Orthologues of the Anaphase-Promoting Complex/Cyclosome Coactivators Cdc20p and Cdhlp Are Important for Mitotic Progression and Morphogenesis in Candida albicans

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The conserved anaphase-promoting complex/cyclosome (APC/C) system mediates protein degradation during mitotic progression. Conserved coactivators Cdc20p and Cdhlp regulate the APC/C during early to late mitosis and G1 phase. Candida albicans is an important fungal pathogen of humans, and it forms highly polarized cells when mitosis is blocked through depletion of the polo-like kinase Cdc5p or other treatments. However, the mechanisms governing mitotic progression and associated polarized growth in the pathogen are poorly understood. In order to gain insights into these processes, we characterized C. albicans orthologues of Cdc20p and Cdhlp. Cdc20p-depleted cells were blocked in early or late mitosis with elevated levels of Cdc5p and the mitotic cyclin Clb2p, suggesting that Cdc20p is essential and has some conserved functions during mitosis. However, the yeast cells formed highly polarized buds in contrast to the large doublets of S. cerevisiae cdc20 mutants, implying a distinct role in morphogenesis. In comparison, cdh1Δ/cdh1Δ cells were viable but showed enrichment of Clb2p and Cdc5p, suggesting that Cdhlp may influence mitotic exit. The cdh1Δ/cdh1Δ phenotype was pleiotropic, consisting of normal or enlarged yeast, pseudohyphae, and some elongated buds, whereas S. cerevisiae cdh1Δ yeast cells were reduced in size. Thus, C. albicans Cdhlp may have some distinct functions. Finally, absence of Cdhlp or Cdc20p had a minor or no effect on hyphal development, respectively. Overall, the results suggest that Cdc20p and Cdhlp may be APC/C activators that are important for mitosis but also morphogenesis in C. albicans. Their novel features imply additional variations in function and underscore rewiring in the emerging mitotic regulatory networks of the pathogen.

Ubiquitin-mediated protein degradation plays a key role in regulating many stages of cell cycle progression. E3 ubiquitin ligases cooperate with E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes to add ubiquitin residues to specific factors, thereby targeting them for degradation via the proteasome (43). The anaphase-promoting complex/cyclosome (APC/C) system represents an E3 ubiquitin ligase system that is crucial for controlling mitotic progression and maintenance of G1 phase (43). The APC/C is required to reduce mitotic cyclin-dependent kinase (CDK) activity, separate sister chromatids, disassemble the mitotic spindle, and load DNA replication origins (66). APC/C substrate specificity is determined by conserved coactivators, including Cdc20p and Cdhlp (43, 66). Cdc20p targets the degradation of proteins associated with the metaphase-to-anaphase transition and mitotic exit. In Saccharomyces cerevisiae, this includes the securin Pds1p (21) and B-type cyclins, such as Clb2p and Clb5p, for example (9, 63, 80). In the absence of Cdc20p, S. cerevisiae cells arrest in metaphase (29) or in later stages of mitosis if Pds1p is also absent (41). Toward the end of mitosis and during G1 phase, APC/C activity is regulated by Cdhlp, which maintains degradation of Clb2p and targets other mitotic regulators for destruction, including Cdc20p, the polo-like kinase Cdc5p, and various spindle factors (17, 31, 35, 57, 64). CDH1 in S. cerevisiae is not essential, due to activity of the Cdc28p/Clb2p inhibitor Sic1p; cdh1Δ sic1Δ cells are not viable (56). However, cdh1Δ cells grow slowly with mild delays in mitotic exit and abnormalities in spindle formation (56, 79). The cells are small and accelerate expression of the ribonucleotide reductase RNR1, demonstrating that Cdhlp has a negative influence on Start (34). Intriguingly, APC/C activity extends beyond mitotic cell cycle control (43, 81), as Cdc20p and Cdhlp functions are also important for the stability of factors involved in developmental processes, including axon growth and dendrite morphogenesis, for example (36, 38).

Candida albicans is one of the most common fungal pathogens of humans, and it exists in different forms, including white-phase yeast, mating-competent opaque-phase yeast, pseudohyphae, hyphae, and chlamydomycoses (82). Differentiation is an important virulence-determining trait, because mutants incapable of switching between cell types are significantly less pathogenic (42, 55). The regulation of developmental events, including the yeast-to-hypha switch, for example, has been extensively investigated and involves a diversity of environmental cues and signaling pathways (8, 13, 16, 59, 82). However, the mechanisms governing basic cell proliferation, including mitotic progression, are much less understood. Transcriptional responses of C. albicans yeast cells passing through mitosis show some similarity to those of S. cerevisiae cells (22, 68), but functional studies on many of the associated genes are lacking. Of the few factors investigated, several show differences in function from their orthologues in S. cerevisiae and
influence morphogenesis. For example, absence of the CDK of bud growth (5, 6), in contrast to the large doublet morphology of C. albicans points are not well defined in C. albicans mitosis in aed genes in CDC5 resulted in transient elongated bud growth (4), while depletion of mitosis and form pseudohyphae. Clb4p was also shown to contrast, Clb4p-depleted cells are delayed at earlier stages of Clb2p also results in elongated buds in form elongated buds. Expression of a nondegradable form of mitotic entry; Clb2p-depleted cells arrest in telophase and SCF activity may be important for the G1/S phases of the cell cycle (40). APC/C activity, on the other hand, has not been investigated to date in C. albicans.

In order to gain more insights into the regulation of mitosis in C. albicans and explore the link between mitotic progression and polarized morphogenesis, we characterized orthologues of APC/C activators Cdc20p and Cdh1p. While both factors show some conservation in mitotic function, their novel features imply additional distinct roles. Overall, our results suggest that the APC/C is important for mitosis and morphogenesis in C. albicans and underscore variations in the mitotic regulatory networks of the pathogen.

### MATERIALS AND METHODS

**Strains, oligonucleotides, plasmids, culture conditions, and RNA extraction.** Strains, oligonucleotides, and plasmids used in this study are listed in Tables 1 and Tables S1 and S2 in the supplemental material, respectively. Strains were grown at 30°C in synthetic minimal medium containing 0.67% yeast nitrogen base, 2% glucose, and all amino acids. For conditional expression of the promoter, minimal inducing medium (-MC) lacking methionine and cysteine or added to minimal or rich medium, and cells were incubated at 37°C. For phenotypic assays, strains were grown overnight, diluted into fresh medium to an optical density at 600 nm (OD600) of 0.2 and collected after the indicated times. Total RNA extraction and Northern blotting were carried out as previously described (5).

**Strain construction.** A strain carrying a single copy of CDC5 under the control of the MET3 promoter was created. The first copy of CDC5 (orf19.6601) was deleted from strain BWP17 by using the URA3 blaster method as previously described (6, 25), followed by loop out the URA3 marker and selection on

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

| Strain   | Genotype                                          | Source                      |
|----------|---------------------------------------------------|-----------------------------|
| BWP17    | ura3Δ::imm434/ura3Δ::imm434 his1Δ::hisG/his1Δ::hisG arg4Δ::hisG/arg4Δ::hisG | Wilson et al. (83a)         |
| HCCA1    | CDC20/cdc20Δ::URA3                               | This study                  |
| HCCA5    | CDH1/cdh1Δ::URA3                                 | This study                  |
| HCCA7    | CDC5/cdc5Δ::hisG                                 | This study                  |
| HCCA26   | cdc1Δ::URA3/MET3::CDH1-HIS1                       | This study                  |
| HCCA45   | cdc1Δ::URA3/cdh1Δ::HIS1                          | This study                  |
| HHCA100  | BWP17 PRM100 (URA3 HIS1)                         | This study                  |
| HHCA109  | cdc20Δ::URA3/MET3::CDC20-HIS1                     | This study                  |
| HHCA118  | cdc1Δ::hisG/MET3::CDC5-ARG4                      | This study                  |
| HHCA131  | cdc1Δ::hisG/MET3::CDC5-ARG4 CDH1/cdh1Δ::URA3     | This study                  |
| HHCA143  | cdc1Δ::hisG/MET3::CDC5-ARG4 cdc1Δ::His1/cdh1Δ::URA3 | This study                  |
| HHCA153  | cdc1Δ::URA3/cdh1Δ::ARG4                          | This study                  |
| AG139    | CLB2/CLB2-HA-HIS1                                | This study                  |
| AG145    | CDC20/cdc20Δ::URA3 CLB2/CLB2-HA-HIS1             | This study                  |
| AG153    | cdc20Δ::URA3/MET3::CDC20-ARG4 CLB2/CLB2-HA-HIS1  | This study                  |
| AG191    | cdc1Δ::His1/cdh1Δ::URA3 CDC5/CDC5-TAP-ARG4       | This study                  |
| AG262    | cdc1Δ::His1/cdh1Δ::URA3 CDC5/CDC5-TAP-ARG4       | This study                  |
| AG268    | cdc1Δ::URA3/cdh1Δ::HIS1 CLB2/CLB2-HA-ARG4        | This study                  |
| AG280    | CDC5/CDC5-TAP-URA3                               | This study                  |

