in *SUM1* and *HST1* derepress MSEs. *LacZ* expression of *HOP1-LacZ* reporter plasmids containing the *SMK1* (pJX43) and *NDT80*(–78) (pMP15), *SPR3*(–14) (pMP38) and *SPR3*(–289) (pMP42) MSEs was measured in wild-type (W303-1A), *sum1* (JXY3) and *hst1* (JXY5) strains during vegetative growth as in Figure 1. The level of expression of the *HOP1-LacZ* reporter plasmid containing the *SMK1* MSE (pJX43) in a *SUM1-1* strain (JRY2456) is also shown.

more directly whether activation by these sites is dependent on Ndt80, we examined *LacZ* expression in a strain in which *NDT80* is ectopically expressed from the *GAL1-10* promoter (Chu and Herskowitz, 1998). We found that under these conditions, all of the MSEs are induced and function as activator sites. However, there are significant differences in the magnitude of Ndt80-dependent activation. Taken together, these results show that although the MSEs contain a common core element, there are distinct classes of MSEs which have different regulatory activities during mitosis and meiosis.

Sum1 and Hst1 are required for MSE-mediated repression

We have shown previously that mutations in *SSN6*, *TUP1*, *SIN3*, *RPD3* or *UME6*, genes known to be involved in repressing some sporulation-specific genes during mitosis, have little effect on the ability of the *SMK1* MSE to repress gene expression in vegetative cells (Pierce *et al*., 1998). To identify genes required for MSE-mediated repression, we performed a genetic screen to isolate mutants that are unable to repress expression of the *HOP1-LacZ* reporter containing the MSE from the *SMK1* promoter. Haploid transformants were mutagenized with ethylmethane sulfonate (EMS) and screened for blue colonies by X-gal filter assays. Twenty-seven independent recessive mutants were isolated that fell into two complementation groups comprised of eight and 19 isolates. The corresponding genes were cloned from a genomic plasmid library by complementation of the *LacZ* expression phenotype using a representative mutant from each group. Subcloning and sequence analysis of the clones obtained in the screen of the library show that *SUM1* is required for complementation of the first group and *HST1* is required for complementation of the second group.

The *SUM1* and *HST1* genes were identified previously based on their involvement in transcriptional silencing at the silent mating type loci. *SUM1* was isolated originally as a dominant allele, *SUM1-1*, which suppresses the defects in silencing at *HMR* and *HML* in *sir2* mutants (Klar *et al*., 1985; Lin *et al*., 1990). Interestingly, *SUM1-1* also suppresses defects in silencing at the silent mating type loci in *sir1*, *sir3* and *sir4* mutants, mutations in the Abf1-, Rap1- or Orc1-binding sites in the *HMR* silencer E-box element, and point mutations in the N-terminal tails of the histone H3 and H4 proteins (Livi *et al*., 1990; Laurenson and Rine, 1991; Chi and Shore, 1996). The *HST1* gene was identified initially as a sequence homolog to *SIR2*, sharing 63% identity and 76% sequence similarity between the entire proteins (Brachmann *et al*., 1995; Derbyshire *et al*., 1996). Although these findings suggest that both proteins may have roles in silencing, *sum1* and *hst1* null mutants do not show any apparent defects in silencing at the silent mating type loci or telomeres and, therefore, the normal functions of these two proteins in the cell were unknown (Brachmann *et al*., 1995; Chi and Shore, 1996; Derbyshire *et al*., 1996).

To verify that Sum1 and Hst1 are required for MSE-mediated repression, we constructed *sum1* and *hst1* null mutants and measured *LacZ* expression from promoter–reporter constructs containing the *SMK1*, *NDT80*(–78) and *SPR3* MSE sites (Figure 2). Expression of *LacZ* from the reporter plasmid containing the *SMK1* MSE in the *sum1* and *hst1* null strains is 170- and 34-fold higher, respectively, than in the wild-type strain. The *LacZ* reporters containing the *NDT80*(–78) and *SPR3* MSEs also show significant derepression in the *sum1* and *hst1* null mutants. In contrast, the *sum1* and *hst1* mutations have no effect on transcriptional repression mediated by the α2-Mcm1 or Ume6 repressor-binding sites (data not shown). These observations indicate that both Sum1 and Hst1 are

specifically required for MSE-mediated transcriptional repression during vegetative growth.

SUM1-1 suppresses the silencing defects in a number of different mutants (Livi *et al*., 1990; Laurenson and Rine, 1991; Chi and Shore, 1996). It was possible that this mutation suppresses the defect in silencing by altering the regulation of genes containing MSEs. We have assayed repression of the *LacZ* reporter construct containing the *SMK1* MSE in a *SUM1-1* mutant and found that it has wild-type levels of repression (Figure 2). This result suggests that the effect of *SUM1-1* on silencing is not an indirect consequence of derepressing MSE-containing promoters.

