Molecular Biology of the Cell
Vol. 10, 3689–3703, November 1999

Genetic and Biochemical Characterization of the Yeast Spo12 Protein

Megan E. Grether* and Ira Herskowitz†

Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143-0448

Submitted January 11, 1999; Accepted September 1, 1999
Monitoring Editor: Thomas D. Fox

We have performed a genetic and biochemical analysis of the *SPO12* gene, which regulates meiotic nuclear divisions in budding yeast. When sporulated, *spo12* mutants undergo a single meiotic nuclear division most closely resembling meiosis II. We observed that Spo12 protein is localized to the nucleus during both meiotic divisions and that Clb1-Cdc28, Clb3-Cdc28, Clb4-Cdc28, and Clb5-Cdc28 kinase activities during meiosis were not affected by a *spo12* mutation. Using two-hybrid analysis, we identified several genes, three of which are meiotically induced, that may code for proteins that interact with Spo12p. We also observed that two genes, *BNS1* (*Bypasses Need for Spo12p*), which has homology to *SPO12*, and *SPO13*, whose mutant phenotype is like that of *spo12*, can partially suppress the meiotic defect of *spo12* mutants when overexpressed. We found that Spo12p is also localized to the nucleus in vegetative cells and that its level peaks during G2/M. We observed that a *spo12* mutation is synthetically lethal in vegetative cells with a mutation in *HCT1*, a gene necessary for cells to exit mitosis, suggesting that Spo12p may have a role in exit from mitosis.

INTRODUCTION

Meiosis is required for the production of haploid gametes from diploid cells. A unique feature of meiosis is that two rounds of chromosome segregation—meiosis I and meiosis II—follow DNA synthesis. In contrast, a single round of nuclear division follows each S phase in mitosis. Although many of the same genes are required for both mitosis and meiosis (Simchen, 1973), their activities must be modified to allow these distinct patterns of nuclear divisions to occur. The yeast gene *SPO12* regulates the meiotic nuclear divisions and may play a role in mitosis as well. Mutations in *SPO12* cause cells to undergo a single meiotic division resembling meiosis II (Klapholz and Esposito, 1980a,b). *SPO12* mRNA is meiotically induced (Malavasic and Elder, 1990), peaking at the time of the meiotic nuclear divisions (Chu *et al.*, 1998). *SPO12* encodes a small protein of ~20 kDa (Malavasic and Elder, 1990) that is novel and has no homologies to proteins of known function.

SPO12 mRNA is also present in vegetative cells (Parkes and Johnston, 1992). During vegetative growth, *SPO12* message is cell cycle regulated, peaking in M phase (Parkes and Johnston, 1992; Cho *et al.*, 1998; Spellman *et al.*, 1998). Although *SPO12* is expressed in vegetative cells, deletion of *SPO12* causes only a mild vegetative phenotype: mutants exhibit a slight G2/M delay (Parkes and Johnston, 1992). *SPO12*, however, does exhibit a number of intriguing genetic interactions with genes required for exit from mitosis. For example, deletion of *SPO12* is synthetically lethal with deletion of *DBF2* (Parkes and Johnston, 1992), which codes for a protein kinase believed to be required for mitotic exit (Toyn and Johnston, 1994). Based on genetic arguments, Toyn and Johnston (1993) hypothesized that Spo12p may be a regulatory subunit for the protein kinases Dbf2 and its relative Dbf20. *SPO12* has also been isolated as a high-copy suppressor of a number of mutations affecting vegetative growth, e.g., *cdc15*, *dbf2-2*, *cdc5-1*, and *tem1-3* (Parkes and Johnston, 1992; Shirayama *et al.*, 1996; Jaspersen *et al.*, 1998). All of these mutant strains arrest late in mitosis, with high Clb-Cdc28 kinase activity. These observations suggest that *SPO12* can facilitate exit from mitosis when overexpressed, perhaps by antagonizing Clb-Cdc28 kinase activity. How *SPO12* functions during mitosis and meiosis is not known. In this article, we describe studies on the level and localization of Spo12p during mitosis and meiosis, two-hybrid partners of Spo12p, high-copy suppression of *spo12* meiotic defects, mutant interactions, and the effects of *SPO12* on Clb-Cdc28 kinase activity during meiosis.

MATERIALS AND METHODS

Media and Growth Conditions

Yeast were grown in YEPD (1% Bacto-yeast extract, 2% Bactopeptone, 2% dextrose) (complete) or minimal (SD; 10.67% yeast nitrogen base without amino acids, 2% dextrose, 1× amino acids)

* Present adress: Incyte Pharmaceuticals, 3160 Porter Drive, Palo Alto, CA 94304.
† Corresponding author. E-mail address: ira@cgl.ucsf.edu.

Table 1. Strain list

Strain number	Genotype	Background	Source
YMG303	**a**/α *SPO12::SPO12(Myc)$_9$-URA3/+*	W303	This study
YMG304	**a**/α *SPO13::SPO13(Myc)$_3$-URA3/+*	W303	This study
YMG548	**a**/α *SPO12(HA)$_2$/SPO12(HA)$_2$*	SK1	This study
YMG530	**a** *SPO12(HA)$_2$*	SK1	This study
EGY48	**a** *ura3 his3 trp1 LexAOp::LEU2*	EGY48	R. Brent
YMG270	**a**/α *spo12::hisG-URA3-hisG/spo12::hisG-URA3-hisG*	W303	This study
YMG294	**a**/α *spo13::hisG-URA3-hisG/spo13::hisG-URA3-hisG*	W303	This study
YMG537	**a** *spo12::hisG-URA3-hisG*	W303	This study
YMG43	α *spo12::hisG-URA3-hisG*	W303	This study
YMG423	**a** *sic1-1::HIS3*	W303	W. Seufert
YMG422	α *sic1-1::HIS3*	W303	W. Seufert
YMG424	**a** *hct1-1::LEU2*	W303	W. Seufert
YMG536	**a** *cdc15-2*	W303	Herskowitz
YMG484	α *cdc15-2*	W303	Herskowitz
YMG485	α *cdc15-2 sic1-1::HIS3*	W303	This study
YMG535	**a** *cdc15-2 spo12::hisG-URA3-hisG*	W303	This study
YMG654	**a**/α *CLB1(HA)$_2$/Clb1(HA)$_2$*	SK1	This study
YMG655	**a**/α *CLB1(HA)$_2$/CLB1(HA)$_2$ spo12::hisG-URA3-hisG/spo12::hisG-URA3-hisG*	SK1	This study
YMG624	**a**/α *CLB3(HA)/CLB3(HA)*	SK1	This study
YMG659	**a**/α *CLB3(HA)/CLB3(HA) spo12::hisG-URA3-hisG/spo12::hisG-URA3-hisG*	SK1	This study
YMG522	**a**/α *CLB4(HA)$_2$/CLB4(HA)$_2$*	SK1	This study
YMG632	**a**/α *CLB4(HA)$_2$/CLB4(HA)$_2$ spo12::hisG-URA3-hisG/spo12::hisG-URA3-hisG*	SK1	This study
YMG998	a/α *CLB5(HA)$_2$/CLB5(HA)$_2$*	SK1	D. Stuart
YMG999	a/α *CLB5(HA)$_2$/CLB5(HA)$_2$ spo12::hisG-URA3-hisG/spo12::hisG-URA3-hisG*	SK1	This study
YMG526	**a** *SIC1(HA)$_2$*	SK1	This study
YMG550	**a**/α *SIC1(HA)$_2$*	SK1	This study

medium, as described by Hicks and Herskowitz (1976). Cells were sporulated in liquid sporulation medium (0.3% potassium acetate and 0.02% raffinose; Kassir and Simchen, 1991) or on sporulation plates (1% potassium, 25 μg/ml zinc acetate, 2% washed agar, and complete amino acids as for SD). Sporulating cultures were synchronized according to the standard regimen described by Padmore *et al.* (1991) with the following modifications. To identify a clone of a particular strain with high sporulation efficiency, four colonies from the strain were grown in liquid YEPD and patched onto a sporulation plate. The sporulation efficiency of each patch was assessed microscopically after ~24 h. Overnight cultures corresponding to the most efficient sporulators were used to inoculate 200 ml of YPA (1% potassium acetate, 2% bactopeptone, 1% yeast extract) cultures (1:100 dilution). After ~15 h of growth in YPA, cells were collected by filtration, washed twice in 200 ml of double-distilled water, and resuspended in 200 ml of sporulation medium. Time zero in each time course is defined as the time of resuspension in sporulation medium.

Genetic and Molecular Biological Methods

Yeast genetic methods were used as described by Rose *et al.* (1990). DNA manipulations were performed as described by Sambrook *et al.* (1989)

Strains and Plasmids

Yeast strains used in this study are described in Table 1. pGal-SPO12, pGal-SIC1 (Jaspersen *et al.*, 1998), pGPD-SPO12, and pGPD-SIC1 were all courtesy of S. Jaspersen (University of California, San Francisco).

Epitope Tagging of Spo12, Spo13, Sic1, and Clb4

Starting plasmids for each construct were the following: pRS306-SPO12, a 0.8-kilobase (kb) *Eco*RI fragment (from L. Johnston, National Institute for Medical Research, London, United Kingdom) cloned into pRS306; pRS306-SPO12(long), a 1.7-kb *Pvu*II fragment (from S. Jaspersen) cloned into pRS306; pRS306-SPO13, a 2.6-kb *Bam*HI/*Nar*I fragment derived from BE806 (from R. Esposito, University of Chicago, Chicago, IL) cloned into pRS306; pRS306-SIC1, a 2.1-kb *Eco*RI/*Bgl*II genomic fragment (from S. Jaspersen) cloned into pRS306; and pRS306-CLB4, a 2.7-kb fragment cloned by PCR amplification with the use of oligonucleotides OMG66 (5′GAAAGCGGCCGCACCGGAATGACCTGGAACCG) and OMG67 (5′GAAAGAATTCGGATACCCAGCATATCAGG). This fragment was cloned into pRS306 with the use of the *Not*I and *Eco*RI sites introduced by PCR amplification. For all of the plasmids listed above, site-directed mutagenesis was used to introduce an in-frame *Bam*HI (*SPO13*, *SIC1*, and *CLB4*) or *Bgl*II (*SPO12*) site. For N-terminal epitope tagging, the new site was introduced just downstream of the translational start. For C-terminal tagging of reading frames, the site was introduced immediately upstream of the translational stop codon. A *Bam*HI fragment containing two copies of an 11-amino acid HA epitope (YPYDVPDYSAL; Wilson *et al.*, 1984) or a multimerized Myc epitope (EQKLISEEDLN; Evan *et al.*, 1985) was inserted into the introduced site.