The Skp/Cullin/F-box (SCF) E3 ubiquitin ligase complex targets degradation of proteins during G1/S to G2/M phases of the cell cycle in most systems, and orthologues of some components have been characterized in C. albicans, including Cdc4p, Grr1p, and Cdc53p (4, 14, 40, 74). Absence of these factors resulted in pseudohyphal and/or hyphal growth. Specific cell cycle defects in the absence of Cdc4p or Cdc53p were not reported, but Grr1p influenced G1 cyclin stability, suggesting that SCF activity may be important for the G1/S phases of the cell cycle (40). APC/C activity, on the other hand, has not been investigated to date in C. albicans.
5′-fluoroorotic acid (Sigma–Aldrich, Oakville, ON, Canada) (6), resulting in strain CDH1. The second copy of CDC5 was then placed under the regulation of the MET3 promoter by using a PCR fusion construction (45, 84). Oligonucleotides HCGS13F and HCGS13R amplified 640 bp of 5′ CDC5-flanking sequence from genomic DNA (gDNA), while oligonucleotides HCGS15F and HCGS15R amplified the CDC5 start site and 518 bp of downstream sequence. Oligonucleotides HCGS14F and HCGS14R amplified an ARG4-MET3 fragment from plasmid pFA-ARG4-MET3 (27). The three resulting fragments were combined and amplified with oligonucleotides HCGS13F and HCGS15R, and the final promoter replacement construct was transformed into strain HCCA1, resulting in strain HCCA118. In order to construct a strain containing a single copy of CDC20 under the control of the MET3 promoter, oligonucleotides HC2F and HC2R containing 80 bp of homology to the 5′ and 3′ CDC20 flanks, respectively, were used to amplify URA3 from pBS-CaURA3. The product was transformed into strain BWP17, generating strain HCCA1. To place the remaining copy of CDC20 under the control of a MET3 promoter, oligonucleotides HCGS1 and HCGS1R amplified 515 bp of CDC20 5′-flanking sequence and oligonucleotides HCGS2F and HCGS4B amplified a fragment containing the CDC20 start site and 720 bp of downstream sequence. A HIS1-MET3 fragment was amplified from plasmid pFA-HIS-MET3 (27) with oligonucleotides HCGS2F and HCGS2R. The products were combined in a fusion PCR with oligonucleotides HIS1C and HCGS10, which were amplified with oligonucleotides HIS1C and HCGS10, respectively, in strain HCCA1 containing an isogenic control strain was created by transforming pPM100 into strain BWP17, creating strain HCCA100. In order to delete both copies of CDH1, oligonucleotides HCF3 and HCF3 containing 80 bp of 5′ and 3′-flanking sequences, respectively, were used to amplify URA3 from plasmid pBS-CaURA3. The resulting deletion construct was amplified from plasmid pBS-CaURA3, generating plasmid pBBP6, amplified with oligonucleotides HCF3 and HCF3, and the final construct was transformed into strain HCCA5. A second deletion strain was constructed in a similar manner, except that the remaining CDH1 allele of strain HCCA5 was replaced with an ARG4 marker, resulting in strain HCCA153. The ARG4-containing fragment was amplified from pBS-CaARG4 with oligonucleotides HCF3 and HCR3. In order to determine if Cdh1p was in the same functional pathway as Cdc5p, one copy of CDH1 was replaced with URA3, as described above, in strain HCCA118, resulting in strain HCCA131. The second copy was replaced with HIS1, as described above, resulting in strain HCCA143. In order to confirm the CDH1 deletion phenotype, a strain carrying a single copy of CDH1 under the control of the MET3 promoter was created. Oligonucleotides HCF5 and HCR5 containing 80 bp of homology to sequences upstream and downstream of the CDH1 start site, respectively, amplified a HIS1-MET3 fragment from pFA-HIS-MET3 (27). The product was transformed into strain HCCA5, resulting in strain HCCA62. All strains were confirmed by PCR. Blot analysis with an anti-GFP antibody was shown. In order to tag Clb2p with three copies of hemagglutinin (HA), HA-HIS1 was amplified from plasmid pFA-HA-HIS1 (39) with oligonucleotides AG4F and AG4R. The product was used as a template in a PCR fusion reaction with oligonucleotides AGSF and AGSR, which contained 100 bp of homology to regions lying upstream and downstream from the stop codon of CLB2, respectively. The fusion construct was transformed into strains BWP17, HCCA1, generating strains A5g39 and A14G5, respectively. The remaining copy of CDC20 in strain A14G5 was placed under the control of a MET3 promoter as described elsewhere, with the exception of using an ARG4-MET3 fragment amplified from plasmid pFA-ARG4-MET3 (27) with oligonucleotides AGHCC2F and HCGS2R. The final fusion product was transformed into strain A14G5, resulting in strain AG153. Clb2p was similarly tagged with HA in strain HCCA5, with the exception of using pFA-HA-ARG4 (39) as a template with oligonucleotides AG4F and AG4R, resulting in strain AG260. In order to tag CDC5, PCR fragments containing either URA3 or ARG4 and 100 bp of homology to sequences immediately up- and downstream from the stop codon of CDC5 were created with oligonucleotides AG1F, AG1R, and plasmids pFA-TAP-URA3 or pFA-TAP-ARG4 (39). PCR constructs were transformed into strains BWP17, HCCA109, and HCCA45, resulting in strains AG280, AG191, and AG262, respectively. All tagged strains were confirmed by PCR and Western blotting. PCRs utilized the Expand long template polymerase (Roche Diagnostics, Laval, QC, Canada). Cells were transformed using lithium acetate (18, 27) with modifications. Transformation mixtures were incubated overnight at 30°C and heat shocked at 43°C for 15 to 60 min prior to plating on selective medium. For increased transformation efficiency in the conditional CDC5 strains, cells were grown overnight in minimal inducing medium lacking methionine and cysteine and then transferred into rich YEPD medium for at least 1 h prior to transformation.

Cell staining and imaging. To visualize nuclei, cells were fixed with 70% ethanol for a minimum of 1 h, stained with 1 μg/ml of 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma–Aldrich) for 20 min, and washed twice with sterile water. To visualize septa, fixed cells were subsequently stained with 2 μg/ml calcofluor white (Sigma–Aldrich) for 10 min. For immunolocalization of α-tubulin, overnight cultures of cells were diluted to an OD600 of 0.2 and incubated for the indicated times in minimal or rich medium. An equal volume of 2× fixative, containing 8% paraformaldehyde, 80 μM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES, pH 7.0), 10 mM MgSO4, 50 mM EGTA, 8 mM AEBSF [4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride], 20 μg/ml leupeptin, and 2 μM aprotinin, was added to cells. Following 20 min of fixation, cells were washed twice with chilled PE buffer (40 mM PIPES, 25 mM EGTA; pH 7.0). Cell walls were digested with chilled PE buffer (40 mM PIPES, 25 mM EGTA; pH 7.0) for 1 h to release cytoplasmic contents. Cells were then incubated with sheep anti-mouse fluorescein isothiocyanate (FITC)–conjugated secondary antibody (1:100; Sigma–Aldrich) in the dark for 2 h at room temperature. Cells were then rinsed with PE buffer and stained with 1 μg/ml DAPI for 20 min. Cells were examined on a Leica DM6000B microscope equipped with a Hamamatsu ORCA ER camera (Hamamatsu Photonics, Hamamatsu City, Japan) using 63× or 100× objectives and DAPI (460-nm) or FITC (520-nm) filters. Images were captured with Openlab software (Improvement Inc., Perkin–Elmer, Waltham, MA).

