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Depletion of the mitotic kinase Cdc5p in *Candida albicans* results in the formation of elongated buds that switch to the hyphal fate over time in a Ume6p and Hgc1p-dependent manner

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ABSTRACT

The fungal pathogen *Candida albicans* differentiates between yeast, hyphae and pseudohyphae in order to enhance survival in the human host. Environmental cues induce hyphal development and expression of hyphal-specific genes. Filaments also result from yeast cell cycle arrest, but the nature of these cells and their mechanisms of formation are less clear. We previously demonstrated that depletion of the mitotic polo-like kinase Cdc5p resulted in the production of filaments under yeast growth conditions that were distinct from hyphae with respect to several criteria, yet expressed hyphal-specific genes at later stages of development. In order to clarify the identity of these growth forms and their relationship to true hyphae, we conducted time course-based investigations of aspects of the polar growth machinery, which can distinguish cell types. During later stages of Cdc5p depletion, the myosin light chain Mlc1p demonstrated a Spitzenkörper-like localization in the tips of some filaments, and the Cdc42p GAP Rga2p became hyper-phosphorylated, as in true hyphae. Hyphal-specific genes *HWP1*, *UME6* and *HGC1* were strongly expressed at approximately the same time. *HWP1* expression was dependent on Ume6p, and absence of Ume6p or Hgc1p influenced late-stage filament morphology and integrity. Finally, polarized growth and *UME6* expression in Cdc5p-depleted cells were independent of the transcription factor Hms1p. Thus, depleting Cdc5p generates elongated buds that switch to a hyphal fate over time through a mechanism that involves *UME6* and *HGC1* induction, possibly in response to maintenance of polarized growth. The results expand on the multiple strategies with which *C. albicans* can modulate growth mode and expression of virulence determinants.

1. Introduction

Candida albicans is an important opportunistic fungal pathogen of humans that exists as a commensal in the gastrointestinal or genitourinary tracts, but can cause a range of infections under immune-compromised conditions (Tong and Tang, 2017). Mortality rates associated with systemic infections can reach as high as 60% (Boonyasiri et al., 2013). One aspect of *C. albicans* biology that is important for virulence is the ability to differentiate into multiple cell types, including yeast, pseudohyphae, and hyphae. Yeast grow via budding that initiates at the G1/S transition of the cell cycle. Initial bud outgrowth is polar, and associated with a high concentration of actin patches, but switches to an isometric mode near mitosis, when the actin patches disperse evenly around the bud (Staebell and Soll, 1985; Hazan et al., 2002). Bud tips contain a polarisome, which regulates actin filament formation at growth sites (Jones and Sudbery, 2010). Later in the cell cycle, polarisome components move to the bud neck, where nuclear division and cytokinesis take place (Sudbery et al., 2004). Pseudohyphae are chains of elongated yeast cells with an extended G2 phase, contain constrictions at septation sites, and are similar to yeast with respect to localization of actin patches, the polarisome, and nuclear division across the bud neck (Sudbery et al., 2004; Sudbery, 2011). Hyphae are distinct in that they maintain polarized growth and actin patches at the tip, undergo the first nuclear division within the germ tube, and lack constrictions at septation sites (Sudbery et al., 2004). Hyphal tips contain a polarisome, but also a proposed vesicle supply center (Crampin et al., 2005), similar to the Spitzenkörper found in hyphal tips of filamentous fungi (Virag and Harris, 2006). The Spitzenkörper is seen as a 3D spot

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in hyphal tips with FM4-64 staining, or localization of the myosin light chain Mlc1p or other proteins (Crampin et al., 2005; Jones and Sudbery, 2010; Bishop et al., 2010). Hyphae also distinctly show hyperphosphorylation of Rga2p, a GTPase activating protein (GAP) for Cdc42p. This results in Rga2p down-regulation, its exclusion from the tip, and maintenance of Cdc42p activity. In contrast, Rga2p localizes to the tips of small and medium yeast and pseudohyphal buds (Zheng et al., 2007; Court and Sudbery, 2007).

