G_{1}/S Transcription Factor Orthologues Swi4p and Swi6p Are Important but Not Essential for Cell Proliferation and Influence Hyphal Development in the Fungal Pathogen Candida albicans

Bahira Hussein,1 Hao Huang,1 Amandeep Glory,1 Amin Osmani,1 Susan Kaminskyj,2 Andre Nantel,3,4 and Catherine Bachewich1*

Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, Quebec H4B 1R6, Canada1; Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan S7N 5E2, Canada2; Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Ave., Montreal, Quebec H4P 2R2, Canada3; and Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec H3A 2B2, Canada4

Received 31 October 2010/Accepted 10 January 2011

The G_{1}/S transition is a critical control point for cell proliferation and involves essential transcription complexes termed SBF and MBF in Saccharomyces cerevisiae or MBF in Schizosaccharomyces pombe. In the fungal pathogen Candida albicans, G_{1}/S regulation is not clear. To gain more insight into the G_{1}/S circuitry, we characterized Swi6p, Swi4p and Mbp1p, the closest orthologues of SBF (Swi6p and Swi4p) and MBF (Swi6p and Mbp1p) components in S. cerevisiae. The mbp1Δ/Δ cells showed minor growth defects, whereas swi4Δ/Δ and swi6Δ/Δ yeast cells dramatically increased in size, suggesting a G_{1} phase delay. Gene set enrichment analysis (GSEA) of transcription profiles revealed that genes associated with G_{1}/S phase were significantly enriched in cells lacking Swi4p and Swi6p. These expression patterns suggested that Swi4p and Swi6p have repressing as well as activating activity. Intriguingly, swi6Δ/Δ swi6Δ/Δ and swi4Δ/Δ mbp1Δ/Δ strains were viable, in contrast to the situation in S. cerevisiae, and showed pleiotropic phenotypes that included multibudded yeast, pseudohyphae, and intriguingly, true hyphae. Consistently, GSEA identified strong enrichment of genes that are normally modulated during C. albicans-host cell interactions. Since Swi4p and Swi6p influence G_{1} phase progression and SBF binding sites are lacking in the C. albicans genome, these factors may contribute to MBF activity. Overall, the data suggest that the putative G_{1}/S regulatory machinery of C. albicans contains novel features and underscores the existence of a relationship between G_{1} phase and morphogenetic switching, including hyphal development, in the pathogen.

* Corresponding author. Mailing address: Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, QC H4B 1R6, Canada. Phone: (514) 848-2424, ext. 5180. Fax: (514) 848-2880. E-mail: cbachewi@alcor.concordia.ca.
† Supplemental material for this article may be found at http://ec.asm.org/.
‡ Published ahead of print on 21 January 2011.

\[384\]
independent of their cell cycle functions (30, 54). Thus, G1 phase is tightly coordinated with development in most systems, and G1/S regulatory factors can play independent roles in controlling developmental events.

*Candida albicans* is one of the most prevalent fungal pathogens in humans. Its ability to differentiate into a variety of cell types, including white phase yeast, mating-competent opaque phase yeast, pseudohyphae, hyphae, or chlamydospores (79), is a crucial virulence-determining trait (49, 66). Thus, it is important to identify the mechanisms underlying basic cell proliferation and differentiation in this organism. However, a detailed picture of the G1/S circuit based on functional analyses is lacking. Cote et al. (26) reported cell cycle-dependent transcription patterns in opaque yeast cells of *C. albicans*, which were most similar to those in *S. cerevisiae*. However, some unique features in putative G1/S circuitry were noted, including the potential involvement of fungal-specific genes (26). More-}

### Table 1. *Candida albicans* strains used in this study

| Strain   | Genotype          | Parent/source |
|----------|-------------------|---------------|
| BWP17    | *ura3*Δ::ln4434/3*his1*Δ::urn434 | BWP17         |
| BH101    | *swi6*Δ::H1/SW1/6  | BWP17         |
| BH104    | *swt4/2*::G-Ura3/His-G/SW14 | BWP17         |
| BH113    | *swt4/2*::His/SW14  | BWP17         |
| BH115    | *swt4/2*::His/SW14  | BWP17         |
| BH120    | *swt6*::H1/SWI6/6::Ura3 | BWP17         |
| BH137    | *mbp1*Δ::H1/SMBP1  | BWP17         |
| BH140    | *swt4/2*::His/SW14  | BWP17         |
| BH150    | *swt4/2*::His/MT3::SW4/4-ARG4 | BWP17         |
| BH160    | *swt4/2*::His/MT3::SW4/4-ARG4 | BWP17         |
| BH180    | *swt4/2*::His/SW1/6  | BWP17         |
| BH185    | *swt4/2*::Ura3/Swi4/2::His1 | BWP17         |
| BH190    | *swt4/2*::His/MT3/4::SW4/4-ARG4 | BWP17         |
| BH261    | *swt4/2*::His/SW1/6::Ura3  | BWP17         |
| BH270    | *swt4/2*::His/MT3/4::SW4/4-ARG4 | BWP17         |
| BH277    | *swt4/2*::His/Swi4/2::Ura3 | BWP17         |
| BH334    | *swt4/2*::His/Swi4/2::Ura3 | BWP17         |
| HH26    | *mbp1*Δ::H1/SMBP1  | BWP17         |
| HH62    | *swt4/2*::His/Swi4/2::Ura3 | BWP17         |
| BH420    | BPW17 (pRM100 pBS-CaARG4) | BWP17         |
| BH440    | BPW17 (pBS-CaHis1 pBS-CaUra3) | BWP17         |
| CB540    | *swt4/2*::Ura3/SW4/4 | BWP17         |
| CB547    | *swt4/2*::Ura3/MT3::SW4/4-ARG4 | BWP17         |
| CB557    | *swt6/2*::Ura3/MT3::SW6/His1 | BWP17         |
| CB600    | *SW16/MT3::SW16/His1* | BWP17         |
| AG160    | *swt4/2*::His/Swi4/2::Ura3 | BWP17         |
| AG168    | *swt4/2*::His/Swi4/2::Ura3 | BWP17         |
| KMCa4a   | *mbp1*::Ura3/MT3::MBP1-ARG4 | BWP17         |

### MATERIALS AND METHODS

#### Media and growth conditions. *Candida albicans* strains were grown at 30°C on solid or in liquid glucose minimal medium (0.67% yeast nitrogen base without amino acids, 2% glucose) supplemented with all amino acids except during selection for Ura3, His1, or Arg444 phototrophs. For MT3 conditional strains, cells were grown in inducing (+MC) or repressing (+MC) minimal medium with or without 2.5 mM methionine and 0.5 mM cysteine, respectively (20). For analysis of cell phenotype, cells were grown overnight in minimal medium, diluted the following day to an optical density at 600 nm (OD600) of 0.1 in fresh medium, and incubated at 30°C.