Protein extraction and Western blotting. Protein extracts were prepared according to the methods described by Bensen et al. (10) with some modifications. For assays involving conditional expression of CDC20, exponential-phase cells grown in minimal inducing (–M) medium were diluted to an OD600 of 0.1 in either minimal repressing (+M) or inducing medium and collected after 3 or 6 h of incubation at 30°C. In order to determine the levels of Clb2p-HA and Cdc5p-TAP in CdH1Δcdh1+ cells, strains were incubated overnight in YEPD medium supplemented with 50 μg/ml uridine. A proportion of the overnight culture was collected, while the remaining cells were diluted to an OD600 of 0.1 in fresh YEPD, incubated at 30°C, and collected at the indicated times. Proportions of budded cells were scored for each time point. Protein was extracted from cell pellets by adding 20 μl of radiomunoprecipitation (RIPA) buffer (10 mM sodium phosphate, 1.0% Triton X-100, 0.1% SDS, 10 mM EDTA, 150 mM NaCl [pH 7.0]) along with 1 mM AEBSF, 5 μg/ml of leupeptin, and 5 μg/ml of aprotinin) and 200 μl of glass beads. Cells were disrupted for three times for 45 s in a bead beater (Biospec Products, Bartlesville, OK) with 2-min intervals on ice. An additional 200 μl of RIPA buffer was added, and the suspension was centrifuged at 15,000×g for 30 min at 4°C. The supernatant was collected and stored at –80°C. Protein was quantified using the Bradford assay (Bio-Rad, Mississauga, ON, Canada), and 30 μg was loaded onto SDS-PAGE gels. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad) and blocked with Tris-buffered saline–Tween (TBST; Tris; pH 7.5; 137 mM NaCl, 0.1% Tween 20) containing 5% skim milk for 1 h. Blots were washed three times for 15 min in TBST and incubated for 1.5 h in 0.4 μg/ml anti-α-hypha antibody (CD2A4; Roche) or 0.05 μg/ml anti-TAP (Thermo Scientific Open Biosystems, Huntsville, AL) diluted in TBST. Blots were rinsed three times for 15 min in TBST and incubated for 1 h in a 1:10,000 dilution of horseradish peroxidase–conjugated secondary antibody. After washing, blots were developed using ECL (GE Healthcare, VWR, Ville Mont-Royal, QC, Canada). Blots were stripped and incubated with 0.2 μg/ml of anti-PSTAIRE (Santa Cruz Biotechnology, Santa Cruz, CA) as a loading control. Western blots were quantified using ImageJ (http://rsb.info.nih.gov/, index.html), according to the methods described at http://hakemillner.org/index.php/2010/11/analyze-gels-and-westernblots-with-image-j/. Briefly, the band density for Cdc5p, Clb2p-HA, or PSTAIRE in each lane of a single blot was divided by that of the first lane for the given protein, in order to determine relative densities. The relative densities of Cdc5p-TAP and Clb2p-HA were then divided by the relative densities of PSTAIRE for the corresponding lane to obtain adjusted relative densities.

Cell size measurements. In order to determine the effect of deleting CDH1 on cell size, strains HHCA45 and HHCA100 were incubated in YEPD medium at 30°C to an OD of 0.8, sonicated, and fixed in 70% ethanol. Approximately 20,000 cells were analyzed by forward light scattering, using a Becton-Dickinson LSRII
analytic flow cytometer. Alternatively, the lengths and widths were measured in approximately 100 cells in the yeast form and multiplied to obtain values in μm². Measurements were taken from mother cells only, where the width corresponded to the widest part of the cell (83). Axial ratios were measured by dividing the length by the width of cells.

RESULTS

Depletion of Cdc20p results in highly polarized growth of yeast buds under yeast growth conditions. In order to further our understanding of how mitosis is regulated and linked to polarized morphogenesis in C. albicans, the potential involvement of APC/C activity was explored by characterizing orthologues of APC/C activators. We first investigated CDC20 (orf19.122; http://www.candidagenome.org/), which shares 51% identity at the protein level with its orthologue in S. cerevisiae. CDC20 function was determined by constructing a strain carrying a single copy under the control of the MET3 promoter (15). Strains HHCA109 (cdc20Δ::URA3/MET3::CDC20-HIS1) and HCC100 (CDC20/CDC20 URA3::HIS1) were used for subsequent analyses. When incubated at 30°C for 2 days, both strains formed normal yeast colonies on solid inducing medium (-MC). However, on repressing medium (+MC) cells depleted of Cdc20p generated filaments, while control cells formed smooth colonies and grew in the yeast form (Fig. 1A). In liquid medium, the majority of cells depleted of Cdc20p for 3 h at 30°C were large budded, many of which contained a short polarized extension. By 7 h, the cells contained highly elongated buds (Table 2; Fig. 1B and 2A), indicating maintenance of polarized growth. After 24 h, some cells contained constrictions and branches similar to pseudohyphae, and many were no longer viable, as shown with propidium iodide staining (see Fig. S1 of the supplemental material).
suggesting that **CDC20** may be an essential gene. In contrast, cells from the control strain (HCCA100) grew in the yeast form in either inducing or repressing medium (Table 2; Fig. 1B). The Cdc20p-depleted phenotype differs from that of **CDC20** null mutants in *S. cerevisiae*, which consist of large doublets (29). However, the phenotype resembles that of Cdc5p-depleted cells, suggesting that Cdc20p and Cdc5p may lie in the same pathway that governs mitotic progression and influences elongated bud growth.

**Cdc20p is required for early and late stages of nuclear division.** Since Cdc20p is required for the metaphase-to-anaphase transition and mitotic exit in *S. cerevisiae* (21, 41), we next determined whether Cdc20p in *C. albicans* influences mitosis. After 3 h of Cdc20p depletion, when most cells were either large doublets or slightly elongated, 28.4% contained two nuclei, while 71.6% contained a single nucleus (Table 2; Fig. 2A). In 72.6% of these cells, the nucleus was located in the mother cell with unsegregated chromosomes, suggesting a metaphase delay. The single nucleus was located in the bud neck in the remaining cells. At 6 h, when most cells were highly elongated, the majority contained two nuclei (Table 2). Nuclei were located exclusively in the filament of approximately 60% of these cells. In contrast, control cells showed normal proportions of budding cells and numbers of nuclei at 6 h (Table 2; Fig. 2A). To confirm the phase of mitosis in which Cdc20p is required, spindle patterns were analyzed by immunolocalizing α-tubulin. After 3 h in repressing medium, approximately 70% of **cdc20/MET3::CDC20** cells were in mitosis, with 52.5% containing short rod-like metaphase spindles and 18.5% containing long telophase spindles (Table 3; Fig. 2B). The remaining 29.0% of cells showed only spindle pole body staining, visualized as single or double spots, indicating cells were in interphase. At 6 h, 70.9% of cells contained long spindles, although the presence of cytoplasmic microtubules interfered with quantification in some cells. In contrast, fewer control cells contained mitotic spindles (Table 3). These results suggest that depletion of Cdc20p leads to an initial delay at metaphase, followed by a block in telophase, implying a role for Cdc20p at both stages of mitosis. In comparison, **CDC20** mutants in *S. cerevisiae* arrest in metaphase with a single nucleus positioned at the bud neck (29). In the absence of the securin Pds1p, however, the cells arrest in later stages of mitosis, consistent with an additional role during mitotic exit (41, 80, 85). Our results further suggest that polarized growth upon Cdc20p depletion is associated with an initial delay in metaphase.

