**Saccharomyces cerevisiae** C-Type Cyclin Ume3p/Srb11p Is Required for Efficient Induction and Execution of Meiotic Development

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The yeast C-type cyclin Ume3p/Srb11p and its cyclin-dependent kinase partner Ume5p/Srb10p repress the transcription of several genes required for meiotic recombination or meiosis I nuclear division. To relieve this repression, Srb11p is destroyed early in meiosis, prior to the first meiotic division. This report identifies two roles for Srb11p in regulating meiotic development. First, *SRB11* is required for the normal exit from the mitotic cell cycle prior to meiotic induction. Specifically, mutants lacking *SRB11* (srb11Δ) uncouple bud growth from chromosome segregation, producing small buds with nuclei. The bud growth defect is most likely due to the failure of srb11Δ mutants to reestablish polarized actin fibers at the bud tip following exposure to sporulation medium. Second, Srb11p is required for the efficient execution of meiosis I. srb11Δ mutants either exhibited a delay in performing meiosis I and meiosis II or skipped meiosis I entirely. This meiotic defect is not due to the activation of the recombination or spindle assembly checkpoint pathways. However, the expression of several meiotic genes is delayed and reduced in the mutant strains. These results suggest a positive role for Srb10-Srb11p in regulating the transcription program. This model is supported by the finding that overexpression of the meiotic inducer *IME2* partially restored the ability of srb11 mutants to perform meiosis I. In conclusion, these findings indicate that Srb11p is required for both entry into and execution of the meiotic program, thus describing multiple roles for a C-type cyclin in the regulation of a developmental pathway.

Meiosis is the process by which diploid organisms produce haploid gametes capable of sexual reproduction. During meiosis, the cell undergoes one round of DNA synthesis (premeiotic S phase) followed by homolog pairing and extensive genetic recombination. Haploidization is achieved through two sequential chromosome divisions (meiosis I and meiosis II) followed by gamete differentiation. In budding yeast cells, entry into meiosis is controlled by both genetic and nutritional pathways (15). Yeast cells that are heterozygous at the mating type alleles enter the meiotic program from the G₁ phase of the cell cycle when starved for nitrogen and a fermentable carbon source (20). Mutations that inappropriately drive the cell through the cell cycle, such as activated *RAS2*, or the overexpression of G₁ cyclins prevents meiotic induction (9, 50). Entry into meiosis is initiated by the transcriptional induction of the *IME1* gene (24). Ime1p, in turn, activates the transcriptional cascade of meiotic genes, including the protein kinase *IME2* (45, 58). Ime2p is required for many aspects of meiosis, including premeiotic S phase (14) and early meiotic gene transcription (37), and has a later, poorly understood role in spore maturation (16, 37). Therefore, the concerted activity of Ime1p and Ime2p is necessary for normal entry and execution of meiosis and spore formation.

As in mitotic cell division, checkpoint pathways are present that monitor the completion of landmark meiotic events. The DNA damage checkpoint pathway involves several proteins that are activated by unreplicated DNA and/or the persistence of DNA breaks (e.g., Rad17p, Rad24p, and Mec1p) (32, 41). Another pathway arrests meiotic progression in pachytene until the final resolution of recombination intermediates (27, 40, 51). Finally, spindle status and chromosome attachment are monitored by a Mad2p-dependent pathway (43). Interestingly, loss of Mad2p activity causes a significant reduction in normal meiosis I disjunction but has little effect on meiosis II segregation, suggesting that the regulation of these two divisions is not identical. Activation of the DNA damage or spindle checkpoint pathways arrests cells in pachytene or metaphase of meiosis I, respectively. The underlying mechanism by which the checkpoint pathway halts meiotic progression is not completely understood. However, the DNA damage checkpoint negatively regulates the Ndt80p transcription factor, which is required for middle meiotic gene expression (8, 18).