The ability to switch between cell types in different environments of the host is crucial for pathogenesis (Lo et al., 1997; Saville et al., 2003). An understanding of the mechanisms that underlie cell differentiation may thus reveal new targets for treating infection. The regulation of the yeast-to-hyphal switch has been extensively investigated, and requires elevated temperature in combination with other environmental cues that are mediated by a diversity of signaling pathways (reviewed in Sudbery, 2011). An important target of many signaling pathways is the transcription factor Efg1p (Lo et al., 1997; Stoldt et al., 1997). Efg1p regulates filament formation and expression of several hyphal-specific genes (HSG's), including the cell wall protein Hwp1p. *UME6* is another central regulator of hyphal development and a target of Efg1p. Yeast cells lacking *UME6* initiate but do not maintain hyphal growth, and *UME6* ovexpression drives hyphal formation under yeast growth conditions (Banerjee et al., 2008; Zeidler et al., 2009). Ume6p in turn maintains expression of *HGC1* (Carlisle and Kadosh, 2010), a cyclin-related factor that is specifically expressed in hyphal cells and required for maintaining hyphal growth (Zheng et al., 2004), in part through regulating Ume6p in a post-transcriptional manner (Mendelsohn et al., 2017).

C. albicans yeast cells can also form filaments in the absence of hyphal-inducing environmental cues. True hyphae can form under yeast growth conditions upon arresting cells in G1 phase through depletion of the G1 cyclin Cln3p (Bachewich and Whiteway, 2005; Chapa y Lazo et al., 2005; Woolford et al., 2016). This may be mediated by Cln3p suppression of hyphal morphogenesis via post-transcriptional inhibition of Ume6p (Mendelsohn et al., 2017). Hyphae also form in the absence of the ubiquitin ligase complex factor Cdc4 (Atir-Lande et al., 2005), also due to post-transcriptional regulation of Ume6p by Cln3p and Hgc1p (Mendelsohn et al., 2017).

Filaments can also form through blocking yeast cells in S, G2 or M phase (Bai et al., 2002; Bachewich et al., 2003, 2005; Bensen et al., 2005; Ciudad et al., 2016; Andaluz et al., 2006; Shi et al., 2007; Shapiro et al., 2009; Trunk et al., 2009; Chou et al., 2011; Senn et al., 2012; Milne et al., 2014) or by reducing expression of various kinases associated with other essential cell cycle or cell growth functions in *C. albicans*. The latter have been referred to as "Essential Process Impairment" (EPI) filaments (Woolford et al., 2016, 2017). Many of these cells also express HSG's. However, the identity of these filaments and the mechanisms underlying their formation largely remain elusive due to lack of comprehensive characterization and complexity in features. For example, cells arrested in mitosis through depletion of the polo-like kinase Cdc5p resemble hyphae in that they maintain polarized growth, lack contrictions along their lengths, move the nucleus from the mother yeast cell into the tube, and express HSG's, including *UME6* (Bachewich et al., 2003, 2005). They contain a constriction at the bud neck due to polar growth originating from the yeast bud that emerges prior to the cell cycle block. However, the filaments are also distinct from hyphae in forming independent of Efg1p, and requiring the spindle checkpoint factor Bub2p for maintenance of polar growth (Bachewich et al., 2003, 2005). Bub2p and the spindle checkpoint factor Mad2p are also required for polar growth under other conditions that arrest mitosis, including depletion of the heat shock factor Hsp90p (Shapiro et al., 2009) or exposure to nocodazole (Bai et al., 2002), respectively, but Bub2p is not required for S phase-arrested filaments (Bachewich et al., 2005). Further, unlike wild-type hyphae, Cdc5p-depleted cells express HSG's only during later stages of elongation (Bachewich et al., 2003, 2005). A novel regulatory pathway involving the transcription factor Hms1p mediates Hsp90p-depleted filament formation and *UME6* expression (Shapiro et al., 2012). In addition, some EPI filaments were only partially dependent on core hyphal regulators, suggesting additional and distinct control of filamentation and HSG expression compared to wild-type filaments (Woolford et al., 2016, 2017). Collectively, this suggests that filaments that form independent of environmental cues in *C. albicans* may not be created in a similar manner. Further, their identity and connection with true hyphae remain unknown (Sudbery, 2011).