#### Strain construction. Strains, oligonucleotides, and plasmids are listed in Table 1, 2, and 3, respectively. In order to construct a strain lacking SW14, alleles were replaced with *URA3* and *HIS1* markers in strain BPW17, using 2-step PCR fusion constructs (62, 82). Fragments approximately 750 bp in length corresponding to the 5’ and 3’ flanks of SW14 were amplified with oligonucleotides BH100F and BH110F and BH140F and BH141R, respectively. A HIS1 fragment from plasmid pBS-CaHIS1 was amplified with oligonucleotides BH13F and BH13R. The products were amplified with oligonucleotides BH100F and BH141R to produce a 2,196-bp product that was transformed into strain BPW17, resulting in strain BH180 (swt4/2::H1/SW1/6). To delete the second copy of SW14, a PCR fusion construct containing the same 5’ and 3’ flanks as described above and a 1,765-bp URA3 fragment, amplified from plasmid pBS-CaUra3 with oligonucleotides BH13F and BH13R, was utilized. The final 2,926-bp construct was transformed into strain BH180, resulting in strain BH185 (swt4/2::His/Swi4/2::Ura3). A prototrophic control strain (BH440) was created by sequentially transforming strain BPW17 with plasmids pBS-CaHIS and pBS-CaR4/3. In order to confirm that the phenotype of strain BH185 was due to the deletion of SW14, a conditional strain carrying a single copy of SW14 under the control of

---

*Candida albicans* is one of the most prevalent fungal pathogens in humans.
the MET3 promoter was created. A 3-kb fragment containing the SWI4 open
reading frame and approximately 1 kb of 5′ and 3′ flanking sequences was
amplified from genomic DNA (gDNA) with oigonucleotides CB119F and
CB119R, respectively. The resulting strain, BH113, was used to amplify the
promoter (20), fragments corresponding to the 5′ and 3′ flanks of SWI4
were amplified using oligonucleotides BH11F and BH11R. The final 4,895-bp
construct was amplified from the three PCR products using oligonucleotides
and 5′-3′ HUSSEIN ET AL. EUKARYOT. CELL

TABLE 2. Oligonucleotides used in this study

| Oligonucleotide | Sequence (5′-3′) |
|-----------------|-----------------|
| BH2F            | GAAGATTCAATGATATGTTGATAGCCAC |
| BH2R            | CCAGCGTTTATAAAGTGAACAGTTCTTC |
| BH3F            | GAGTCGACCGTCTATCATATAAACACGGTGATAGGGCGATATTGGAGCTC |
| BH3R            | CACGGGAATTAGTAGTTATACGTTCTGCTGCGCATGTAACGGTCAGC |
| BH4F            | CGAACACGTCATATCTCATTATCTCCCCCTTG |
| BH4R            | TCCACATCCATACTAAATCTTATACAGG |
| BH7F            | GTAACATACCTTATCGAGGATTTACCCAC |
| BH7R            | GATGTGATGTTGAATGACTGTGTTGTCAAGTCACCAATGTGTATA |
| BH8F            | CCGTCAATCTTATTTACACCCCTATCAATTTAGGGCGATATTGGAGCTC |
| BH8R            | GTTTCCTATTATCTCATGTTGCTTGGTCGCA |
| BH10F           | AGCAGTATCTACATGGAATTAATCGAG |
| BH10R           | TTGGTATAAATCATTTGAGTATGGT |
| BH11F           | AGCTACACTCTCAAAATGTTGAATACCCAGGATCCCCCTTTTATAGGAA |
| BH11R           | ACACCTCTCATAATGAGTATATACGTTT |
| BH12F           | AGCGTTAAGTCCTAATTGGCAATGTAAG |
| BH12R           | ACCAACCAAACTCATTATCTCAGG |
| BH13F           | CGCTACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| BH13R           | ATGGTGGCAGCGTATCGAGAGGAGG |
| BH14F           | CCAATGACAAATATATGAGGAGTAGT |
| BH14R           | GATTTAATGGGATTAGATGAGTAGG |
| CB115F          | CCAATAGGGAATTAGAAAGTATACATG |
| CB115R          | CATTTATCAACGATGTTGCGATAG |
| CB119F          | AGCTACACTCTCAAAATGTTGAATACCCAGGATCCCCCTTTTATAGGAA |
| CB119R          | ACACCTCTCATAATGAGTATATACGTTT |
| CB120F          | CCACGGTTAATGAGTATATACGTTT |
| CB120R          | ATGTGATGGGTTGATAAATGAAATGAGCG |
| CB122F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB122R          | CATTTATCAACGATGTTGCGATAG |
| CB127F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB127R          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB128F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB128R          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB129F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB129R          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB130F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB130R          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB131F          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |
| CB131R          | ATGTCACCTACTCAAATGTATGTTATACCAAGGATCCACCCAAAT |

Primers CB120F and CB120R were then used to amplify the flanking and vector
sequences from pCB181, into which the BamHI/BglII hisG-URA3-hisG cassette
(p5921) (33) was cloned, resulting in plasmid pCB181. The SWI4 deletion con-
struct was liberated using SalI and SacI restriction enzymes and transformed into
strain BWP17. The resulting strain, BH104, was grown overnight in yeast extract-
peptone-dextrose (YPD) medium and then plated onto 5-fluoroorotic acid (5-
FOA) to select for URA3 auxotrophs. PCR screening confirmed strain BH113
which was transformed into strain BWP17, resulting in strain CB540