Many genes that are required for the execution of landmark meiotic events in yeasts are expressed in both transient and temporal fashions (36). Different classes of meiotic genes (early, middle, and late) have been established based on the timing of their expression (7, 34). As expected, genes expressed early are required for premeiotic S phase and progression through meiotic prophase I. Middle genes are involved in both meiotic divisions and spore wall assembly, while late genes are required for spore wall maturation. The repression of early meiotic genes (e.g., *SPO13* and *HOP1*) in vegetative cells requires the concerted action of several genes, including those for the histone deacetylation complex Sin3p-Ume6p-Rpd3p (23, 48, 53, 54), which functions through the URS1 promoter element (4, 52). In addition to Sin3p-Ume6p-Rpd3p, another set of transcriptional repressors, encoded by *UME2*, *UME3*, and *UME5*.
functions through a different mechanism. UME2, UME3, and UME5 also called SBR9, SBR11, and SBR10, respectively) copurify with the RNA polymerase II holoenzyme (17, 30). Ume3/Srb11p is a C-type cyclin that is required for activation of the cyclin-dependent kinase Ume5p/Srb10p (10, 47). Unlike those of cyclins that regulate mitotic cell division, Srb11p levels do not vary throughout the cell cycle (10). Rather, Srb11p is rapidly destroyed early in development, prior to meiosis I. This destruction is important, as a degradation-resistant allele of *SRB11* reduces *SPO13* transcript accumulation (10). These findings suggest that Srb11p may play a role in regulating the early stages of meiotic development.

To further investigate the role of Srb11p in controlling meiosis, the effect of a null allele on meiotic induction and progression was analyzed. These studies identified two functions for Srb11p in normal meiotic development. First, Srb11p is required for the efficient execution of meiosis I. Mutants lacking this cyclin either performed the first division late or skipped the event entirely, forming two-spore ascii. This delay was also observed at the level of gene expression, as mRNA levels were reduced and expression from several genes was delayed, providing a possible explanation for the meiotic defect. This hypothesis is supported by the finding that overexpression of the *IME2* meiotic inducer partially restored the ability of an srb11 mutant to undergo meiosis I. In addition, Srb11p couples bud growth with nuclear division in the last cell cycle prior to meiotic entry, as mutants produce small buds with a nucleus. Taken together, these results define new roles for the Srb11p-Srb10p kinase in both the cellular response to sporulation signals and the execution of meiotic development itself.

**MATERIALS AND METHODS**

**Strains, media, and plasmids.** The strains used in this study are listed in Table 1. These strains are derived from crosses between rapidly sporulating SK1 and W303, which provided efficient sporulation without premature meiotic induction. Yeast strains were grown and induced to undergo meiosis as described previously (10). The *IME2* overexpression construct pMR1 was a gift from E. Winter (Thomas Jefferson University, Philadelphia, Pa.); this construct contains the *IME2* gene from pH5400 (44) inserted into pRS426 (26). Disruption of *RAD17* was accomplished by using pAAA19 (a gift from T. Weinert, University of Arizona). The MAD2 disruption was generated by using oligonucleotide-directed recombination as described previously (31). The *srbl1::TRP1* allele was constructed by replacing the internal *Sph1-BglII* fragment of *SRBl1* with an *Sph1-BglII* *TRP1* DNA fragment derived from pJ2380 (22).

**Northern analysis.** Northern blot analyses were performed as described previously (35) with 25 μg of total RNA. Probes were obtained from PCR amplification of the gene in question and labeled with [32P]-UTP by using a Primer II random priming kit (Stratagene). Signals were quantitated by phosphorimaging (Fuji Inc.). The same blot was stripped and reprobed for various transcripts as previously described (10).

**Meiotic cell synchrony.** Synchronous meiotic cultures were generated as described previously (25). To monitor the execution of meiosis I and meiosis II, samples were taken at various times, fixed in 70% ethanol, and then stained with the nucleic acid-specific stain 4′,6′-diamidino-2-phenylindole (DAPI). For each time point, at least 200 cells were assayed for the appearance of bi- and tetranucleated cells to indicate the execution of meiosis I and meiosis II, respectively. Flow cytometric analyses of sporulating cultures were conducted as described previously (42). Briefly, yeast cells were harvested and fixed in 70% ethanol. The cells were washed briefly with water, sequentially treated with RNase A and pepsin, and then stained with propidium iodide (10 μg/ml). Cells were washed, lightly sonicated to disrupt clumps, and sorted by using a BECton Dickinson FACStar®PLUS. At least 50,000 cells were analyzed per sample. All meiotic time courses were conducted at least twice.

