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Minireview

MINIREVIEW

Transcriptional control of hyphal morphogenesis in *Candida albicans*

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ABSTRACT

Candida albicans is a multimorphic commensal organism and opportunistic fungal pathogen in humans. A morphological switch between unicellular budding yeast and multicellular filamentous hyphal growth forms plays a vital role in the virulence of *C. albicans*, and this transition is regulated in response to a range of environmental cues that are encountered in distinct host niches. Many unique transcription factors contribute to the transcriptional regulatory network that integrates these distinct environmental cues and determines which phenotypic state will be expressed. These hyphal morphogenesis regulators have been extensively investigated, and represent an increasingly important focus of study, due to their central role in controlling a key *C. albicans* virulence attribute. This review provides a succinct summary of the transcriptional regulatory factors and environmental signals that control hyphal morphogenesis in *C. albicans*.

Keywords: *C. albicans*; hyphae; morphogenesis; transcription factor(s); environmental signals

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INTRODUCTION

Candida albicans is a commensal and opportunistic fungal pathogen that resides within the gastrointestinal tract (Hoffmann *et al.* 2013; Noble, Gianetti and Witchley 2017), the female reproductive system (Drell *et al.* 2013; Merenstein *et al.* 2013) and the oral cavity of healthy individuals (Ghannoum *et al.* 2010). In humans, *C. albicans* can cause infections ranging from superficial thrush to disseminated and invasive infections (Colman-Lerner, Chin and Brent 2001; Noble, Gianetti and Witchley 2017). While superficial infections can often be successfully treated with antifungal agents, those patients who suffer from recurrent infections, or disseminated *Candidiasis*, often encounter a high degree of morbidity and mortality (Delaloye and Calandra 2014; Chang *et al.* 2017; Rodrigues, Rodrigues and Henriques 2019). The most common antifungal drugs used to treat *C. albicans* infections are limited to three main drug classes: azoles, polyenes and echinocandins (Colman-Lerner, Chin and Brent 2001; Kelly *et al.* 2004; Chang *et al.* 2017; Perfect 2017; Hart *et al.* 2019). Although topical antifungal treatments are often effective at treating acute mucosal infections (Bondaryk, Kurzatkowski and Staniszewska 2013; Pappas *et al.* 2016), treatment of chronic or disseminated *Candidiasis* can be complicated by drug toxicity associated with high doses of these antifungal agents and the increasing resistance to these drugs acquired by *C. albicans* strains (Bray 2015; Benitez and Carver 2019; Sanglard 2019). This suggests that developing novel antifungal drugs is imperative for the treatment of *C. albicans* associated infections (Bray 2015; Du Toit 2017; Perfect 2017; Cully 2018; Hart *et al.* 2019).

C. albicans has the ability to differentiate into multiple distinct morphological states, including, but not limited to, unicellular budding yeast, filamentous pseudo-hyphae, true hyphae, specialized mating-competent 'opaque' cells, a commensal-specific 'GUT' phenotype, as well as a unique chlamydospore morphology (Sudbery, Gow and Berman 2004; Lu, Su and Liu 2014; Gow and Yadav 2017). While each of these morphological forms play an interesting role in *C. albicans* biology, this review will focus on the reversible morphological transition from yeast to hyphae as it plays a central role in the virulence of this fungal pathogen. *C. albicans* hyphae can attach to, and penetrate through, the epithelial cell layers of an infected host, using the coordinated release of hydrolytic enzymes and the generation of hydrostatic pressure to propel hyphae deeper into damaged tissue, ultimately leading to disseminated candidiasis (Felk *et al.* 2002; Gow, Brown and Odds 2002; Naglik *et al.* 2003; Dalle *et al.* 2010). Once disseminated, *C. albicans* is capable of invading many of the host's internal organs, leading to serious life-threatening infections (Filler and Sheppard 2006; Phan *et al.* 2007; Dalle *et al.* 2010; Zhu and Filler 2010). The yeast-to-hyphal transition also plays a critical role in the ability of *C. albicans* to escape from, and simultaneously destroy macrophages which would otherwise aid in clearing the fungal infection (Schroppel *et al.* 2001; Lorenz, Bender and Fink 2004; Ghosh *et al.* 2009).