Construction of CLB3HA

Starting with a pGal-CLB3HA clone constructed by S. Jaspersen, a *Bam*HI/*Xba*I fragment containing the *CLB3HA* reading frame was cloned into pRS306. PCR was used to amplify a 1-kb 5′ flanking sequence (with the use of oligonucleotides OMG60 [5′CCGGAAATTCACCACGTTTGACAC] and OMG61 [5′CCGGGGATCCAGTCCTGTGTTTAAG]) and a 1-kb 3′ flanking sequence (with the use of oligonucleotides OMG62 [5′ CCGGTCTAGAAAAGCCTCAGCTCGAGACAT] and OMG63 [5′GAAAGCGGCCGCGAACTAATCGTCCTGTTACC]). These fragments were sequentially cloned upstream (5′ flanking sequence) and downstream (3′ flanking sequence) of the ORF in pRS306 with the use of the restriction sites

introduced by PCR amplification. Finally, a 2.2-kb *Eco*RI/*Bgl*II fragment derived from genomic clone A5 (Fitch *et al.*, 1992) was used to substitute for the equivalent fragment in the CLB3HA genomic clone; thus, only sequences 3′ to the HA epitope tag were derived from PCR-amplified DNA.

Construction of CLB1HA in pRS306

A 2.5-kb fragment including the *CLB1HA* gene was PCR amplified with the use of oligonucleotides OMG55 (5′GAAAGAGCTCGATTTCCTCATTCGTCTTCC) and OMG56 (5′GAAAGTCGACGTCGAAGGAATTAGGAAGGC) from a strain containing *CLB1HA* (from M. Tyers, Mount Sinai Hospital, Toronto, Ontario, Canada). The resulting fragment was then cloned into pRS306 with the use of *Spe*I and *Sac*I sites introduced by PCR amplification.

Disruption of SPO12

Starting with the *spo12::TRP1* construct of Parkes and Johnston (1992; courtesy of L. Johnston), an *Xba*I/*Bam*HI fragment containing the *hisG-URA3-hisG* cassette (Alani *et al.*, 1987) was substituted for the *Xba*I/*Bam*HI fragment of *spo12::TRP1*, which contained most of the *TRP1* sequence, yielding *spo12::hisG-URA3-hisG*.

Disruption of BNS1

A 1.5-kb genomic fragment containing the *BNS1* ORF and flanking sequences was PCR amplified from genomic DNA with the use of oligonucleotides OMG23 (5′CCGGGAATTCGTGCTTTAGGGCTTGGTGCC) and OMG24 (5′CCGGACTAGTTCGCCAGAGTTTACGAACGC) and cloned into pBluescript KS$^+$ with the use of *Eco*RI and *Spe*I sites introduced in the oligonucleotides. Oligonucleotides OMG25 (5′GGCCGGATCCCGCAAATCTGTTACTTTTTTTTAGCGC) and OMG26 (5′GGCCGGATCCTTTAACGGATGATGATCATAAATTGGC) were then used to amplify sequences 5′ and 3′ to the ORF as well as the pBluescript backbone. This fragment was then cut with *Bam*HI (introduced in OMG25 and OMG26) and religated, resulting in a circularized plasmid in which a *Bam*HI site was present in place of the *BNS1* ORF. The resulting plasmid was then cut with *Bam*HI, and the *Bam*HI/*Bgl*II fragment of pNKY51 (Alani *et al.*, 1987) containing the *hisG-URA3-hisG* cassette was inserted, resulting in the *bns1::hisG-URA3-hisG* construct.

Construction of pLexA-SPO12

A pRS306-SPO12 clone containing a *Bgl*II site introduced immediately downstream of the translational start (see above) was cut with *Asp*718 (filled in with the Klenow fragment of DNA polymerase) and *Bgl*II and cloned into pEG203 cut with *Xho*I (filled in with the Klenow fragment of DNA polymerase) and *Bam*HI to yield pLexA-SPO12.

Immunofluorescence and DAPI Staining

Cells were processed for immunofluorescence as described by Sil and Herskowitz (1996). DAPI staining of sporulating cells was carried out by fixing cells in 70% ethanol or formaldehyde, rinsing once in 1× PBS, incubating for 12 min in 1 μg/ml DAPI, washing once in 1× PBS, and spotting on a slide for microscopic examination.

Cell Extracts and Immunoblot Analysis

Cell extracts were prepared for immunoblot analysis essentially as described by Sanders and Herskowitz (1996) with the following modifications. Samples were fractionated with 12.5% SDS-polyacrylamide gels and dry blotted with the use of a dry blot apparatus (E&K Scientific Products, Saratoga, CA) according to the manufacturer's instructions. Filters were blocked for 1–16 h in TBS containing 0.1% Triton X-100 and 2% nonfat dry milk and decorated with a 1:1000 dilution of HA11 or 12CA5 anti-HA antibodies (Babco, Richmond, CA). A 1:2000 dilution of goat anti-mouse immunoglobulin G heavy and light chain coupled to HRP (Bio-Rad, Richmond, CA) was used as the secondary antibody. Spo12-HA protein was visualized with the use of the Amersham (Arlington Heights, IL) ECL protein detection kit.

Synchronization of Cells with α-Factor

A total of 200 ml of **a** *SPO12-HA* cells (YMG530) were grown to OD_{600} = 0.3 at 30°C and arrested with 20 μg/ml α-factor (Bio-Synthesis, Lewisville, TX) for 3–3.5 h. Cells were collected by filtration and washed twice with 200 ml of 30°C YEPD. Cells were resuspended in 200 ml of 30°C YEPD and allowed to reenter the cell cycle. Samples (10 ml) were taken every 10 min. One milliliter was fixed for microscopic examination of budding index, and the remainder was processed for immunoblot analysis.

Two-Hybrid Analysis

Two-hybrid analysis was performed as described by Gyuris *et al.* (1993). Yeast strain EGY48 containing the reporter plasmid pSH18-34 was cotransformed with pLexA-SPO12 and a library of *Saccharomyces cerevisiae* genomic plasmids in a pJG4-5 vector containing transcriptional activation domain fusions (courtesy of R. Brent, Massachusetts General Hospital, Boston, MA). Of 385,000 independent transformants screened, 886 colonies were identified that allowed growth on SD-LEU as a result of the activation of the LexAop::LEU2 reporter construct. These were colony purified and tested for the ability to activate Gal-inducible transcription of two reporter constructs (LexAop::LEU2 and pSH18-34; Gyuris *et al.*, 1993) in the presence of pLEXA-SPO12. Fourteen clones were able to do so. The specificity of the interaction of these clones with pLexA-SPO12 was tested by analyzing the activation potential of these clones when coexpressed with an unrelated LexA DNA-binding domain fusion (pRFHM1; Gyuris *et al.*, 1993). DNA sequencing of plasmids was done with the use of an Applied Biosystems (Foster City, CA) sequencing machine (Biomolecular Resource Center facility, University of California, San Francisco).

High-Copy Suppression Analysis

A library of *S. cerevisiae* genomic plasmids in the 2-μm vector YEp13 (Nasmyth and Reed, 1980) was transformed into a strain homozygous for *spo12*::hisG-URA3-hisG. Individual transformants were patched onto selective medium to select for the library plasmid and replica plated to sporulation medium. Cells from each patch of sporulating cells were microscopically examined for tetrads. Approximately 8,000 independent transformants were analyzed. Plasmids with suppressing activity were rescued from yeast and retransformed into a *spo12* deletion mutant. All five positive clones were tested for the presence of *SPO12* or *BNS1* by PCR. For the two clones that did not contain *SPO12* or *BNS1*, end sequencing was performed to map the genomic inserts. The inserts of these clones were found to overlap in a small region containing just two complete ORFs, *SPO13* and *ARD1*. *SPO12*, *BNS1*, and *SPO13* were each subcloned into pRS425 and tested for the ability to suppress *spo12* or *spo13* mutations.

Histone H1 Kinase Assays from Sporulating Cultures

Ten-milliliter aliquots were collected from 200-ml cultures of cells undergoing synchronous meiosis at 30- or 60-min intervals after resuspension in sporulation medium. One milliliter was fixed for DAPI analysis, and the remainder was processed for kinase assays as described below. Cells were collected by centrifugation, washed once with water, and frozen in liquid nitrogen until processing. Pellets were then allowed to thaw on ice and were resuspended in 500 μl of IP buffer (20 mM Tris, pH 7.4, 100 mM NaCl, 50 mM NaF,

50 mM β-glycerophosphate, 5 mM EDTA, 0.2% Triton X-100, 1 mM PMSF, 2 μg/ml aprotinin, 2 mM benzamidine, 0.1 mM Na_3VO_4). Cells were lysed by bead beating twice for 1 min in a Biospec Products (Bartlesville, OK) mini bead beater according to the manufacturer's instructions. Supernatant was collected and centrifuged for 10 min at 4°C. Supernatant was then transferred to a fresh tube, and protein concentration was determined by the Bradford assay (Bio-Rad). ClbHA-Cdc28p kinase complexes were immunoprecipitated from 500 μg of total protein per sample with 1 μl of 12CA5 antibody (Babco) and 40 μl of a 1:1 slurry of protein A–CL4B Sepharose (Pharmacia, Piscataway, NJ) equilibrated in IP buffer by rocking for 1–3 h at 4°C. Immunoprecipitates were collected by centrifugation and washed three times in IP buffer. Immunoprecipitates were then washed once in 50 mM HEPES, pH 7.4, 1 mM DTT. Excess liquid was removed, and immunoprecipitates were resuspended in 20 μl of kinase assay reaction mixture (50 mM HEPES, pH 7.4, 2 mM $MgCl_2$, 100 μM ATP, 1 mM DTT, 5 μg of histone HI [Fluka Chemical, Buchs, Switzerland], 2.5 μCi [^{32}P]γATP) and incubated at room temperature for 20 min. Reactions were stopped with 10 μl of 4× sample buffer. Samples were boiled for 10 min and fractionated on a 12.5% SDS-polyacrylamide gel. Gels were fixed for 20 min in destain (25% methanol, 7.5% glacial acetic acid), dried, and exposed for ~16 h.