We next examined the expression of a series of sporulation-specific genes in *sum1*-Δ or *hst1*-Δ mutants during vegetative growth by hybridization analysis (Figure 3A). The levels of *SMK1* and *SPR3* mRNAs are at least 50-fold higher in the *sum1* mutant than in the wild-type strain. In contrast, *SMK1* is not derepressed in the *hst1* mutant strain. *SPR3* is derepressed in the *hst1* mutant 7- to 9-fold, significantly less than the level of derepression seen in the *sum1* mutant. *NDT80* is not derepressed in either mutant despite the fact that it contains an MSE that is repressed in a Sum1- and Hst1-dependent fashion in the *LacZ* reporter assay (Figure 2). The level of *SMK1* and *SPR3* expression in a *sum1* mutant is indistinguishable from that seen in the *sum1 ndt80* double mutant (data not shown). These data show that derepression of *SMK1* or *SPR3* is not an indirect effect of derepressing the Ndt80 transcriptional activator.

The absence of derepression of *NDT80* upon deletion of *SUM1* or *HST1* does not necessarily mean that its promoter is not subject to Sum1 or Hst1 regulation. In addition to the two MSEs, the *NDT80* promoter contains elements at –158 and –296 that conform to the URS1 consensus sequence (Luche *et al*., 1990). The Ume6 protein binds to URS1 sites and functions as a repressor in vegetative cells by recruiting the Sin3–Rpd3 histone deacetylase complex to the promoter (Kadosh and Struhl, 1997, 1998a,b). To test the possibility that *NDT80* is repressed in vegetative cells through both the Sum1- and Ume6-dependent pathways, *NDT80* mRNA levels were assayed in *ume6* and *sum1 ume6* mutant strains (Figure 3C). While *NDT80* mRNA is not expressed in either *sum1* or *ume6* mutants, it is expressed in the *sum1 ume6* double mutant. This result indicates that Sum1 can regulate the expression of *NDT80* under conditions where the Ume6-dependent repression is derepressed.

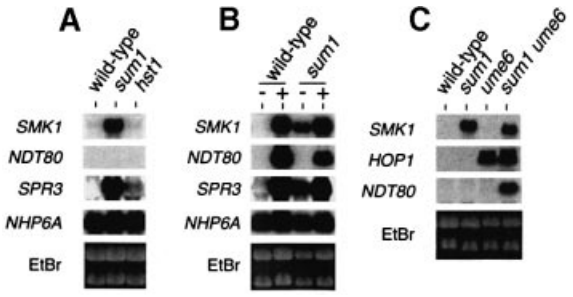

Fig. 3. Sum1 and Hst1 repress expression of middle-sporulation-specific genes. (**A**) Expression of mid-sporulation-specific genes under vegetative conditions. RNA was prepared from wild-type (W303-1A), *sum1*-Δ (JXY3) and *hst1*-Δ (JXY5) strains grown under vegetative conditions. The same blot was hybridized with radiolabeled DNA fragments specific for the coding regions of the *SMK1, NDT80, SPR3* and *NHP6A* genes. An ethidium bromide-stained gel before transfer is shown as a control for RNA loading. (**B**) Expression of mid-sporulation genes in *NDT80*-expressing strains. RNA was prepared from wild-type (W303-1A) or *sum1* (JXY3) strains grown in galactose-containing media. Strains contained (+) or lacked (–) the *GAL1-10* promoter fused to *NDT80* as indicated (Chu and Herskowitz, 1998; Chu *et al*., 1998). (**C**) Expression of *SMK1, NDT80* and the early meiotic *HOP1* gene in wild-type (W303-1A), *sum1* (JXY3), *ume6* (RSY431) and *sum1 ume6* (JXY15) strains grown under vegetative conditions.

Ectopic expression of *NDT80* has been shown to activate a relatively large number of mid-sporulation genes, and Ndt80 has also been shown to interact with MSE DNA (Chu and Herskowitz, 1998). We compared the ability of *NDT80* to activate several middle genes in wild-type and *sum1* mutant strains (Figure 3B). *NDT80* driven by the galactose-inducible *GAL1* promoter activated *SMK1* and *SPR3* expression in vegetative wild-type and in *sum1* mutant backgrounds. These results show that Sum1 is not required for MSE-dependent transcriptional activation by Ndt80 and, furthermore, demonstrate that high-level expression of *NDT80* is able to bypass SUM1-dependent repression.

Sum1 represses SMK1 but not adjacent genes

Under some conditions, the Sum1 and Hst1 proteins are involved in the transcriptional silencing of large regions of the chromosome (Klar *et al*., 1985; Lin *et al*., 1990; Livi *et al*., 1990; Laurenson and Rine, 1991; Brachmann *et al*., 1995; Chi and Shore, 1996; Derbyshire *et al*., 1996). If Sum1 and Hst1 function in the same manner as the Sir proteins to repress transcription, then other genes in the vicinity of the MSEs may also be silenced. The *NHP6A* gene is transcribed divergently from *SMK1*, and their translation initiation sites are separated by only 306 bp. Although *SMK1* is repressed by Sum1 during vegetative growth, *NHP6A* is expressed at comparable levels in wild-type, *sum1* and *hst1* strains (Figure 3A). These results suggest that unlike silencing at the telomeres, rDNA and silent mating type loci, repression mediated by Sum1 through the MSEs is gene specific, highly localized and does not spread to neighboring genes.

