A Role for the Swe1 Checkpoint Kinase During Filamentous Growth of *Saccharomyces cerevisiae*

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Manuscript received November 29, 2000
Accepted for publication February 21, 2001

ABSTRACT

In this study we show that inactivation of Hsl1 or Hsl7, negative regulators of the Swe1 kinase, enhances the invasive behavior of haploid and diploid cells. The enhancement of filamentous growth caused by inactivation of both genes is mediated via the Swe1 protein kinase. Whereas Swe1 contributes noticeably to the effectiveness of haploid invasive growth under all conditions tested, its contribution to pseudohyphal growth is limited to the morphological response under standard assay conditions. However, Swe1 is essential for pseudohyphal differentiation under a number of nonstandard assay conditions including altered temperature and increased nitrogen. Swe1 is also required for pseudohyphal growth in the absence of Tec1 and for the induction of filamentation by butanol, a related phenomenon. Although inactivation of Hsl1 is sufficient to suppress the defect in filamentous growth caused by inactivation of Tec1 or Flo8, it is insufficient to promote filamentous growth in the absence of both factors. Moreover, inactivation of Hsl1 will not bypass the requirement for nitrogen starvation or growth on solid medium for pseudohyphal differentiation. We conclude that the Swe1 kinase modulates filamentous development under a broad spectrum of conditions and that its role is partially redundant with the Tec1 and Flo8 transcription factors.

ORGANISMS are able to respond to changes in environmental conditions via a broad spectrum of cellular responses. The nature and magnitude of those responses differ depending upon the type of cell and the degree to which it must individually cope with those changes. The demands upon free-living unicellular organisms to adapt to environmental change are greater than those of metazoan cells, which may be buffered by homeostatic mechanisms provided by the organism as a whole. Fungi, as free-living organisms, have developed the flexibility to deal with substantial changes in their environment including the quantity, quality, and location of nutrients. The capacity of many species of fungi to switch between a cellular yeast form and filamentous forms in response to environmental changes is one example of that flexibility that is well documented (reviewed in Madhani and Fink 1998 and Borges-Walmsley and Walmsley 2000). In some fungal pathogens of plants and animals the ability to switch between the two forms is thought to be an important determinant of pathogenicity (reviewed in Madhani and Fink 1998 and Borges-Walmsley and Walmsley 2000). In the budding yeast *Saccharomyces cerevisiae* the differentiation from the yeast form to the filamentous form is thought to allow otherwise sessile cells to forage for nutrients (Gimeno *et al.* 1992).

Although most laboratory strains of *S. cerevisiae* are incapable of filamentous differentiation (Liu *et al.* 1996), invasive growth has now been documented in many wild strains and some laboratory strains. The differentiated state is known as haploid invasive growth in haploid cells (Roberts and Fink 1994) and as pseudohyphal growth in diploid cells (Gimeno *et al.* 1992). Despite the significant differences between both the nature of the invasive forms and the environmental stimuli that lead to their differentiation, haploid and diploid states share significant similarities. The differentiation of both types of invasive cells apparently involves the activation of a number of signaling pathways. Among those, the mitogen-activated protein kinase (MAPK) signaling pathway acting through the Tec1/Ste12 transcription factor and the cyclic-AMP/protein kinase A (cAMP/PKA) pathway acting through the Flo8 transcription factor are the best characterized (reviewed in Madhani and Fink 1998 and Borges-Walmsley and Walmsley 2000). There is, at least, partial overlap in the targets of the two transcription factors (Lo and Dranginis 1998; Madhani *et al.* 1999; Pan and Heitman 1999; Rupp *et al.* 1999).

A number of features of the cellular responses of haploid and diploid cells to filamentous differentiation signals are similar, although manifested to a different extent by each cell type (Gimeno *et al.* 1992; Roberts and Fink 1994). One of the most obvious differences between filamentous cells and the equivalent yeast form

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cells is in cell morphology. First, cells adopt a unipolar budding pattern characterized by the emergence of a new bud predominantly at one end of the cell. In daughter cells the new bud site is distal from the site of cytokinesis whereas in mothers that site is adjacent to the prior bud site. Furthermore, invasive cells adopt an elongated morphology. Finally, abscission of daughter cells from mothers is either delayed or suppressed. Together these changes lead to the formation of the filaments of cells that are characteristic of invasive development. Despite rather extensive characterization of these phenotypes, the extent to which these phenotypic changes affect the capacity of cells to invade solid medium is not entirely clear.

The differentiation of invasive cells is thought to involve significant alteration of cell cycle dynamics (Kron *et al.* 1994). However, much of the evidence for alteration of the cell cycle derives from studies of populations of cells that have been induced to acquire the invasive phenotype via mutations that activate one of the pathways thought to transduce the differentiation signal. When the transcription factor, *PHD1*, is ectopically expressed, it induces pseudohyphal differentiation (Gimeno and Fink 1994) and an associated shortening of G1 phase and elongation of G2 phase. Furthermore, enhancing the activity of G1 cyclins (*CLN1* and *CLN2*) or decreasing the activity of mitotic cyclins (*CLB1* and *CLB2*) via ectopic expression or mutation leads to similar changes in cell cycle dynamics as well as a number of morphological changes similar to those observed in pseudohyphal growth (Kron *et al.* 1994; Lew *et al.* 1997; Ahn *et al.* 1999; Edgington *et al.* 1999; Loeb *et al.* 1999b). Finally, single amino acid changes in Cdc28 itself can lead to all of the aforementioned morphogenetic changes (Edgington *et al.* 1999). Taken together these observations suggest that many and perhaps all of the changes in cell morphology and budding pattern associated with invasive growth are a consequence of changes in the activity of the Cdc28 cyclin-dependent kinase (CDK).

The morphogenetic changes associated with invasive growth are most consistent with hyperaccumulation of G1 cyclins and/or delayed activation of mitotic forms of the CDK. Although there is little reason *a priori* to exclude other mechanisms, inhibitory phosphorylation has received the most attention and is, therefore, the mechanism for which there is the best experimental understanding. Manipulation of Swe1, the budding yeast homolog of the fission yeast Wee1 protein kinase that catalyzes inhibitory tyrosine phosphorylation of Clb-associated forms of the Cdc28 CDK (Booher *et al.* 1993), is normally activated in response to morphogenetic aberrations (Lew and Reed 1995; Sia *et al.* 1996). Its activity leads to a delayed mitotic progression resulting in elongation of G2 phase and to the acquisition of hyperpolarized cell morphology. Indeed, ectopic activation of Swe1 either by overexpression or by inactivation of its negative regulators, Hsl1 and Hsl7, has been shown to promote morphological changes reminiscent of invasive cells (Ma *et al.* 1996; Barral *et al.* 1999; Edgington *et al.* 1999; McMillan *et al.* 1999a; Shulewitz *et al.* 1999). Furthermore, inactivation of Hsl1 has been shown to promote invasive growth in a noninvasive yeast strain (Edgington *et al.* 1999). Finally, Hsl7 has been proposed to repress pseudohyphal growth via inhibition of the Ste20 MAPK kinase (Fujita *et al.* 1999). On the other hand, inactivation of Swe1 has been shown to have different effects on the invasive properties of haploid and diploid strains. Inactivation of *SWE1* inhibits haploid invasive growth (Edgington *et al.* 1999), whereas it has no effect on pseudohyphal differentiation in diploid cells (Kron *et al.* 1994; Ahn *et al.* 1999).

We have reexamined the role of Swe1 and its regulators in filamentous differentiation using the well-characterized invasive strain of *S. cerevisiae*, Σ1278b (Gimeno *et al.* 1992; Grenson *et al.* 1966). We have extended previous observations (Edgington *et al.* 1999) by demonstrating that inactivation of either Hsl1 or Hsl7 enhances the invasive behavior of both haploid and diploid cells and that the enhancement is mediated via the Swe1 protein kinase. Whereas Swe1 contributes noticeably to the effectiveness of haploid invasive growth under all conditions tested, its contribution to pseudohyphal growth is limited to morphological differentiation under standard assay conditions consistent with the recent report of Ahn and colleagues (Ahn *et al.* 1999). However, we find that Swe1 is essential for all aspects of pseudohyphal differentiation under a number of nonstandard assay conditions as well as in the absence of Tec1. Moreover, Swe1 is required for the induction of filamentation of both haploid and diploid cells promoted by butanol, a recently described phenomenon related to pseudohyphal development (Lorenz *et al.* 2000). Finally, inactivation of Hsl1 is insufficient to promote filamentous growth in the absence of both Tec1 and Flo8 or to bypass the requirement for nitrogen starvation or growth on solid medium for pseudohyphal differentiation. We conclude that Swe1 modulates the effectiveness of invasive growth under a broad spectrum of conditions.