Hyphal morphogenesis in *C. albicans* is tightly controlled by dozens of transcription factors (TFs) that contribute to the activation or repression of the hyphal transcriptional program. The activity of these regulatory TFs is controlled by a wide range of environmental signals and nutritional cues, including: pH (Buffo, Herman and Soll 1984), N-acetylglucosamine (GlcNAc) (Simonetti, Strippoli and Cassone 1974), serum (Taschdjian, Burchall and Kozinn 1960), CO_2 (Klengel *et al.* 2005), temperature (Shapiro *et al.* 2009), nutritional deprivation (Lu *et al.* 2011) and hypoxia (Klengel *et al.* 2005). Moreover, the switch from yeast to hyphae is highly dependent on many secondary signaling pathways, such as the Efg1-mediated cAMP pathway and the Cph1-mediated MAPK pathway (Biswas, Van Dijck and Datta 2007) which are highly regulated by specific transcription factor(s) (Ishii *et al.* 1997; Cao *et al.* 2006; Kaplan and Kaplan 2009). These secondary signaling pathways play an important role in hyphal formation and contributes immensely to the virulence of *C. albicans* (Cao *et al.* 2006; Whiteway and Bachewich 2007). Although TFs are typically not targeted for antifungal drug development, understanding the role of TFs in the filamentation process would provide valuable data on genes and pathways that could be amenable for therapeutic avenues. Recent advances in drugs targeting protein–protein and protein–DNA interactions suggest that TFs may also serve as antifungal targets (Bahn 2015; McCarthy *et al.* 2017). For example, small molecules were identified that inhibited the DNA binding of Upc2p transcriptional induction of sterol gene expression which may serve as leads for antifungal drug development (Gallo-Ebert *et al.* 2014). In addition, a lead compound (iKIX1) was identified as an inhibitor of drug resistance transcription factor (Pdr1) and Gal11A domains in *Candida glabrata* (Nishikawa *et al.* 2016).

The TFs and signals involved in *C. albicans* hyphal morphogenesis are discussed in this review with a comprehensive analysis in Table 1, Figs 1 and 2. All 'unassigned' TFs, with no known environmental signals are presented only in the table. Many of these TFs overlap in their regulation of target genes, so we examined the potential gene target network associated with hyphal growth for the TFs outlined in Table 1 using the PathoYeastract database (Monteiro *et al.* 2017). PathoYeastract generated a list of documented regulations for these TFs and these interactions were visualized as a network highlighting the overlapping target genes. The interactions were visualized using Gephi software (https://gephi.org/) and presented in Fig. 1. Although we set stringent filters to only display documented interactions associated with hyphal growth, the network is dense and demonstrates the complexity of TF networks including inter-TF interactions.

ENVIRONMENTAL SIGNALS THAT REGULATE HYPHAL MORPHOGENESIS

Serum

Serum is a potent inducer of hyphal growth in *C. albicans*. Within minutes of exposure to serum, yeast-form cells cultured in liquid YPD medium at 37°C will initiate the hyphal program. Albumin is dispensable for serum-induced filamentation (Feng *et al.* 1999), indicating that other components or derivatives of serum are primarily responsible for the induction of filamentation. N-acetylglucosamine (GlcNAc) and proline, which are derived by the breakdown of serum glycoproteins, and are independently capable of inducing filamentation, may be central to serum's ability to induce hyphal formation (Mattia *et al.* 1982; Holmes and Shepherd 1987; Ernst 2000). Serum regulates hyphal formation through various TFs including *EFG1* (Lo *et al.* 1997), *UME6* (Banerjee *et al.* 2008), *BRG1* (Su *et al.* 2018), *NGS1* (Naseem *et al.* 2017) and *RON1* (Naseem *et al.* 2017), and through signaling pathways including the cAMP-PKA pathway (Ernst 2000; Sudbery 2011; Noble, Gianetti and Witchley 2017).

EFG1 regulates hyphal morphogenesis through cAMP-PKA pathway and interactions with other regulators such as *FLO8*

Table 1. List of TFs involved in *C. albicans* hyphal morphogenesis.