**Recombination assays.** JX212 and JX213 were induced to enter meiosis by using standard protocols. At various times, samples were taken and lightly sonicated, and the cell numbers were determined by direct counting. The cells were serially diluted and then plated on solid rich medium to determine total viable cell numbers and minimal medium lacking leucine to monitor prototrophy due to intergenic recombination or gene conversion. The plates were incubated for 3 days at 30°C, and the numbers of colonies were determined.

**RESULTS.**

Srb11p is required for the efficient execution of meiosis I. To assess the role that Srb11p plays in regulating meiotic development, a homozygous diploid harboring an *SRB11* null allele (*srbl1Δ*) was constructed (RYS389). This strain was induced to enter meiosis, and samples were taken at various times. First, we monitored the progression through meiosis I and meiosis II by monitoring nuclear divisions by using DAPI staining. The mutant strain exhibited two significant phenotypes in this assay. First, there was an approximate 3-h delay in the appearance of bi- and tetranucleated cells indicative of cells under-
going meiosis I and meiosis II, respectively (Fig. 1A; quantitated in Fig. 1B). In addition, the maximum sporulation level was achieved 9 h earlier in the wild type than in the mutant (15 and 24 h, respectively). These results indicate that Srb11p is required for the timely execution of the first and second meiotic divisions.

The second phenotype observed for the mutant strain was the dramatic increase in the final percentage of two-spore ascii or dyads compared to the percentage in the wild-type strain (52 versus 6%) (Table 2 and Fig. 1A, 24-h time point). Dyads are formed when one of the two meiotic divisions fails to occur, producing two diploid spores instead of the normal four haploid products. To analyze the srb11Δ dyads further, they were dissected onto rich medium, and spore clones were obtained. No significant loss in viability was observed (data not shown), indicating that chromosome segregation was not significantly affected. To investigate which of the two divisions occurred in the dyads, a mating test was performed with the resulting spore colonies. If the reductional, meiosis I division occurred, then a majority of the cells would be genetically MATA/α or MATA/α at the mating type locus due to linkage of the MAT locus to the centromere on chromosome III. These cell types would be competent for mating. Conversely, a single equational, meiosis II division would produce MATA/α cells that would be unable to mate. These mating studies revealed that 83% of the spore colonies (22 dyads tested) were unable to mate and indicated that the srb11Δ mutant strain bypassed meiosis I and completed equational (meiosis II) division. The genetic background used in these initial experiments was derived from SK1, a strain known to exhibit rapid sporulation kinetics. To determine if elevated dyad formation was dependent on the strain background, these experiments were repeated with isogenic strains derived from W303. Identical results were obtained (data not shown), indicating that the failure to fully execute the first meiotic division was strain independent. Taken together, these data indicate that Srb11p is required for the timely and efficient execution of the first meiotic division.

**Srb11p is required for normal recombination kinetics.** The studies presented above indicate that mutants lacking Srb11p exhibit either a delay in executing meiosis I or skip the division entirely. To more precisely define the stage of meiosis that requires Srb11p, two early landmark events were examined. First, premeiotic S phase was monitored by using flow cytometry (see Materials and Methods for details). No significant differences were detected in the timing or efficiency of premeiotic S phase (data not shown), indicating that Srb11p is dispensable for bulk DNA synthesis during meiosis. We next asked whether Srb11p was required for meiotic recombination. To address this question, two diploid strains were constructed either wild type or mutant for **SRB11** and containing hetero-

### Table 2. Phenotypic analysis of an srb11Δ srb11Δ homozygous diploid during meiosis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Budding index (%) of bi- and tetranucleated cells (at the following h after shift)</th>
<th>% Binucleated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSY335</td>
<td>SRB11/RSB11</td>
<td>0 3 15 36</td>
<td>78 6</td>
</tr>
<tr>
<td>RSY389</td>
<td>srb11Δ/ srb11Δ</td>
<td>28 22 22 20</td>
<td>73 52</td>
</tr>
</tbody>
</table>

*The budding index was calculated as the fraction of cells containing buds in the total population during vegetative growth (0 h) and at 3, 15, and 36 h after a shift to sporulation medium.