MATERIALS AND METHODS

Yeast media: Growth, manipulation, and construction of yeast strains was performed by standard procedures unless otherwise indicated. Synthetic low ammonia dextrose plate (SLAD) contained 50 μm ammonium sulfate, 6.8 g/liter yeast nitrogen base without amino acids or ammonium sulfate, 2% dextrose, and 2% washed agar (Gimeno *et al.* 1992). Synthetic low ammonia raffinose-galactose plates were prepared with 1% raffinose and 1% galactose instead of 2% dextrose as carbon source. Synthetic leucine dextrose plate and synthetic proline dextrose plate were prepared with 0.75 mg/ml leucine and 0.1 mg/ml proline, respectively, as the only nitrogen source. Agar was washed five times as 2% wt/vol suspension with deionized water for 30 min each wash. After the final

TABLE 1

***S. cerevisiae* strains used in this study[a]**

Strains	Genotype	Source and reference
L5528	*MAT***a** *ura3-52 his3::hisG*	Roberts and Fink (1994)
RLVsc68	*MAT***a** *ura3-52 his3::hisG hsl1::URA3*	This study
RLVsc69	*MAT***a** *ura3-52 his3::hisG tec1::Kan^r*	This study
RLVsc70	*MAT***a** *ura3-52 his3::hisG flo8::hisGURA3Kan^{r}hisG*	This study
RLVsc166	*MAT***a** *ura3-52 leu2::hisG flo8::hisGURA3Kan^{r}hisG hsl1::URA3*	This study
RLVsc227	*MAT***a** *ura3-52leu2::hisG tec1::Kan^r hsl1::URA3*	This study
RLVsc361	*MAT***a** *ura3-52 tec1::Kan^r swe1::URA3 hsl1::URA3*	This study
RLVsc382	*MAT***a** *ura3-52 flo8::hisGURA3Kan^{r}hisG tec1::Kan^r hsl1::URA3*	This study
RLVsc176	*MAT***a** *ura3-52 his3::hisG tec1::Kan^r swe1::URA3*	This study
L5487	*MAT*α *ura3-52 leu2::hisG*	Roberts and Fink (1994)
RLVsc46	*MAT*α *ura3-52 leu2::hisG hsl1::URA3*	This study
RLVsc47	*MAT*α *ura3-52 leu2::hisG swe1::URA3*	This study
RLVsc190	*MAT*α *ura3-52 leu2::hisG hsl7::URA3*	This study
RLVsc274	*MAT*α *ura3-52 his3::hisG hsl1::URA3 hsl7::URA3 swe1::URA3*	This study
RLVsc275	*MAT*α *ura3-52 hsl1::LEU2 hsl7::URA3*	This study
RLVsc258	*MAT*α *ura3-52 hsl7::URA3 swe1::LEU2*	This study
RLVsc116	*MAT*α *ura3-52 leu2::hisG hsl1::URA3 swe1::URA3*	This study
RLVsc291	*MAT***a**/α *ura3-52/ura3-52 (Ycplac33)*	This study
RLVsc63	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 his3::hisG/HIS3 hsl1::URA3/hsl1::URA3*	This study
RLVsc182	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 his3::hisG/HIS3 hsl7::URA3/hsl7::URA3*	This study
RLVsc284	*MAT***a**/α *ura3-52/ura3-52 hsl1::LEU2/hsl1::LEU2 hsl7::URA3/hsl7::URA3*	This study
RLVsc64	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 his3::hisG/HIS3 swe1::URA3/swe1::URA3*	This study
RLVsc297	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/leu2::hisG hsl1::URA3/hsl1::URA3swe1::URA3/swe1::URA3*	This study
RLVsc285	*MAT***a**/α *ura3-52/ura3-52 hsl7::URA3/hsl7::URA3 swe1::LEU2/swe1::LEU2*	This study
RLVsc296	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 his3::hisG/HIS3 hsl7::URA3/hsl7::URA3hsl1::URA3/hsl1::URA3swe1::URA3/swe1::URA3*	This study
RLVsc294	*MAT***a**/α *ura3-52/ura3-52 his3::hisG/HIS3 tec1::Kan^r/tec1::Kan^r (Ycplac33)*	This study
RLVsc66	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 his3::hisG/HIS3 flo8::hisGURA3Kan^{r}hisG/flo8::hisGURA3Kan^{r}hisG*	This study
RLVsc295	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 flo8::hisGURA3 Kan^{r}hisG/flo8::hisGURA3Kan^{r}hisG hsl1::URA3/hsl1::URA3*	This study
RLVsc286	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 tec1::Kan^r/tec1::Kan^r hsl1::URA3/hsl1::URA3*	This study
RLVsc392	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2 flo8::hisGURA3 Kan^{r}hisG/flo8::hisGURA3Kan^{r}hisG hsl1::URA3/hsl1::URA3 tec1::Kan^r/tec1::Kan^r*	This study
RLVsc323	*MAT***a**/α *ura3-52/ura3-52 leu2::hisG/LEU2his3::hisG/his3::hisG tec1::Kan^r/tec1::Kan^r swe1::URA3/swe1::URA3 (pRS413)*	This study

[a] All strains are in the Σ1278b background (Grenson *et al.* 1966).

wash, it was sterilized by autoclaving at 4% wt/vol in deionized water and diluting to 2% final concentration with 2× liquid media filter sterilized. Yeast extract, peptone, yeast nitrogen base are from Difco (Detroit), and agar is from Angus. Other reagents were obtained from Sigma (St. Louis).

Yeast strains and plasmids: All yeast strains were derived in the Σ1278b background (Grenson *et al.* 1966; Gimeno *et al.* 1992). Yeast strains were as described in Table 1. Standard genetic methods were used for genetic manipulation of *S. cerevisiae*. Marker segregation or PCR assay determined genotypes. Strain RLVsc69 was constructed by using a PCR-mediated disruption method with the use of the G418 resistance cassette of plasmid pFA6-kanMX2 (Wach *et al.* 1994). Plasmids are described in Table 2.

Filamentous assay conditions: The Σ1278b yeast strain is the prototypical strain for analysis of both haploid invasive and pseudohyphal growth (Gimeno *et al.* 1992). We refer to both of these responses using the general descriptions, filamentous or invasive growth.

For haploid invasive growth, the strains were patched on

TABLE 2

Plasmid list

Plasmid	Description	Source or reference
pRS413	*CEN HIS3*	Sikorski and Hieter (1989)
YcpLAC33	*CEN URA3*	Geitz and Sugino (1988)
pHL129	*flo8::hisG::URA3::Kan^{r}hisG*	Liu *et al.* (1996)
pNE39	*hsl1::LEU2*	Edgington *et al.* (1999)
pNE38	*hsl1::URA3*	A. Myers, unpublished data
pswe1::URA3	*swe1::URA3*	Edgington *et al.* (1999)
pSWE1-10g	*swe1::LEU2*	Booher *et al.* (1993)
phs17::URA3	*hsl7::URA3*	Ma *et al.* (1996)

YEPD and pregrown at 30° for 2 days. Cells from these plates were plated in small patches on YEPD, YEP-GAL, or SC plates using a round glass rod to avoid disturbing the surface of the agar and incubated for 2 days at 30°. Haploid invasive growth was evaluated based upon three criteria. First, we analyzed the capacity of cells to resist removal from the surface of the plate under a stream of water (water washed). Next, the ability of cells to invade the agar was evaluated following "scrubbing" the surface of the plate with a gloved finger while washing under a stream of water (mechanically washed). Third, the cells remaining in the plate after mechanical washing were subjected to microscopic examination (invasive growth). Although these are qualitative assays, together they provide a number of criteria to judge the capacity of cells to differentiate. These include the ability of the cells to penetrate the agar, changes in cell morphology, and conversion of the budding pattern of cells from axial to unipolar budding.

For pseudohyphal development, strains were pregrown for 2 days on SD medium at 30° and then transferred to SLAD or modified SLAD plates, as indicated. To avoid disturbing the agar surface and the colony density-dependent inhibition of pseudohyphae formation (Wright *et al.* 1993), single unbudded cells were carefully placed at 1 cm from each other by using the needle of a dissecting microscope. A total of 20–100 colonies, each derived from a single unbudded cell, were analyzed for each strain. The pictures shown are of representative colonies. Pseudohyphal growth was evaluated by multiple criteria. First, colony and cell morphology was monitored after one day of growth on SLAD plates (an assay of the extent of early morphological differentiation). The length/width ratio of cells forming filaments was evaluated after 1 day of growth on SLAD at 30° by measuring both dimensions of cells in imaged filaments. Second, cell shape and budding pattern were evaluated as described by Mosch and Fink (1997), using cells that were washed off plates and analyzed by microscopic examination. Third, the cell and colony morphology was evaluated after 5 days of growth on SLAD before (total growth) and after mechanically washing the noninvasive cells from the plate surface (invasive growth). Butanol-induced filamentous differentiation was evaluated as previously described (Lorenz *et al.* 2000).

Microscopy and imaging: Microcolonies and colonies growing on plates were imaged from below through the agar and plastic petri dish with a Nikon Labophot microscope. Pixera VCS image-acquisition software and Pixera charge-coupled device camera were used to capture images at 1280 × 1024 resolution. Images were copied in Adobe Photoshop converted to gray scale, enhanced in contrast, and filtered to remove noise. Cells from suspension cultures were imaged with a Nikon Eclipse E800 microscope by using IPLAB Spectrum software and a Photmometrix Quantix camera. Images were flattened and copied into Photoshop. The size of the final images was reduced and then the cropped images were assembled into figures using Canvas 6 (Deneba).