Overexpression of SIR2 partially suppresses defects in repression of an hst1 mutant

Overexpression of *HST1* partially suppresses the effects on silencing of a *sir2* mutant, suggesting that the two proteins have related activities (Brachmann *et al*., 1995; Derbyshire *et al*., 1996). We have performed the converse experiment and found that overexpression of *SIR2* partially suppresses the MSE-repression defect of an *hst1* mutant (Figure 4). The suppression by *SIR2* is specific for *hst1* because overexpression of *SIR2* does not alter expression of a reporter lacking an MSE and it does not suppress the defect of a *sum1* mutant (data not shown). In addition, overexpression of *SIR4* has no effect on the repression defect in an *hst1* mutant (data not shown). These data further support the idea that Sir2 and Hst1 have similar activities.

Since overexpression of *SIR2* can partially restore the repression to an *hst1* mutant, it was possible that Sir2 normally is involved in MSE-mediated repression. However, the level of the MSE-mediated repression of the

LacZ reporter promoter containing the *SMK1* MSE in a *sir2* mutant is approximately the same as in the wild-type strain and the level of repression in a *sir2 hst1* mutant is the same as in the *hst1* strain. We also found that the level of *SPR3* mRNA is comparable between *hst1* and *hst1 sir2* strains during vegetative growth (data not shown). These data suggest that while Hst1 and Sir2 are functionally related, they play non-overlapping roles in repressing mid-sporulation genes and in silencing, respectively, in wild-type cells. This finding is supported further by the observation that mutations in *sir3* and *sir4*, which affect silent mating type loci and telomere silencing, as well as mutations in *SIR2* homologs, *hst2*, *hst3* and *hst4*, do not affect MSE-mediated repression of our reporter constructs (data not shown).

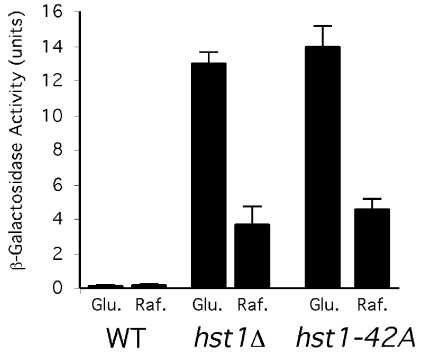

Fig. 4. Overexpression of *SIR2* partially suppresses the *hst1* mutant phenotype. The *SMK1* MSE reporter vector, pJX43, was co-transformed with a high copy p*GAL10-SIR2* plasmid, pAR14 (Holmes *et al*., 1997), into wild-type (W303a), *hst1*-Δ (JXY5) and *hst1-42A* (JXY2) strains. The level of β-galactosidase activity was measured during vegetative growth in 3% raffinose (derepressing conditions) or 2% glucose (repressing conditions). A *LacZ* reporter vector lacking the MSE expresses the same level of β-galactosidase in wild-type and mutant strains grown in glucose or *raffinose*.

Sum1 binds specifically to the SMK1 MSE

The Sum1 and Hst1 proteins do not show sequence similarity to a known DNA-binding motif. To determine whether either protein binds directly to the *SMK1* MSE, an electrophoretic mobility shift assay (EMSA) was developed to monitor binding to the site. Two slowly migrating MSE-binding activities are detectable in cell extracts from the wild-type strain (Figure 5A). The slower migrating MSE-binding complex (arrow) is competed specifically by unlabeled MSE DNA, but not by non-specific DNA or an MSE containing a mutation in the consensus sequence (lanes 2 and 3; data not shown). The MSE-specific shift is missing in extracts prepared from