* (Total number of binucleated cells/total number of bi- and tetranucleated cells) × 100.
lelic markers at the leu1 locus (JX212 or JX213, respectively) (Table 1). Three independent isolates of each strain were induced to enter meiosis. Aliquots were taken at various times and plated on rich medium to determine overall viability and defined minimal medium to detect leucine prototrophs due to intergenic recombination at the leu1 locus (see Materials and Methods for details). The timing of appearance of Leu+ prototrophs was similar in both strains (Fig. 2, top panel) although the wild type produced recombinants with a slightly higher frequency and completed the process 4 h before the mutant. The reduction in colonies on leucine-lacking medium observed with the mutant was not due to a general loss in viability, as determined by plating on rich medium (Fig. 2, bottom panel). Therefore, these data suggest a delay and perhaps a reduction in the efficiency of recombination events.

The meiosis I defect in srb11 mutants is independent of RAD17 DNA damage or recombination or MAD2 spindle checkpoint pathways. The findings described above suggest that srb11 mutants exhibit a small delay in finishing recombination. Persistent recombination intermediates trigger a RAD17-dependent checkpoint pathway that leads to pachytene arrest (32), providing a possible explanation for the srb11 mutant meiotic delay. To test this possibility, the ability of srb11 mutants to undergo meiosis in a rad17 strain defective for the DNA damage checkpoint was examined. Isogenic strains were constructed with a deletion of rad17 and with or without the wild-type SRB11 allele. These strains were induced to enter meiosis, and samples were taken as described above. Deleting RAD17 alone (RSY503) continued to display the delayed kinetics (Fig. 3, top panel) and the aberrant production of binucleated cells (Fig. 3, bottom panel) observed for the srb11Δ single mutant. These results suggest that the meiotic defect observed in srb11 mutants is not due to the accumulation of recombination intermediates. In addition to the recombination checkpoint, Rad17p is involved in the general DNA damage response (57), suggesting that the srb11 mutant does not generate this type of damage. These experiments were repeated with the srb11 mad2 strain (RSY770) to determine whether srb11 mutants possess a problem with chromosome capture by the spindle. Like previous results, the mad2 mutation did not rescue the srb11Δ-induced delay, indicating that spindle checkpoint activation was also not involved (data not shown). These findings argue against the observed meiotic defect being due to accumulated cellular damage and suggest a more direct role for Srb11p in the regulation of meiotic development.

Srb11p is required for normal recombination during meiosis. Wild-type JX212 (SRB11) and mutant JX213 (srb11Δ) strains containing heteroalleles at leu1 were induced to enter meiosis. (Top) Three independent samples were taken at the indicated times following the shift and plated on medium to select for recombinants. Error bars indicate standard deviations. (Bottom) Overall cell viability was determined by plating the appropriate dilutions of the same samples on rich medium without selection.

FIG. 2. Srb11p is required for normal recombination during meiosis. Wild-type JX212 (SRB11) and mutant JX213 (srb11Δ) strains containing heteroalleles at leu1 were induced to enter meiosis. (Top) Three independent samples were taken at the indicated times following the shift and plated on medium to select for recombinants. Error bars indicate standard deviations. (Bottom) Overall cell viability was determined by plating the appropriate dilutions of the same samples on rich medium without selection.

FIG. 3. The srb11Δ meiotic defect is independent of checkpoint pathways. Wild-type strain RSY335 (SRB11), mutant strain RSY389 (srb11Δ), or double-mutant strain RSY304 (srb11Δ rad17Δ) was induced to enter meiosis, and samples were taken at the indicated times. The samples were fixed, stained with DAPI, and examined for the production of binucleated and tetranucleated cells. A total of 200 cells were counted for each time point. (Top) Total percentage of the population completing either meiotic division plotted against time in sporulation medium. (Bottom) Percentage of the population containing only binucleated cells.

Srb11p is required for normal meiotic transcription program. The results described above indicate that Srb11p is required for normal progression through meiosis I. Since Srb11p is involved in transcriptional regulation, we next examined the fate of the transcription program in wild-type and...
mutant cells. The mRNA accumulation profiles of several genes representing the various expression classes were examined. Specifically, **IME1**, the master regulatory switch for meiosis, is induced as the cell enters the program (24). Genes representative of the early (**IME2**) (45), early-middle (**NDT80**) (8), middle (**SPS2**) (39), and late (**SPS100**) (39) classes were also examined. RSY335 and RSY389 were induced to enter meiosis, and samples were taken at various times. Total RNA samples prepared from each time point were blotted and subjected to Northern analysis (see Materials and Methods for details).