RESULTS

Inactivation of Hsl1 and Hsl7 enhances filamentous differentiation via activation of the Swe1 protein kinase: The similarity between the morphological phenotype of filamentous cells and that resulting from a decrease in the activity of mitotic forms of CDK has been well documented (Kron *et al.* 1994; Lew *et al.* 1997; Ahn *et al.* 1999; Edgington *et al.* 1999; Loeb *et al.* 1999b). Furthermore, mutations that reduce the activity of Cdc28 or enhance the activity of Swe1, a CDK inhibitory kinase, have been shown to promote haploid invasive growth in a laboratory yeast strain that is only modestly invasive (Edgington *et al.* 1999). This prompted us to assess the importance of Swe1 activity for pseudohyphal and haploid invasive growth using the well-characterized invasive yeast strain, Σ1278b (Gimeno *et al.* 1992).

Diploid cells carrying disrupted alleles of either *HSL1* or *HSL7*, which encode negative regulators of Swe1 (Ma *et al.* 1996; Barral *et al.* 1999; Edgington *et al.* 1999; McMillan *et al.* 1999a; Shulewitz *et al.* 1999), or carrying disrupted alleles of both genes, were constructed and their behavior in pseudohyphal growth assays was examined (Figure 1A). Early in the process of pseudohyphal development on SLAD plates the *hsl1*Δ/*hsl1*Δ, *hsl7*Δ/*hsl7*Δ, and *hsl1*Δ/*hsl1*Δ *hsl7*Δ/*hsl7*Δ diploid cells were substantially more filamentous and exhibited more robust pseudohyphal differentiation than wild-type cells grown on the same medium (Figures 1A and 2C; Table 3). This is an adaptive response as demonstrated by the analysis of cell shape and budding pattern of the same cells in liquid YEPD (Table 3). We conclude that the *hsl1*Δ and *hsl7*Δ mutations enhance not only the cellular phenotype of the filamentous cells but also the penetrance of that phenotype in both haploid and diploid cells. Moreover, the two mutations do not have additive effects consistent with the epistasis analysis of these mutations in vegetative cells, indicating that *HSL1* and *HSL7* lie in the same pathway (McMillan *et al.* 1999a;

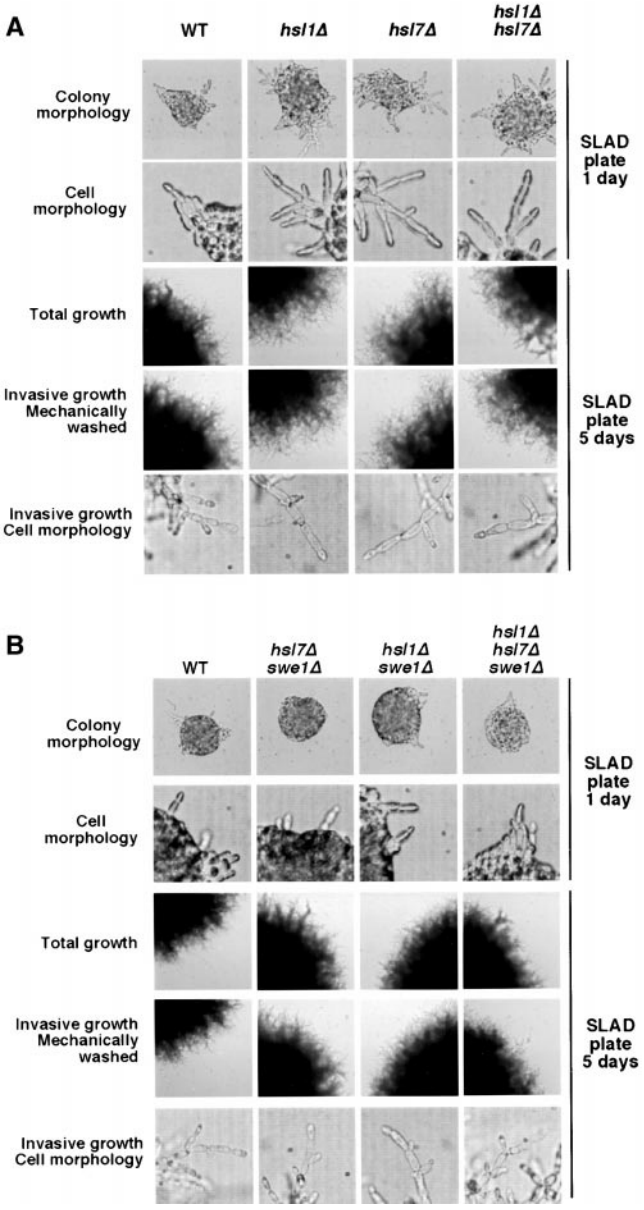


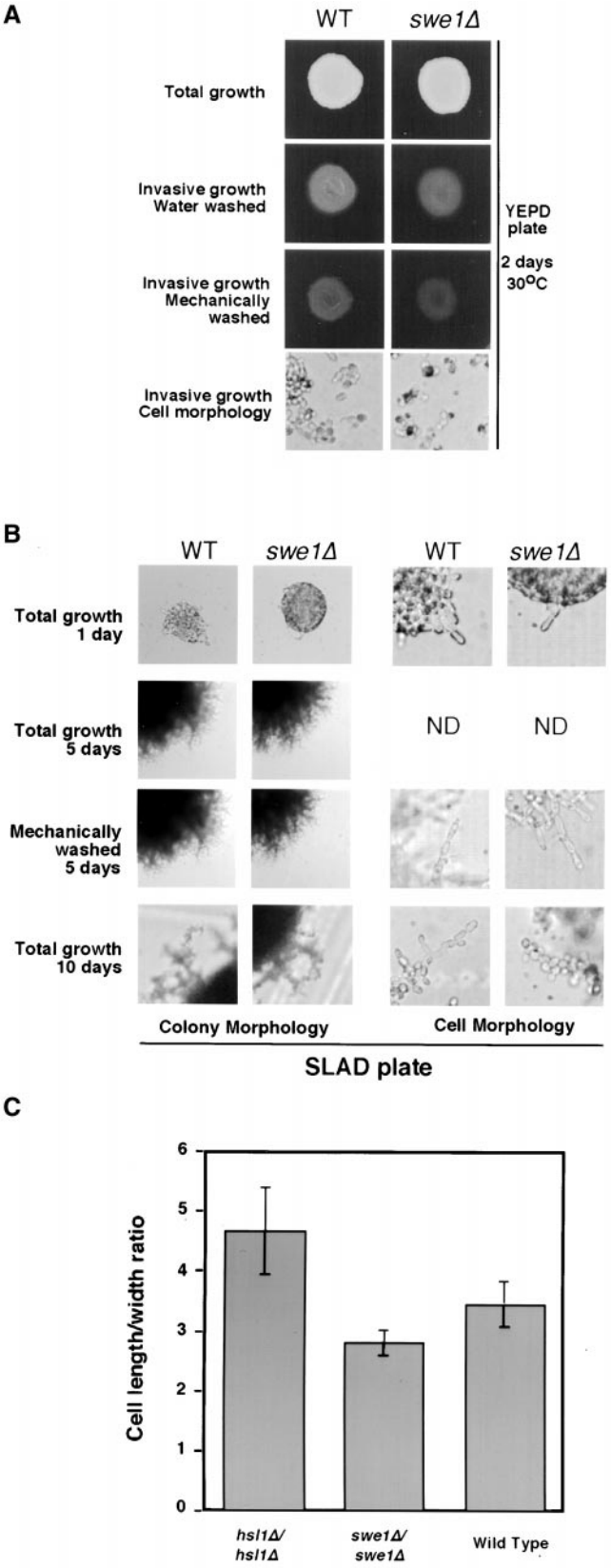
FIGURE 1.—Inactivation of *HSL1* and *HSL7* enhances filamentous growth via a *SWE1*-dependent pathway. (A) Enhancement of pseudohyphal growth by inactivation of *HSL1* and *HSL7*. Cell and colony morphology of the indicated strains is shown after 1 and 5 days of growth on a SLAD plate at 30° before and after mechanical washing of the plate to remove noninvasive cells. Wild-type (WT, RLVsc291), *hsl1*Δ/*hsl*Δ (RLVsc63), *hsl7*Δ/*hsl7*Δ (RLVsc182), and *hsl1*Δ/*hsl1*Δ *hsl7*Δ/hsl7Δ (RLVsc284) strains are shown. (B) Inactivation of SWE1 suppresses the enhancement of pseudohyphal growth caused by *hsl1*Δ and *hsl7*Δ. Pseudohyphal growth of wild-type (RLVsc291), *hsl1*Δ/*hsl1*Δ *swe1*Δ/*swe1*Δ (RLVsc297), *hsl7*Δ/*hsl7*Δ *swe1*Δ/*swe1*Δ (RLVsc285) and *hsl1*Δ/*hsl1*Δ *hsl7*Δ/*hsl7*Δ *swe1*Δ/*swe1*Δ (RLVsc296) strains.

SHULEWITZ *et al.* 1999). We obtained similar results with haploid mutant strains; however, the enhancement of filamentous differentiation was less dramatic than that caused by the same mutations in diploid strains (data not shown).