TF Genes	Description	*In vivo* relevance	References
ACE2	Transcription factor that stimulates the pseudohyphal formation and the morphological switch in *C. albicans*. Ace2p is prominently expressed during the G2 phase of the cell cycle, therefore, upon entry into the nucleus the protein is present only in daughter cells.	Mutants grow in either pseudohyphal or hyphal form but have reduced virulence *in vivo*.	(Colman-Lerner, Chin and Brent 2001; Kelly *et al.* 2004)
ADR1	Regulates vital genes that express the proteins of carbon generating pathways that are required for the morphological switch. Non-functional Adr1p affects the virulence potential of *C. albicans*.	Mutant strains showed reduced virulence *in vivo*.	(Ramirez and Lorenz 2009)
AFT2	Imperative for maintaining iron homeostasis as well as the regulation of iron dependent pathways. *AFT2* deletion has serious effects on morphology and significantly impairs filamentous growth.	*AFT2* gene mutation attenuates cell surface ferric reductase activity and virulence in a mouse model.	(Kaplan and Kaplan 2009; Liang *et al.* 2010; Castells-Roca *et al.* 2011; Xu *et al.* 2013)
AHR1/ZCF37	Zinc cluster transcription factor, acts as a cofactor for Mcm1p regulon. The Mcm1p-Ahr1p complex directly activates the expression of adhesion genes required for both cell adhesion and hyphal growth.	*AHR1* deletion showed reduced virulence in a mouse model.	(Askew *et al.* 2011)
ARG81	Oxidative burst generated by macrophages triggers *C. albicans* to stimulate the biosynthetic arginine pathway genes including *ARG81*, and promotes filamentous growth.		(Jimenez-Lopez *et al.* 2013)
ASG1	Positive regulator in *C. albicans* hyphal switch. *C.albicans* mutants lacking the Asg1p factor had no effects on fluconazole susceptibility. Further screening and investigation of these *C. albicans* mutants concluded that the Asg1p factor is needed for growth to be accomplished with non-fermentative carbon sources.		(Coste *et al.* 2008)
ASH1	*ASH1* gene encodes regulatory proteins that are necessary for the morphological switch of *C. albicans*. Ash1p is localized in the daughter cell nuclei of the yeast form of *C. albicans* as well as on the tip of the hyphae once the transition occurs. Moreover, *C. albicans* mutants that lack the *ASH1* gene revealed defects in filamentous growth along with reduced virulence under environmental conditions that stimulate hyphal growth.	Required for virulence *in vivo*.	(Inglis and Johnson 2002)
BRE1	Hyperfilamentous growth occurred in the heterozygous mutant strain in nutrient rich conditions.		(Uhl *et al.* 2003)
BRG1/GAT2	Zinc finger, GATA transcription factor, represses hyphal regulator Nrg1p. Overexpression of Brg1p overcomes Nrg1p and induces hyperfilamentation.	Over-expression of *BRG1* attenuates virulence *in vivo*.	(Uhl *et al.* 2003; Cleary *et al.* 2012; Du *et al.* 2012)
CAS2/FGR15	Transposon insertion location alters filament growth. Insertion at +384 location induced less filamentation, whereas +216 insertion induced hyperfilamentation.		(Uhl *et al.* 2003; Bruno *et al.* 2006; Homann *et al.* 2009)
CAS3/ADA2	Pleiotropic transcriptional coactivator in many pathways, including the SAGA complex and Cas5p pathways. Cas3p deficient strains demonstrated poor hyphal growth.	Mutant showed reduced hyphae formation *in vivo*.	(Bruno *et al.* 2006; Pukkila-Worley *et al.* 2009; Sellam *et al.* 2009)
CAS5	MADS-box transcriptional factor. Part of the protein kinase C mediated MAP kinase pathway. Coactivator with Cas3p for gene regulation.	*CAS5* mutants has decreased virulence and filamentation in the *C. elegans* infection model.	(Bruno *et al.* 2006; Chamilos *et al.* 2009; Pukkila-Worley *et al.* 2009)
CPH1	MAPK cascade gene required for filamentous growth. Heterozygous mutants show stunted growth while homozygous deletion mutants show no filamentous growth. Overexpression leads to hyperfilamentation.	*CPH1* mutants are virulent in a mouse model. However, *CPH1* and *EFG1* double mutants are avirulent and unable to form filaments.	(Liu, Kohler and Fink 1994; Lo *et al.* 1997; Csank *et al.* 1998; Du *et al.* 2012)
CPH2	Basic HLH transcription factor of the Myc subfamily. Required for pathways involving *ECE1*, *HWP1*, *HYR1*, *RBT1*, *RBT4*, *TEC1* and *SAPs* 4–6. Represses *RBT4* and *SAP5* in YPD medium. Medium dependent transcription factor, homozygous null displays abnormal hyphal growth in Lee's medium regardless of carbohydrate source.	Strains lacking Cph2p showed reduced colonization in the mouse gastrointestinal tract.	(Lane *et al.* 2001a,.b; Rosenbach *et al.* 2010)
CRZ1	Crz1p is a calcineurin-dependent transcription factor and has a positive effect on hyphae development. Crz1p deletion leads to decrease in sinusoidal hyphae formation.	*CRZ1* mutants does not show attenuated virulence in a murine model of disseminated candidiasis.	(Onyewu *et al.* 2004; Brand *et al.* 2009)