TABLE 3. Plasmids used in this study

| Plasmid        | Description               | Reference or source |
|----------------|---------------------------|---------------------|
| pBS-CaURA3     | pBluescript CaURA3        | A. J. P. Brown      |
| pBS-CaHIS1     | pBluescript CaHIS1        | Bachewich           |
| pBS-CaARG4     | pBluescript CaARG4        | Bachewich           |
| pFA-MET3p-CaURA3 | pFA-MET3p-CaURA3     | 36                   |
| pFA-MET3p-CaHIS1 | pFA-MET3p-CaHIS1     | 36                   |
| pFA-MET3p-CaARG4 | pFA-MET3p-CaARG4     | 36                   |
| p5921          | pUC18-hisG-URA3-hisG     | W. A. Fonzi          |
| pRM100         | pUC19 HUSSEIN ET AL. EUKARYOT. CELL\n
In order to delete one copy of MBP1, a similar PCR fusion strategy was utilized.
PCR fragments corresponding to the 5′ and 3′ flanks of MBP1 were
amplified using oligonucleotides BH7F and BH7R and BH9F and BH9R, respectively. A HIS1 fragment was amplified from plasmid pH8s-CaHIS1 using oligonucleotides BH8F and BH8R. The final 3,016-bp fusion product was transformed into strain BH137, resulting in strain BH140 (swi4::HIS1/mbp1). The second copy of MBP1 was replaced with a PCR fusion construct containing a UR43 fragment that was amplified from plasmid pH8s-CaURA3 using oligonucleotides BH8F and BH9R. The final 3,104-bp fusion product was transformed into strain BH137, resulting in strain BH263 (swi4::HIS1/swi6::URA3). To create a strain with a deletion of HIS1, a HIS1-containing construct containing 80 bp complementary to the 5′ and 3′ flanks of SW6 was amplified with oligonucleotides CB115F and CB115R and CB131F and CB131R. The final 3,000-bp fusion construct was amplified with oligonucleotides BH2F and BH4R and transformed into strain BH101, resulting in strain BH120 (swi6::HIS1/swi6::URA3). The second copy of SW6 was deleted using a PCR fusion construct. Oligonucleotides BH2F and BH2R and BH4F and BH4R amplified the 5′ and 3′ flanking fragments of SW6, respectively. The UR43 marker from pH8s-CaURA3 was amplified with oligonucleotides BH3F and BH3R. The final 2,721-bp fusion construct was amplified with oligonucleotides BH2F and BH4R and transformed into strain BH101, resulting in strain BH120 (swi6::HIS1/swi6::URA3). A strain containing a single conditional copy of SW6 was created by placing one allele of SW6 under the control of the MET3 promoter, using a construct created with oligonucleotides BH3F and BH3R (11) that contained a 36-bp fragment immediately up- and downstream of the SW6 start codon, respectively, and 20 bp homologous to plasmid pFA-MET3-HIS1 (36). The final product was transformed into strain BW171, producing strain CB600. The second allele of SW6 was deleted using a PCR fusion construct. Oligonucleotides CB129F and CB129R and CB130F and CB130R amplified the 5′ and 3′ flank of SW6, respectively. The second copy of SW6 was deleted from strain BH113 by replacing the SW6::URA3 fragment (26). The first copy of SW6 was deleted from strain BH113 using a PCR fusion construct created with oligonucleotides CB115F and CB115R, as described above. The final 1,208-bp product was transformed into strain BH115, resulting in strain BH140 (swi4::hisG/swi4::URA3). The second copy of SW6 was then placed under the control of the MET3 promoter using a PCR fusion construct created with oligonucleotides BH101F and BH101R, BH111F and BH111R, and BH12F and BH12R, as described above. The final 4,895-bp product was transformed into strain BH140, resulting in strain BH160 (swi4::hisG/MET::SW6-HIS1/swi4::URA3). The second copy of SW6 was deleted using a PCR fusion construct created from oligonucleotides BH2F and BH2R, BH3F and BH3R, and BH4F and BH4R as described above, and the final 2,721-bp product was transformed into strain BH160, resulting in strain BH190 (swi4::hisG/MET::SW6-HIS1/URA3/swi6::URA3). A prototrophic phenotype was selected by growth on YPD plates. For transcription profiling of strains lacking Swi4p and Swi6p, overnight cultures of strains BH190 and BH2420 were diluted to an OD540 of 0.2 in 10 ml of repressing medium and collected after 7 h of incubation at 30°C. Cell pellets were frozen and stored at −80°C until use. Microarray hybridizations were performed with four pairs of RNA preparations produced from independent cultures. RNA from cell pellets was extracted using a MasterPure yeast RNA purification kit (Epicentre Biotechnologies, InterScience, Markham, ON, Canada). Total RNA (40 μg) was used in direct labeling with dCTP linked to Cy3 or Cy5. Sample labeling and hybridizations to oligonucleotide microarrays were performed as described previously (57). Slides were scanned and quantified with an Axon GenePix pro 4.0 scanner (Axon Instruments, Inc., Sunnyvale, CA). Data normalization using LOWESS and statistical analyses were performed with Genespring, version 7.3 (Agilent Technologies, Santa Clara, CA). Genes with a significant change in transcript abundance were identified in a Volcano plot using a 1.7-fold-change cutoff with an r test function using as the confidence level a P value of <0.05. To construct pie chart distributions of the total set of modulated genes, the data were manually sorted into select categories. The data were also sorted according to biological processes using the gene ontology (GO) Slim Mapper at Candida Genome Database (CGD) (http://www.candidagenome.org/cgi-bin/GO/goTermMapper). Significant enrichment of genes within a particular process was determined by comparing the number of modulated genes within Swi4p- and Swi6p-depleted cells that grouped to a particular process to the total number of genes within the genome that sorted to the same process, using the Fisher exact test. Alternatively, we used gene set enrichment analysis (GSEA) (56, 72) to compare a ranked list of genes modulated in the Swi4p- and Swi6p-depleted cells to 29 lists of 64 to 558 genes that exhibit cell cycle-dependent periodic expression in C. albicans opaque cells (26) or significant changes during polar morphogenesis, including yeast-to-hypha transitions and the production of highly elongated buds due to M or S phase arrest (5, 34, 43, 53, 58, 71). We used the “classic” enrichment statistic setting and calculated the false discovery rate (FDR) by performing 10,000 permutations. Selected result graphs are shown, and the complete GSEA output folder is included as GSEA data file S1 in the supplemental material.

Cell imaging. Nuclei and septa were visualized by fixing cells in 70% ethanol for 1 h, followed by incubation in 1 μg/ml 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma) for 20 min. After rinsing with double-distilled water, cells were incubated in 1 μg/ml calcifluor white (Sigma) for 10 min. Nomarski differential interference contrast (DIC) and fluorescent images were obtained with a Leica DMI6000B microscope (Leica Microsystems Canada, Inc., Richmond Hill, ON, Canada) equipped with a Hamamatsu ORCA ER camera (Hamamatsu Photonics, Hamamatsu City, Japan) using 63× or 100× objectives and DAPI (460-nm) filter sets. Images were captured with Openlab software (Improvision, Inc., PerkinElmer).

RESULTS

Cells lacking SW4 or SW6 demonstrate drastic changes in growth pattern, unlike cells lacking MBP1. Candida albicans open reading frames (ORFs) 19.5855, 19.4545, and 19.4725 are annotated as MBP1, SW14, and SW16, respectively, in the Candida Genome Database (http://candidagenome.org) and are 29, 23, and 26% identical to orthologues in S. cerevisiae at the protein level. The factors contain ankyrin repeat domains, similar to SBF/MBF components in other fungi (9). However, leucine zippers found in Swi4p and Swi6p of S. cerevisiae (69) are lacking, and all three C. albicans factors contain a KIA-N DNA binding domain, which is absent in Swi6p of S. cerevisiae.

The functions of MBP1p, Swi4p, and Swi6p in C. albicans were determined by sequentially replacing alleles with UR43 and HIS1 markers in strain BW171, creating strains BH261, BH1185, and BH120, respectively. An isogenic control strain,
FIG. 1. Deletion of SWI4 or SWI6 results in dramatic changes in growth pattern, including cell enlargement and induction of filaments, in contrast to deletion of MBP1. (A) Cells from strains BH440 (MBP1/MBP1 SWI6/SWI6 SWI4/SWI4 UR43+ HIS1+), BH261 (mbp1Δ:URA3/mbp1Δ:URA3), BH120 (swi6Δ:URA3/swi6Δ:HIS1), and BH185 (swi4Δ:URA3/swi4Δ:HIS1) were incubated in liquid glucose minimal medium overnight and then diluted into fresh medium and incubated for 7 h at 30°C. Strain BH190 (swi6Δ:URA3/swi6Δ:HIS1 swi4Δ:hisG/MET3::URA3/ARG4) was incubated in repressing medium for 7 h.

(B) Strains BH120, BH185, and BH440 at higher magnification. Bar = 10 μm.