Hsl1 and Hsl7 are thought to act as negative regulators of Swe1 by virtue of their ability to recruit Swe1 to the bud neck and regulate its phosphorylation and subsequent degradation (MCMILLAN *et al.* 1999a; SHULEWITZ *et al.* 1999). The finding that inactivation of negative regulators of Swe1 enhances filamentous growth suggested to us that either these regulators or Swe1 might be a target of the filamentous growth signaling pathway. We asked whether the enhancement of haploid invasive and pseudohyphal growth observed in the *hsl1*Δ and *hsl7*Δ mutants was mediated via *SWE1*. To do so, homozygous diploid *hsl1*Δ *swe1*Δ, *hsl7*Δ *swe1*Δ, and *hsl1*Δ *hsl7*Δ *swe1*Δ strains were compared to the wild-type strain in pseudohyphal growth assay (Figure 1B). The enhancement of filamentous differentiation resulting from inactivation of *HSL1*, *HSL7*, or both was suppressed by inactivation of Swe1. The *hsl1*Δ *swe1*Δ, *hsl7*Δ *swe1*Δ, and *hsl1*Δ *hsl7*Δ *swe1*Δ mutants all exhibited a substantial reduction in cell elongation, extent of unipolar budding, and filamentation relative to the same strains having wild-type *SWE1* (Figure 1 and data not shown). However, despite the fact that inactivation of *SWE1* suppressed the enhancement of filamentation caused by the *hsl1*Δ and the *hsl7*Δ mutations, the capacity of homozygous diploid *hsl1*Δ *swe1*Δ, *hsl7*Δ *swe1*Δ, and *hsl1*Δ *hsl7*Δ *swe1*Δ strains to undergo pseudohyphal differentiation appeared to be unaffected relative to the wild-type strain (Figure 1B). We have shown that *swe1*Δ suppresses the enhancement of haploid invasive growth observed in *hsl1*Δ, *hsl7*Δ, and *hsl1*Δ *hsl7*Δ mutants (data not shown). Thus, the enhancement of both forms of invasive growth by inactivation of *HSL1* and/or *HSL7* appears to be a consequence of hyperactivation of *SWE1*.

Swe1 contributes to filamentous growth under standard assay conditions: The finding that inactivation of *SWE1* blocked the enhancement of pseudohyphal growth caused by inactivation of *HSL1* or *HSL7* without diminishing that capacity relative to wild-type cells suggested that *SWE1* is not required for pseudohyphal differentiation. Although a significant effect of *swe1*Δ on the haploid invasive behavior of wild-type cells in the D273 background was reported by EDGINGTON *et al.* (1999), those conclusions were based on microscopic examination since a relatively small number of those cells remain in the agar. Thus, the effect of inactivation of *SWE1* on filamentous behavior of the more invasive Σ1278b background warranted further examination. We evaluated the behavior of haploid and diploid *swe1*Δ mutants in the haploid invasive and pseudohyphal growth assays. *swe1*Δ mutants show a modest but reproducible reduction in the effectiveness of haploid invasive growth based upon visual examination (Figure 2A). Consistent with previous results (EDGINGTON *et al.* 1999), the cells are more rounded and a smaller proportion of the cells remain in the agar following gentle or mechanical washing. In contrast, diploid wild-type and *swe1*Δ/*swe1*Δ strains appear equally invasive after 5 and 10 days of

growth (Figure 2B), consistent with the findings of Ahn *et al.* (1999). However, there is a noticeable and reproducible difference in the extent of cell elongation and unipolar budding in the *swe1*Δ/*swe1*Δ cells as compared to the wild-type control (Table 3; Figure 2C). The length to width ratio of *swe1*Δ/*swe1*Δ cells forming filaments on SLAD plate is lower than that of wild-type cells. Moreover, *swe1*Δ/*swe1*Δ cells growing on SLAD exhibit less cell elongation and a lower frequency of unipolar budding in response to the pseudohyphal growth signals (nitrogen starvation and plate contact; Table 3). We conclude that *swe1*Δ is required for a full morphological response but not for substrate invasion during pseudohyphal growth and contributes to all aspects of haploid invasive growth under standard assay conditions.



Relationship between Swe1 and the MAPK and cAMP/PKA pathways: Inactivation of *SWE1* suppresses the strong enhancement of filamentous differentiation caused by *hsl1*Δ and *hsl7*Δ mutations and reduces the extent of morphological differentiation of otherwise wild-type pseudohyphal cells but does not appear to play a fundamental role in their invasive properties. There are two possible explanations for these results: either activation of Swe1 enhances the extent of filamentous differentiation via a pathway that is distinct from that used by wild-type cells or Swe1 activation modulates a pathway normally used to promote filamentous differentiation. To distinguish between these possibilities we examined the relationship between Swe1 and the signal transduction pathways involved in filamentous growth.

The cAMP and the MAPK pathways are known to play roles in transduction of the signals leading to differentiation of both pseudohyphal and haploid invasive cells (reviewed by Madhani and Fink 1998 and Borges-Walmsley and Walmsley 2000). Inactivation of *HSL1* and/or *HSL7*, presumed negative regulators of *SWE1*, stimulates both types of filamentous growth, presumably through repression of mitotic forms of the CDK (the targets of Swe1). We, therefore, asked whether inactivation of *HSL1* or *HSL7* was sufficient for filamentous differentiation or whether its activity simply modulates the effects of those pathways. The genes encoding the Flo8 or Tec1 transcription factors, downstream targets of the cAMP and MAPK pathways (Gavrias *et al.* 1996; Mosch and Fink 1997; Lorenz and Heitman 1998;

Figure 2.—The role of *SWE1* during filamentous development. (A) Swe1 modulates the extent of haploid invasive growth. Invasive growth assay of wild-type (L5487) and *swe1*Δ (RLVsc47) strains. Yeast strains were pregrown on YEPD plates and patched onto fresh YEPD plates. Patches were photographed before and after water or mechanical washing to evaluate the extent of invasion and morphological changes following 2 days of incubation at 30°. (B) *SWE1* is dispensable for the invasiveness of diploid strains undergoing pseudohyphal differentiation. Pseudohyphal growth of wild-type (RLVsc291) and *swe1*Δ/*swe1*Δ (RLVsc64) strains. (C) SWE1 is required for cell elongation during pseudohyphal differentiation. The ratio of length to width of cells forming filaments was evaluated as described in materials and methods.

TABLE 3

Analysis of morphological differentiation of diploid cells under inducing and noninducing conditions for pseudohyphal growth

Growth medium	Genotype	% of cells exhibiting cell shape			% of cells exhibiting budding pattern	
		Long PH	Oval YF	Round YF	Bipolar	Unipolar
SLAD	Wild type	23	64	13	59	41
plate	*hsl1*Δ/*hsl1*Δ	38	58	4	51	49
	*swe1*Δ/*swe1*Δ	15	52	33	74	26
YEPD	Wild type	2	44	54	76	24
liquid	*hsl1*Δ/*hsl1*Δ	5	48	47	73	27
	*swe1*Δ/*swe1*Δ	0	40	60	79	21

Rupp *et al.* 1999), respectively, were individually inactivated in either wild-type or *hsl1*Δ-deficient strains and then the resulting strains were evaluated for their capacity to undergo filamentous differentiation (Figure 3). As previously reported, inactivation of either *FLO8* or *TEC1* resulted in a loss or substantial reduction in the capacity of both haploid and diploid cells to grow invasively (Gavrias *et al.* 1996; Liu *et al.* 1996; Mosch and Fink 1997; Lorenz and Heitman 1998). Furthermore, either the *tec1*Δ or the *flo8*Δ mutation results in a substantial reduction in the invasiveness of haploid *hsl1*Δ strains (Figure 3A). However, despite the comparable effect of those mutations on invasiveness, there was a noticeable difference in the effect of the *hsl1*Δ mutation on haploid *flo8*Δ and *tec1*Δ strains (Figure 3A). Inactivation of *FLO8* had little effect on the morphology of haploid *hsl1*Δ mutants growing on solid rich media whereas inactivation of *TEC1* strongly suppressed the morphological response of the *hsl1*Δ cells.

Inactivation of *HSL1* or *HSL7* was much more effective in suppressing the defect in pseudohyphal differentiation resulting from inactivation of either *TEC1* or *FLO8* than it was in suppressing the defect in haploid invasive growth caused by the same mutations (Figure 3B). Whereas both *tec1*Δ/*tec1*Δ strains and *flo8*Δ/*flo8*Δ strains exhibited a substantial defect in the capacity to grow invasively (Gavrias *et al.* 1996; Liu *et al.* 1996; Mosch and Fink 1997; Lorenz and Heitman 1998), the same cells exhibited near-wild-type levels of pseudohyphal differentiation when *HSL1* was also inactivated. This was most dramatically illustrated by comparison of the *flo8*Δ/*flo8*Δ mutants to the *hsl1*Δ/*hsl1*Δ *flo8*Δ/*flo8*Δ mutants. Whereas the former shows neither morphological differentiation nor invasive growth, the latter is both highly elongated and highly invasive (Figure 3B). In contrast to the *flo8*Δ/*flo8*Δ mutants, the *tec1*Δ/*tec1*Δ strain retained some capacity to invade the agar and form filaments (Mosch and Fink 1997; Lorenz and Heitman 1998). However, invasive cells were apparent only after the plate was washed (there were no invasive cells on the periphery of the colony). Furthermore, the extent of morphological differentiation and the frequency of invasive cells were significantly reduced relative to that observed with wild-type cells. In contrast, inactivation of *HSL1* in a *tec1*Δ mutant suppressed both the morphological defect and the inability to invade (Figure 3B). Finally, the suppression of the filamentous growth defect of both haploid and diploid *tec1*Δ and *flo8*Δ strains caused by inactivation of *HSL1* is dependent upon *SWE1* (data not shown).