**Cdc20p-depleted cells express HWP1 at later stages of growth.** We previously demonstrated that cells depleted of Cdc5p expressed some hypha-associated genes, including **HWP1**, at later stages of growth (5, 6). In order to determine whether Cdc20p-depleted cells showed a similar response, **HWP1** expression was investigated by Northern blotting. When incubated in inducing medium, exponential-phase **cdc20Δ/MET3::CDC20** yeast cells did not demonstrate expression of **HWP1** (Fig. 3). Incubation in repressing medium for 6 h, which resulted in highly elongated buds, also did not induce **HWP1** expression. However, after 9 h in repressing medium, **HWP1** was strongly expressed in **cdc20Δ/MET3::CDC20** cells, in contrast to control cells (**CDC20/CDC20**) under identical conditions (Fig. 3). Thus, depletion of Cdc20p also results in delayed induction of a hypha-associated gene.

**Cells lacking Cdh1p have a pleiotropic phenotype.** Cdh1p regulates APC/C activity during late stages of mitosis and into G1 phase (56, 79, 80, 86). orf19.2084 of *C. albicans* is annotated as **CDH1** (http://www.candidagenome.org/) and shares 43.8% identity at the protein level with Cdh1p from *S. cerevisiae*. In order to investigate **CDH1** function, the alleles were sequentially replaced with **URA3** and **HIS1** markers, resulting in strain HCCA45. When incubated on solid YEPD medium at 30°C for 2 days, **cdh1Δ/cdh1Δ** colonies contained uneven edges and were smaller in diameter and more elevated than control colonies (Fig. 4A). When grown in liquid YEPD medium for 7 h, approximately 70% of **cdh1Δ/cdh1Δ** cells were in a yeast form, while the remaining cells consisted of pseudohyphae and irregular-shaped elongated cells, as well as a small proportion of elongated buds (Fig. 4B; Table 4). A few cells (2.5%) showed narrow diameters, similar to true hyphae. In contrast, 97.5% of **CDH1/CDH1** cells grew in a normal yeast form (Fig. 4B; Table 4). To confirm that the phenotype was due to the absence of Cdh1p, a strain containing a single copy of **CDH1** under the control of the **MET3** promoter was created (HHCA26). When incubated in repressing medium for 8 h, **cdh1Δ/MET3::CDH1** cells showed a pleiotropic phenotype, similar to **cdh1Δ/cdh1Δ** cells, whereas the control strain (HCCA100) formed normal yeast (see Fig. S2 in the supplemental material). Some filamentation and abnormal cell morphologies were also observed in **cdh1Δ/MET3::CDH1** cells in inducing medium, but at lower

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**TABLE 2. Number of nuclei and cell morphology in Cdc20p-depleted cells**

| Strain and incubation time (h) | % of cells with indicated no. of nuclei | % of cells with indicated morphology |
|-------------------------------|----------------------------------------|------------------------------------|
|                               | 1  | 2  | 3  | Frag<sup>b</sup> | Unbudded/small bud | Large bud | Elongated bud |
| **cdc20/MET3::CDC20**         |    |    |    |               |                   |           |              |
| 0 h (236)                     | 81.8| 18.2| 0  | 0              | 91.1              | 8.9       | 0            |
| 3 h (173)                     | 71.6| 28.4| 0  | 0              | 12.7              | 53.1      | 34.1         |
| 6 h (216)                     | 22.2| 54.2| 2.8| 20.8           | 3.0               | 2.0       | 95.0         |
| **CDC20/CDC20**              |    |    |    |               |                   |           |              |
| 0 h (145)                     | 95.0| 4.8 | 0  | 0              | 93.1              | 6.9       | 0            |
| 3 h (120)                     | 82.5| 12.5| 0  | 0              | 70.0              | 26.7      | 3.3          |
| 6 h (132)                     | 84.0| 16.0| 0  | 0              | 74.2              | 23.5      | 2.3          |

<sup>a</sup> Cells from strains HCCA109 (**cdc20::URA3/MET3::CDC20::HIS1**) and HCCA100 (**CDC20/CDC20 URA3** **MET3**) were incubated in repressing medium (+MC) at 30°C and collected at the indicated time points. Cells were fixed and stained with DAPI.

<sup>b</sup> Frag, fragmented chromosomes.
frequencies than under repressing conditions, which may reflect overexpression of \(CDH1\). In \(S. \text{cerevisiae}\), cells lacking Cdh1p are greatly reduced in size (34). However, the length-by-width measurements of \(C. \text{albicans}\) cells only in the yeast form were greater in the absence of Cdh1p (28.7 ± 1.5 \(\mu\)m\(^2\) \([n = 110]\) for HHCA45 versus 18.0 ± 0.6 \(\mu\)m\(^2\) \([n = 99]\) for HHCA100; means ± standard errors of the means [SEM]) (see Fig. S3 in the supplemental material). Forward light scattering confirmed that absence of Cdh1p did not result in a subpopulation of smaller cells; rather, cells were either similar or larger in size than control cells (Fig. 4C). Although the mean axial ratios did not greatly differ (1.31 ± 0.03 \([n = 110]\) for \(cdh1\Delta/cdh1\Delta\) versus 1.41 ± 0.03 \([n = 99]\) for \(CDH1/CDH1\) cells), more \(cdh1\Delta/cdh1\Delta\) cells demonstrated identical length and width measurements (19.1% \([n = 110]\) for \(cdh1\Delta/cdh1\Delta\) versus 7.0% \([n = 99]\) for \(CDH1/CDH1\) cells), indicating a subpopulation of cells were more round. Overall, the difference in phenotype from \(S. \text{cerevisiae cdh1}\Delta\)
cells (34) suggests that *C. albicans* Cdh1p may have some distinct functions.

**Cdh1p is important, but not essential, for nuclear division and septation.** Cdh1p in *S. cerevisiae* is important for mitotic exit; its absence results in a higher number of cells with separated nuclei and elongated spindles (56, 79), as well as abnormalities in chromosome segregation and spindle structure (52).

To determine whether Cdh1p influences mitosis in *C. albicans*, cells were incubated in YEPD for 7 h, fixed, processed for immunolocalization of α-tubulin, and/or stained with DAPI. In the absence of Cdh1p (HCCA45), slightly more cells contained longer spindles than did control cells (HCCA100). Consistently, cells were difficult to score due to abundant cytoplasmic microtubules (Fig. 5A). Nuclear division was also deregulated in 14.8% (n = 216) of cdh1Δ/cdh1Δ cells. For example, two or more nuclei were present in unbudded yeast cells (5.1%), as well as in compartments of pseudohyphae and elongated buds (5.1%). Another proportion of pseudohyphal cells (4.6%) lacked nuclei in some compartments. In contrast, 100% of control cells contained a normal number of nuclei. Notably, in 6.9% of cdh1Δ/cdh1Δ cells containing a single nucleus, the organelle was located across the bud neck (Fig. 5B), compared to 2.2% of control cells. The fact that Cdh1p is required for nuclear division and septation, coupled with the moderate increase in the number of telophase spindles, suggests that Cdh1p in *C. albicans* may influence the regulation of mitotic exit.