BH440, and conditional strains for MBP1 (KMCa4a), SWI4 (BH150), and SWI6 (CB557), respectively, were also constructed. To determine effects on cell growth, overnight cultures were diluted into fresh minimal medium and incubated for 7 h at 30°C. Most mbp1ΔΔ cells (BH261) were normal in morphology, but 7.4% (n = 309) were elongated, compared to 0.3% (n = 343) in the control strain (Fig. 1). When the conditional MBP1 strain KMCa4a was incubated in inducing (–MC) or repressing (+MC) medium, most cells were in a normal yeast form (see Fig. S1 in the supplemental material).

Our results agree with large-scale screens of C. albicans mutants which showed that mbp1ΔΔ cells (20%) showed normal cell growth and phenotype defects in repressing medium, although the effects were not as severe in strain CB557 (see Fig. S1 in the supplemental material). Thus, Mbp1p is not essential for normal cell growth and has only a mild influence on morphology in C. albicans.

In contrast, cells lacking SWI4 (BH185) or SWI6 (BH120) showed a dramatic and pleiotropic change in growth pattern, including enlarged budding yeast cells and a diversity of elongated and filamentous cells (Fig. 1). Length-to-width measurements of yeast cells in strains lacking SWI4 (30.6 ± 1.3 μm², n = 102) and SWI6 (30.9 ± 1.2 μm², n = 100) were greater than those of control cells (18.5 ± 0.5 μm², n = 101) (Fig. 2). In addition, approximately 46% (n = 193) and 44% (n = 144) of cells from strains BH185 and BH120, respectively, were elongated or filamentous. Similarly, in large-scale phenotypic screens (40, 61), swi4ΔΔ colonies and cells showed growth defects, although colony morphology on solid medium was unaffected. We also found that swi4ΔΔ and swi6ΔΔ colonies did not show dramatic changes in morphology on solid medium (not shown), which suggests that environmental conditions, including liquid versus solid medium, can influence the phenotype. Consistent with the deletion phenotypes, conditional SWI4 (BH150) and SWI6 (CB557) strains grew predominantly in the yeast form under inducing conditions and showed growth and phenotype defects in repressing medium, although the effects were not as severe in strain CB557 (see Fig. S1 in the supplemental material).

Thus, Swi6p and Swi4p influence yeast size and growth patterns in a similar manner, whereas Mbp1p has only a minor effect. The dramatic increase in yeast cell size raises the possibility of a delay in G1 phase and a role for Swi6p and Swi4p in G1/S regulation. In comparison, swi4Δ or swi6Δ cells in S. cerevisiae also demonstrated cell enlargement, bud defects, slow growth, and some elongation but did not produce filaments as seen in the C. albicans mutants (32, 51, 60).

Cells lacking both Swi6p and Swi4p or Mbp1p are viable and do not arrest in G1 phase. Factors comprising MBF in S. pombe or SBF/MBF in S. cerevisiae are crucial for cell cycle entry; cells lacking either Cdc6, Res1, and Res2, Swi4p and Swi6p, or Swi4p and Mbp1p are inviable and/or
In order to obtain additional evidence that Swi4p and Swi6p play a role in G1/S regulation in *C. albicans*, we created a conditional strain lacking both copies of *SWI6* and carrying a single copy of *SWI4* under the control of the *MET3* promoter (BH190), as well as an isogenic control strain (BH420). Overnight cultures of cells were incubated in fresh inducing or repressing medium for 7 h at 30°C. Under repressing conditions, strain BH190 was viable and showed yeast cell enlargement comparable to that in the single deletion strains, based on length-to-width measurements (33.1 ± 1.3 μm², n = 100) (Fig. 2 and 3A). However, the number of elongated or filamentous cells was moderately higher in repressing medium than in inducing medium (66%, n = 150, versus 37%, n = 230, respectively). In contrast, control cells (BH420) were in a normal yeast form (Fig. 3A). In confirmation of the phenotype, subsequent deletion of both *SWI6* and *SWI4* (AG168) resulted in similar effects (Fig. 3B). Thus, in contrast to the situation in *S. cerevisiae*, the combined functions of Swi4p and Swi6p are important but not essential for cell proliferation in *C. albicans*.

We next investigated whether the combined functions of Swi4p and Mbp1p were essential for growth. A conditional strain (BH277) lacking both copies of *MBP1* and carrying a single copy of *SWI4* under the control of the *MET3* promoter was created. In inducing medium, few cells (6.9%, n = 153) showed elongation, comparable to *mbp1Δ* cells (Fig. 3A). In repressing medium, cells remained viable and appeared similar to *swi4Δ* cells (Fig. 3A), with 37% (n = 230) in an elongated or filamentous form. Since strain BH277 was viable, a strain with *SWI4* and *MBP1* deleted was constructed (HH62). The phenotype was similar to that of strain BH277 under repressing conditions, with 30.8% (n = 221) elongated or filamentous...
The slight reduction in filamentation compared to that seen in other mutants could reflect a mild synergistic effect. Collectively, these data show that Mbp1p plays a minor role in growth, that Swi4p and Mbp1p are not highly redundant in function, and that cells lacking both factors can progress through the cell cycle, unlike the situation in *S. cerevisiae* (44).

The gene expression patterns in cells lacking Swi6p and Swi4p suggest that these factors influence G1/S progression. Cote et al. (26) reported the transcription profiles of synchronized opaque phase cells as they pass through the cell cycle, which consisted of four waves of expression. Each wave was represented by a specific set of keynote genes, many of which resembled those expressed at similar cell cycle stages in *S. cerevisiae* (40). The four waves were also associated with putative core transcription factor regulators, including Fkh2p (S/G2), Mcm1p (G2/M), and Ace2p (M/G1). The wave of G1/S-associated gene expression was suggested to be mediated by a single ankyrin motif-containing MBF complex, as seen in *S. pombe*, since MCB elements were present in promoters of the G1/S cluster of genes and the *C. albicans* genome lacked SCB elements (26). Since Swi4p and Swi6p in *C. albicans* have pronounced and similar effects on cell size and growth pattern, our results raise the possibility that these factors may be critical components of MBF in *C. albicans*, with Mbp1p playing only a minor role. To obtain additional evidence for this model, we first investigated whether the MCB-containing G1 cyclins *PCL2* and *CCN1* (15, 26) were downregulated in cells lacking Swi4p and Swi6p. Overnight cultures of swi6Δ/swi6Δ swi4Δ/*MET3::SWI4* (BH190) and SWI4/SWI4 SWI6/SWI6 (BH420) cells were incubated in fresh repressing medium for 7 h at 30°C, and RNA was extracted for Northern blot analyses. Both cyclins were repressed in strain BH190 (Fig. 4A), suggesting that Swi4p and Swi6p influence G1/S progression.