We have shown that the effect of inactivation of *HSL1* upon both haploid invasive growth and pseudohyphal development is dependent upon *SWE1* (Figure 1). Furthermore, it is sufficient to bypass the requirement for either *TEC1*, the primary output of the MAPK pathway, or *FLO8*, the primary output of the cAMP pathway, in mediating pseudohyphal differentiation (Figure 3B). Indeed, both haploid and homozygous diploid *hsl1*Δ *tec1*Δ *swe1*Δ and *hsl1*Δ *flo8*Δ *swe1*Δ strains were unable to invade or to undergo significant morphological differentiation (data not shown). Together, these results suggested that inactivation of *HSL1* might be sufficient to promote pseudohyphal differentiation even in the absence of the filamentous signaling pathways. However, those same mutations in a *tec1*Δ *flo8*Δ strain are insufficient to promote filamentous differentiation in either haploid or diploid strains (Figure 3, A and B). In addition, we found that homozygous diploid *hsl1*Δ mutants were unable to undergo pseudohyphal differentiation, even after 20 days of growth if the SLAD medium was supplemented with a high level of nitrogen (Figure 3C). Finally, although *hsl1*Δ/*hsl1*Δ cells are modestly elongated in liquid medium (Table 3), they still exhibit a dramatic morphological response to low nitrogen and solid growth medium (Figures 1A and 2C; Table 3). Thus, although inactivation of *HSL1* is able to fully suppress the defect in the invasiveness of diploid cells caused by inactivation of either *TEC1* or *FLO8*, *hsl1*Δ cells still depend upon the activity of at least one of those pathways (MAPK or cAMP/PKA) as well as

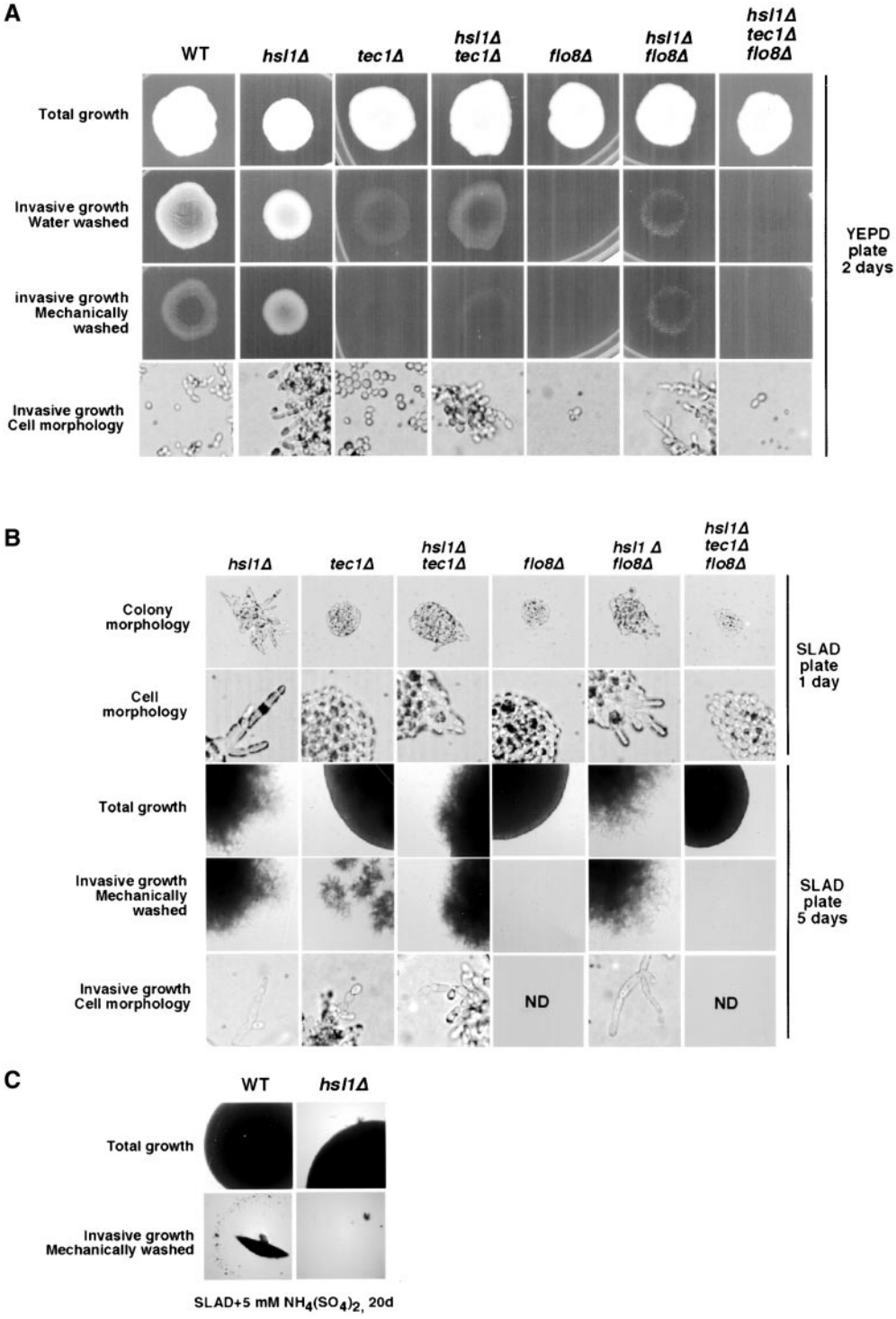


FIGURE 3.—Relationship between Swe1 activation and the filamentous signaling pathways. (A) Inactivation of *HSL1* can partially rescue either the *tec1*Δ or *flo8*Δ defect in haploid invasive growth. Invasive growth of wild-type (WT, L55-28), *tec1*Δ (RLVsc69), *hsl1*Δ *tec1*Δ (RLVsc227), *flo8*Δ (RLVsc70), *hsl1*Δ *flo8*Δ (RLVsc166), and *hsl1*Δ *tec1*Δ *flo8*Δ (RLVsc382) strains. (B) Inactivation of *HSL1* can fully complement the pseudohyphal growth defect of either *tec1*Δ/*tec1*Δ or *flo8*Δ/*flo8*Δ. Pseudohyphal growth of wild-type (WT, RLVsc291), *tec1*Δ/*tec1*Δ (RLVsc294), *hsl1*Δ/*hsl1*Δ *tec1*Δ/*tec1*Δ (RLVsc286), *flo8*Δ/*flo8*Δ (RLVsc66), *hsl1*Δ/*hsl1*Δ *flo8*Δ/*flo8*Δ (RLVsc295), and *hsl1*Δ/*hsl1*Δ *tec1*Δ/*tec1*Δ *flo8*Δ/*flo8*Δ (RLVsc392) strains. (C) Pseudohyphal development promoted by inactivation of *HSL1* still requires the normal nitrogen starvation signal. Pseudohyphal growth assay of wild type (WT, RLVsc291) and *hsl1*Δ/*hsl1*Δ (RLVsc63) on SLAD plate supplemented with a high concentration of ammonium sulfate (5 mM).

on the normal stimuli for filamentous differentiation (contact with solid substrate and nitrogen starvation).

***SWE1* is required for invasiveness of haploid and diploid cells in the absence of *TEC1*:** The findings described above suggested that activation of *SWE1* might also be important for the residual invasive behavior exhibited by *tec1*Δ mutants. To determine whether that is the case, we analyzed invasive behavior of both haploid and diploid *tec1*Δ mutants having or lacking functional *SWE1*. In both cases, the modest invasiveness of *tec1*Δ mutants was found to depend upon *SWE1* (Figure 4). This was most apparent when comparing the frequency of invasive *tec1*Δ/*tec1*Δ cells to that of *tec1*Δ/*tec1*Δ *swe1*Δ/*swe1*Δ cells on washed SLAD plates but could also be observed by comparing washed plates of *tec1*Δ and *tec1*Δ *swe1*Δ haploid strains. Furthermore, the extent of morphological differentiation appeared to be more compromised in *tec1*Δ *swe1*Δ mutants than in cells having a *tec1*Δ