**Cib2p and Cdc5p are elevated in Cdc20p-depleted cells and Cdh1p-depleted cells.** Cdc20p directs APC/C-dependent degradation of the cohesin Pds1p in *S. cerevisiae* and contributes to initial degradation of the mitotic cyclin Cib2p (9, 85). Cdh1p, on the other hand, activates APC/C-dependent degradation of Cib2p toward the end of mitosis and into G1 phase and targets degradation of the polo-like kinase Cdc5p as well as other factors, including Cdc20p (9, 17, 56, 57, 64, 77). To determine whether Cdc20p and Cdh1p influence mitotic progression in *C. albicans* through similar means, we tagged Cib2p with HA and Cdc5p with TAP in strains lacking Cdc20p or Cdh1p. Following depletion of Cdc20p (+MC), Cib2p-HA was elevated relative to levels in the same strain (AG153) under Cdc20p-inducing conditions (-MC), particularly at 3 h, and also relative to control cells (AG139) grown in either inducing or repressing medium (Fig. 6A). In addition, Cdc5p-TAP was enriched in repressing versus inducing medium in cdc20Δ/MET3::CDC20 cells (AG191) and compared to that in control cells (AG280) in either medium (Fig. 6A). Untagged control strain BWP17 did not show any signal for either protein. These results suggest that Cdc20p influences the stability of Cib2p, as well as Cdc5p, consistent with the notion that Cdc20p-depleted cells are blocked in mitosis. Since Cdh1p maintains degradation of Cib2p and Cdc5p into G1 phase in *S. cerevisiae*, we next investigated the levels of Cib2p-HA and Cdc5p-TAP in overnight cultures of cdh1Δ/cdh1Δ (AG268 and AG262) and CDH1/CDH1 (AG139 and AG280) cells that were semisynchronized in an unbudded state and at subsequent time points after inoculation into fresh medium. In overnight cultures, when approximately 90% of the cells were unbudded, the control strains showed very little signal for Cdc5p-TAP or Cib2p-HA. However, as cells proceeded to bud, the levels of both proteins increased (Fig. 6B), consistent with both factors peaking in expression later in the cell cycle (12, 22). In contrast, overnight cultures of strains lacking Cdh1p, which contained similar proportions of unbudded cells as the control strains, demonstrated strong enrichment of Cdc5p-TAP and Cib2p-HA (Fig. 6B). Cdc5p-TAP levels continued to accumulate as more cells proceeded through the cell cycle, as expected if Cdh1p were important in targeting its degradation. However, Cib2p-HA levels did not increase over time (Fig. 6B). In comparison, both factors are strongly elevated in cdh1Δ cells of *S. cerevisiae* (17, 56, 77, 79, 80). These results suggest that *C. albicans* Cdh1p

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**TABLE 3. Spindle patterns in Cdc20p-depleted cells**

| Strain and incubation time (h) | Interphase | Early mitosis | Late mitosis |
|-------------------------------|------------|---------------|--------------|
| cdc20/MET3::CDC20 3 h (200)  | 29.0       | 52.5          | 18.5         |
| 6 h (320)                   | 16.3       | 12.8          | 70.9         |
| CDC20/CDC20 3 h (179)       | 83.2       | 3.9           | 12.8         |
| 6 h (154)                   | 77.2       | 13.0          | 9.7          |

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\(^a\) Cells from strain HCCA109 (cdc20::URA3/MET3::CDC20-HIS1) and HCCA100 (CDC20/CDC20 UR43::HIS1+) were incubated in repressing medium (+MC) for the indicated times at 30°C and processed for immunofluorescence of α-tubulin.

\(^b\) Cells containing single or double spots of α-tubulin representing spindle pole bodies without spindles indicated cells were in interphase. Cells containing short rod-like spindles were in early mitosis, while those with longer spindles were in later stages of mitosis.

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**FIG. 3.** *HWP1* is expressed in Cdc20p-depleted cells at later stages of growth. The Northern blot contained 20 µg of total RNA extracted from exponential-phase cells of strain HHC1A09 (cdc20::URA3/MET3::CDC20-HIS1) incubated in inducing medium (-MC) or transferred to repressing medium (+MC) and incubated for the indicated times. Control strain BWP17 (CDC20/CDC20) was incubated in repressing medium for 9 h. *ACT1* was used as a loading control.
influences degradation of similar mitotic targets as its orthologue in *S. cerevisiae*. However, the fact that Clb2p did not continue to accumulate with longer incubation periods suggests that additional factors may contribute to Clb2p degradation in the presence and/or absence of Cdh1p.

The absence of Cdh1p does not influence Cdc5p-depleted polarized growth. Cdh1p lies downstream of Cdc5p function in *S. cerevisiae*, as the latter is a component of the FEAR and MEN pathways, which act to release Cdc14p from the nucleolus and ultimately activate Cdh1p (48, 65, 70, 78). Cdh1p then acts back on Cdc5p by targeting it for degradation (17, 64). Since a small proportion of elongated buds were observed in *C. albicans* cells lacking *CDH1*, it is possible that Cdh1p contributes in part to the polarized growth response observed in Cdc5p-depleted cells. In order to address this question, both copies of *CDH1* were deleted from a strain carrying a single copy of *CDC5* under the control of the *MET3* promoter (HCCA143). After incubation in repressing medium for 7 h, 80.2% of cells lacking *CDH1* and depleted of Cdc5p contained elongated buds, compared to 91% of cells depleted of Cdc5p only (HCCA126) (Table 5). Elongated cells in the two strains appeared similar, even after 24 h of Cdc5p depletion (Fig. 7). In contrast, only 11.7% of cells lacking *CDH1* (HHCA45) or 2.0% of cells containing both *CDH1* and *CDC5* (HHCA100) demonstrated elongated buds (Table 5). Thus, the absence of Cdh1p did not result in synergistic effects with Cdc5p deple-

FIG. 4. Cells lacking Cdh1p demonstrate a pleiotropic phenotype. (A) Strains HCCA45 (cdh1Δ::URA3/cdh1Δ::HIS1) and HCCA100 (CDH1/CDH1::URA3 URA3 "HIS1") were incubated on YEPD plates for 48 h at 30°C. (B) Strains incubated in liquid YE PD medium for 7 h and fixed. Bar, 10 µm. (C) Strains incubated as described for panel B were sonicated, fixed, and subjected to forward (FSC-A) light scattering.
tion, suggesting that the factors may lie in the same functional pathway in *C. albicans*.

**Cdc20p does not influence serum-induced hyphal growth, while Cdh1p has a moderate effect.** Since Cdc20p and Cdh1p influence morphogenesis, we next investigated whether the factors were important for serum-induced hyphal formation. The *cdc20/MET3::CDC20* and control strains were first incubated in repressing medium at 30°C for 2 h in order to deplete Cdc20p before addition of serum. Cells were then transferred to fresh repressing medium with 10% serum and incubated at 37°C for 3 h. Prior to the addition of serum, the majority of cells were either large doublets or slightly polarized (Table 2). Exposure to serum resulted in the emergence of hyphae from elongated daughter buds or from one bud of large doublets (Fig. 8). Hyphae resembled those of control cells and were only moderately shorter in length

| Strain (n) | % of cells with indicated morphology |
|-----------|-------------------------------------|
|           | Yeast | Pseudohypha | Elongated bud |
| Δcdh1Δ/cdh1 (212) | 68.9 | 19.4 | 11.7 |
| CDH1/CDH1 (202) | 96.5 | 3.5 | 0 |

*a* Overnight cultures of strains HCCA45 (*cdh1::URA3/cdh1::HIS1*) and HCCA100 (*CDH1/CDH1 URA3::HIS1*) were diluted into fresh YPD medium and incubated at 30°C for 7 h.

*b* Includes irregular-shaped, elongated cells and standard pseudohyphal cells (72).
FIG. 6. Cbl2p and Cdc5p levels in cells lacking Cdc20p or Cdh1p. Western blot assays were performed to determine the levels of Cbl2p-HA and Cdc5p-TAP. (A) Exponential-phase cultures of strains AG191 (cdc20::URA3/MET3::CDC20/HIS1/CDC5/CDC5-TAP-ARG4), AG153 (cdc20::URA3/MET3::CDC20-ARG4 CLB2/CLB2-HA-HIS1), AG139 (CLB2/CLB2-HA-HIS1), and AG280 (CDC5/CDC5-TAP-URA3) grown in inducing medium (-MC) were diluted into fresh inducing or repressing (+MC) medium for 6 h and included as untagged controls. (B) Strains AG139 (CLB2/CLB2-HA-HIS1), AG280 (CDC5/CDC5-TAP-URA3), AG268 (cdh1::URA3/cdh1::HIS1 CLB2/CLB2-HA-ARG4), and AG262 (cdh1::HIS1/cdh1::URA3 CDC5/CDC5-TAP-ARG4) were collected after overnight incubation in YEPD medium (time 0) or after diluting into fresh YEPD medium and incubating for 1, 2, or 3 h. Anti-PSTAIRE was used as a loading control. Proportions of budded cells are indicated. Density values represent adjusted relative densities (see Materials and Methods).