In order to gain more evidence that Swi4p and Swi6p contribute to G1/S regulation, we used oligonucleotide microarrays to measure the transcription profiles of Swi4p- and Swi6p-depleted cells, despite the fact that asynchronous growth and a pleiotropic phenotype could dilute relevant cell cycle stage-specific expression patterns. Indeed, when a similar approach was used with swi4Δ cells of *S. cerevisiae*, very few SBF targets were identified, which was attributed in part to the lack of cell synchrony (42). However, it is difficult to obtain synchronous cell populations and subsequent time course-based transcription profiles in *C. albicans* (26), and some expression patterns may be strong enough to overcome the barriers imposed by asynchrony and pleiotropic phenotypes. Overnight cultures of swi6Δ/swi6Δ swi4Δ/*MET3::SWI4* and SWI4/SWI4 SWI6/SWI6 cells were thus incubated in repressing medium for 7 h at 30°C and processed for microarray analysis. Significantly modulated genes were initially obtained using a 1.7-fold cutoff and a t test function with a P value of <0.05 (see Table S1 in the supplemental material for a complete list of genes). Based on this method, a small proportion (11.0%) of genes was found to overlap with the total set of periodically expressed cell cycle-regulated genes of Cote et al. (26) (Fig. 4B). Of these, 31.0% corresponded to the G1/S cluster, while 25.0, 22.5, and 21.5% corresponded to S/G2, G2/M, and M/G1 clusters, respectively (see Table S2 in the supplemental material).

To confirm whether G1/S cluster genes were significantly enriched in Swi4p- and Swi6p-depleted cells, we used gene set enrichment analysis (GSEA) (56, 72), a computational method that determines whether defined sets of genes exhibit a statistically significant bias in their distribution within a ranked gene list. Since GSEA is a rank-based method that is not limited to an arbitrarily defined set of significantly modulated genes, it allows us to detect more subtle changes in transcript profiles. In addition to calculating a P value for any observed enrichment, this method also calculates a false discovery rate (FDR) by performing a permutation analysis on 10,000 randomly distributed datasets. The Broad Institute maintains an extensive web page on this tool, including a detailed guide on interpreting GSEA results (http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html). We thus compared a ranked list of the 5,675 genes whose transcript profiles were measured in cells lacking Swi4p and Swi6p to the distribution of the four cell cycle gene clusters defined by Cote et al. (26), as well as 25 gene sets representing morphogenetic switching, including the yeast-to-hypha transition, in *C. albicans* (5, 34, 43, 53, 58, 71).

As seen in Fig. 5 and in Table S3 in the supplemental material, genes of the G1/S cluster showed a highly significant bias toward upregulation (normalized enrichment score \[\text{NES} = 3.41, P < 0.0001, \text{FDR} < 0.01\%\]) in cells lacking Swi4p and Swi6p. Fifty-three percent of the G1/S cluster genes were present in the leading edge, which is defined as genes that were ranked at an earlier position than the maximal enrichment score. We also observed an enrichment of the upregulated G2/M cluster genes, although it was not as strong as the G1/S cluster. There was no significant enrichment of the S/G2 and M/G1 cluster genes. These results suggest that cultures lacking Swi4p and Swi6p have a greater proportion of cells in the G1/S phase than a culture of control cells, supporting a role for Swi4p and Swi6p in G1/S regulation. Impaired progression through G2/M is also possible but not as clear.

Of the genes that were strongly modulated and in common with the periodic cell cycle set of Cote et al. (26), the MCB-containing G1 cyclin *PCL2* was the most strongly downregulated gene (see Tables S1 and S2 in the supplemental material). *CCN1* was not included in the list, in contrast to the Northern blot results. However, its exclusion was due to the fact that it has a very low expression level and was only detected on two of the microarray experiments, where it was strongly downregulated. Additional downregulated MCB-containing factors include the G1/S keynote genes *HCM1* and *GIN4* (26), the chromatin cohesin factor *MCD1*, and genes of unknown function. More G1/S-associated genes were upregulated, in agreement with the GSEA results, including the keynote gene *YOX1* (26), a target of SBF in *S. cerevisiae*, and factors associated with DNA synthesis and DNA repair (*RNR1, DUT1, PMS1, MLH1*, and *TERT*) and other functions (see Table S2 in the supplemental material). These expression patterns raise the possibility that Swi4p and Swi6p have repressing and inducing activities. In support of this, MFB is a transcriptional repressor of genes predominantly associated with DNA replication and repair in *S. cerevisiae*; the absence of *MBP1* or *SWI4* and *MBP1* resulted in elevated levels of DNA repair genes (12, 28). Moreover, the absence of Res1 or Res2 in *S. pombe* resulted in the repression or induction, respectively, of MBF target genes (11). Few other MCB-containing genes which were not identified by Cote et al. (26) were modulated in cells lacking Swi4p and Swi6p. These included un-
characterized genes (orf19.6048, orf19.4664, and orf19.413), a putative ion exchanger (orf19.2397), and a transcriptional repressor of hyphal formation that is responsive to DNA damage (RFX2). Of the S/G2 cluster genes, the putative central G2/M regulator FKH2 (26) was repressed, as well as histones (HHO1 and HTA3), gamma tubulin (TUB4), and other factors, most of which had unknown functions. The strongly modulated G2/M-associated genes did not include cell cycle regulatory factors, with the exception of a spindle midzone-associated protein (ASE1) (see Table S2 in the supplemental material). M/G1 factors notably included the key gene CDC6, encoding a component of the prereplicative complex (see Table S2). Intriguingly, the putative central regulator of the M/G1 cluster genes, MCM1, was also repressed. Thus, the expression profiles revealed modulation of some key MCB-containing genes, as well as several core putative regulators of subsequent cell cycle phases. Modulation of genes associated with other cell cycle stages could reflect additional functions for Swi4p and Swi6p, particularly in G2/M phase, based on GSEA results. Alternatively, this may represent indirect effects of the initial impairment in G1/S progression; cell cycle-dependent transcription patterns are regulated by a hierarchy of sequentially expressed networks of transcription.
factors (64), and in S. cerevisiae, SBF/MBF indirectly act on genes in subsequent cell cycle stages through their transcription factor targets and subsequent effectors (41). Cells lacking Swi4p and Swi6p demonstrated strong modulation of additional genes associated with many other cellular processes (Fig. 4C; also see Table S1 in the supplemental material). Although the majority of these genes lack MCB elements and the responses likely reflect indirect effects, some of the expression patterns further support an influence on G1 phase. For example, ribosome biogenesis and RNA metabolism genes were downregulated (see Table S1) and identified by gene ontology (GO) term analysis as being the most significantly enriched in Swi4p- and Swi6p-depleted cells (Table 4). In addition, orthologues of TOR (target of rapamycin) pathway-dependent regulators of cell size, proliferation, and ribosome biogenesis, including SFP1, SCH9 (48), and TBF1 (39), were repressed in Swi4p- and Swi6p-depleted cells (see Table S1). Regulation of ribosome biogenesis is not clear in C. albicans, but it is intriguing that ribosome biogenesis is sensed and initiated at start in S. cerevisiae (16). PES1, a pescadillo orthologue important for yeast growth in C. albicans (67) but not identified in the periodic data set of Cote et al. (26), was also downregulated (see Table S1). Paradoxically, the G1 cyclin CLN3 was induced in cells lacking Swi4p and Swi6p (see Table S1). While Yox1p negatively regulates CLN3 expression in S. cerevisiae (41), both of these factors were induced in Swi4p- and Swi6p-depleted cells of S. cerevisiae (41), both of these factors were induced in Swi4p- and Swi6p-depleted cells of C. albicans, demonstrating another example of subtle rewiring within the otherwise generally similar framework of cell cycle expression patterns in the two organisms (26). Collectively, these results demonstrate that Swi4p and Swi6p influence G1/S phase progression and, thus, may contribute to MBF activity.