RESULTS

Localization and Levels of Spo12p during Meiosis

To characterize Spo12p during meiosis, we constructed both Myc and HA epitope-tagged versions of Spo12p, which were used for immunofluorescence and immunoblotting, respectively. Both epitope-tagged versions of Spo12p were able to complement the meiotic defect of a homozygous *spo12* deletion mutant when integrated in single copy (our unpublished results).

Immunofluorescence was used to determine the cellular localization of Spo12p during meiosis. Progress through meiosis in these experiments was monitored by DAPI staining. By immunolocalization, we saw Spo12p staining predominantly in cells that were undergoing meiosis I or meiosis II (Figure 1A). Cells with small, round nuclei that exhibited Spo12p staining were occasionally observed (data not shown). It was not possible to determine by immunofluorescence whether these were sporulating cells or vegetative cells that had not yet entered the sporulation program (Spo12p is also expressed during the mitotic cell cycle; see below). In all cases, Spo12p colocalized with DAPI staining, indicating that it is predominantly if not exclusively nuclear. By the time the meiotic nuclear divisions were complete, Spo12p was no longer detectable.

We used immunoblot analysis to determine the time course of Spo12p expression during meiosis (Figure 1B). For these experiments, we used the SKI strain background, which progresses through meiosis relatively synchronously (Padmore *et al.*, 1991). Progress through meiosis was monitored by DAPI staining. Spo12p first became detectable as cells started to enter the meiotic divisions (~5 h) and was most prominent when the cells were in meiosis I or meiosis II. Once meiosis was complete, Spo12p was no longer detectable. The lack of Spo12p at the beginning of the meiotic time course and in cells arrested in YPA medium suggests that the cells observed by immunofluorescence with small round nuclei that contained Spo12p are likely to be residual vegetative cells in the sporulating culture.

Localization and Presence of Spo12p during Vegetative Growth

Although the phenotype of *spo12* mutants is most striking in sporulating cells, *SPO12* does have a role in vegetative growth; *spo12* mutants exhibit a slight G2/M delay (Parkes and Johnston, 1992). *SPO12* mRNA is present in vegetative cells, and its message is one of many genes known to exhibit cell cycle periodicity, peaking in M phase (Cho *et al.*, 1998; Spellman *et al.*, 1998). Using the same epitope-tagged constructs as in the meiotic studies, we determined the localization of Spo12p during vegetative growth and its expression throughout the mitotic cell cycle (Figure 2). As in meiosis, Spo12p was predominantly nuclear during vegetative growth. Staining was most prominent in large-budded cells that were going through mitosis, although staining was also observed in unbudded cells and in cells with small buds.

To obtain a more precise profile of the level of Spo12p throughout the mitotic cell cycle, we used α-factor to synchronize a population of cells containing epitope-tagged Spo12p in G1. The cells were then released from α-factor arrest, and aliquots were taken every 10 min and analyzed for Spo12p by immunoblotting. Progress through the cell cycle was monitored by scoring budding index (Figure 2B). We found that Spo12p was expressed with some periodicity, peaking as cells were in G2/M and at its lowest levels during G1 and S (Figure 2C). The pattern of protein expression parallels the cell cycle regulation of *SPO12* mRNA observed by Parkes and Johnston (1992). In addition, Spo12p levels are at their highest at the same point in the cell cycle that seems to be affected in the *spo12* deletion mutant, i.e., G2/M.

Two-Hybrid Analysis

Two-hybrid analysis was used to identify proteins that may physically interact with Spo12p. We constructed an in-frame fusion of the entire *SPO12* ORF to the LexA DNA-binding domain and demonstrated that this construct (pLexA-SPO12) had no intrinsic transcriptional activity when transformed into strains containing reporter constructs regulated by LexA DNA-binding sites (not shown). To identify proteins that can interact with LexA-Spo12p to activate transcription, a library of *S. cerevisiae* genomic clones in the pJG4-5 vector (courtesy of R. Brent) was transformed into EGY48, which contained pLexA-SPO12 and appropriate reporter constructs. Because a genomic library was used in this study, in principle it should have been possible to identify both meiotic and vegetative partners of Spo12p. Of 385,000 independent transformants, 14 were able to activate transcription of two independent reporter constructs in the presence of pLexA-SPO12. These same clones were unable to induce transcription when coexpressed with an unrelated LexA DNA-binding domain fusion (pRFHM1; Gyuris *et al.*, 1993), indicating that the interaction with LexA-SPO12 was specific. All 14 clones were sequenced to determine the nature of the library activation domain fusion proteins.

Three of the 14 clones contained very short fusion protein products that did not correspond to identified ORFs in the *S. cerevisiae* genome. The remaining 11 corresponded to novel or known genes (Table 2). Ten of the clones were isolated only once in the screen; the plasmid containing the fusion to

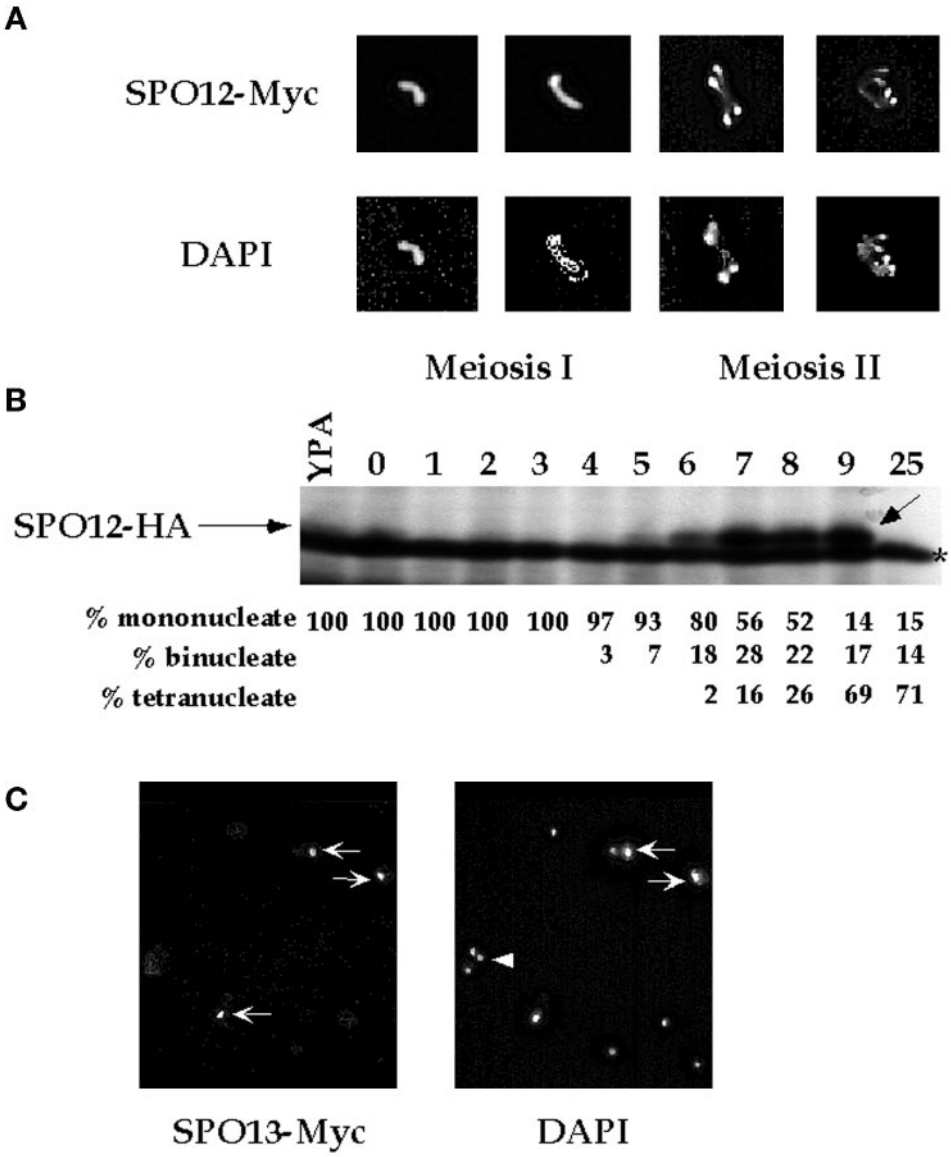

Figure 1. (A) Localization of Spo12p in meiotic cells. Cells expressing Spo12-$(Myc)_9$ were sporulated and costained with the anti-Myc mAb 9E10 and DAPI to visualize nuclei. Spo12-$(Myc)_9$ protein colocalized with DAPI staining and was visible only in cells undergoing meiotic divisions. (B) Time course of expression of Spo12p during meiosis. Cells homozygous for a gene replacement of Spo12-$(HA)_2$ (YMG548) were induced to undergo synchronous meiosis. Aliquots were removed at hourly intervals and processed for immunoblot analysis and DAPI staining. The same amount of total protein was loaded per lane. Spo12-$(HA)_2$ protein is indicated by an arrow. A background band present in all samples (including in untagged strains; data not shown) is indicated by an asterisk. The YPA sample was collected from cells grown overnight in YPA medium immediately before resuspension in sporulation medium. The 0-h time point was taken immediately after cells were resuspended in sporulation medium. Progress through meiosis was monitored by DAPI staining. % mononucleate indicates the percentage of cells that had not entered the meiotic divisions; % binucleate indicates the percentage of cells that were actively undergoing meiosis I or had completed meiosis I but had not entered meiosis II; % tetranucleate indicates the percentage of cells that were undergoing or had completed meiosis II. (C) Localization of Spo13p during meiosis. Cells expressing Spo13-$(Myc)_3$ were costained with the anti-Myc mAb 9E10 and DAPI. Arrows point to the nuclei of three mononucleate cells in which Spo13p colocalized with DAPI staining. The arrowhead shows the nucleus of a cell undergoing meiosis II in which Spo13p is not detectable.