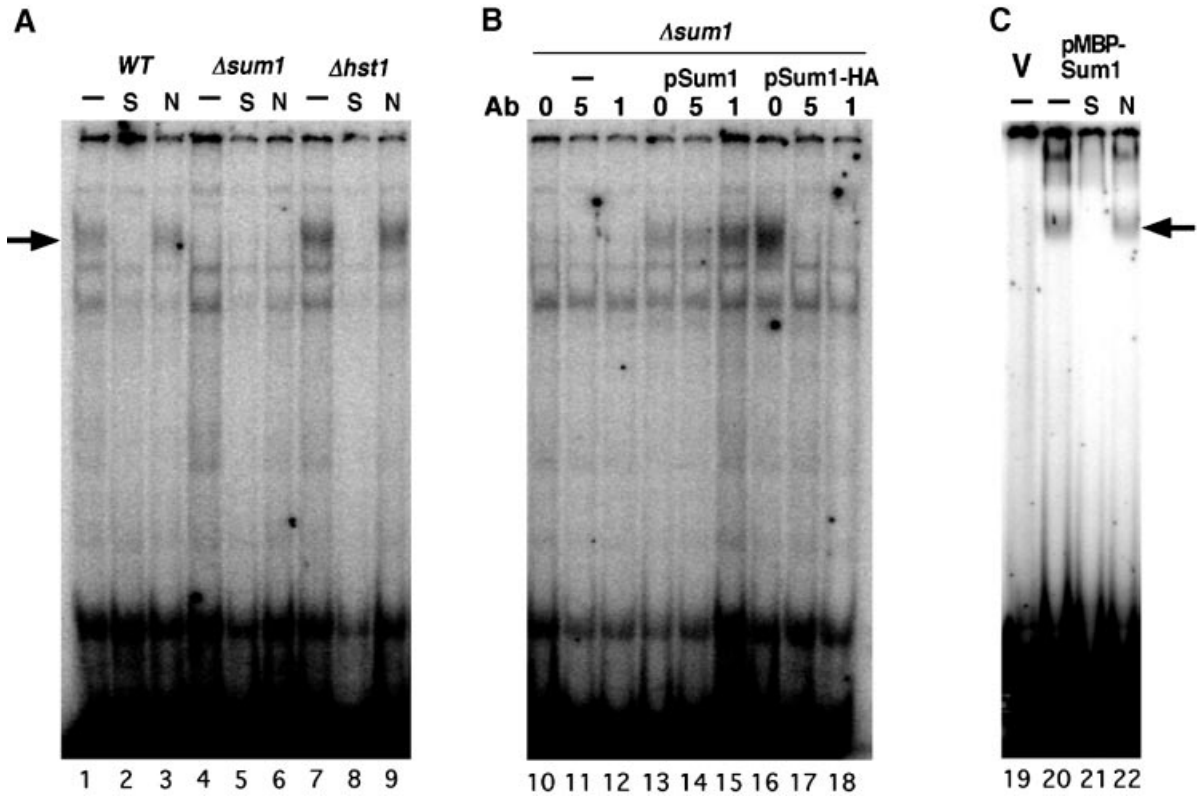

Fig. 5. Binding activity of the *SMK1* MSE. (**A**) An EMSA of a 23 bp double-stranded labeled oligonucleotide containing the *SMK1* MSE site with crude extracts from wild-type (W303-1A, lanes 1–3), *sum1* (JXY3, lanes 4–6) and *hst1* (JXY5, lanes 7–9) strains (Gailus-Durner *et al*., 1997). Binding competition experiments were performed by pre-mixing an excess of unlabeled MSE DNA (S, lanes 2, 5 and 8) or a non-specific site (N, lanes 3, 6 and 9) before the addition of the extract. No competitor (–) was added in lanes 1, 4 and 7. The minor increase in complex formation seen in the *hst1* samples is not reproducible. (**B**) An antibody supershift experiment to an HA-tagged version of Sum1. Extracts were made from JXY3 (*sum1*-Δ) transformed with either a negative control vector (pRS415, lanes 10–12), wild-type *SUM1* (pSUM1, lanes 13–15) or *SUM1* tagged with three copies of the HA epitope (pSUM1-HA, lanes 16–18) (Chi and Shore, 1996). A 1 μl aliquot of HA antibody was added to the reaction at a 1:5 dilution in lanes 11, 14 and 17, and a 1:25 dilution in lanes 12, 15 and 18. No antibody was added to lanes 10, 13 and 16. (**C**) DNA-binding activity of an MBP–Sum1 fusion to the *SMK1* MSE. Lanes 19–22 show shifts by partially purified bacterial extracts from strains containing a pMAL-C2 blank expression vector (V, lane 19) or an MBP–Sum1 expression vector (lanes 20–22). Lane 21 contains an excess of unlabeled MSE DNA and lane 22 contains a similar quantity of a non-specific site.

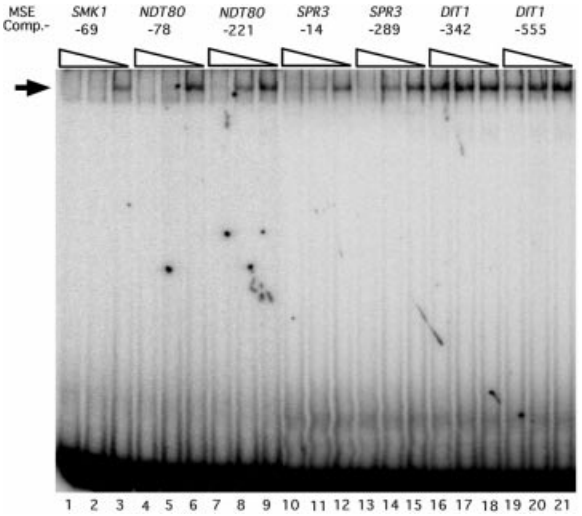

Fig. 6. Competition for Sum1 binding to different MSEs. An EMSA is shown of MBP–Sum1 partially purified from bacterial extracts binding to a labeled *SMK1* MSE in the presence of different amounts of cold competitor MSE DNA. MSEs from *SMK1*(–69) (lanes 1–3), *NDT80*(–78) (lanes 4–6), *NDT80*(–221) (lanes 7–9), *SPR3*(–14) (lanes 10–12), *SPR3*(–289) (lanes 13–15), *DIT1*(–342) (lanes 16–18) and *DIT1*(–555) (lanes 19–21) were used as cold competitors for Sum1 binding. Cold competitor was added at 333-fold (lanes 1, 4, 7, 10, 13, 16 and 19), 33-fold (lanes 2, 5, 8, 11, 14, 17 and 20) and 3-fold (lanes 3, 6, 9, 12, 15, 18 and 21) excess to labeled wild-type *SMK1* MSE.

the *sum1* null mutant but is present in *hst1* null mutant extracts (lanes 4 and 7). This result indicates that Sum1 is required for the MSE-specific shift.

To determine whether Sum1 is in the shifted complex, an extract from a *sum1* strain harboring a plasmid expressing an epitope-tagged Sum1-hemagglutinin (HA) fusion protein was incubated with HA antiserum and DNA-binding activity was monitored in an EMSA. The presence of the HA antibody inhibited the formation of the MSE-specific complex with HA-tagged Sum1 (Figure 5B, lanes 17 and 18) but not with the untagged protein (lanes 14 and 15). This result indicates that Sum1 is a component of the MSE-specific shifted complex.