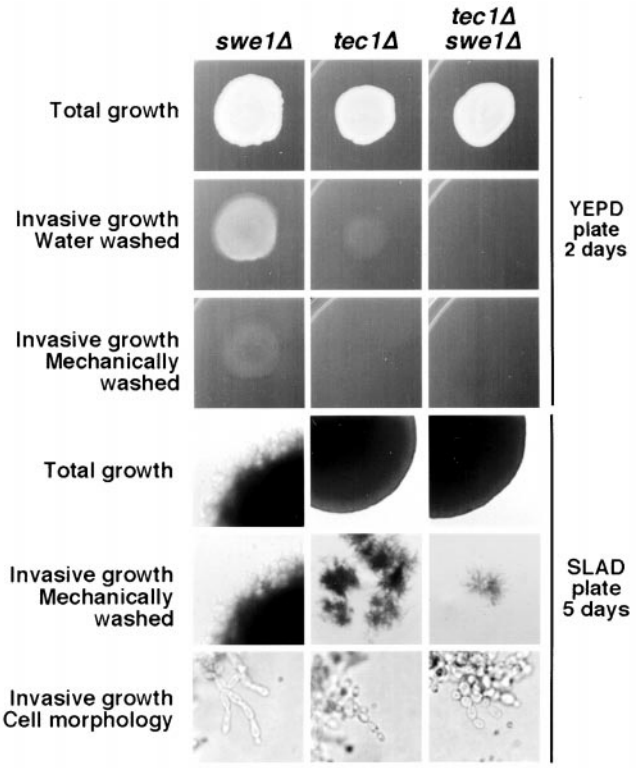


FIGURE 4.—Both *SWE1* and *TEC1* contribute to the same aspects of filamentous development. Top section: *SWE1* contributes to haploid invasive growth in the absence of *TEC1*. Invasive growth assay of wild-type (WT, L5528), *tec1*Δ (RLVsc69), and *swe1*Δ *tec1*Δ (RLVsc176) strains. Bottom section: Swe1 inactivation leads to a decrease in the residual invasive capacity of *tec1*Δ/*tec1*Δ strain under pseudohyphal growth conditions. Pseudohyphal growth of wild-type (WT, RLVsc291), *tec1*Δ/*tec1*Δ (RLVsc294), and *swe1*Δ/*swe1*Δ *tec1*Δ/*tec1*Δ (RLVsc323) strains.

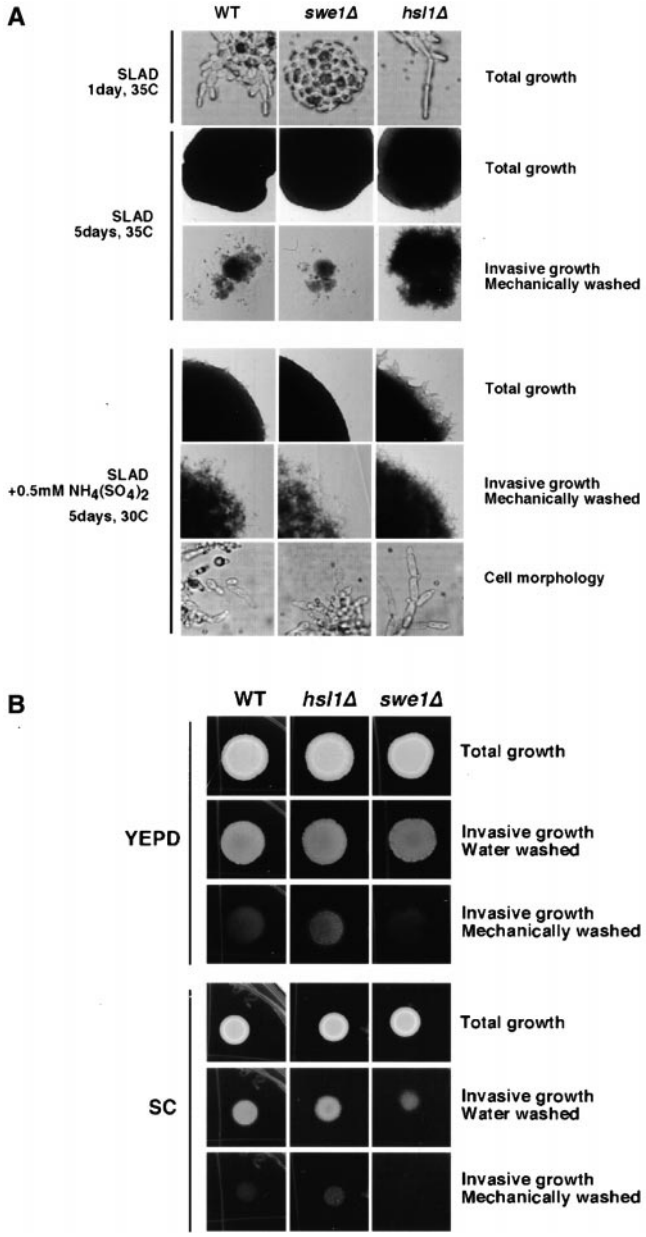


FIGURE 5.—*SWE1* is essential for filamentous differentiation in suboptimal conditions. (A) SWE1 inactivation leads to inhibition of pseudohyphal development at elevated temperatures or at moderate nitrogen levels. Pseudohyphal growth of wild-type (WT, RLVsc291), *hsl1*Δ/*hsl1*Δ (RLVsc63), and *swe1*Δ/*swe1*Δ (RLVsc64) strains on SLAD plates at 35° and on SLAD plates supplemented with a moderate level of ammonium sulfate (0.5 mM). (B) Inactivation of Swe1 abolishes haploid invasive growth on SC plates. Invasive growth of wild-type (WT, L5487), *hsl1*Δ (RLVsc46), and *swe1*Δ (RLVsc47) strains on the indicated solid growth medium.

mutation alone. Thus, despite our failure to detect a contribution of *SWE1* to the invasive behavior of otherwise wild-type strains, we have observed a substantial effect of the *swe1*Δ mutation upon the invasiveness of cells that are partially compromised in that capacity.

Swe1 is required for filamentous growth under suboptimal induction conditions: The finding that *SWE1* contributes to both haploid invasive and pseudohyphal growth in cells lacking *TEC1* suggested that the contribution of *SWE1* might be most significant when the signals for filamentous differentiation are compromised. Although pseudohyphal growth is most often assayed on minimal medium containing 50 μM ammonium sulfate (SLAD) at 30°, we also detect filamentous differentiation and invasiveness in the presence of 10-fold higher ammonium sulfate as well as on SLAD plates at lower or higher temperatures. To evaluate whether *SWE1* is more important for filamentous growth under these "suboptimal" conditions, the effect of inactivation of *SWE1* on the invasive behavior of cells was analyzed.

Inactivation of *SWE1* has little if any effect upon the extent of pseudohyphal differentiation in otherwise wild-type cells under standard conditions. In contrast to its dispensability in cells growing on SLAD medium at 30° (Figure 2B), inactivation of *SWE1* compromised pseudohyphal growth on the same medium at either 20° (data not shown) or 35° (Figure 5A). This is most obvious when the extent of morphological differentia-

tion of the cells is examined. The *swe1*Δ mutants exhibit no elongation or filament formation after 1 day of growth. There is also a noticeable effect on the invasiveness and morphological differentiation of the cells observed after 5 days of growth as evidenced by the frequency of cells retained on the plate after washing and microscopic examination. Similarly, pseudohyphal growth is compromised in *swe1*Δ mutants growing at 30° on medium containing 500 μM (rather than 50 μM) ammonium sulfate (Figure 5A). However, the morphogenesis defect of *swe1*Δ mutants grown at higher nitrogen levels is less apparent. Furthermore, we have examined the effect of alternative carbon sources (raffinose/galactose) and nitrogen sources (leucine and proline) on pseudohyphal growth of wild-type and *swe1*Δ mutant cells. In all cases, inactivation of *SWE1* had a readily detectable effect on the ability of cells to undergo pseudohyphal differentiation (data not shown). Finally, under all conditions tested inactivation of *hsl1*Δ led to the enhancement of pseudohyphal differentiation (Figure 5A and data not shown).

We obtained similar results with haploid mutant strains growing on solid rich medium containing either glucose (YEPD) or galactose (YEPGal, data not shown) as a carbon source as well as on synthetic complete medium with glucose as carbon source (SC; Figure 5B). Whereas on YEPD medium *swe1*Δ mutants were only slightly compromised in their capacity to invade, no invasive growth was observed under the nonstandard conditions. In all cases, hyperactivation of *SWE1* caused a modest but noticeable increase in invasiveness. We conclude that Swe1 is essential for filamentous differentiation under a variety of nonstandard induction conditions.

Swe1 is essential for induction of filamentous growth by butanol: It has been recently shown that fusel alcohols, notably butanol, induce filamentous growth in haploid cells on both solid and liquid media (Dickinson 1996; Lorenz *et al.* 2000). This phenomenon is dependent upon the MAPK/Tec1 pathway, but not on Flo8, and results in alterations in cell morphology similar to pseudohyphal differentiation. We, therefore, addressed whether *SWE1* is required for alcohol-induced filamentous differentiation. As reported previously (Lorenz *et al.* 2000), wild-type haploid cells grown in either rich liquid medium or solid SLAD medium containing 1% butanol exhibited elongated cell morphology and unipolar budding. In liquid this leads to the formation of chains of elongated cells clustered in florets (Lorenz *et al.* 2000 and Figure 6A). These features were even more pronounced in the *hsl1*Δ strain (data not shown). Interestingly, the *swe1*Δ strain was substantially compromised in its capacity to undergo butanol-induced differentiation. This was manifested by an inability to differentiate into elongated cells despite acquisition of the unipolar budding pattern in at least some of the cells (Figure 6A). Although haploid cells do not normally undergo invasive differentiation on SLAD plates, they do so in the presence of 1% butanol (Lorenz *et al.* 2000 and data not shown). Inactivation of Swe1 suppresses most of the phenotypic response observed in those cells (data not shown).