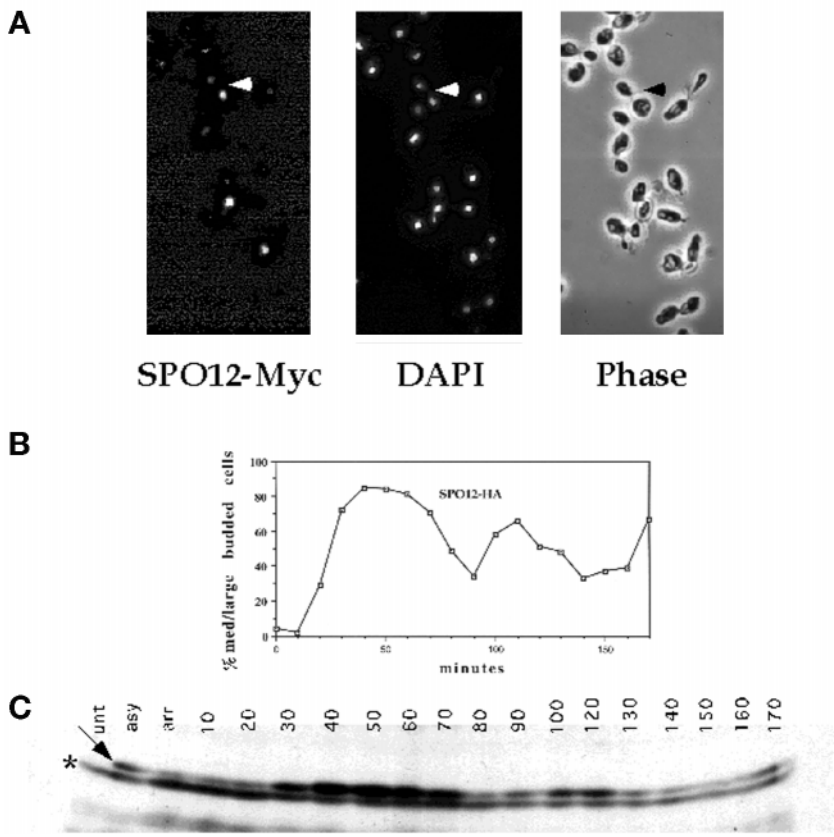

Figure 2. (A) Localization of Spo12p in vegetative cells. An asynchronous population of cells expressing Spo12-$(Myc)_9$ (strain YMG303) was costained with the anti-Myc mAb 9E10 and DAPI. The same field is shown with anti-Myc staining, DAPI staining, and a phase image. Spo12p protein was detectable in the nuclei of most but not all cells. The arrowheads point to a large-budded cell that exhibits Spo12p staining. (B and C) Time course of expression of Spo12p in vegetatively growing cells. Cells expressing Spo12-$(HA)_2$ (YMG530) were arrested with α-factor. After release from α-factor arrest, samples were taken at 10-min intervals. Spo12-$(HA)_2$ protein is indicated by an arrow. A background band present in all samples is indicated by an asterisk. Synchrony was monitored by counting budding index (B). In C, the same amount of total protein was loaded per lane. unt, untagged control; asy, sample from the asynchronous population of cells before α-factor arrest; arr, cells arrested in α-factor.

YDR267C was isolated twice. Three of the genes identified in the two-hybrid screen are induced during meiosis (Chu *et al.*, 1998). They are *MEC3*, which plays a role in a DNA damage checkpoint gene in mitotic cells (Weinert *et al.*, 1994); YLR132C, a novel ORF; and *FUR1*, which encodes uracil phosphoribosyltransferase (Kern *et al.*, 1990).

High-Copy Plasmid Suppression of a spo12 Mutation during Meiosis

To identify genes that can function in place of or downstream of *SPO12* during meiosis, we carried out a screen for high-copy plasmids that could compensate for a complete

Table 2. Genes identified in a two-hybrid screen using pLEXA-SPO12 as bait

Gene	Gene information	Extent of fusion[a]	Induced during meiosis?[b]
YDR267C	Contains WD40 repeats, similar to TAFII100	C-terminal two-thirds of protein	No
YHR073W	Similar to human oxysterol-binding protein	Starts at amino acid 201	No
YPR118W	Similar to *Methanococcus jannaschi* translation initiation factor EIF-2B	C-terminal half of protein	No
YLR016C	Novel	C-terminal half of protein	No
MEC3	Known role in DNA-damage checkpoint	C-terminal third of protein	Yes
FIR1	Found to interact with REF-2 in another two-hybrid screen	Amino acids 259–817	No
YLR132C	Novel	Contains the whole ORF	Yes
EZL1	Known transcription factor	C-terminal quarter of protein	No
BAT2	Branched-chain amino acid transaminase	C-terminal half of protein	No
FUR1	Uracil phosphoribosyltransferase	C-terminal half of protein	Yes
YPR152W	Similar to *Mus musculus* formin-binding protein	C-terminal two-thirds of protein	No

[a] Segment of each ORF fused to the transcriptional activation domain constructs.
[b] According to Chu *et al.* (1998).

Table 3. High-copy suppression of *spo12*Δ/*spo12*Δ and *spo13*Δ/*spo13*Δ strains

	Tetrads (%)	Dyads (%)	Sporulation (%)
Plasmid (in *spo12*Δ/*spo12*Δ)			
YEp13	0.3 ± 0.6	99.7 ± 0.6	44.8 ± 2.3
pRS425	0.3 ± 0.7	99.7 ± 0.7	61.5 ± 14.9
21–36 (YEp13-SPO12)	79.6 ± 2.8	20.4 ± 2.8	52.6 ± 7.1
47-32 (YEp13-SPO12)	72.8 ± 2.8	27.2 ± 2.8	58.3 ± 8.3
pRS425-SPO12	52.5 ± 2.4	47.8 ± 2.4	76.2 ± 1.1
Analysis of *SPO13* (in *spo12*Δ/*spo12*Δ)			
17-1 (YEp13-SPO13)	9.1 ± 5.9	90.9 ± 5.9	45.9 ± 5.0
30-10 (YEp13-SPO13)	17.7 ± 4.7	82.3 ± 4.7	47.4 ± 3.2
pRS425-SPO13	7.1 ± 1.9	92.9 ± 1.9	70.1 ± 4.4
Analysis of *BNS1* (in *spo12*Δ/*spo12*Δ)			
32-1 (YEp13-BNS1)	23.8 ± 5.5	76.2 ± 5.5	54.4 ± 7.3
pRS425-BNS1	8.8 ± 3.3	91.2 ± 3.3	71.9 ± 5.7
Plasmid (in *spo13*Δ/*spo13*Δ)			
YEp13	5.3 ± 2.4	94.7 ± 2.4	50.1 ± 3.4
pRS425	6.6 ± 3.0	93.4 ± 3.0	52.7 ± 7.6
17-1 (YEp13-SPO13)	68.4 ± 4.4	31.6 ± 4.4	65.5 ± 5.1
30-10 (YEp13-SPO13)	76.6 ± 4.2	23.4 ± 4.2	68.9 ± 7.1
pRS425-SPO13	44.2 ± 4.5	55.8 ± 4.5	61.1 ± 8.5
Analysis of *SPO12* (in *spo13*Δ/*spo13*Δ)			
21-36 (YEp13-SPO12)	23.0 ± 6.1	77.0 ± 6.1	53.9 ± 8.0
47-32 (YEp13-SPO12)	18.3 ± 5.9	81.7 ± 5.9	51.3 ± 6.8
pRS425-SPO12	11.4 ± 2.7	88.6 ± 2.7	54.3 ± 5.4
Analysis of *BNS1* (in *spo13*Δ/*spo13*Δ)			
32-1 (YEp13-BNS1)	8.7 ± 5.7	91.3 ± 5.7	52.7 ± 2.7
pRS425-BNS1	9.8 ± 6.4	90.2 ± 6.4	39.9 ± 6.0

Plasmids were transformed into an **a**/α *spo12*Δ/*spo12*Δ strain (YMG270) or an **a**/α *spo13*Δ/*spo13*Δ strain (YMG294). These strains were sporulated on plates, and 200 cells from each of four independently derived transformants per plasmid were analyzed for efficiency of sporulation and for asci. Tetrad values are the number of tetrads divided by the number of tetrads plus the number of dyads. Dyad values are the number of dyads divided by the number of tetrads plus the number of dyads. Sporulation values are the number of tetrads plus dyads divided by the total number of cells counted in each case (200).

deletion of *SPO12*. A library of *S. cerevisiae* genomic clones in the 2-μm vector YEp13 (Nasmyth and Reed, 1980) was transformed into a strain homozygous for a *spo12* deletion (YMG270). Individual transformants were patched onto selective medium to select for the library plasmid and then replica plated to sporulation medium. Cells from each patch of sporulating cells were microscopically examined for the presence of tetrads, which indicated restoration of *SPO12*-like activity. Of ~8000 independent transformants, 7 clones allowed the *spo12* deletion strain to form some tetrads (9–80% of total asci; Table 3). Homozygous *spo12* mutants carrying YEp13 formed virtually no tetrads (<0.3%; Table 3). Plasmids with suppressing activity were rescued from yeast and retransformed into the *spo12* deletion strain. Of these, five retained the ability to suppress the *spo12* deletion at least partially. These clones were analyzed further to determine the sequences responsible for the suppressing activity.

The two clones with the strongest ability to suppress the deletion phenotype, 21-36 and 47-32, encoded *SPO12* itself (Table 3). A third clone, 32-1, contained YGR230W, an ORF with homology to *SPO12* (Figure 3). We have named this ORF *BNS1* for *Bypasses Need for Spo12p*. In addition, two independent clones contained *SPO13*, another gene required for progression through the meiotic nuclear divisions (see below). Each of these three ORFs (*SPO12*, *BNS1*, and *SPO13*) was subcloned into another high-copy vector (pRS425) to determine whether they were responsible for the complementing activity. Suppression was observed in each case, but the magnitude of suppression was 1.5- to 3-fold lower than that of the original library clone. The reduced suppression may have been due to a slightly lower copy number of clones in pRS425 than in YEp13 or to the presence of additional ORFs on the original plasmids that contribute to the suppression, although this seems unlikely. In any case, it is clear that overexpression of *SPO12*, *SPO13*, and *BNS1* allows a homozygous *spo12* deletion strain to sporulate to form some tetrads instead of dyads.