Swi4p and Swi6p influence morphogenesis, including differentiation of hyphae. G1/S circuit components can regulate developmental processes independent of their cell cycle function. For example, E2F and pRb in mammals are linked to neuronal and adipocyte differentiation (54), whereas the SBF targets Tos4p and Yox1p in S. cerevisiae regulate genes associated with mating and pseudohyphal growth, respectively (41). However, the absence of SBF alone does not lead to changes in cell fate in the latter. To determine whether Swi4p and Swi6p also influenced developmental events in C. albicans, the phenotypes of cells lacking these factors were examined more closely. Strains lacking SWI4 (BH185) or SWI6 (BH120) or both

![Gene set enrichment analysis (GSEA)](image)

FIG. 5. Gene set enrichment analysis (GSEA) of the transcriptional profile of Swi4p- and Swi6p-depleted cells. A ranked list of genes modulated in Swi4p- and Swi6p-depleted cells was compared to 29 gene sets of 64 to 558 genes that exhibit cell cycle-dependent periodic expression in C. albicans opaque cells (26) or significant changes during polar morphogenesis, including yeast-to-hypha transitions and the production of highly elongated buds due to M or S phase arrest (5, 34, 43, 53, 58, 71). Genes in the ranked list are organized along the x axis with upregulated genes to the left and downregulated genes to the right. The positions of the genes in each gene set are illustrated by the vertical black bars, while the green curve represents the cumulative value of the enrichment score (y axis). Graphs of selected results are shown, while the complete GSEA output folder is included as GSEA data file S1 in the supplemental material. BMDM, bone marrow-derived monocytes; RHE90, reconstituted human epithelial cells; HU, hydroxyurea.

#### TABLE 4. GO term analysis of significantly modulated genes in cells lacking Swi4p and Swi6p

| GO category          | P value | GO genes in set (834 total) | GO genes in genome (6,804 total) |
|----------------------|---------|----------------------------|----------------------------------|
| Ribosome biogenesis  | 2.7E−13 | 88                         | 746                              |
| Biological process   | 8.3E−7  | 263                        | 2,741                            |
| RNA metabolic process| 1.7E−6  | 123                        | 627                              |
| Response to chemical stimul | 4.5E−3  | 93                         | 741                              |
| Carbohydrate metabolism | 2.3E−3  | 47                         | 787                              |

* Strains analyzed were BH190 (lacking Swi4p and Swi6p) and BH420 (wild type).
* The GO type was biological process.
* Significance of overrepresentation of GO categories represented in significantly modulated genes based on the Fisher exact test.
* Number of genes within total significantly induced gene set of Swi6p- and Swi6p-depleted cells that are associated with the select GO category.
* Total number of genes within the genome that associate with the selected GO category.
AG168 and BH190) demonstrated a pleiotropic phenotype. Of the yeast cells, many were multibudded (Fig. 1 and 6Ad and g), suggesting defects in cell separation similar to those of swi4/Sw6 cells of *S. cerevisiae* (60). DAPI staining demonstrated that most cells contained a single nucleus, but a proportion showed multinucleation (11.4%, *n* = 158, strain BH185; 9.4%, *n* = 180, strain BH190; and 3.8%, *n* = 160, strain BH420) (Fig. 6Ad and e), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases. Intriguingly, some yeast cells were bean shaped, resembling opaque phase cells, and contained four nuclei (Fig. 6Ae and f), indicating deregulation of nuclear division in some cases.
PHRI, and IHD1, for example, at high levels (21, 43, 58) (see Table S4 in the supplemental material). This provides additional evidence that true hyphae can form upon the depletion of Swi4p and Swi6p. Some hyphal regulators were simultaneously repressed, including CZF1, TPK1, and PDE2 (see Table S4). When we used GSEA to compare the transcriptional profiles of the Swi4p- and Swi6p-depleted cells to the results of other microarray-based experiments based on morphogenetic switching, including yeast-to-hyphal cells, the strongest enrichments were seen with gene sets obtained from Cdc5p-depleted or hydroxyurea-treated cells, which form hypha-like elongated buds (5) (Fig. 5). Intriguingly, the analysis also revealed very strong enrichments of gene sets from the profiles of C. albicans interacting with host cells, such as primary mouse macrophages (53), reconstituted oral human epithelium (71), or polymorphonuclear leukocytes (34). In contrast, gene sets from laboratory-induced hyphae (43, 58) were not as enriched, mostly due to the fact that their upregulated genes tended to cluster at both the top and the bottom of the ranked gene list of Swi4p- and Swi6p-depleted cells (data not shown). These results suggest that Swi4p and Swi6p can influence the signaling associated with host cell-induced morphogenetic switching, including hyphal induction.

**DISCUSSION**

The G1/S transition serves as the gateway to cell proliferation but is not well characterized in C. albicans. Ankyrin-repeat motif proteins, including Swi4p, Swi6p, and Mbp1p in S. cerevisiae and Cdc10, Res1, and Res2 in S. pombe (9), comprise the major components of G1/S transcription factor complexes in fungi studied to date and are crucial for growth. Here, we show that the closest orthologues of Swi4p and Swi6p in C. albicans are important for cell proliferation and, thus, may contribute to MBF activity but that their combined function is, surprisingly, not essential for growth. We also show that these factors strongly influence morphogenesis, including hyphal differentiation, and expression patterns normally induced by interactions with host cells, supporting the existence of an important relationship between G1/S phase of the cell cycle and hyphal development in C. albicans.