![Image](https://example.com/fig4.png)

**FIG. 4.** Meiotic gene expression kinetics in the wild type and an **srb11Δ** mutant. Wild-type RSY335 (**SRB11**) and mutant RSY389 (**srb11Δ**) cultures were induced to enter meiosis, and samples were taken at the indicated times. Total RNA was prepared, and transcript levels for **IME1**, **IME2**, **NDT80**, **SPS2**, and **SPS100** were determined (see Materials and Methods for details). **ENO1** mRNA levels were used to standardize loadings.

Overexpression of **IME2** partially relieves the **srb11Δ** meiotic defect. **Ime2p** plays an important role in promoting early meiotic gene transcription, including its own (37). Therefore, the reduction of **IME2** mRNA levels may provide an explanation for the **srb11Δ** mutant phenotype. To determine whether increased **IME2** mRNA levels can compensate for the loss of **SRB11**, the **IME2** gene was placed in a high-copy-number plasmid (pMR1; see Materials and Methods for details). This plasmid and a vector control were introduced into the diploid **srb11Δ/H9004** mutant (**RSY389**), and the transformants were induced to enter meiosis. Northern blot analysis revealed that **IME2** mRNA was indeed elevated to levels equal to or greater than those observed when the gene is fully induced (data not shown). DAPI analysis revealed that the final percentages of cells undergoing at least one meiotic division were similar in the strains overexpressing **IME2** (83%) and the vector control (79%) (Table 3). Importantly, the percentages of **srb11Δ** cells that overexpressed **IME2** and that were able to undergo both divisions (percentages of tri- and tetranucleated cells) increased significantly compared to the results obtained with the vector control (62 versus 37%). These findings suggest that the **srb11Δ** meiotic defect may be due at least in part to reduced **Ime2p** activity. However, since the mutant phenotype was not completely rescued by **IME2** overexpression, **Srb11p** may also regulate meiosis through an **IME2**-independent pathway.

**Srb11p** is required for coupling bud growth with nuclear division in cells entering the meiotic program. During the studies described above, we observed that a significant percentage of **srb11Δ** cells maintained their buds throughout the sporulation time course. When transferred to sporulation medium, wild-type cells complete their current cell cycle and enter
TABLE 3. IME2 overexpression partially suppresses the srb11 meiotic defect

<table>
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<tr>
<th>Overexpression plasmid</th>
<th>% of cells that were</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mononucleated</td>
<td>Binucleated</td>
</tr>
<tr>
<td>SRB11</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Vector</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>IME2</td>
<td>17 ± 6</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

a RSY389 (srb11Δ) was transformed with the indicated plasmids: SRB11, pKC342 (10); vector, pRS425; pMR1, IME2 inserted into a 2 µm high-copy-number vector (see Materials and Methods for details). Transformants were subjected to a standard sporulation regimen in liquid medium. Cells were fixed and stained with DAPI 24 h after a shift to sporulation medium. Three independent cultures harboring pMR1 were assayed; data are means and standard deviations.

the meiotic program early in G1 (unbudded) (20). Accordingly, we found that the percentage of wild-type cells with buds decreased rapidly following transfer to sporulation medium (Fig. 1A; quantitated in Table 2). However, even after 36 h in sporulation medium, 20% of mutant cells still retained buds. Since the budding frequencies were similar in both strains during vegetative culturing, these results suggest that Srb11p is required for the normal completion of the last cell cycle prior to entry into the meiotic program.

Normally, bud development and nuclear division are coupled through the activity of the cyclin-Cdc28 protein kinase (reviewed in reference 28). Bud emergence occurs early in S phase, and bud growth continues throughout the remainder of the cell cycle. Nuclear division commences only when the bud reaches an appropriate size (reviewed in reference 19). Surprisingly, we found that many small buds which persisted in sporulation medium were equivalent in size to those indicative of S phase and contained a nucleus-sized DAPI-stained body (Fig. 1A). As indicated in the 24-h micrograph, the mother of the abnormal bud was still able to undergo meiosis, indicating that the cell was not significantly crippled by this characteristic. These findings suggest that nuclear division was not altered in the srb11 mutant but rather that bud growth was defective. Taken together, these findings indicate that nuclear division and bud growth have become uncoupled in the srb11 mutant.