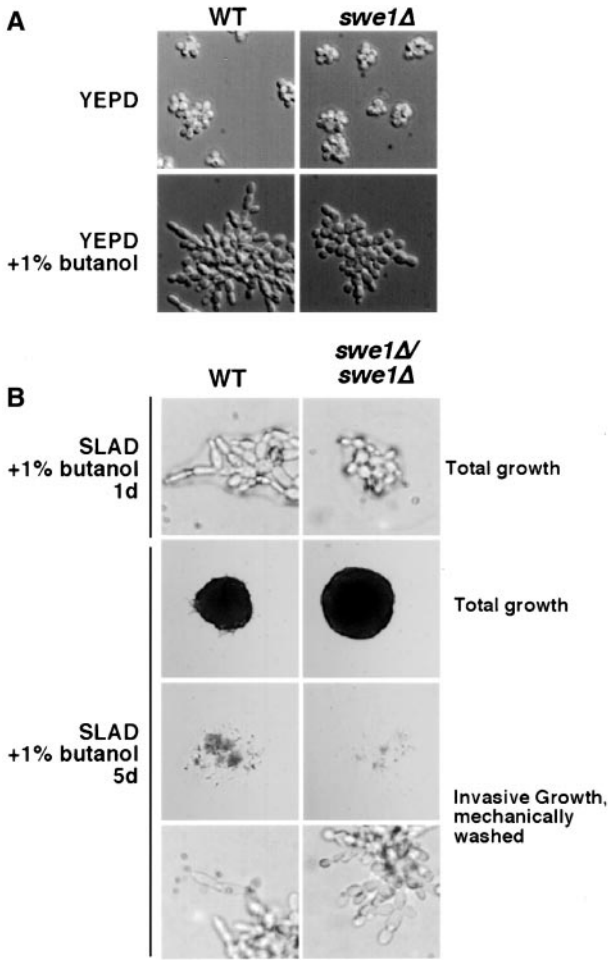


Figure 6.—*SWE1* is essential for the butanol-mediated induction of filamentous growth. (A) Inactivation of Swe1 leads to a decrease in the filamentous response of haploid cells growing in liquid media supplemented with butanol. Wild-type (WT, L5487) and *swe1*Δ (RLVsc47) strains are shown during growth in YEPD with or without 1% butanol. (B) Inactivation of Swe1 diminishes the invasiveness and cell elongation of diploid cells induced by growth on butanol-containing SLAD plates. Pseudohyphal development of wild-type (WT, RLVsc291) and *swe1*Δ/*swe1*Δ (RLVsc64) strains growing on SLAD plate supplemented with 1% butanol.

In contrast to its effect on haploid cells, diploid cells growing on SLAD plates containing 1% butanol exhibited a partial inhibition of pseudohyphal development (Figure 6B). As in other nonstandard conditions for pseudohyphal differentiation that we have examined, inactivation of *SWE1* resulted in a strong reduction in invasive growth in the presence of butanol (Figures 1 and 6B). That reduction, as in butanol-induced filamentation in haploid cells, was accompanied by failure

to develop elongated cell morphology and the retention of unipolar budding. Although ethanol was also shown to stimulate pseudohyphal development of diploid strains (Lorenz *et al.* 2000), we found no reduction in the effectiveness of ethanol-induced pseudohyphal differentiation caused by inactivation of *SWE1* (data not shown). Thus, butanol induces filamentation under a wide range of conditions in both haploid and diploid cells and most of those responses are suppressed by inactivation of Swe1.

DISCUSSION

We have performed an in-depth investigation of the role of the Swe1 protein kinase, a negative regulator of Clb-associated CDK activity (Booher *et al.* 1993; Lew and Reed 1995; Sia *et al.* 1996) in filamentous differentiation in the budding yeast. Two approaches were taken to the manipulation of Swe1 activity. Swe1 was either activated by elimination of its negative regulators Hsl1 and Hsl7 (Ma *et al.* 1996; Barral *et al.* 1999; Edgington *et al.* 1999; McMillan *et al.* 1999a; Shulewitz *et al.* 1999) or inactivated directly by disruption of the *SWE1* gene (Booher *et al.* 1993). Inactivation of either Hsl1 or Hsl7 has been shown to promote polarized growth of yeast cells (Ma *et al.* 1996; Edgington *et al.* 1999; McMillan *et al.* 1999a; Shulewitz *et al.* 1999) and to lead to a unipolar budding pattern and invasiveness (Edgington *et al.* 1999). In the absence of those proteins Swe1 fails to localize to the bud neck and is no longer properly regulated with regard to bud emergence (McMillan *et al.* 1999a; Shulewitz *et al.* 1999). As a consequence, Clb-associated CDK activity fails to be activated in a timely manner, leading to polarized growth promoted by persistence of G1 cyclin-associated CDK activity (McMillan *et al.* 1999a; Shulewitz *et al.* 1999). Our study extends previous observations (Kron *et al.* 1994; Ahn *et al.* 1999; Edgington *et al.* 1999; Loeb *et al.* 1999a) by establishing that inactivation of either Hsl1 or Hsl7 enhances all aspects of filamentous growth in both haploid and homozygous diploid strains. When compared to wild-type cells, the mutant cells are more invasive, more elongated, and more filamentous. In that context, it is noteworthy that two of the mutations identified by Myers and colleagues (Blacketer *et al.* 1995) that promote both haploid invasive and pseudohyphal growth of the D273 background are allelic with *HSL1* and *HSL7* (*elm2* and *elm5*, respectively; A. Myers, personal communication). However, we show that, despite their enhanced filamentous behavior, the *hsl1*Δ and *hsl7*Δ mutant strains still depend upon the normal filamentous growth signals (contact with a solid substrate, nitrogen starvation, and a functional MAPK or cAMP/PKA pathway). Moreover, we show that the enhancement of both haploid invasive growth and pseudohyphal growth that occurs in response to inactivation of either Hsl1 or Hsl7 is dependent upon a functional Swe1. Thus, activation of Swe1 is sufficient to explain the enhancement of invasive growth resulting from Hsl1 and Hsl7 inactivation.

Swe1 is likely to exert its effect on filamentous differentiation via its capacity to phosphorylate and inactivate the Cdc28 CDK (Sia *et al.* 1996), its only known target. However, as others have observed using the same mutation (Sia *et al.* 1996; Edgington *et al.* 1999), we have found that eliminating the Swe1 phosphorylation site on Cdc28 has a less dramatic effect on the capacity of cells to differentiate in butanol-containing medium than does inactivation of Swe1 (data not shown). Although this appears to support a role for Swe1 independent of CDK phosphorylation, it has been reported that Swe1 can inhibit the activity of their target CDKs without phosphorylation (McMillan *et al.* 1999b). Consequently, this issue remains to be resolved.

It was recently reported that inactivation of Hsl7 enhances pseudohyphal growth whereas overexpression leads to its repression (Fujita *et al.* 1999). That study found that the effect of Hsl7 overexpression was suppressed by overexpression of Ste20, the Pak family kinase of the MAPK signaling pathway that targets Tec1 (Fujita *et al.* 1999). Furthermore, the enhancement of polarized growth resulting from inactivation of Hsl7 was suppressed by inactivation of Ste20 in both haploid and diploid cells. Although our data does not specifically address the effect of Hsl7 overexpression, we demonstrate that the effect of Hsl7 inactivation can be explained entirely by the activation of Swe1. Since inactivation of Hsl7 in cells lacking Swe1 results in no enhancement of invasive growth, we conclude that the inhibitory effect of Hsl7 on Ste20 does not contribute significantly to invasive growth under standard assay conditions. In fact, there was no condition in which *hsl7*Δ *swe1*Δ mutants were more invasive than wild-type cells. Nevertheless, the two findings might not be contradictory. It is known that Swe1 activation, via inhibition of mitotic forms of the CDK, prolongs the period during which G1 cyclin-associated CDK remains active and, thereby, the extent of polarized growth (Lew and Reed 1993). It is possible that the contribution of hyperactive Swe1 to morphogenesis is a consequence of the capacity of G1 cyclin-associated CDK to modulate Ste20 activity (Oehlen and Cross 1998).