In an attempt to understand how *BNS1* and *SPO13* can partially compensate for the lack of Spo12p, we characterized these ORFs further. *BNS1* mRNA is not induced during meiosis (Chu *et al.*, 1998). *BNS1* is predicted to encode a 137-amino acid protein of 15.8 kDa that is homologous to Spo12p (40% identical over 80 amino acids; Figure 3A). Like Spo12p, the protein encoded by *BNS1* is very basic (predicted isoelectric point of 11.22 compared with an isoelectric point of 10.19 for Spo12p). Neither Spo12p nor Bns1p contains any recognizable structural motifs, nor are they similar to Spo13p. The highest degree of identity between Spo12p and Bns1p is a segment of 19 amino acids that is also homologous to at least three other hypothetical ORFs, one from *Schizosaccharomyces pombe*, one from *Caenorhabditis elegans*, and, intriguingly, an expressed sequence tag isolated from a human testis cDNA library (Figure 3B). The functional significance of this homology is not known.

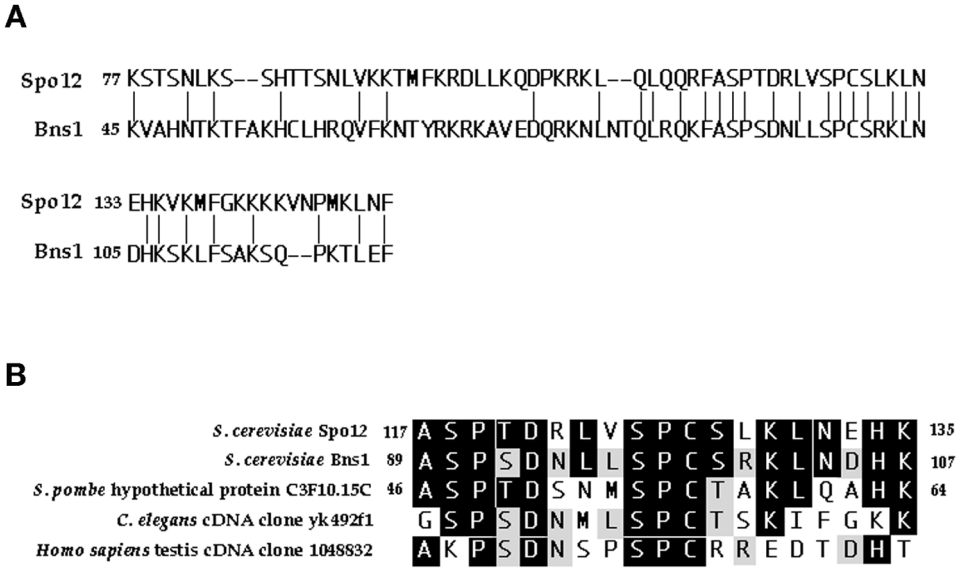

Figure 3. (A) Homology between Spo12 and Bns1. Lines indicate identical amino acids. Numbers correspond to the amino acid positions in each protein. (B) Domain of homology shared by Spo12 and other ORFs. Amino acids identical to those found in Spo12 are shaded black. Those in common among other ORFs but not in common with *SPO12* are shaded gray. Numbers correspond to amino acid positions in each protein where known.

To determine whether *BNS1* plays a role during meiosis or vegetative growth, we replaced its coding sequence with the *hisG-URA3-hisG* cassette (Alani *et al.*, 1987). Strains homozygous for the *bns1* deletion were fully viable and sporulated as efficiently as wild-type strains. We also determined the phenotype of a *spo12*Δ *bns1*Δ double mutant. These strains were also viable and did not display any obvious vegetative growth defects. Furthermore, when sporulated, the *spo12*Δ *bns1*Δ double mutant formed dyads with the same efficiency as a *spo12* single mutant. Thus, although *BNS1* can partially compensate for a lack of *SPO12* function when overexpressed, it does not appear to play any role in controlling meiotic nuclear divisions.

Analysis of Spo13p

SPO13, like *SPO12*, is clearly important for programming the meiotic nuclear divisions. A homozygous *spo13* deletion mutant sporulates to form dyads with high efficiency, and as in *spo12*, the single meiotic division observed in *spo13* most closely resembles meiosis II (Klapholz and Esposito, 1980a,b). To gain some additional information regarding when and where Spo13p might act, we epitope tagged the *SPO13* ORF with the Myc epitope. This protein was able to complement the meiotic defect of a homozygous *spo13* deletion mutant when integrated at single copy (our unpublished results). We found that like Spo12p, Spo13p is also predominantly nuclear (Figure 1C). However, Spo13p was detectable only before meiosis I and not during meiotic nuclear divisions. Spo13p was seen in mononucleate cells with large nuclei with diffuse DAPI staining. We hypothesize that these correspond to cells immediately before meiosis I, probably in the late stages of or after the completion of premeiotic DNA synthesis. Because we were unable to detect Spo13p in vegetative cultures, it is unlikely that the Spo13p-staining cells in sporulating cultures were contaminating vegetative cells. The temporal profiles of Spo13p and Spo12p during meiosis suggest that *SPO13* acts upstream of *SPO12*.

Because of the similarity between Spo12p and Bns1p, we tested the hypothesis that *SPO13* was able to high-copy suppress *spo12* mutants by activating *BNS1*. If this were true, overexpression of *SPO13* should not be able to suppress a *spo12 bns1* double mutant. High-copy *SPO13*, however, was able to suppress the double mutant as efficiently as it suppressed *spo12* single mutants (our unpublished results).

Because high-copy *SPO13* was able to partially suppress the phenotype of a *spo12* deletion mutant, we tested whether the reciprocal was also true, i.e., whether overexpression of *SPO12* could suppress the phenotype of a *spo13* mutant. High-copy plasmids encoding *SPO12* or *BNS1* were transformed into a homozygous *spo13* deletion mutant, and the strains were sporulated (Table 3). Empty-vector and high-copy *SPO13* plasmids were used as controls. High-copy *SPO13* was, as predicted, the strongest suppressor. High-copy *SPO12* could weakly suppress the *spo13* mutant phenotype. Although the total number of tetrads seen in high-copy suppression of the *spo13* mutant by *SPO12* was comparable to that seen by high-copy suppression of *spo12* by *SPO13*, the magnitude of the suppression was quite different (~3-fold versus ~37-fold; Table 3) as a result of differences in the background number of tetrads observed in *spo12* versus *spo13* mutants. In *spo12* mutants, one virtually never sees any tetrads formed, whereas in *spo13* mutants, we observed a background level of tetrads that could reach as high as ~8%. High-copy *BNS1* did not cause statistically significant suppression of a *spo13* deletion. Thus, overex-

Table 4. Segregation analysis from crossing *spo12::hisG-URA3-hisG* to either *hct1-1::LEU2* or *sic1-1::HIS3*

Viable:inviable	Number of tetrads	*hct1Δ spo12Δ* spores observed
hct1-1::LEU2 × spo12::hisG-URA3-hisG		
4:0	3	0
3:1	14	0
2:2	5	0
1:3	0	0
0:4	0	0

Viable:inviable	Number of tetrads	*sic1Δspo12Δ* spores observed
sic1-1::HIS3 × spo12::hisG-URA3-hisG		
4:0	12	13
3:1	7	2
2:2	0	0
1:3	1	0
0:4	0	0

Two independent diploids were dissected for each cross.

pression of *BNS1* can partially bypass the need for *SPO12* but not *SPO13* function.

Genetic Interactions of spo12 with hct1 and sic1

Overexpression of *SPO12* can suppress the late mitotic arrest phenotype of a number of mutants that arrest with high Clb-Cdc28 kinase activity (Parkes and Johnston, 1992; Shirayama *et al.*, 1996; Jaspersen *et al.*, 1998), suggesting that *SPO12* may play a role in exit from mitosis. To determine whether Spo12p may act to decrease Clb-Cdc28 kinase activity, we tested whether deletion of *SPO12* was synthetically lethal with *hct1* or *sic1* mutants. *HCT1* encodes a substrate-specific regulator of the anaphase-promoting complex (APC) and is required for degradation of Clb2p during exit from mitosis (Schwab *et al.*, 1997; Visintin *et al.*, 1997). *SIC1* encodes a cyclin-dependent kinase inhibitor (CKI) that is expressed from late mitosis into G1 (Donovan *et al.*, 1994). Although a strain containing deletion of *HCT1* or *SIC1* alone is viable, *hct1 sic1* double mutants are inviable. This result has been interpreted to mean that as long as at least one of the two pathways for inactivating Clb-Cdc28 kinase activity is intact (through proteolysis mediated by Hct1p or by inhibition of Clb-Cdc28 by Sic1p), cells are able to progress through the cell cycle relatively normally. Inactivation of both pathways simultaneously, however, causes cells to arrest with large buds, presumably with high Clb-Cdc28 kinase activity (Schwab *et al.*, 1997). We found that whereas *spo12 sic1* double mutants were viable, *spo12 hct1* double mutants were inviable (Table 4). Thus, if Spo12p inhibits Clb-Cdc28 kinase activity, it does so in an APC-independent manner and may facilitate the activity of a CKI, presumably Sic1p.

To investigate this hypothesis further, we found by immunoblot analysis that Spo12p and Sic1p show similar levels throughout the mitotic cell cycle (data not shown). We also tested whether the ability of *SPO12* to suppress *cdc15-2* when overexpressed (Jaspersen *et al.*, 1998) was dependent on *SIC1* function. Although overexpression of *SPO12* did suppress the growth defect of a *cdc15-2* mutant, it did not suppress the defect of a *cdc15-2 sic1* double mutant (Figure 4A). In addition, the ability of *SIC1* to suppress *cdc15* when overexpressed (Mendenhall, 1993; Schwob *et al.*, 1994; Toyn *et al.*, 1997; Jaspersen *et al.*, 1998) was dependent on having an intact copy of *SPO12* in the genome (Figure 4B). These results suggest that Spo12p and Sic1p may act together in vegetative cells to facilitate exit from mitosis.