(41.2 ± 1.2 μm [n = 56] compared to 45.2 ± 2.2 μm [n = 52]), demonstrating that Cdc20p is not important for hypha formation. Since germ tubes emerged from yeast cells that were blocked in mitosis, and preferentially from a preexist-
tant for mitotic exit and may target conserved factors during late stages of mitosis and G1 phase (9, 17, 56, 57, 64, 77). Since CDH1 is not essential, additional factors must be required for mitotic exit. In S. cerevisiae, the CDK inhibitor Sic1p contributes to this function; Δcdh1 Δsic1 cells are not viable and arrest in a large-budded state (56). The C. albicans Sic1p homologue Sol1p is also not essential (4). However, its role in mitosis was not determined, and the deletion phenotype consisted of most cells containing elongated buds when in exponential phase but reverting to a yeast growth mode at higher cell density, unlike cdh1Δ/Δ cells. Sic1p and Cdh1p are activated by Cdc14p phosphatase in S. cerevisiae (69), which in turn is regulated by the FEAR and MEN pathways (48, 65, 70, 73, 78). Although Cdc14p is also important for Clb2p degradation and mitotic exit in C. albicans (20), its relationship with Cdh1p is not clear, since cdc14Δ/cdc14Δ cells were defective in cell separation and did not form elongated buds. Similarly, the MEN kinase Dbf2p does not appear to be tightly coordinated with Cdc14p, unlike in S. cerevisiae (28).

### TABLE 5. Morphologies of cells lacking Cdc5p and Cdh1p

| Strain          | Period (h) of incubation (n) | Yeast | Enlarged yeast | Pseudohypha | Elongated bud |
|-----------------|------------------------------|-------|----------------|-------------|--------------|
| cdh1/cdh1       | 0 (234)                      | 80.3  | 14.5           | 0           | 5.2          |
| cdc5/MET3::CDC5 | 7 (268)                      | 10.1  | 9.7            | 0           | 80.2         |
| CDH1/CDH1       | 0 (202)                      | 92.6  | 4.5            | 0           | 2.9          |
| cdc5/MET3::CDC5 | 7 (178)                      | 8.4   | 0.6            | 0           | 91.0         |
| cdh1/cdh1       | 0 (376)                      | 75.8  | 18.1           | 1.9         | 4.2          |
| CDC5/CDC5       | 7 (238)                      | 57.6  | 11.3           | 19.4        | 11.7         |
| CDH1/CDH1       | 0 (232)                      | 97.0  | 3.0            | 0           | 0            |
| CDC5/CDC5       | 7 (248)                      | 94.4  | 3.6            | 0           | 0            |

*Overnight cultures of strains HCCA143 (cdh1::URA3/cdh1::HIS1 cdc5::hisG/MET3::CDC5-ARG4), HCCA126 (CDH1/CDH1 cdc5::hisG/MET3::CDC5-ARG4), HHCA45 (cdh1::URA3/cdh1::HIS1 CDC5/CDC5), and HHCA100 (CDH1/CDH1 CDC5/CDC5 URA3c HIS1c) were diluted in repressing medium (+MC) and fixed after 0 or 7 h of incubation at 30°C.

FIG. 7. Absence of Cdh1p does not influence polarized growth of Cdc5p-depleted cells. Strains HCCA143 (cdh1::URA3/cdh1::HIS1 cdc5::hisG/MET3::CDC5-ARG4) and HCCA126 (CDH1/CDH1 cdc5::hisG/MET3::CDC5-ARG4) were incubated in repressing medium at 30°C for 24 h and fixed. Bar, 10 μm.
Doublet morphology. The polarized phenotype could arise in *S. cerevisiae* (56). This suggests that *Cdh1p* contributes to *C. albicans* cell size but resulted in yeast cell enlargement, in striking contrast to the situation in *S. cerevisiae* (34). This phenotype is not consistent with a role in negatively regulating Start, since the cells can express virulence factors (2, 5), and Mad2p is dispensable for yeast and hyphal growth (5, 7), while Rad53p plays an additional role in hyphal development (62). It’s not clear if the different checkpoints converge on a similar pathway or process to influence polarized bud growth (12), and the downstream targets remain elusive. Since Cdc20p is a target of the checkpoint factor Mad2p in other systems (87), it may influence the stability of factors important for the polarized response in *C. albicans*. It will be informative to determine whether inactivation of Cdc20p is a common feature of elongated bud growth induced by other conditions or if it plays only an indirect role. In *S. cerevisiae*, apical bud growth is associated with Cdc28p/G1 cyclin activity, while a switch to isometric growth occurs when Cdc28p associates with B-type cyclins like Clb2p (49, 54). Downregulation of Cdc28p/Clb2p activity, through activation of Swe1p-dependent inhibitory phosphorylation of Cdc28p or other means, results in cell elongation (49, 54). However, yeast cell elongation in *C. albicans* may involve some different mechanisms, since Clb2p stabilization or absence induces the response (10). Clb2p was elevated in Cdc20p-depleted cells, Swe1p is only partly required for elongated growth of Rad52p-depleted or hydroyurea (HU)-treated cells (2, 23), and absence of Cdc20p and Cdh1p did not induce polarized growth in *S. cerevisiae*. If the response is due to defects in switching to isometric bud growth, it is not clear why hypha-associated genes, such as *HWP1* (2, 5), become strongly induced. It is possible that cell fate changes occur during later stages of polar growth (6), or checkpoints may activate a separate pathway with hypha-like characteristics (12). Indeed, specific *RAD52* mutations prevent cell elongation but not cell cycle arrest in response to HU, implying a direct role in polarized growth (62). Although the underlying mechanisms remain unclear, polarized bud growth may be important for pathogenesis (12), since the cells can express virulence factors (2, 5), and absence of *MAD2* (7), *SWE1* (26), or *TRX1* (23) reduces virulence.

Cdh1p may contribute in part to cell elongation through its influence on mitotic progression. However, our results suggest an additional role in hyphal morphogenesis. The small proportion of *cdh1Δ*/*cdh1Δ* cells unresponsive to securin could reflect cell-to-cell variations in the elevated levels of Clb2p, as overexpression of Clb2p or Clb4p compromises true hyphal growth (10). Consistently, other factors important for mitotic exit in *C. albicans* influenced hyphal growth, albeit in a stronger manner (20, 28). Although a pseudohyphal state can preclude hyphal differentiation, as seen in cells lacking Gin4p, Hsl1p, and Fkh2p (11, 83), staining from a defect in the ability of yeast buds to switch from apical to isometric growth (10), and Cdc20p may contribute to this process in *C. albicans*. Other conditions that arrest mitosis, S, or G2/M phase, or depletion of Hsp90p, result in similar elongated cells (2, 5–7, 10, 12, 23, 24, 30, 58, 61). The common response is mediated by different cell cycle checkpoint factors where investigated (12). For example, the DNA damage and replication checkpoint kinase Rad53p mediates filamentous growth in response to H2O2 and genotoxic stresses (23, 62), while the spindle checkpoint factors Bub2p and Mad2p are important for polarized growth in response to mitotic arrest (5, 7). Intriguingly, Bub2p and Mad2p are dispensable for yeast and hyphal growth (5, 7), while Rad53p plays an additional role in hyphal development (62).