Here, we further characterize filaments that form in response to a mitotic block induced by Cdc5p depletion in order to clarify their identity and mechanisms of formation. Through conducting the first time-course based investigations of aspects of the polar growth machinery and other hyphal-diagnostic features, we provide evidence that Cdc5p-depleted cells initially represent elongated buds, but switch to the hyphal fate over time in a manner that involves Ume6p and Hgc1p, but is independent of Hms1p. Our results extend the array of mechanisms that *C. albicans* utilizes for modulating growth form and HSG gene expression, which are important for virulence.

2. Materials and methods

2.1. Strains, oligonucleotides, plasmids and culture conditions

Strains, oligonucleotides and plasmids used in this study are listed in Tables 1–3, respectively. Strains were incubated in synthetic medium (0.67% yeast nitrogen base, 2.0 g adenine, 2.5 g uridine, 2.0 g tryptophan, 1.0 g histidine, 1.0 g arginine, 1.0 g methionine, 1.5 g tyrosine, 1.5 g isoleucine, 7.45 g valine, 1.5 g lysine, 2.5 g phenylalanine, 5.0 g glutamic acid, 10.0 g threonine and 3.0 g leucine per 50 L) containing either 2.0% glucose (SD) or 2.0% sodium succinate (SS) to repress or

Table 1
Strains used in this study.

Strain	Genotype	Source
RM1000	*ura3Δ:: imm434/ura3Δ::1 imm434 his1Δ::hisG/his1Δ::hisG*	Negredo et al. (1997)
BWP17	*ura3Δ::imm434/ura3Δ::imm434 his1Δ::hisG/his1Δ::hisG arg4Δ::hisG/arg4Δ::hisG*	Wilson et al. (1999)
SC5314	*URA3/URA3, HIS1/HIS1*	Fonzi and Irwin (1993)
CB104	*cdc5Δ::hisG/cdc5Δ::HIS1 PCK1::CDC5-URA3*	Bachewich et al. (2003)
CB105	*cdc5Δ::hisG/cdc5Δ::HIS1 PCK1::CDC5-hisG*	Bachewich et al. (2003)
CB400	RM1000 (pRM100 *URA3+, HIS1+*)	Bachewich et al. (2003)
CDC5-25	*CDC5/cdc5Δ::hisG*	This study
AG240	*cdc5Δ::hisG/cdc5Δ::HIS1 PCK1::CDC5-hisG MLC1/MLC1-GFP-URA3*	This study
AG332	*MLC1/MLC1-GFP-URA3*	This study
AG374	*RGA2/RGA2-HA-URA3*	This study
AG379	*cdc5Δ::hisG/cdc5Δ::HIS1 PCK1::CDC5-hisG RGA2/RGA2-HA-URA3*	This study
AG500	*cdc5Δ::hisG/MET3::CDC5-ARG4*	This study
AG509	*cdc5Δ::hisG/MET3::CDC5-ARG4*	This study
AG518	*cdc5Δ::hisG/MET3::CDC5-ARG4, UME6/ume6Δ::URA3*	This study
AG530, 531	*cdc5Δ::hisG/MET3::CDC5-ARG4, ume6Δ::URA3/ume6Δ::HIS1*	This study
AG536, 540	*cdc5Δ::hisG/MET3::CDC5-ARG4, HGC1/hgc1Δ::URA3*	This study
AG547	AG509 pRM100 (*URA3+HIS1+*)	This study
AG553	AG500 pRM100 (*URA3+HIS1+*)	This study
AG574, 577	*cdc5Δ::hisG/MET3::CDC5-ARG4, hgc1Δ::URA3/hgc1Δ::HIS1*	This study
AG570, 572	*cdc5Δ::hisG/MET3::CDC5-ARG4, HMS1/hms1Δ::URA3*	This study
AG579-581	*cdc5Δ::hisG/MET3::CDC5-ARG4, hms1Δ::URA3/hms1Δ::HIS1*	This study