Effect of Spo12p on Clb-Cdc28 Kinase Activity during Meiosis

We examined the levels of Sic1p during meiosis to test the hypothesis that Spo12p might interact with Sic1p during meiosis to modulate Clb-Cdc28 kinase activity and thus control progress through the meiotic nuclear divisions. We were unable to detect any Sic1p in meiotic cells, although high levels of Sic1p were found in cells arrested in YPA presporulation medium (Dirick *et al.*, 1998; our unpublished results). Thus, if Spo12p interacts with a CKI during meiosis, it is unlikely to be Sic1p.

To test more directly the hypothesis that Spo12p modulates Clb-Cdc28 kinase activity during meiosis, we followed levels of kinase activity during meiosis in the wild type and in *spo12* mutants (Figure 5). For this experiment, we constructed HA epitope-tagged versions of the five Clb proteins expressed during meiosis—Clb1p, Clb3p, Clb4p, Clb5p, and Clb6p (Grandin and Reed, 1993; Chu and Herskowitz, 1998; Stuart and Wittenberg, 1998)—and introduced them individually as homozygous gene replacements into wild-type SK1 cells or homozygous *spo12* deletion mutants in the SK1 strain background. These strains were then sporulated synchronously, and aliquots were taken at intervals of 30–60 min over a period of 9 h, at which point most of the cells had completed the meiotic divisions. Progress through meiosis was monitored by DAPI staining (Table 5). Clb-associated kinases were immunoprecipitated from the same amount of total protein from each culture, and in vitro kinase assays were performed with histone H1 as substrate. We were unable to detect any H1-associated kinase activity in wild-type or *spo12* mutant strains containing Clb6-HA (data not shown). For the remaining strains, no significant differences between the wild type and the *spo12* mutants in the timing or the level of activation of the specific Clb-Cdc28 kinases were detected (Figure 5).

DISCUSSION

Spo12p plays an important role in meiosis and appears to have a role in the mitotic cell cycle as well. We have made several observations on *SPO12* that bear on its meiotic and mitotic roles.

Presence and Localization of Spo12p

We found that Spo12p is localized to the nucleus during meiosis and mitosis. Spo12p is present during the meiotic nuclear divisions but is not detectable by immunoblot or

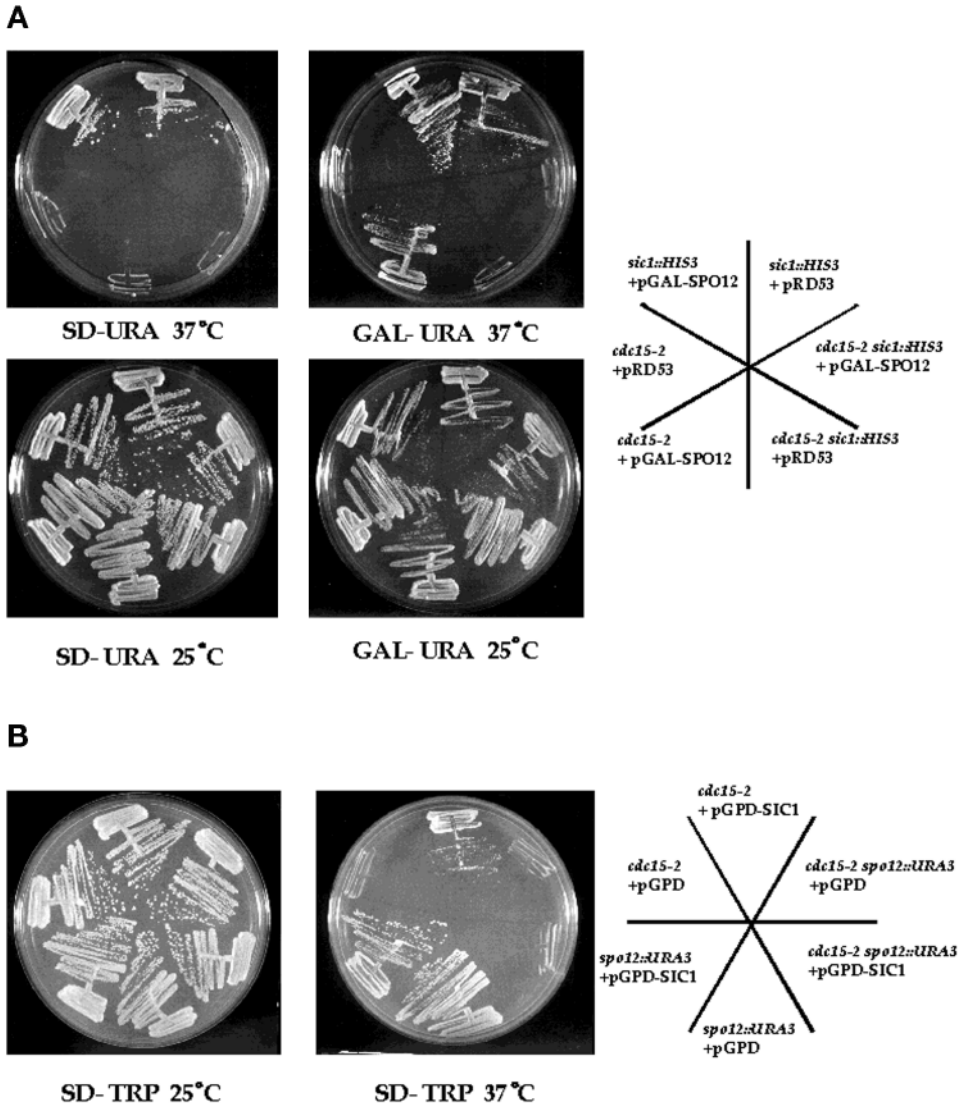

Figure 4. (A) High-copy suppression of *cdc15-2* but not *cdc15-2 sic1-1::HIS3* by pGAL-SPO12. Strains of the genotypes *sic1-1::HIS3*, *cdc15-2*, and *cdc15-2 sic1-1::HIS3* were transformed with pGAL-SPO12 or the vector control pRD53. They were then streaked for single colonies on SD medium or SGAL (0.67% yeast nitrogen base without amino acids, 1× amino acids, 2% galactose, 2% Bacto-agar) medium (to induce expression of *GAL-SPO12*), and plates were incubated at 25 or 37°C (the nonpermissive temperature for *cdc15-2*). Strains were YMG422, YMG484, and YMG485. (B) Partial high-copy suppression of *cdc15-2* but not *cdc15-2 spo12* by pGPD-SIC1. Strains of the genotypes *cdc15-2*, *spo12::hisG-URA3-hisG*, and *cdc15-2 spo12::hisG-URA3-hisG* were transformed with pGPD-SIC1 (the GPD promoter is constitutively active) or a pGPD vector control, streaked for single colonies, and grown at 25 or 37°C to test the ability of pGPD-SIC1 to restore growth to strains containing the *cdc15-2* mutation. Strains were YMG536, YMG537, and YMG535.

immunofluorescence at other times, making it unlikely that it is present but delocalized in these cells. *SPO12* mRNA levels have a similar profile during meiosis (Malavasic and Elder, 1990; Chu *et al.*, 1998). Immunoblot analysis of a synchronized vegetative cell population showed that Spo12p is present throughout the mitotic cell cycle and peaks in G2/M. *SPO12* mRNA levels have also been shown to peak at this stage of the cell cycle (Parkes and Johnston, 1992; Cho *et al.*, 1998; Spellman *et al.*, 1998).

Proteins That Interact with Spo12p

Candidate proteins that interact with Spo12p were identified with the use of the two-hybrid system. At least three of the genes identified in this screen are induced to some level during meiosis (Chu *et al.*, 1998) and clearly warrant further study. They are YLR132C, *MEC3*, and *FUR1*. YLR132C is a novel ORF with no known homologies. Its transcript exhibits an expression pattern during sporula-