Moreover, these cells (i.e., small buds with a nucleus) were not observed in vegetative cultures, suggesting that this phenotype is restricted to cells induced to enter meiosis. Srb11p is required for actin repolarization following exposure to sporulation medium. The above findings indicated that bud growth was defective in srb11 mutants only entering meiosis. Bud emergence and growth require actin polarization to efficiently deliver necessary cell wall components to the growing bud tip (1). To determine whether polarization was affected in srb11A strains, a fluorochrome-labeled phalloidin was used to monitor actin patch localization (see Materials and Methods). Patches represent actin and actin binding protein foci that associate with the membrane. They are dispersed in unbudded cells but congregate at the newly selected bud site and in the growing bud itself (reviewed in reference 33). For this analysis, the cells were divided into those containing small buds (≤10% the mother size) and large buds that had not undergone nuclear division (as determined by DAPI staining; data not shown). This distinction was made due to the observation that the actin cytoskeleton behaved in different ways in large and small buds in response to heat shock (13).

During vegetative growth, the wild type displayed actin patches localized to both small and large growing buds (0 h) (Fig. 5A, panels 1 and 2; quantitated in Fig. 5B). However, within 30 min following transfer to sporulation medium, significant actin depolarization was observed in both bud types (Fig. 5A, panels 5 and 6). Within 60 min, the wild type relocalized actin patches to the large buds (Fig. 5A, panel 10) but not the small buds (panel 9) (compare the small-budded cell on the left in panel 10 with the large-budded cell on the right). Small-budded cells began to regain oriented actin patches by 120 min (Fig. 5A, panel 13) and were back to nearly vegetative levels by 240 min (panel 17). It should be noted that the presence of small-budded cells in wild-type sporulation cultures after 4 h was very rare. These findings indicate that yeast cells depolarize their actin cytoskeleton in response to sporulation medium. Moreover, a difference in repolarization kinetics was observed between cells with large buds and those with small buds.

Like the wild-type strain, the srb11A mutant displayed localized actin patch patterns in both large- and small-budded vegetative cells (Fig. 5A, panels 3 and 4) that were rapidly lost upon exposure to sporulation medium (panels 7 and 8). Polarization returned to large-budded mutant cells with kinetics somewhat slower than those for the wild-type control (Fig. 5A, panels 12, 16, and 20). However, small buds failed to reestablish a substantial degree of polarized staining, even by 4 h (Fig. 5A, panels 11, 15, and 19) (see a direct comparison in panel 20). Further incubation (24 h) did not significantly alter these results, suggesting that this phenotype was terminal and not the result of slowed kinetics (data not shown). These findings indicate that Srb11p is required for the efficient recovery of polarized actin, at least in cells harboring small buds. Moreover, this observation, combined with the DAPI analysis, suggests that Srb11p couples bud growth to nuclear division, but only in cells switching from mitotic cell growth to meiotic development. These results define a new function for the C-type cyclin-Cdk kinase in regulating cell fate decisions (see Discussion).

DISCUSSION

The Ume3p/Srb11p-Ume5p/Srb10p kinase represses the transcription of several diverse gene sets, including those for sucrose utilization (SUC2) (26), the stress response (SSA1) (10), and meiosis (SPO13) (47). This study demonstrates a new requirement for Srb11p in normal entry into the meiotic program and the execution of meiosis I itself. Following exposure to sporulation medium, Srb11p is required for the completion of the last cell cycle prior to entry into meiosis. Specifically, Srb11p is important for reestablishing polarized actin necessary for the continued growth of new buds. Following entry into the meiotic program, Srb11p is also necessary for the efficient execution of meiosis I. A mutant lacking SRB11 either exhibits a delay in the execution of meiosis I or skips the event entirely. These phenotypes are independent of both the recombination and the spindle damage checkpoint pathways, arguing against the cause of the meiotic defect being cellular damage. Rather, a delay and a reduction in the mRNA levels of several
meiotic genes were observed, suggesting that the underlying defect lies in the transcription program. This model is supported by the result that the overexpression of IME2, a critical inducer of meiotic gene expression, can partially suppress the srb11 meiotic defect. Taken together, these findings reveal new roles for C-type cyclins in controlling entry into and execution of a developmental process.