To test whether Sum1 can bind to MSE DNA directly *in vitro*, we constructed an *MBP-SUM1* expression vector and purified the fusion protein from bacteria. The maltose binding protein (MBP)–Sum1 fusion protein produces a specific shift of the *SMK1* MSE, while MBP purified from extracts lacking the fusion does not (Figure 5C, lanes 19–22). These results show that Sum1 can bind to MSE DNA directly and specifically.

We have shown that MSEs from the promoters of different sporulation-specific genes vary in their ability to repress transcription (Figure 1). To determine whether the ability of these different sites to repress transcription correlates with their Sum1-binding affinities, we conducted competition EMSAs with unlabeled MSE duplex oligonucleotides (Figure 6). MSEs that function as strong repressor sites *in vivo*, such as *SMK1* and *NDT80*(–78), function as strong competitors for Sum1 binding. In contrast, the *DIT1* sites, which do not function as repressor sites *in vivo*, are unable to compete for Sum1 binding to the *SMK1* MSE. These data demonstrate that the ability of Sum1 to bind to an MSE *in vitro* correlates with the ability of the site to repress transcription *in vivo*.

Discussion

The MSE was first identified as an element required for the activation of the mid-sporulation-specific *SPS4* and *SPR3* genes (Hepworth *et al*., 1995; Ozsarac *et al*., 1997). Analysis of the genome-wide transcriptional program during sporulation (Chu *et al*., 1998) showed that sequence elements conforming to the MSE consensus proposed by Ozsarac (gNCRCAAA$^{A}/_{T}$) are found in the promoters of most middle sporulation genes. In addition, Ndt80 has been shown to bind directly to MSE DNA, and ectopic expression of *NDT80* in vegetative cells has been shown to transcriptionally activate many MSE-containing promoters (Chu and Herskowitz, 1998; Chu *et al*., 1998).

In this report, we have shown that a subclass of MSEs can function as potent repressor sites (Figure 1). Not all MSEs are strong repressor sites and, even within a single promoter with multiple MSEs, some are strong repressor sites while others are not (e.g. the *NDT80* –78 and –221 MSEs; Figure 1). Although repressing and non-repressing MSEs conform to the consensus sequence, the different regulatory activities of the sites suggest that the sequence specificity of MSE recognition is more complex than previously proposed. Differences of non-conserved positions in the core element and base pairs flanking this site must therefore be important for contributing to the specificity of Ndt80 and Sum1 binding and/or their regulatory activities.

Our results suggest that there can be two occupancy states at certain MSE sites: a Sum1-bound transcriptionally inactive state, which is associated with mitotic growth, and an Ndt80-bound transcriptionally active state, which is associated with mid-sporulation. *NDT80* is expressed slightly before most mid-sporulation-specific genes (Hepworth *et al*., 1998). Thus, it is likely that during the interval between induction (early sporulation) and meiotic chromosome segregation (mid-sporulation), the ratio of Sum1:Ndt80 binding activity decreases, leading to the derepression, as well as activation, of MSE-containing promoters. Comparison of the repression and activation properties of the different MSEs in the heterologous promoter assay (Figure 1) suggests that different MSEs have different relative affinities for Sum1 and Ndt80. We have shown that the differences in the repression *in vivo* correlate with differences in Sum1-binding affinity *in vitro*. These differences, in combination with variations over time in the ratio of the two proteins with opposing transcriptional regulatory effects, could provide a simple binary mechanism to vary both the precise timing and the magnitude of expression of a large number of target genes. Indeed, there is considerable variation in the timing of expression of genes containing MSEs during the middle stages of sporulation (Chu *et al*., 1998).

The idea that DNA-binding proteins with opposing transcriptional effects can determine developmental choices is firmly established in the life cycle of λ phage by the Cro or cI proteins, which bind to the same sites but have different regulatory activities (Ptashne, 1992). Our results raise the possibility that the regulated competition for occupancy at key MSEs by Sum1 and Ndt80 may control the irreversible commitment step of meiotic chromosome segregation and subsequent spore morphogenesis. Additional layers of complexity that could be applied to this model would include regulated changes in Sum1 or Ndt80 activities in response