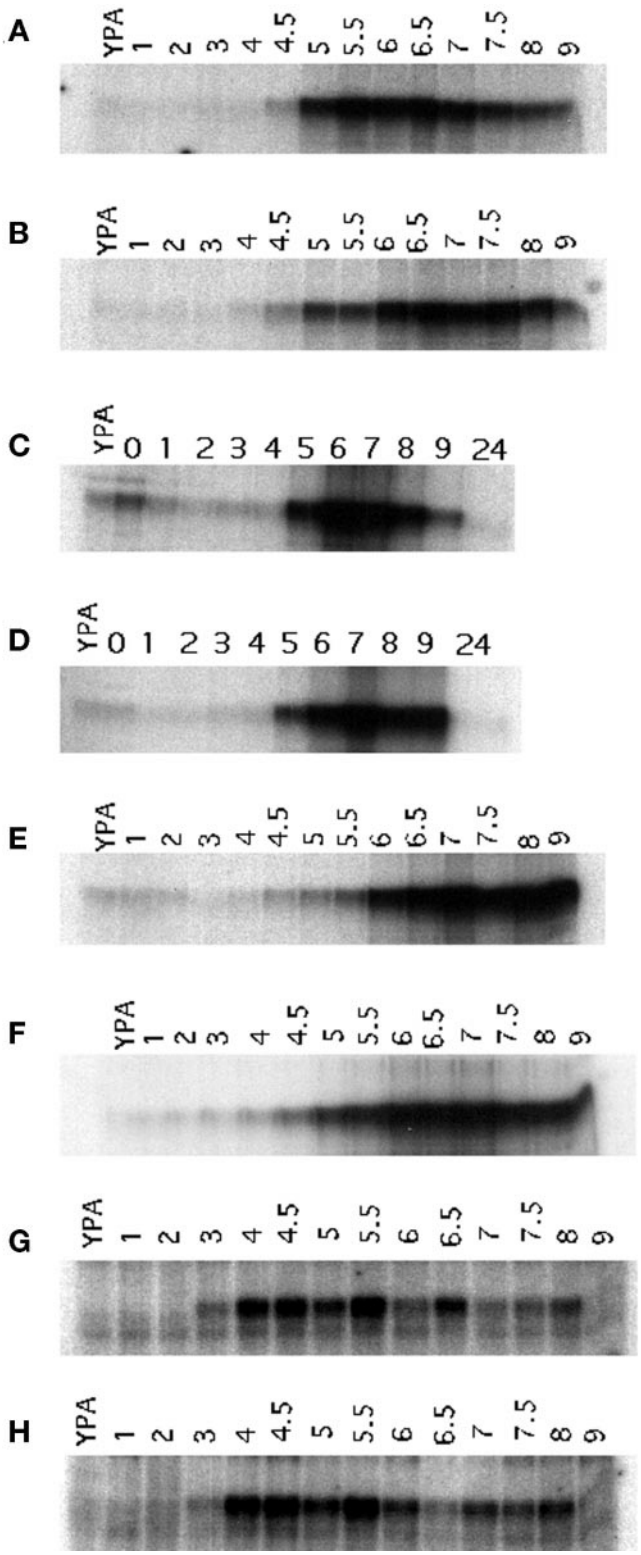

Figure 5. Histone H1 kinase activity in strains undergoing synchronous sporulation. Wild-type and *spo12* strains homozygous for gene replacements of *CLB1HA*, *CLB3HA*, *CLB4HA*, and *CLB5HA* (*CLB1/CLB1* [A], *CLB1/CLB1 spo12*Δ*/spo12*Δ [B], *CLB3/CLB3* [C], *CLB3/CLB3 spo12*Δ*/spo12*Δ [D], *CLB4/CLB4* [E], *CLB4/CLB4 spo12*Δ*/spo12*Δ [F], *CLB5/CLB5* [G], and *CLB5/CLB5 spo12*Δ*/spo12*Δ [H]) were induced to undergo synchronous meiosis. Aliquots were collected at hourly or half-hourly intervals as indicated. Active Clb-Cdc28p kinase complexes were immunoprecipitated from the same amount of total protein in each lane with the use of anti-HA antibodies. Kinase activity of these complexes was measured by an in vitro kinase assay with histone H1 as a substrate. Progress through meiosis was monitored by DAPI staining (Table 5). In each case, the onset of histone H1 kinase activity corresponded to the onset of the meiotic divisions. Strains were YMG654, YMG655, YMG624, YMG659, YMG522, YMG632, YMG998, and YMG999.

tion that is similar to that of *SPO12* (Chu *et al.*, 1998), suggesting that their proteins may be present at the same time during sporulation and have the opportunity to interact physically.

MEC3 is required for the DNA damage checkpoint response in mitosis and is probably required for the meiotic recombination checkpoint (Weinert *et al.* 1994; Lydall *et al.*, 1996; Lydall and Weinert 1997). Transcription of *MEC3* is induced twofold when cells enter the meiotic divisions (Chu *et al.*, 1998). Perhaps interaction between Spo12p and Mec3p signals a meiotic cell that recombination is complete and that the cell can execute meiosis I.

FUR1 encodes uracil phosphoribosyltransferase, which produces UMP from uracil (Kern *et al.*, 1990). Transcription of *FUR1* is induced when cells enter meiotic divisions (Chu *et al.*, 1998). Although a link between Spo12p and pyrimidine metabolism is not obvious, *FUR1* is notable because of a previous functional connection between *FUR1* and *SPO12*: overexpression of either gene can suppress mitotic arrest resulting from overexpression of stabilized Clb2 (Glotzer, 1993). Overexpression of *SPO12* or *FUR1* suppresses point mutations but not deletions in the *CLB2* destruction box.

The other candidate partners for Spo12p might function with Spo12p during vegetative growth. Whether any of these proteins physically interact with Spo12p remains to be determined

Overexpression of BNS1 and SPO13 Can Partially Suppress the spo12 Meiotic Phenotype

We used high-copy suppression of a *spo12* deletion to identify proteins that can drive the two meiotic nuclear divisions in the absence of Spo12p. The rationale was that these proteins might be downstream targets of Spo12p or might bypass the requirement of Spo12p for the meiotic divisions. We found two genes, *BNS1* and *SPO13*, that could partially compensate for a complete lack of Spo12p during meiosis.

BNS1 is the only ORF in the yeast genome with significant homology to *SPO12*. Their proteins are 40% identical over ~80 amino acids, with one segment containing 14 of 19 identical residues and 4 of 19 conservative changes. Proteins containing this segment are also found in a number of other organisms, including *S. pombe*, *C. elegans*, and humans. The degree of similarity with Spo12p does not extend significantly beyond this region. In at least one other circumstance, *BNS1* can function like *SPO12*: both *BNS1* and *SPO12* can suppress the arrest phenotype of *cdc15-2* mutants when overexpressed (Jaspersen *et al.*, 1998). Despite the similarity

Table 5. Progress through meiosis of strains in Figure 5.

		Time												
CLB1/CLB1	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	94	67	38	36	22	13	8	8
Percent binucleate	0	0	0	0	0	0	6	29	34	22	23	12	4	80
Percent tetranucleate	0	0	0	0	0	0	0	4	28	42	55	75	88	84
		Time												
CLB1/CLB1 spo12Δ/spo12Δ	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	100	84	63	55	46	19	15	14
Percent binucleate	0	0	0	0	0	0	0	16	37	45	54	81	85	86
		Time												
CLB3/CLB3	YPA	0	1	2	3	4	5	6	7	8	9	24		
Percent mononucleate	100	100	100	100	100	100	85	48	13	12	6	7		
Percent binucleate	0	0	0	0	0	0	14	25	15	4	6	13		
Percent tetranucleate	0	0	0	0	0	0	1	27	72	84	88	80		
		Time												
CLB3/CLB3 spo12Δ/spo12Δ	YPA	0	1	2	3	4	5	6	7	8	9	24		
Percent mononucleate	100	100	100	100	100	100	100	71	28	16	14	13		
Percent binucleate	0	0	0	0	0	0	0	29	72	84	86	87		
		Time												
CLB4/CLB4	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	100	95	87	83	68	54	22	17
Percent binucleate	0	0	0	0	0	0	0	5	13	15	28	22	34	20
Percent tetranucleate	0	0	0	0	0	0	0	0	0	2	4	24	44	63
		Time												
CLB4/CLB4 spo12Δ/spo12Δ	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	100	99	81	62	47	32	36	21
Percent binucleate	0	0	0	0	0	0	0	1	19	38	53	68	34	79
		Time												
CLB5/CLB5	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	73	31	20	9	15	6	7	3
Percent binucleate	0	0	0	0	0	0	27	46	33	22	12	10	7	3
Percent tetranucleate	0	0	0	0	0	0	0	23	47	69	73	84	86	94
		Time												
CLB5/CLB5 spo12Δ/spo12Δ	YPA	1	2	3	4	4.5	5	5.5	6	6.5	7	7.5	8	9
Percent mononucleate	100	100	100	100	100	100	97	78	41	33	17	17	7	6
Percent binucleate	0	0	0	0	0	0	3	22	59	67	83	83	93	94

Progress through meiosis was monitored by DAPI staining. Time is indicated in hours.

between *SPO12* and *BNS1*, *BNS1* does not appear to have a role in meiosis: *BNS1* mRNA is not induced during meiosis (Chu *et al.*, 1998), null mutants for *bns1* exhibited no meiotic phenotype, and *spo12 bns1* double mutants were viable with no obvious growth defects. Spo12p protein has been proposed to be a cofactor for the protein kinase Dbf2p (Toyn and Johnston, 1993). Perhaps Bns1p acts to facilitate the activity of the Dbf2-related protein kinase Dbf20p.

Although both Spo12p and Spo13p are nuclear, Spo13p is present before Spo12p. How then can suppression of *spo12* by *SPO13* be explained? Overexpression of *SPO13* during meiosis has been reported to delay meiosis I (McCarroll and Esposito, 1994). *spo12* mutants skip meiosis I and execute only meiosis II (Klapholz and Esposito, 1980a,b). Perhaps overexpression of *SPO13* in *spo12* mutants delays meiotic divisions long enough that a compensatory mechanism can trigger meiosis I. Another possibility is that overexpression of *SPO13* in *spo12* mutants somehow promotes a second round of nuclear division that follows the meiosis II–like division that normally occurs in *spo12* mutants. Assessment of the patterns of chromosome segregation or spore viability under conditions of suppression might make it possible to distinguish between these two possibilities.

We have also observed that *SPO12* can partially suppress *spo13* mutants when overexpressed. This suppression can be explained if *SPO12* acts downstream of *SPO13*, as is indicated by their temporal patterns of expression. The magnitude of suppression might be low because Spo13p activates targets other than Spo12p for efficient sporulation.

Synthetic Lethality of spo12 and hct1 Further Supports a Role for SPO12 in Exit from Mitosis

A critical event for exit from mitosis is the inactivation of Clb-Cdc28 kinase activity. Although the phenotype of *spo12* mutants in vegetative cells is subtle, overexpression of *SPO12* can suppress late mitotic arrest of several different mutants. All of these mutants arrest with high Clb-Cdc28 kinase activity, leading to the hypothesis that Spo12p inhibits Clb-Cdc28 kinase activity in some way (Shirayama *et al.*, 1996). The fact that Spo12p is expressed in late mitosis and that *spo12* mutants experience a G2/M delay indicates that *SPO12* normally has a role at this time.

We observed that *spo12* and *hct1* are synthetically lethal. *HCT1* is a component of the APC that is required for degradation of Clb2p and subsequent inactivation of Clb-Cdc28 kinase activity (Schwab *et al.*, 1997; Visintin *et al.*, 1997). Synthetic lethality of *spo12* with *hct1*, a known component of the APC, suggests that if Spo12p acts to inhibit Clb-Cdc28 kinase activity, it does not do so via an APC-dependent mechanism.