Swi6p and Swi4p are important for yeast cell proliferation and may contribute to MBF activity. Our results provide functional evidence that orthologues of Swi4p and Swi6p are important for yeast cell proliferation in C. albicans, influence G1/S progression and, thus, may contribute to MBF activity. First, key MCB-containing G1/S genes (26) were modulated in cells lacking Swi4p and Swi6p, including the G1 cyclins PCL2 and CCN1 (15), the transcription factor YOX1, and factors associated with DNA synthesis/repair, including RNR1. G1/S cluster genes were significantly enriched and represented the highest proportion of periodically expressed cell cycle genes modulated in the cells. The G2/M cluster genes were also enriched but to a lesser extent, which could imply a role for Swi4p and Swi6p in regulating additional cell cycle stages, given the rewiring in the cell cycle circuitry of the pathogen (15, 26). Alternatively, G2/M gene modulation may be an indirect response to a G1/S delay, since G1/S events influence transcription in subsequent cell cycle stages in S. cerevisiae (41), and the basic cell cycle expression program in C. albicans is very similar (26). The total number of MCB-containing genes modulated in cells lacking Swi4p and Swi6p was not high, which could imply that Swi4p and Swi6p do not function via interaction with MCB elements. Alternatively, asynchronous growth, pleiotropy in phenotype, and/or redundancy from other unknown factors could mask specific expression patterns, particularly those that are subtle. Indeed, transcription profiles of Δswi4 cells in S. cerevisiae did not reveal modulation of many SBF targets, which was attributed in part to asynchronous growth (42) and could also be due to compensation from Mbp1p (12). Our use of GSEA circumvented these problems in part, revealing significant enrichment of G1/S genes. Second, cells lacking Swi4p and/or Swi6p showed similar growth defects, including a dramatic increase in yeast cell size, suggesting a delay in G1 phase. Although an increase in size could occur by slowing G2/M phase, our results suggest that G1 phase is a major and possibly primary target of Swi4p and Swi6p. Third, additional expression patterns in cells lacking Swi6p and Swi4p, including repression of ribosome biogenesis genes, suggest a role for these factors in G1/S regulation. Ribosome biogenesis, controlled in part by the TOR pathway, is coordinated with cell cycle initiation in other organisms (46, 65) and feeds back on start in S. cerevisiae via Whi5p (16). Finally, the ability to form true hyphae upon depletion of Swi4p and Swi6p also supports a role for these factors in G1 phase progression. Previous work demonstrated that true hyphae with active cell cycles could form when G1 phase was blocked through depletion of the G1 cyclin CLN3 (7, 22). True hyphae also formed in the absence of the SCF ubiquitin ligase F-box protein Cdc4p, which has a G1/S-associated function in S. cerevisiae (2). In contrast, blocking or slowing other cell cycle phases in C. albicans results in elongated buds and/or pseudohyphal growth (15). Collectively, the results strongly suggest that Swi4p and Swi6p influence G1/S progression and, thus, may contribute to MBF activity. Future investigations involving chromatin immunoprecipitation-microarray (ChIP-chip) analysis will help to clarify the precise binding sequences and targets of these factors.

Swi4p, Swi6p, and Mbp1p show unique features compared to orthologues in other systems. Our results highlight key differences in the proposed functions of Swi4p and Swi6p compared to orthologues in other systems. A central finding is that the combined function of C. albicans Swi4p and Swi6p is not essential for cell proliferation. In contrast, the absence of both orthologues in S. cerevisiae results in nonviable cells; Mbp1p does not compensate (17, 44). Although we cannot rule out whether Mbp1p compensates for the lack of Swi4p and Swi6p in C. albicans, it plays a limited role in growth and shows low redundancy with Swi4p, based on the different phenotypes of swi4ΔΔ and mbp11ΔΔ cells and the lack of synergistic effects in the absence of both genes. This suggests that other factors may be involved in controlling cell proliferation. The viability of swi4ΔΔ mbp11ΔΔ cells of C. albicans is also novel and contradicts the lethal effects resulting from the absence of both orthologues in S. cerevisiae (44) or of both Res1p and Res2p in S. pombe (11, 55). It is possible that Swi6p in C. albicans can compensate under these conditions, since it contains a KiA-N DNA binding domain, unlike Swi6p in S. cerevisiae. However, the Swi6p orthologue Cdc10 in S. pombe also contains this domain but does not bind DNA directly or compensate for loss
of Res1 and Res2 (78). Thus, additional elements may be involved in G_{1}/S control in *C. albicans*. Candidate factors include the APSES domain-containing proteins Elf1p or Elf1p, which can bind MluI sites (63). However, there is currently no evidence supporting a role for either factor in regulating cell proliferation. Additional ankyrin-repeat domain proteins of unknown function and low homology to Swi4p, Swi6p, or Mbp1 exist in *C. albicans*, and we are currently exploring their potential contributions.

Another difference in the putative functions of Swi4p and Swi6p includes the possibility that they possess both activating and repressing activity, based on the repression and induction of G_{1}/S cluster genes. More genes were induced, and these included factors associated with DNA synthesis and repair. Intriguingly, MBF in *S. cerevisiae* represses its targets, which include DNA synthesis and repair factors, in cell cycle phases other than G_{1}/S with the assistance of the corepressor Nrm1p (27). In contrast, the SBF complex activates its targets. In *S. pombe*, the heteromeric MBF has two constitutive DNA binding elements, Res1 and Res2, but G_{1} transcripts increased or decreased in res1Δ versus res2Δ cells, respectively (11). Since the absence of Swi4p or Swi6p in *C. albicans* produced a similar phenotype, it is not likely that one is an activator while the other is a repressor. The putative dual function of Swi4p and Swi6p in regulating targets may be mediated by cofactors. In support of this, a candidate Nrm1p homologue exists in *C. albicans* that genetically interacts with and antagonizes putative Swi4p/Swi6p function (D. Kornitzer, personal communication).

If Swi4p, Swi6p, and other factors contribute to MBF activity, the resulting organization of this complex in *C. albicans* is not clear. The absence of Swi4p and Swi6p resulted in synergistic effects on filamentation, suggesting that these factors may act separately. However, this did not extend to cell cycle function, as the single and double mutants showed similar increases in yeast cell size. In addition, Swi4p and Swi6p physically interact in *C. albicans* (C. Bachewich, unpublished observations). Given that *S. cerevisiae* contains two G_{1}/S transcription units as a consequence of undergoing a whole-genome duplication (26), it is unexpected that *C. albicans*, with its more simplified genome and absence of SCB elements, would also require several G_{1}/S transcription complexes. The potential contribution of Mbp1p is also not clear, but it may be more important under different conditions or in different cell types, since Res2 of *S. pombe* contributes to mitotic cell proliferation but plays a stronger role during meiotic division (4, 55, 84). Overall, our results highlight potential variations in the putative G_{1}/S regulatory circuit in *C. albicans* versus other fungi.

**Swi4p and Swi6p play additional roles in morphogenesis and influence hyphal differentiation.** The absence of Swi4p and Swi6p resulted in pleiotropic morphologies, due in part to defects in budding. The pleiotropy could reflect variability in the levels of G_{1} cyclins and/or other regulatory factors which influence the timing of progression through G_{1}/S phase and, perhaps, subsequent cell cycle stages, generating different cell shapes and types. The phenotype of cells lacking Swi4p and Swi6p was reminiscent of that of Grr1p mutants (47), with the exception that true hyphae could also form, based on strong modulation of several hyphal-associated genes and the presence of unconstricted septa. The relationship between hyphal development and G_{1} phase in *C. albicans* is complex, since there is conflicting information on whether hyphal initiation can occur at later cell cycle stages (15, 37, 70). A G_{1} phase-dependent bias for hyphal initiation could exist (15, 70), but strong environmental inducers, such as serum, may be able to override this relationship, allowing hyphal formation at other cell cycle stages (37). Consistent with this, hyphae and pseudohyphae form when yeast cells of *C. albicans* are arrested in G_{1} phase through depletion of the G_{1} cyclin Cln3p (7, 22). In contrast, arresting or slowing other cell cycle phases results in elongated buds or pseudohyphae, respectively (15). The presence of true hyphae within the filamentous population of cells lacking Swi6p and Swi4p suggests that these factors may mediate, in part, the effect of Cln3p on hyphal development. The transcription profiles of Swi4p- and Swi6p-depleted cells, which show strong enrichment of gene sets from profiles of *C. albicans* interacting with a diversity of host cells, further suggest that Swi4p and Swi6p function may contribute to host cell-induced morphogenetic switching, including hyphal induction. The factors may lie downstream of additional pathways, since Swi4p is a mitogen-activated protein kinase (MAPK) target (8, 45, 52). Alternatively, it is possible that hyphal growth is a general response to aspects associated with a G_{1} phase delay and that Swi4p and Swi6p play only an indirect role. The initial presence of pseudohyphal followed by hyphal characteristics in some cells supports this point. However, true hyphae could also form directly from yeast, and intriguingly, G_{1}/S transcription regulators can independently influence developmental gene expression in other systems (41, 54). Many yeast cells lacking Swi4p and Swi6p also resembled opaque phase cells. Since a downstream target of SBF in *S. cerevisiae* functions as a repressor of mating, it is possible that Swi4p and Swi6p function and/or the G_{1}/S circuit influence additional developmental pathways in *C. albicans*, which is currently under investigation.