Table I. Yeast strains

Name	Genotype	Source
W303-1A	*MAT**a** ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112*	L.Neigeborn
W303-1B	*MATα ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112*	L.Neigeborn
W1011-3B	*MATα ade2-1 trp1-1 can1-100 ura3-1 leu2-3,112*	L.Neigeborn
W1346-3C	*MAT**a** trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112*	L.Neigeborn
RSX2-7B	*MAT**a**/MATα trp1-hisG/trp1-hisG ura3-SK1/ura3-SK1 leu2-hisG/leu2-hisG lys2-SK1/lys2-SK1 gal80-LEU2/gal80-LEU2 IME1-14-TRP1/+ ho::LYS2/ho::LYS2*	L.Neigeborn
RSY431	*MAT**a** ade2 ade6 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3-112 ume6Δ::HIS3*	R.Strich
JXY1	isogenic to W1011-3B *sum1-50A*	this study
JXY2	isogenic to W1011-3B *hst1-42A*	this study
JXY3	isogenic to W303-1A *sum1Δ::kanMX4*	this study
JXY5	isogenic to W303-1A *hst1Δ::kanMX4*	this study
JXY15	isogenic to RSY431 *sum1Δ::kanMX4*	this study
JXY19	isogenic to W303-1A *sir2Δ::TRP1*	this study
JXY20	isogenic to W303-1A *sir2Δ::TRP1 hst1Δ::kanMX4*	this study
THC13	*MAT**a** HMLα HMRa ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 sir3Δ::HIS3*	M.Gartenberg
THC18	*MAT**a** HMLα hmraΔ::lys2Δ ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 sir4Δ::HIS3*	M.Gartenberg
YCB466	*MAT**a** hst1ΔLEU2 hst2Δ::TRP1 hst3Δ::HIS3 hst4Δ::URA3*	J.Boeke
YCB547	*MATα hst3Δ3::HIS3 hst4Δ1::URA3*	J.Boeke
JXY21	isogenic to YCB466 *ura3*	this study
JXY22	isogenic to YCB547 *ura3*	this study
JXY25	isogenic to W303-1B *sum1Δ::kanMX4 ndt80Δ::kanMX4*	this study
MPY2	isogenic to W303-1A *HIS3::pGAL1-10-NDT80*	this study
JRY2456	*MATα ade2-101 his3Δ200 leu2 lys1 lys2-801 SUM1-1 ura3-52*	J.Rine

to nutritional or perhaps meiotic checkpoint signals that regulate progression of sporulation.

Chu and Herskowitz (1998) previously demonstrated that Ndt80 can activate its own expression, presumably through the MSEs found in its promoter. It has been proposed that Ndt80 may be expressed at a low level early during sporulation (e.g. by a URS1-dependent mechanism), which in turn could lead to high levels of Ndt80 during mid-sporulation through a positive (MSE-dependent) feedback loop (Chu and Herskowitz, 1998; Hepworth *et al*., 1998). Our results show that *NDT80* is derepressed in a double *ume6 sum1* mutant but not in a *ume6* or a *sum1* single mutant background. These observations show that *NDT80* is regulated through both the Ume6 and Sum1 pathways and are consistent with the transcriptional cascade model for *NDT80* induction.

The sequence similarity of the Hst1 and Sir2 proteins, the genetic interactions between *SUM1* and *SIR* genes, and our demonstration that *sum1* and *hst1* mutants are defective in MSE repression suggest that mechanisms used to repress mid-sporulation-specific genes in vegetative cells may be partially shared with those used for silencing. The deletion of *SUM1* leads to more robust derepression than the deletion of *HST1* for all MSEs tested in the *LacZ* reporter assay. In addition, *SMK1* and *SPR3* are expressed at high levels in vegetative *sum1* mutants, while in *hst1* mutant backgrounds *SMK1* is not expressed and *SPR3* is expressed at only moderate levels. Furthermore, the level of derepression of the *SMK1* and *SPR3* MSEs in the *LacZ* expression assay, as well as expression of these mRNAs in a *sum1 hst1* mutant strain, is comparable with that seen in the single *sum1* mutant (data not shown). These data show that Sum1 is able at least partially to repress transcription in the absence of Hst1. Therefore, while Sum1 may function to recruit Hst1 to MSE-containing promoters, it also has an intrinsic ability to repress expression in the absence of Hst1.

SUM1 was first identified as a dominant mutant, *SUM1-1*, that suppresses the effects of *sir2*, as well as other mutants, in silencing of the silent mating type loci. We have shown that the *SUM1-1* mutant retains wild-type levels of MSE-mediated repression. Therefore, it is likely that the *SUM1-1* mutation does not alter its normal DNA-binding specificity. Our data suggest that Sum1 and Hst1 may interact, and raise the possibility that the *SUM1-1* mutation causes alterations in binding affinity and/or specificity towards other Sir proteins. If so, these gain-of-activity interactions might play a role in recruiting Hst1, which is known to suppress *sir2* defects partially when overexpressed, to silencing complexes.

Materials and methods

Plasmids

Oligonucleotides containing MSEs from the indicated promoters were cloned into the *Xho*I site of the *HOP1-LacZ* reporter vector, pAV124, as described previously (Pierce *et al*., 1998). The sequences of the MSEs are shown in Figure 1, and each of these sites is flanked with TCGA ends to facilitate its cloning into a *Xho*I site of the vector.

The MBP–Sum1 fusion was constructed by cloning a PCR-generated fragment containing the entire *SUM1* open reading frame (ORF) between the *Bam*HI and *Pst*I sites of pMAL-C2 (New England Biolabs). The PCR fragment encodes amino acid residues 2–1062 of Sum1 and includes 28 bp 3′ to the termination codon. Clones were screened by restriction analysis and confirmed by their ability to express an MBP fusion protein as monitored by Western analysis.