One possibility is that Spo12p activates a CKI. Sic1p is present from late M into G1 and is required to keep Clb5- and Clb6-Cdc28 kinases inactive until cells are ready to replicate their DNA (Donovan *et al.*, 1994). Sic1p may also play a role in exit from mitosis (Donovan *et al.*, 1994; Toyn *et al.*, 1997). Thus, one attractive possibility is that Spo12p may facilitate Sic1p activity during vegetative growth. Genetic observations indicate that this hypothesis may be correct. The ability of *SPO12* when overexpressed to suppress *cdc15-2*, which arrests late in mitosis with high Clb-Cdc28 kinase activity, requires functional *SIC1*. In addition, the ability of *SIC1* to suppress *cdc15-2* requires functional *SPO12*. Therefore, these proteins may act together. For example, interaction between Spo12p and Sic1p may stabilize Sic1p and allow it to decrease Clb-Cdc28 kinase activity. Another possibility is that Spo12p acts to inhibit SCF, which is required for degradation of Sic1p (Feldman *et al.*, 1997; Skowyra *et al.*, 1997).

Spo12p Does Not Affect Clb1-, Clb3-, Clb4-, or Clb5-Cdc28 Kinase Activity during Meiosis

The experiments in mitotic cells described above lead to the possibility that Spo12p might regulate Clb-Cdc28 kinase activity during meiosis. Five of the six B-type cyclins are expressed during meiosis (all but *CLB2*), and deletion of the *CLB* genes either alone or in combination can lead to the reduction or complete absence of the meiotic nuclear divisions, depending on the mutant combination (Grandin and Reed, 1993; Dahmann and Futcher, 1995; Chu and Herskowitz, 1998; Stuart and Wittenberg, 1998). Because Spo12p may inhibit Clb-Cdc28 kinase activity in vegetative cells, and because of the clear importance of the Clb proteins for meiosis, we hypothesized that Spo12p might modulate Clb-Cdc28 kinase levels during meiosis.

We first considered the possibility that Spo12p regulates Clb-Cdc28 kinase activity during meiosis through Sic1p; however, Sic1p was not detectable in meiosis after the very early stages and in particular was absent during the meiotic divisions, when Spo12p is believed to be required. Our observations provide no support for the hypothesis that Spo12p acts via Sic1p in meiosis. We then examined Clb1-, Clb3-, Clb4-, Clb5-, and Clb6-Cdc28 kinase activities directly in the wild type and in *spo12* mutants. The levels and timing of specific Clb-Cdc28 kinase activities (as assayed by measuring histone H1 kinase activity in vitro) were indistinguishable for wild-type and *spo12* mutant strains, indicating that Spo12p does not regulate these species.

So what is Spo12p doing? It remains an open question whether Spo12p regulates Clb-Cdc28 activity during meiosis. Perhaps Spo12p acts with Sic1p during mitosis, but during meiosis its relevant partner is an as yet unidentified meiotic CKI. Another possibility is that Spo12p alters Clb-Cdc28 kinase activity phosphorylation. We cannot exclude the possibility that Spo12p regulates meiotic divisions via a completely distinct mechanism. For example, perhaps Spo12p (and Spo13p) are required to ensure cosegregation of sister chromatids during meiosis I.

In summary, *SPO12* is crucial for the proper regulation of the meiotic nuclear divisions and possibly exit from mitosis as well. The observations described here should provide information that will ultimately contribute to the understanding of the function of Spo12 protein.

ACKNOWLEDGMENTS

We thank Sue Jaspersen, Roger Brent, Rochelle Esposito, Bruce Futcher, Leland Johnston, Wolfgang Seufert, Dave Stuart, and Mike Tyers for reagents and Sue Jaspersen, Julia Charles, and members of the Herskowitz laboratory for valuable discussions. This work was supported by grants from the National Institutes of Health to I.H. and a Damon Runyon-Walter Winchell postdoctoral fellowship to M.E.G.

REFERENCES

Alani, E., Cao, L., and Kleckner, N. (1987). A method for gene disruption that allows repeated use of a *URA3* selection in the construction of multiply disrupted yeast strains. Genetics *116*, 541–545.

Cho, R.J., *et al.* (1998). A genome-wide transcriptional analysis of the mitotic cell cycle. Mol. Cell. *2*, 65–73.

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.

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

Dahmann, C., and Futcher, B. (1995). Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics *140*, 957–963.

Dirick, L., Goetsch, L., Ammerer, G., and Byers, B. (1998). Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science *281*, 1854–1857.

Donovan, J.D., Toyn, J.H., Johnson, A.L., and Johnston, L.H. (1994). $P40^{SDB25}$, a putative CDK inhibitor, has a role in the M/G1 transition in *Saccharomyces cerevisiae*. Genes Dev. *74*, 1640–1653.

Evan, G.I., Lewis, G.K., Ramsay, G., and Bishop, J.M. (1985). Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. Mol. Cell. Biol. *5*, 3610–3616.

Feldman, R.M., Correll, C.C., Kaplan, K.B., and Deshaies, R.J. (1997). A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. Cell *91*, 221–230.

Fitch, I., Dahmann, C., Surana, U., Amon, A., Nasmyth, K., Goetsch, L., Byers, B., and Futcher, B. (1992). Characterization of four B-type cyclin genes of the budding yeast. Mol. Biol. Cell *7*, 805–818.

Glotzer, M. (1993). The Destruction of Mitotic Cyclins. Ph.D. Thesis. San Francisco, CA: University of California, San Francisco.

Grandin, N., and Reed, S.I. (1993). Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol. Cell. Biol. *13*, 2113–2125.

Gyuris, J., Golemis, E., Chertkov, H., and Brent, R. (1993). Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell *75*, 791–803.

Hicks, J.B., and Herskowitz, I. (1976). Interconversion of yeast mating types. Direct observations of the action of the homothallism (*HO*) gene. Genetics *83*, 245–258.

Jaspersen, S.L., Charles, J.F., Tinker-Kulberg, R.L., and Morgan, D.O. (1998). A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerevisiae*. Mol. Biol. Cell *9*, 2803–2817.

Kassir, Y., and Simchen, G. (1991). Monitoring meiosis and sporulation in *Saccharomyces cerevisiae*. Methods Enzymol. *194*, 94–110.

Kern, L., de Montigny, J., Jund, R., and Lacroute, F. (1990). The *FUR1* gene of *Saccharomyces cerevisiae*: cloning, structure and expression of wild-type and mutant alleles. Gene *88*, 149–157.

Klapholz, S., and Esposito, R.E. (1980a). Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics *96*, 567–588.

Klapholz, S., and Esposito, R.E. (1980b). Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics *96*, 589–611.

Lydall, D., Nikolsky, Y., Bishop, D.K., and Weinert, T. (1996). A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature *383*, 840–843.

Lydall, D., and Weinert, T. (1997). G2/M checkpoint genes of *Saccharomyces cerevisiae*: further evidence for roles in DNA replication and/or repair. Mol. Gen. Genet. *256*, 638–651.

Malavasic, M.J., and Elder, R.T. (1990). Complementary transcripts from two genes necessary for normal meiosis in the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol. *10*, 2809–2819.

McCarroll, R.M., and Esposito, R.E. (1994). *SPO13* negatively regulates the progression of mitotic and meiotic nuclear division in *Saccharomyces cerevisiae*. Genetics *138*, 47–60.

Mendenhall, M.D. (1993). An inhibitor of $p34^{CDC28}$ protein kinase activity from *Saccharomyces cerevisiae*. Science *259*, 216–219.

Nasmyth, K.A., and Reed, S.I. (1980). Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. Proc. Natl. Acad. Sci. USA *77*, 2119–2123.

Padmore, R., Cao, L., and Kleckner, N. (1991). Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell *66*, 1239–1256.

Parkes, V., and Johnston, L.H. (1992). *SPO12* and *SIT4* suppress mutations in *DBF2*, which encodes a cell cycle protein kinase that is periodically expressed. Nucleic Acids Res. *20*, 5617–5623.

Rose, M.D., Winston, F., and Hieter, P. (1990). Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sanders, S.L., and Herskowitz, I. (1996). The Bud4 protein of yeast, required for axial budding, is localized to the mother/bud neck in a cell cycle-dependent manner. J. Cell Biol. *134*, 413–427.

Schwab, M., Lutun, A.S., and Seufert, W. (1997). Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. Cell *90*, 683–693.

Schwob, E., Bohm, T., Mendenhall, M.D., and Nasmyth, K. (1994). The B-type cyclin kinase inhibitor $p40^{SIC1}$ controls the G1 to S transition in *S. cerevisiae*. Cell *79*, 233–244.

Shirayama, M., Matsui, Y., and Toh-e, A. (1996). Dominant mutant alleles of yeast protein kinase gene *CDC15* suppress the *lte1* defect in termination of M phase and genetically interact with CDC14. Mol. Gen. Genet. *251*, 176–185.

Sil, A., and Herskowitz, I. (1996). Identification of an asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast *HO* gene. Cell *84*, 711–722.

Simchen, G. (1973). Are mitotic functions required in meiosis? Genetics *76*, 745–753.

Skowyra, D., Craig, K.L., Tyers, M., Elledge, S.J., and Harper, J.W. (1997). F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell *91*, 209–219.

Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., and Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol. Biol. Cell *9*, 3273–3297.

Stuart, D., and Wittenberg, C. (1998). *CLB5* and *CLB6* are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. *12*, 2698–2710.

Toyn, J.H., Johnson, A.L., Donovan, J.D., Toone, W.M., and Johnston, L.H. (1997). The Swi5 transcription factor of *Saccharomyces cerevisiae* has a role in exit from mitosis through induction of the cdk-inhibitor Sic1 in telophase. Genetics *145*, 85–96.

Toyn, J.H., and Johnston, L.H. (1993). Spo12 is a limiting factor that interacts with the cell cycle protein kinases Dbf2 and Dbf20, which are involved in mitotic chromatid disjunction. Genetics *135*, 963–971.

Toyn, J.H., and Johnston, L.H. (1994). The Dbf2 and Dbf20 protein kinases of budding yeast are activated after the metaphase to anaphase cell cycle transition. EMBO J. *13*, 1103–1113.

Visintin, R., Prinz, S., and Amon, A. (1997). *CDC20* and *CDH1*: a family of substrate-specific activators of APC-dependent proteolysis. Science *278*, 460–463.

Weinert, T.A., Kiser, G.L., and Hartwell, L.H. (1994). Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. *8*, 652–655.

Wilson, I.A., Niman, H.L., Houghten, R.A., Cherenson, A.R., Connolly, M.L., and Lerner, R.A. (1984). The structure of an antigenic determinant in a protein. Cell *37*, 767–778.