In summary, we have demonstrated that Swi4p and Swi6p are important for cell proliferation and influence G_{1}/S progression, suggesting that they may contribute to MBF-like activity in *C. albicans*. Our results also suggest that the emerging G_{1}/S regulatory circuit in *C. albicans* has unique features compared to those in other ascomycetes and is linked to aspects of hyphal development, possibly through Swi4p and Swi6p function. Future work addressing the composition, regulation, and targets of MBF and the G_{1}/S regulatory machinery in *C. albicans* will significantly advance our understanding of the thematic variations in how cells regulate basic proliferation and coordinate this process with developmental events, which are critical for virulence in an important fungal pathogen of humans.

**ACKNOWLEDGMENTS**

We thank M. Whiteway (Health Sector, Biotechnology Research Institute, NRC) and D. Kornitzer (Technion, IIT) for comments on the manuscript and P. Cote (Health Sector, Biotechnology Research Institute, NRC) for sharing bioinformatic data.

This work was supported by Canadian Institutes of Health Research Operating Bridge grant IG1-78908 (C.B.) and, in part, through Canadian Institutes of Health Research Team grant CTP79843 (C.B. and A.N.).
Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat. Genet. 42:590–598.

62. Noble, S. M., and A. D. Johnson. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot. Cell 4:298–309.

63. Noffz, C. S., V. Liedschulte, K. Lengeler, and J. F. Ernst. 2008. Functional mapping of the Candida albicans Efg1 regulator. Eukaryot. Cell 7:881–893.

64. Orlando, D. A., et al. 2008. Global control of cell-cycle transcription by coupled CKI and network oscillators. Nature 453:944–947.

65. Pestov, D. G., Z. Strezoska, and L. F. Lau. 2001. Evidence of p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G1/S transition. Mol. Cell. Biol. 21:4246–4255.

65a. Rose, M. D., F. Winston, and P. Hieter. 1990. Methods in yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

66. Saville, S. P., et al. 2006. Inhibition of filamentation can be used to treat disseminated candidiasis. Antimicrob. Agents Chemother. 50:3312–3316.

67. Shen, J., L. E. Cowen, A. M. Griffin, L. Chan, and J. R. Kohler. 2008. The Candida albicans pescadillo homolog is required for normal hypha-to-yeast morphogenesis and yeast proliferation. Proc. Natl. Acad. Sci. U. S. A. 105:20918–20923.

68. Shi, Q. M., Y. M. Wang, X. D. Zheng, R. T. Lee, and Y. Wang. 2007. Critical role of DNA checkpoints in mediating genotoxic-stress-induced filamentous growth in Candida albicans. Mol. Biol. Cell 18:815–826.

69. Sidorova, J., and L. Breeden. 1993. Analysis of the SW14/SWI6 protein complex, which directs G1/S-specific transcription in Saccharomyces cerevisiae. Mol. Cell. Biol. 13:1069–1077.

70. Soll, D. R., M. A. Herman, and M. A. Staebell. 1985. The involvement of cell wall expansion in the two modes of mycelium formation of Candida albicans. J. Gen. Microbiol. 131:2367–2375.

71. Spiering, M. J., et al. 2010. Comparative transcript profiling of Candida albicans and Candida dubliniensis identifies SFL2, a C. albicans gene required for virulence in a reconstituted epithelial infection model. Eukaryot. Cell 9:251–260.

72. Subramanian, A., et al. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. 102:15545–15550.

73. Sudbery, P., N. Gow, and J. Berman. 2004. The distinct morphogenetic states of Candida albicans. Trends Microbiol. 12:317–324.

74. Sudbery, P. E. 2001. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. Mol. Microbiol. 41:59–31.

75. Trusk, K., et al. 2009. Depletion of the cullin Cdc53p induces morphogenetic changes in Candida albicans. Eukaryot. Cell 8:756–767.

76. Umeyama, T., A. Kaneko, M. Niimi, and Y. Uehara. 2006. Repression of CDC28 reduces the expression of the morphology-related transcription factors, Efg1p, Nrg1p, Rbf1p, Rim101p, Fkh2p and Tec1p and induces cell elongation in Candida albicans. Yeast 23:537–552.

77. Verma, R., J. Smiley, B. Andrews, and J. L. Campbell. 1992. Regulation of the yeast DNA replication genes through the MluI cell cycle box is dependent on SW16. Proc. Natl. Acad. Sci. U. S. A. 89:9479–9483.

78. Whitehall, S., P. Stacey, K. Dawson, and N. Jones. 1999. Cell cycle-regulated transcription in fission yeast: Cdc10-Res protein interactions during the cell cycle and domains required for regulated transcription. Mol. Biol. Cell 10:3705–3715.

79. Whiteway, M., and C. Bachewich. 2007. Morphogenesis in Candida albicans. Annu. Rev. Microbiol. 61:529–553.

80. Wightman, R., S. Bates, P. Amornrattanapan, and P. Sudbery. 2004. In Candida albicans, the Nim1 kinases Gin4 and Hsl1 negatively regulate pseudohypha formation and Gin4 also controls septin organization. J. Cell Biol. 164:581–591.

81. Wittenberg, C., and R. La Valle. 2003. Cell-cycle-regulatory elements and the control of cell differentiation in the budding yeast. Bioessays 25:856–867.

82. Yang, L., et al. 2004. Rapid production of gene replacement constructs and generation of a green fluorescent protein-tagged centromeric marker in Aspergillus nidulans. Eukaryot. Cell 3:1359–1362.

83. Zheng, X., Y. Wang, and Y. Wang. 2004. Hgc1, a novel hypha-specific G1 cyclin-related protein regulates Candida albicans hyphal morphogenesis. EMBO J. 23:1845–1856.

84. Zhu, Y., T. Takeda, S. Whitehall, N. Peat, and N. Jones. 1997. Functional characterization of the fission yeast Start-specific transcription factor Res2. EMBO J. 16:1023–1034.