Isolation and analysis of mutants defective in MSE repression

A list of the strains used in this study is shown in Table I. Strains W1011-3B and W1346-3C, derivatives of W303 harboring pJX43, a *hop1-LacZ* reporter vector with the *SMK1* MSE site (Pierce *et al*., 1998), were mutagenized with 3% EMS to 20% survival, and 2.4×10^4 colonies were screened for *LacZ* expression by X-gal filter assays. The mutants were rescreened for repression and sorted into complementation groups by pairwise mating between the mutants and screening for the inability to repress the *LacZ* reporter promoter. Genes that complement the mutations were cloned by co-transforming a *CEN*/*LEU2* plasmid *S.cerevisiae* genomic library (ATCC 77163) with pJX43 into a mutant from each complementation group (JXY1 and JXY2) and screening the transformants for white colonies in X-gal filter assays. The complementing library plasmids were purified, reassayed for repression and the end points of each insert were determined by sequencing. Four and seven independent clones were

isolated which complement the *sum1-50A* and *hst1-42A* mutants, respectively. All of the clones contained a full-length copy of their respective genes. Subclones of the individual ORFs contained on the complementation plasmids were constructed in pRS415, transformed back into the mutant strains and assayed for complementation of repression by X-gal filter assay.

To construct *sum1*-Δ and *hst1*-Δ null mutants, PCR-generated DNA fragments containing the *KanMX4* gene (Wach, 1996) with short flanking regions containing homology to the target gene on both ends were transformed into a W303 diploid strain and transformants were selected by plating on YEPD plates containing G418 (200 mg/l) (Gibco-BRL). Colonies were rescreened for growth on G418 plates and the integration was verified by PCR using primer pairs that hybridize within and outside of the transformed fragment. Haploid *sum1*-Δ (JXY3) and *hst1*-Δ (JXY5) null mutant strains were obtained by dissection of sporulated heterozygous diploid strains. JXY3 and JXY5 were mated with JXY1 and JXY2, respectively, and sporulated. The diploid strains and dissected spores all show derepression of the *lacZ* reporter containing the *SMK1* MSE, indicating that strains JXY1 and JXY2 contain mutations in the *SUM1* and *HST1* genes, respectively.

Liquid β-galactosidase assay

β-galactosidase activities of the *HOP1-LacZ* constructs containing different MSE sites were determined in W303A for repression activity in vegetative cells and RSX2-7B for activation during mid-sporulation. To measure the suppression of the *hst1* mutant by overexpression of *SIR2*, strain JXY5 (*hst1*-Δ) that was co-transformed with pAR14, a 2μ p*GAL10-SIR2* vector (Holmes *et al*., 1997), and pJX43 were grown in SD –ura –leu medium to saturation, diluted 1:25 into SRaf –ura –leu after being washed once in water, grown overnight and then assayed for β-galactosidase activity. All β-galactosidase activity assays in liquid were performed as described previously (Gailus-Durner *et al*., 1997).

Electrophoretic mobility shift assays

Yeast cell extracts were prepared from wild-type (W303-1A), *sum1*-Δ (JXY3) and *hst1*-Δ (JXY5) strains as described previously (Gailus-Durner *et al*., 1997). Oligonucleotides containing the *SMK1* MSE were end-labeled with [γ-^{32}P]ATP using polynucleotide kinase and purified by Nensorb columns (NEN) according to the manufacturer. The oligonucleotides were made double-stranded by mixing with a 3-fold excess of the matching strand, incubating at 90°C for 20 min and slowly cooling to 25°C overnight in a water bath. Binding reactions for the various protein preparations were carried out in 10 mM Tris–HCl pH 7.5, 40 mM NaCl, 4 mM $MgCl_2$, 6% (w/v) glycerol, 10 μg/ml of sonicated salmon sperm DNA and ^{32}P-labeled oligonucleotide pairs (10 000 c.p.m.) in a total volume of 20 μl at room temperature for 20 min. Competition experiments were performed by premixing an excess of unlabeled MSE DNA or a non-specific site before the addition of the extract. Protein dilutions were made in 20 mM Tris–HCl pH 8, 50 mM NaCl, 1 mM EDTA, 1 mg/ml bovine serum albumin (BSA), 5 mM β-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (PMSF). In the antibody supershift experiment, extracts were made from strain JXY3 transformed with either a negative control vector (pRS415), wild-type *SUM1 (*pSUM1) or *SUM1* tagged with three copies of the HA epitope (pSUM1-HA) (Chi and Shore, 1996). A 1 μl aliquot of antibody to the HA epitope (Boehringer Mannheim) was added to the binding reaction at a 1:5 or 1:25 dilution and incubated for 30 min. Samples were analyzed on a 6% polyacrylamide gel (run in 0.5× TBE buffer for 60 min at 200 V). Gels were dried after electrophoresis, exposed to a phosphor screen and scanned on a Model 425E Molecular Dynamics phosphorimager.

Acknowledgements

We are deeply indebted to Lenore Neigeborn, Randy Strich, Jasper Rine, David Shore, Marc Gartenberg and Jef Boeke for providing strains, plasmids and helpful advice for the genetic screens and analysis. The authors would like to thank Randy Strich and David Norris for comments on the manuscript. This work is supported by Busch Postdoctoral and Predoctoral Fellowships to V.G.D. and M.P., respectively, a grant from the National Science Foundation (E.W.) and grants from the American Cancer Society (E.W. and A.K.V.).

References

Brachmann,C.B., Sherman,J.M., Devine,S.E., Cameron,E.E., Pillus,L. and Boeke,J.D. (1995) The *SIR2* gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression and chromosome stability. *Genes Dev.*, **9**, 2888–2902.

Chi,M.H. and Shore,D. (1996) *SUM1-1*, a dominant suppressor of *SIR* mutations in *Saccharomyces cerevisiae*, increases transcriptional silencing at telomeres and HM mating-type loci and decreases chromosome stability. *Mol. Cell. Biol.*, **16**, 4281–4294.