**FIG. 8.** Hyphal formation in the absence of Cdc20p or Cdh1p. Strains HCCA109 (cdc20::URA3/MET3::CDC20/HIS1) and HCCA100 (CDC20/CDC20 URA3::HIS1) were incubated in repressing medium for 2 h at 30°C prior to transfer into fresh repressing medium containing 10% fetal bovine serum. Cells were grown for 3 h at 37°C. Strains HCCA45 (cdh1::URA3/cdh1::HIS1) and HCCA100 (CDH1/CDH1) were incubated in YEPD medium containing 10% fetal bovine serum for 3 h at 37°C. Bar, 10 μm.

Despite its conserved features, Cdh1p also demonstrated some variations in function. First, Clb2p was enriched in un budded *cdh1Δ/cdh1Δ* cells but did not accumulate with longer incubation periods, in contrast to that seen with Cdc5p-TAP or for Clb2p levels in *CDH1* mutants of *S. cerevisiae* (56). This suggests that Cdh1p contributes to Clb2p degradation in *C. albicans* but that another factor(s) may be involved and/or become more important in the absence of Cdh1p. First, deletion of *CDH1* did not reduce cell size but resulted in yeast cell enlargement, in striking contrast to the situation in *S. cerevisiae* (34). This phenotype is not consistent with a role in negatively regulating Start, unlike that demonstrated with *S. cerevisiae* Cdh1p. The *C. albicans cdh1Δ/cdh1Δ* phenotype shared more similarity with *S. cerevisiae* cells overexpressing CDH1 or carrying a constitutively active mutant (51, 56). Thus, the results suggest that *C. albicans* Cdh1p has conserved and possibly novel functions, and they underscore the notion that the pathways governing mitotic progression in *C. albicans* involve distinct features (6, 20, 28). Future investigations of Cdh1p targets and regulation will provide further insights into its roles and the mitotic circuitry of the pathogen.

**Cdc20p and Cdh1p influence yeast morphogenesis and polar growth patterns.** Our results demonstrate that elongated bud growth is coupled to mitotic defects caused by depletion of Cdc20p. In contrast, absence of *CDC20* (29) or other conditions that arrest mitosis in *S. cerevisiae* result in a yeast doublet morphology. The polarized phenotype could arise...
cdh1Δ/cdh1Δ cell walls prior to incubation in serum revealed that unresponsive cells were not all originally pseudohyphae (data not shown). Thus, Cdh1p influences polarized morphogenesis in a complex manner. The phenotype of cdh1Δ/cdh1Δ cells could be a secondary response to defects in cell cycle progression, but it is intriguing that Cdh1p function is independently linked to developmental regulators in metazoans and in S. cerevisiae (43, 81).

Overall, we have identified key factors that contribute to the regulatory networks governing mitosis and associated polar morphogenesis in C. albicans. Our results highlight additional functional variations in important mitotic regulators compared to other systems, consistent with the emerging theme of cell cycle rewiring in the pathogen (12, 22).

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REFERENCES

1. Ahonen, L. J., et al. 2005. Polo-like kinase 1 creates the tension-sensing 3F5/2 phosphoepitope and modulates the association of spindle-checkpoint proteins at kinetochores. Curr. Biol. 15:1078–1089.
2. Andaluz, E., T. Ciudad, J. Gomez-Raja, R. Calderone, and G. Larriba. 2005. Rad52 depletion in Candida albicans triggers both the DNA-damage checkpoint and filamentation accompanied by but independent of expression of hypha-specific genes. Mol. Microbiol. 59:1452–1472.
3. Archambault, V., and D. M. Glover. 2009. Polo-like kinases: conservation and divergence in their functions and regulation. Nat. Rev. Mol. Cell Biol. 10:265–275.
4. Atir-Lande, A., T. Gildor, and D. Kornitzer. 2005. Role for the SCFCDC4 ubiquitin ligase in Candida albicans morphogenesis. Mol. Biol. Cell 16:2772–2782.
5. Bachewich, C., A. Nantel, and M. Whiteway. 2005. Cell cycle arrest during S or M phase generates polarized growth via distinct signals in Candida albicans. Mol. Microbiol. 57:942–959.
6. Bachewich, C., D. Y. Thomas, and M. Whiteway. 2003. Depletion of a polo-like kinase in Candida albicans activates cyclase-dependent hyphal-like growth. Mol. Cell. Biol. 14:2163–2180.
7. Bai, C., N. Ramanan, Y. M. Wang, and Y. Wang. 2016.ENDA2 mutations cause SWE1-mediated cell cycle phenotypes in Candida albicans and Saccharomyces cerevisiae. Microbiology 155:3847–3859.
8. Ciosk, R., et al. 2006. The Cdc4p phosphatase affects late cell-cycle events and morphogenesis in Candida albicans. J. Cell Sci. 119:1130–1143.
9. Cohen-Fix, O., J. M. Peters, M. W. Kirschner, and D. Koshland. 1996. Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. 10:3816–3833.
10. Cote, P., H. Hugues, and M. Whiteway. 2009. Transcriptional analysis of the Candida albicans cell cycle. Mol. Biol. Cell 20:3633–3737.
11. Finley, K. R., K. J. Bouchonville, A. Quick, and J. Berman. 2008. Dysindependent nuclear dynamics affect morphogenesis in Candida albicans by phosphorylation of the Bub2p spindle checkpoint. J. Cell Sci. 121:466–476.
12. Finley, K. R., K. J. Bouchonville, A. Quick, and J. Berman. 2008. Dysindependent nuclear dynamics affect morphogenesis in Candida albicans by phosphorylation of the Bub2p spindle checkpoint. J. Cell Sci. 121:466–476.
13. Huang, X., R. Hatcher, J. P. York, and P. Zhang. 2005. Securin and separase phosphorylation act redundantly to maintain sister chromatid cohesion in mammalian cells. Mol. Biol. Cell 16:4725–4732.
14. Hartwell, L. H., R. K. Mortimer, J. Culotti, and M. Culotti. 1973. Genetic control of the cell division cycle in yeast. V. Genetic analysis of cde mutants. Genetics 74:267–286.
15. Hazan, I., M. Sepulveda-Becerra, and H. Liu. 2002. Hyphal elongation is regulated independently of cell cycle in Candida albicans. Mol. Biol. Cell 13:134–145.
16. Hildebrandt, E. R., and M. A. Hoy. 2001. Cell cycle-independent degradation of the Saccharomyces cerevisiae spindly motor Cdc10p requires APC(Cdc11) and a bipartite degradation sequence. Mol. Biol. Cell 12:3402–3416.
17. Holland, A. J., and S. S. Taylor. 2008. Many facets of separate regulation. SEB Exp. Biol. Ser. 59:99–112.
18. Huang, X., R. Hatcher, J. P. York, and P. Zhang. 2005. Securin and separase phosphorylation act redundantly to maintain sister chromatid cohesion in mammalian cells. Mol. Biol. Cell 16:4725–4732.
19. Jorgensen, P., J. L. Nishikawa, B. J. Breitkreutz, and M. Tyers. 2002. Systematic identification of pathways that couple cell growth and division in yeast. Science 297:395–400.
20. Jiang, Y. L., et al. 1997. APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. Science 275:1311–1314.
21. Kim, A. H., and A. Bonni. 2007. Thinking within the D box: initial identification of Cdh1-APC substrates in the nervous system. Mol. Cell. Neurosci. 34:281–287.
22. Kitada, K., A. L. Johnson, L. H. Johnston, and A. Stuigno. 1993. A multiplicity suppressor gene of the Saccharomyces cerevisiae G1 cell cycle mutant gene cdc14 encodes a protein kinase and is identified as CDC5. Mol. Cell. Biol. 13:4445–4457.
23. Konishi, Y., J. Stegmueller, T. Matsuda, S. Bonni, and A. Bonni. 2004. Cdh1p-APC controls axonal growth and patterning in the mammalian brain. Science 303:1026–1031.
24. Lavoie, H., A. Sellam, C. Askew, A. Nantel, and M. Whiteway. 2008. A toolbox for epitope-tagging and genome-wide location analysis in Candida albicans. BMC Genomics 9:578.
25. Li, W., et al. 2006. The F-box protein Gril regulates the stability of Ccn1, Cln3 and Hof1 and cell morphogenesis in Candida albicans. Mol. Microbiol. 62:212–226.
26. Lim, H. H., P. Y. Goh, and U. Surana. 1998. Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Cbi2 during M phase in budding yeast. Curr. Biol. 8:231–241.
27. Lo, H. J., et al. 1997. Nonfilamentous C. albicans mutants are avirulent. Cell 90:939–949.
28. Manchado, E., M. Eguren, and M. Malumbres. 2009. SLA2 mutations cause SWE1-mediated cell cycle arrest in Candida albicans: rapid and efficient gene targeting using 100 bp of flanking homology region. Yeast 26:1339–1347.
29. Moshe, Y., J. Boulaire, M. Pagano, and A. Hershko. 1996. Isogenic strain construction and gene mapping in Candida albicans. Genetics 134:717–728.
30. Moshe, Y., J. Boulaire, M. Pagano, and A. Hershko. 1996. Isogenic strain construction and gene mapping in Candida albicans. Genetics 134:717–728.
gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot. Cell 4:298–309.