We demonstrate that deregulation of Swe1 strongly enhances filamentous growth. Although cells with activated Swe1 still depend upon the normal signals for filamentous growth, Swe1 activation is sufficient to promote invasive behavior even in the absence of either Tec1 or Flo8, downstream targets of the MAPK and cAMP/PKA pathways, respectively. Together with previous studies, these findings support a model in which modulation of CDK activity plays a central role in filamentation (Kron *et al.* 1994; Ahn *et al.* 1999; Edgington *et al.* 1999; Loeb *et al.* 1999a; Madhani *et al.* 1999). Indeed, Swe1, most likely acting through its capacity to

inhibit Clb/CDK activity, can override a defect in either the MAPK or cAMP/PKA pathways, suggesting that either or both of these pathways may act, at least in part, by inhibiting Clb/CDK activity. Although it is possible that in cells lacking Tec1 or Flo8 the signal for filamentous growth is transduced via Swe1, we consider it more likely that Swe1 activation suppresses the deficiency in those pathways by providing an alternative or supplementary mechanism for Clb/CDK inhibition. Indeed, Swe1 activation is unable to promote filamentous differentiation when both the Tec1 and Flo8 transcription factors have been inactivated, suggesting that the morphogenesis checkpoint pathway provides a modulatory function as opposed to being a primary pathway for transduction of the signals for filamentous differentiation.

Despite the capacity of hyperactivated Swe1 to effectively suppress the defect in invasiveness caused by inactivation of either Flo8 or Tec1, there are striking differences between the effect of *flo8*Δ and *tec1*Δ mutants on filamentous differentiation. First, in diploid cells, *TEC1*, but not *FLO8*, is required for cell elongation (Mosch and Fink 1997; Pan and Heitman 1999). Conversely, cell-cell adhesion is defective in the noninvasive *flo8*Δ mutant population (Pan and Heitman 1999) but appears to be unaffected in *tec1*Δ mutants (Mosch and Fink 1997; Pan and Heitman 1999). We find that although activation of Swe1 suppresses the defect in cell elongation in *tec1*Δ mutants, that suppression is inefficient. This suggests that Swe1 cannot activate but, instead, modulates the Tec1-dependent filamentous signal that promotes cell elongation. Furthermore, Swe1 activation induces cell-cell adhesion in an otherwise defective *flo8*Δ strain, perhaps indirectly through modulation of a target of Tec1 such as Flo11 (Gavrias *et al.* 1996; Mosch and Fink 1997; Lorenz and Heitman 1998; Rupp *et al.* 1999). Thus, cell-cell adhesion appears to be a consequence of activation of a downstream element that can act as a target for the MAPK, cAMP/PKA, and the morphogenesis checkpoint pathway.

Our results demonstrate that Swe1 contributes to pseudohyphal and haploid invasive growth under standard assay conditions. Although inactivation of Swe1 has a noticeable effect on the efficiency of invasive growth of haploid cells and on morphological differentiation of diploid cells, the invasiveness of diploid *swe1*Δ mutants is largely indistinguishable from that of wild-type cells (Ahn *et al.* 1999). We have furthered prior observations by demonstrating a requirement for either Swe1 or Tec1 in the standard assay. Indeed, inactivation of Swe1 eliminates the residual invasive growth observed under optimal conditions in both haploid and homozygous diploid *tec1*Δ mutants. This suggests that these two proteins act coordinately to induce filamentous differentiation. Moreover, consistent with previous observations (Madhani *et al.* 1999), we find that the well-established invasive growth assays may not be sufficient to measure all of the properties important for filamentous differentiation. As in that earlier analysis, we have shown that alteration of the standard assay conditions reveals important factors for differentiation that were not revealed under the standard conditions. We have established a requirement for *SWE1* for both forms of filamentous growth under nonstandard conditions (suboptimal temperature and quality or quantity of nutrients). Furthermore, we found that Swe1 is essential for butanol-mediated induction of filamentous growth, a related phenomenon (Dickinson 1996; Lorenz *et al.* 2000). We have confirmed that the Flo8 transcription factor is largely dispensable for butanol-induced filamentation in both haploid and diploid cells. This suggests that, even under those conditions, *FLO8* is not required for development of polarization or a unipolar budding pattern (Lorenz *et al.* 2000 and data not shown). In contrast, haploid and homozygous diploid cells carrying *swe1*Δ (Figure 6) or *tec1*Δ (Lorenz *et al.* 2000 and data not shown) mutations exhibit unipolar budding and cell-cell adhesion but are not elongated when growing on either liquid and solid butanol media. Our findings lead to the conclusion that inactivation of Swe1 has a dramatic effect in terms of both kinetics and extent of filamentous differentiation of haploid and diploid cells under many conditions.

It has been suggested that CDKs are the targets of pseudohyphal growth signals (Ahn *et al.* 1999). The modulatory function of Swe1 on filamentous growth may not be evident under optimal conditions because its contribution is masked by activation of another pathway, such as that involving TEC1. However, the Swe1 pathway may become more important when repression of Clb-associated CDK activity via those other pathways becomes limiting. Our experiments support a model in which at least three distinct pathways (MAPK, cAMP/PKA, and the morphogenesis checkpoint pathway) play a role in filamentous differentiation of both haploid and diploid strains with each pathway influencing the extent of the phenotypic responses. Moreover, the extent of signaling via each pathway may be strongly influenced by differences in physiological conditions. Indeed, under a variety of conditions, inactivating each of these pathways has a striking consequence in terms of specific phenotypic responses. For example, inactivation of Swe1 results in defects in cell elongation and budding pattern under optimal conditions and affects a broad range of responses under the nonstandard induction conditions that were analyzed. Thus, the effect of Swe1 inactivation on haploid invasive growth may be more readily apparent simply because the strength of one or both of the other signals is lower under haploid invasive growth conditions than it is in diploid cells undergoing pseudohyphal differentiation. Indeed, we, as well as others (Edgington *et al.* 1999 and data not shown), have noticed that inactivation of Swe1 has a readily detectable effect on the weak haploid invasive behavior of noninvasive laboratory strains. On the basis of our results, we suggest that when filamentous signaling via the MAPK/

Tec1 pathway or the cAMP/Flo8 pathway is diminished, Swe1-dependent inhibition of Clb/CDK function becomes essential for filamentous differentiation.

The model proposed above raises important questions. Is the Swe1 protein kinase regulated in response to signals that promote invasive growth and, if so, via what mechanism? This question has yet to be adequately addressed. Both direct and indirect mechanisms by which invasive growth signals might enhance Swe1 activity can be envisioned. Since *SWE1* is a target of the G1-specific transcription system (Lew and Reed 1995), alteration of cell cycle kinetics can substantially alter the accumulation of the *SWE1* transcript. Specifically, mechanisms that delay the accumulation of B-type cyclin-associated CDK activity (including Swe1, itself) can enhance the accumulation of G1-specific transcripts. Swe1 is also regulated at the level of turnover via the Cdc34-SCF^{Met30} ubiquitin ligase (Kaiser *et al.* 1999). It is not clear whether its capacity to act as a substrate is controlled via regulation of the Hsl1 protein kinase or via other mechanisms. Alternatively, the activity of the Swe1 kinase may be regulated via nutritional mechanisms.

The ability to switch from a yeast form to a filamentous form is also typical of many fungal pathogens (reviewed in Madhani and Fink 1998 and Borges-Walmsley and Walmsley 2000). The multiple signaling pathways that control dimorphism are conserved among evolutionarily distant fungi and are required for virulence and infection of fungal pathogens. In *Ustilago maydis*, a smut fungus that infects and induces tumors in corn, both the MAPK (Banuett and Herskowitz 1994) and the cAMP/PKA (Gold *et al.* 1994, 1997) pathways have been shown to be essential for filamentous development and pathogenesis. In the same way, the two pathways are essential for the appressorium formation, a specialized infectious structure, and subsequent infection of rice by the fungus *Magnaportha grisea* (Mitchell and Dean 1995; Xu and Hamer 1996). Finally, the cAMP and MAPK pathways have been shown to be essential for dimorphism and pathogenicity of *Criptococcus neoformans* (Alspaugh *et al.* 1997) and *Candida albicans* (Liu *et al.* 1994; Singh *et al.* 1994, 1997; Kohler and Fink 1996; Leberer *et al.* 1996, 1997) respectively, two human opportunistic fungal pathogens that are seen with greatly increased prevalence in immunocompromised hosts and frequently lead to elevated morbidity and mortality (Imam *et al.* 1990; Cassone 1996). Here we propose that *SWE1* kinase plays an important modulatory role in filamentous differentiation of *S. cerevisiae*. Precedence for a role of a Swe1-like kinase in cell differentiation has recently been reported in the fungus *Aspergillus nidulans* where the unphosphorylatable CDK $NIMX^{cdc2AF}$ has been shown to block conidia formation (Ye *et al.* 1999). Consistent with the high conservation of the other signaling pathways involved in dimorphism we propose also that *SWE1* could play a relevant role in that process and, thereby, in the virulence of fungal pathogens. Indeed, cyclin-dependent protein kinases have been demonstrated to play an important modulatory role in the hyphal development and the virulence of *C. albicans* (Loeb *et al.* 1999b).

We thank Anne Dranginis, Tracie Bierwagen, Alan Myers, Danny Lew, Monique Smeets, Steven Reed, Jonathan Loeb, and Haoping Liu for strains and plasmids. We also thank Alan Myers and members of the TSRI Cell Cycle Group (the S. Reed, P. Russell, C. McGowan, and C. Wittenberg laboratories) for their comments and support during the course of this research. R.L.V. is supported by an AIDS fellowship from the National AIDS Program, Istituto Superiore di Sanità, Italy. Support for this project was from US Public Health Service grant GM-43487 to C.W.

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Communicating editor: S. Sandmeyer