Chu,S. and Herskowitz,I. (1998) Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. *Mol. Cell*, **1**, 685–696.

Chu,S., DeRisi,J., Eisen,M., Mulholland,J., Botstein,D., Brown,P.O. and Herskowitz,I. (1998) The transcriptional program of sporulation in budding yeast. *Science*, **282**, 699–705.

Derbyshire,M.K., Weinstock,K.G. and Strathern,J.N. (1996) *HST1*, a new member of the *SIR2* family of genes. *Yeast*, **12**, 631–640.

Gailus-Durner,V., Chintamaneni,C., Wilson,R., Brill,S.J. and Vershon,A.K. (1997) Analysis of a meiosis-specific URS1 site: sequence requirements and involvement of replication protein A. *Mol. Cell. Biol.*, **17**, 3536–3546.

Hepworth,S.R., Ebisuzaki,L.K. and Segall,J. (1995) A 15-base-pair element activates the *SPS4* gene midway through sporulation in *Saccharomyces cerevisiae. Mol. Cell. Biol.*, **15**, 3934–3944.

Hepworth,S.R., Friesen,H. and Segall,J. (1998) *NDT80* and the meiotic recombination checkpoint regulate expression of middle sporulation-specific genes in *Saccharomyces cerevisiae. Mol. Cell. Biol.*, **18**, 5750–5761.

Holmes,S.G., Rose,A.B., Steuerle,K., Saez,E., Sayegh,S., Lee,Y.M. and Broach,J.R. (1997) Hyperactivation of the silencing proteins, Sir2p and Sir3p, causes chromosome loss. *Genetics*, **145**, 605–614.

Kadosh,D. and Struhl,K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell*, **89**, 365–371.

Kadosh,D. and Struhl,K. (1998a) Histone deacetylase activity of Rpd3 is important for transcriptional repression *in vivo. Genes Dev.*, **12**, 797–805.

Kadosh,D. and Struhl,K. (1998b) Targeted recruitment of the Sin3–Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin *in vivo. Mol. Cell. Biol.*, **18**, 5121–5127.

Klar,A.J., Kakar,S.N., Ivy,J.M., Hicks,J.B., Livi,G.P. and Miglio,L.M. (1985) *SUM1*, an apparent positive regulator of the cryptic mating-type loci in *Saccharomyces cerevisiae. Genetics*, **111**, 745–758.

Krisak,L., Strich,R., Winters,R.S., Hall,J.P., Mallory,M.J., Kreitzer,D., Tuan,R.S. and Winter,E. (1994) *SMK1*, a developmentally regulated MAP kinase, is required for spore wall assembly in *Saccharomyces cerevisiae. Genes Dev.*, **8**, 2151–2161.

Kupiec,M., Byers,B., Esposito,R. and Mitchell,A. (1997) Meiosis and sporulation in *Saccharomyces cerevisiae.* In Pringle,J., Broach,J. and Jones,E. (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 3, pp. 889–1036.

Laurenson,P. and Rine,J. (1991) *SUM1-1*: a suppressor of silencing defects in *Saccharomyces cerevisiae. Genetics*, **129**, 685–696.

Lin,C.I., Livi,G.P., Ivy,J.M. and Klar,A.J. (1990) Extragenic suppressors of *mar2 (sir3*) mutations in *Saccharomyces cerevisiae. Genetics*, **125**, 321–331.

Livi,G.P., Hicks,J.B. and Klar,A.J. (1990) The *sum1-1* mutation affects silent mating-type gene transcription in *Saccharomyces cerevisiae. Mol. Cell. Biol.*, **10**, 409–412.

Luche,R.M., Sumrada,R. and Cooper,T.G. (1990) A *cis*-acting element present in multiple genes serves as a repressor protein binding site for the yeast *CAR1* gene. *Mol. Cell. Biol.*, **10**, 3884–3895.

Ozsarac,N., Straffon,M.J., Dalton,H.E. and Dawes,I.W. (1997) Regulation of gene expression during meiosis in *Saccharomyces cerevisiae*: *SPR3* is controlled by both *ABFI* and a new sporulation control element. *Mol. Cell. Biol.*, **17**, 1152–1159.

Pierce,M., Wagner,M., Xie,J., Gailus-Durner,V., Six,J., Vershon,A.K. and Winter,E. (1998) Transcriptional regulation of the *SMK1* mitogen-activated protein kinase gene during meiotic development in *Saccharomyces cerevisiae. Mol. Cell. Biol.*, **18**, 5970–5980.

Ptashne,M. (1992) *A Genetic Switch.* Cell Press, Cambridge, MA.

Wach,A. (1996) PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *S.cerevisiae. Yeast*, **12**, 259–265.

Wagner,M., Pierce,M. and Winter,E. (1997) The CDK-activating kinase *CAK1* can dosage suppress sporulation defects of *smk1* MAP kinase mutants and is required for spore wall morphogenesis in *Saccharomyces cerevisiae. EMBO J.*, **16**, 1305–1317.

Wagner,M., Briza,P., Pierce,M. and Winter,E. (1999) Distinct steps in yeast spore morphogenesis require distinct *SMK1* MAP kinase thresholds. *Genetics*, **151**, 1327–1340.

Received June 6, 1999; revised and accepted September 22, 1999