46. Ofir, A., and D. Kornitzer. 2010. Candida albicans cyclin Cib4 carries S-phase cyclin activity. Eukaryot. Cell 9:1311–1319.

47. Park, C. J., et al. 2008. Requirement for the budding yeast polo kinase Cdc5 in proper microtubule growth and dynamics. Eukaryot. Cell 7:444–453.

48. Pereira, G., C. Manson, G. Grindlay, and J. Schiebel. 2002. Regulation of the Bfa1p-Bub2p complex at spindle pole bodies by the cell cycle phosphatase Cdc14p. J. Cell Biol. 157:367–379.

49. Pryne, D., and A. Bretscher. 2000. Polarization of cell growth in yeast. J. Establishment and maintenance of polarity states. J. Cell Sci. 113:365–375.

50. Reimann, J. D., et al. 2001. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. Cell 105:645–655.

51. Robbins, J. A., and F. R. Cross. 2010. Requirements and reasons for effective inhibition of the anaphase promoting complex activator CDH1. Mol. Biol. Cell 21:914–925.

52. Ross, K. E., and O. Cohen-Fix. 2003. The role of Cdh1p in maintaining genomic stability in budding yeast. Genetics 165:489–503.

53. Rottmann, M., S. Dieter, H. Brunner, and S. Rupp. 2003. A screen in Saccharomyces cerevisiae identified CaMCM1, an essential gene in Candida albicans crucial for morphogenesis. Mol. Microbiol. 47:943–959.

54. Rua, D., B. T. Tohe, and S. J. Kron. 2001. Cell cycle control of yeast filamentous growth. Curr. Opin. Microbiol. 4:720–727.

55. Saville, S. P., A. L. Lazzell, C. Monteagudo, and J. L. Lopez-Ribot. 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection. Eukaryot. Cell 2:1053–1060.

56. Schwab, M., A. S. Latum, and W. Seufert. 1997. Yeast Hct1 is a regulator of Cdc2 cyclin proteolysis. Cell 90:683–693.

57. Schwab, M., M. Neutznner, D. Mocke, and W. Seufert. 2001. Yeast Hct1 recognizes the mitotic cyclin Cib2 and other substrates of the ubiquitin ligase APC. EMBO J. 20:5165–5175.

58. Shapiro, R. S., et al. 2009. Hsp90 orchestrates temperature-dependent Candida albicans morphogenesis via Ras1-PAK signaling. Curr. Biol. 19:621–629.

59. 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.

60. Sherman, F. 1991. Getting started with yeast. Methods Enzymol. 194:3–21.

61. Sherwood, R. K., and R. J. Bennett. 2008. Microtubule motor protein Kar3 is required for normal mitotic division and morphogenesis in Candida albicans. Eukaryot. Cell 7:1460–1474.

62. 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.

63. Shirayama, M., A. Toth, M. Galova, and K. Nasmyth. 1999. APC(Cdc20) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. Nature 402:203–207.

64. Shirayama, M., W. Zachariae, R. Ciosk, and K. Nasmyth. 1998. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/Izzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae. EMBO J. 17:1336–1349.

65. Shou, W., et al. 1999. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nuclear RENT complex. Cell 97:233–244.

66. Simpson-Lavy, K. J., et al. 2010. Fifteen years of APC/cyclosome: a short and impressive biography. Biochem. Soc. Trans. 38:78–82.

67. Snead, J. L., et al. 2007. A coupled chemical-genetic and bioinformatic approach to Polo-like kinase pathway exploration. Chem. Biol. 14:1261–1272.

68. Spellman, P. T., et al. 1998. Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. Mol. Biol. Cell 9:3273–3297.

69. Stegemeier, F., and A. Amon. 2004. Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu. Rev. Genet. 38:203–232.

70. Stegemeier, F., R. Visintin, and A. Amon. 2002. Separase, polo kinase, the kinetochore protein Sic19, and Spc12 function in a network that controls Cdc14 localization during early anaphase. Cell 108:207–220.

71. Stemmann, O., I. H. Gorr, and D. Boos. 2006. Anaphase top-y-turvo: Cdk1 a securin, separase a CKI. Cell Cycle 5:11–13.

72. Stay, P., N. Gow, and J. Berman. 2004. The distinct morphological states of Candida albicans. Trends Microbiol. 12:317–324.

73. Sullivan, M., and F. Uhmann. 2003. A non-proteolytic function of separase links the onset of anaphase to mitotic exit. Nat. Cell Biol. 5:249–254.

74. Trunk, K., et al. 2009. Depletion of the cullin Cdc5p induces morphogenetic changes in Candida albicans. Eukaryot. Cell 8:756–767.

75. Uhmann, F., F. Lottspeich, and K. Nasmyth. 1999. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature 400:37–42.

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, Bfb1p, Rim101p, Pck2p and Tec1p and induces cell elongation in Candida albicans. Yeast 23:557–552.

77. Visintin, C., et al. 2008. APC/C-Cdh1-mediated degradation of the Polo kinase Cdc5 promotes the return of Cdc14 into the nucleolus. Genes Dev. 22:79–90.

78. Visintin, R., E. S. Hwang, and A. Amon. 1999. Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature 398:818–823.

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

80. Wasch, R., and F. R. Cross. 2002. APC-dependent proteolysis of the mitotic cyclin Cib2 is essential for mitotic exit. Nature 418:550–562.

81. Wasch, R., J. A. Robbins, and F. R. Cross. 2010. The emerging role of APC/C in controlling differentiation, genomic stability and tumor suppression. Oncogene 29:1–10.

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

83. Wightman, R., S. Bates, P. Amorurutanaporn, and P. Sudbery. 2004. In Candida albicans, the Nin1 kinases Gin4 and Hsl1 negatively regulate pseudohypha formation and Gin4 also controls septin organization. J. Cell Biol. 164:381–391.

84. Wilson, R. B., D. Davis, and A. P. Mitchell. 1999. Rapid hypothesis testing with Candida albicans through gene disruption with short homology regions. 181:1868–1874.

85. 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.

86. Yeong, F. M., H. H. Lim, C. G. Padmasheer, and U. Surana. 2000. Exit from mitosis in budding yeast: biphasic inactivation of the Cdc28-Cib2 mitotic kinase and the role of Cdc20. Mol. Cell 5:501–511.

87. Zachariae, W., T. H. Shin, M. Galova, B. Obermaier, and K. Nasmyth. 1996. Identification of subunits of the anaphase-promoting complex of Saccharomyces cerevisiae. Science 274:1201–1204.

88. Zick, J., and K. G. Hardwick. 2010. Getting down to the phosphorylated ‘nuts and bolts’ of spindle checkpoint signalling. Trends Biochem. Sci. 35:18–27.