This study presents evidence that srb11/H9004 mutants uncouple nuclear division from bud growth. Specifically, small buds stop growing, while nuclear division does not stop, resulting in a small bud containing a nucleus. Since small buds containing DAPI masses are not observed in vegetative cells, the requirement of Srb11p for this process appears to be restricted to cells that are switching from mitotic to meiotic cell division. Moreover, small buds fail to reassemble their actin cytoskeleton following transfer to sporulation medium, providing a likely mechanism for this phenotype. Why are small buds more likely to stop growing than large buds during the final cell cycle prior to meiosis? During vegetative growth, bud site selection occurs in G1, with emergence initiating as the cell enters S phase (reviewed in reference 28). Bud site selection and initial bud emergence require the G1 cyclin-Cdc28 kinase (29). Specifically, actin localization to the growing bud tip is dependent on Cln-Cdc28 activity. As the bud continues to grow, it shifts from apical to isotropic growth, which requires the B-type cyclins Clb1p and Clb2p. However, G1 cyclins antagonize meiotic induction by inhibiting Ime1p function (9). Therefore, the G1 cyclins are rapidly downregulated in cells exposed to sporulation conditions to promote meiotic induction. This system effectively excludes continuation of the mitotic cell cycle and funnels cells toward meiosis. However, the downregulation of the G1 cyclins may have an adverse effect on cells harboring recently emerged buds, since actin disorganization accompanies the shift to sporulation medium. One model consistent with our data is that the Srb11-Srb10p kinase fills this gap by providing Cdk activity, which allows the continuation of bud growth until B-type cyclin-Cdc28 activity takes over. It is interesting that the large-budded cells, which no longer required Cln-Cdc28 activity, although delayed, were able to reorganize their actin in the srb11 mutant strain. This strategy would allow efficient entry into meiosis without interference from Cln-Cdc28 or sacrifice of newly born buds.

This report demonstrates that exposure to sporulation medium results in a transient depolarization of actin fibers. Similarly, vegetative cells exposed to heat shock undergo a transient depolymerization of actin that halts bud growth (13). As the cells adapt to the higher temperature, polarized actin fibers return and cell division resumes. These findings suggest that the cell may view exposure to sporulation medium as a "stress response." Does Srb11-Srb10p also control actin polarization...
in vegetative cells exposed to stress? Delley and Hall showed that actin polarization returned to cells approximately 120 min following heat shock (13). Previous studies from our laboratory found that Srb11p is destroyed in response to several types of stress, including heat shock (10, 11). With heat shock, the reduction in Srb11p levels is also transient. Within 10 min following heat shock at 37°C, Srb11p levels are reduced to below the limits of detection, but they return to pre-heat shock concentrations within 120 min. Therefore, the kinetics of Srb11p destruction and accumulation mirror those reported for depolarizing-rewarping actin filaments. These findings are consistent with Srb11p also playing a role in actin reorganization during vegetative growth. Preliminary experiments do suggest a role for Srb11p in this process, as mutant strains displayed a 20% delay in actin reorganization kinetics compared to the results for the wild type following exposure to heat shock (L. H. Rutkowski and R. Strich, unpublished results). However, if Srb11p-Srb10p does indeed control actin reorganization following heat shock, its role is modest, and additional regulatory factors must be involved.

The most pronounced phenotype associated with the loss of Srb11p activity is the 10-fold increase in dyad formation, with these cells predominantly skipping meiosis I. How does Srb11p-Srb10p regulate meiosis I? A possible answer to this question was provided by the findings that the mRNA levels of many meiotic genes were reduced and that their peak accumulation was delayed in the srb11 mutant. The reduction or delay in the expression of key meiotic genes required for meiosis I may lead to the exclusive execution of meiosis II by cells. To test this possibility, we monitored meiotic S phase by 15N incorporation of S phase by 

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mRNA levels in the srb11 mutant and the ability of IME2 overexpression to partially suppress the meiotic defect. This possibility is currently under investigation. Since Srb11p degradation begins at approximately the time when Ime2p accumulation peaks, it is possible that the combination of these two events leads to the full induction of SPO13 and the rest of the early genes. This model would also predict that the downregulation of Srb11p provides a mechanism to attenuate early meiotic gene expression by reducing Ime2p-dependent induction of its own expression. This mechanism could be important to allow the normal transition from the first meiotic prophase to the later stages of meiotic development.

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