Table 1. Continued

TF Genes	Description	*In vivo* relevance	References
CSR1/ZAP1	Csr1p has a positive effect on hyphae development in a zinc dependent manner and plays a role in zinc homeostasis in *C. albicans*. Mutation in Csr1p causes a decrease in hyphal formation in a zinc-limited growth condition.	*ZAP1* mutants develop biofilms in a rat model of catheter-associated infection,	(Kim *et al.* 2008; Nobile *et al.* 2009)
CTA4	Mutation in Cta4p reduced filamentation under hyphae inducing conditions containing serum. Cta4p mutants produced smooth colonies on serum-supplemented media and had no hyphae growth in liquid media.	*CTA4* mutants showed attenuated virulence in a mouse model of systemic candidiasis.	(Chiranand *et al.* 2008; Vandeputte *et al.* 2011)
CUP9	Transcriptional repressor that inhibits *SOK1* expression, which is required for degradation of Nrg1p. Deletion of Cup9p induces filamentation in white cells and opaque cells of *C. albicans*.	CUP9 mutants demonstrated production of longer and denser filaments in the oral mucosa.	(Guan *et al.* 2013; Lu, Su and Liu 2014; Meir *et al.* 2018)
CWT1	*CWT1* mutants exhibit smaller colonies with increased wrinkled morphology. *CWT1* mutants do not affect filamentation in liquid medium but decrease hyphae filamentation on solid medium.	*CWT1* heterozygous mutants are avirulent in a mouse model of systemic infection.	(Moreno *et al.* 2010; Vandeputte *et al.* 2011)
CZF1	Zinc-finger-containing protein. Ectopic expression of Czf1p accelerates hyphae filamentation in embedded cells. Overexpression of Czf1p stimulates filamentation in growth media lacking glucose. *CZF1* gene deletion has moderate effects on hyphae filamentation.		(Brown *et al.* 1999; Ernst 2000; Giusani, Vinces and Kumamoto 2002)
EFG1	Efg1p promotes hyphae filamentation under serum and GlcNAc conditions. Under low-temperatures and in embedded conditions, it act as a repressor. Efg1p promotes and downregulates filamentation under normoxic and hypoxic conditions respectively.	*EFG1* mutants unable to form hyphae *in vivo*.	(Stoldt *et al.* 1997; Brown *et al.* 1999; Riggle *et al.* 1999; Ernst 2000; Leng *et al.* 2001; Giusani, Vinces and Kumamoto 2002; Whiteway and Bachewich 2007; Desai *et al.* 2018)
EFH1	Overexpression of Efh1p leads to pseudohyphal formation, whereas *EFH1* deletion analysis revealed no specific phenotypic expression. Efh1p seems to activate gene expression and supports the function of the Efg1p, which is the primary essential regulator and illustrates an important role in the regulation of *C. albicans* morphogenesis.	*EFH1* null mutant has increased intestinal colonization whereas *EFH1* over-expression showed reduced intestinal tract and oral cavity colonization.	(Doedt *et al.* 2004; White *et al.* 2007)
FGR17	The *FGR17* gene codes for a filamentous growth regulator 17 protein (Fgr17p) that contains a DNA binding zinc cluster motif, and it is known to be a negative regulator for the morphological switch of *C. albicans*. Mutation in this gene affects the filamentous hyphal growth of *C. albicans*.		(Vandeputte *et al.* 2011)
FGR27	Involved in the cell adherence of *C. albicans* to a silicone substrate, thus contributes to the biofilm formation along with the filamentous morphological transition.		(Uhl *et al.* 2003; Vandeputte *et al.* 2011; Finkel *et al.* 2012)
FKH2	Fkh2p is required for the formation of true hyphal growth and plays a vital role in the virulence factor of *C. albicans*. Fkh2p protein acts in a downstream pathway or in parallel to Efg1p and Cph1p.		(Bensen, Filler and Berman 2002)
FLO8	Flo8p regulates the cAMP/PKA pathway which plays an essential role in *C. albicans* virulence by regulating hyphal growth. Flo8p deleted mutants showed complete suppressing effects on hyphal growth.	*FLO8* mutants unable to form hyphae *in vivo* in *saccharomyces* infection model.	(Cao *et al.* 2006; Pukkila-Worley *et al.* 2009; Ryan *et al.* 2012; Polvi *et al.* 2019).
GPR1	*GPR1*, a G-protein-coupled receptor, and *GPA2*, a Gα subunit, induce hyphae formation and morphogenesis in a cAMP-dependent manner.	*GPR1* mutants are virulent in mouse model of infection.	(Miwa *et al.* 2004)
GRF10	Homeobox transcription factor. Expression increased in stationary phase and during filamentation. Overexpression induces filamentation. Severely decreased filamentation in homozygous null mutants was observed.		(Romanowski *et al.* 2012; Ghosh *et al.* 2015; Wangsanut *et al.* 2017)
HAP5	Component of the CCAAT-binding transcription factor that inhibits hyphal growth of *C. albicans*. *C.albicans* that lack this factor display significant defects in the hyphal formation and have decreased virulence. Furthermore, *HAP5* deleted mutants had an increased expression of specific respiratory enzymes that are encoded by *CYC1* and *COX5*. This indicates that *C. albicans* CCAAT-binding factor may play an imperative role in mitochondrial components along with carbon metabolism.		(Johnson *et al.* 2005)

Table 1. Continued

TF Genes	Description	*In vivo* relevance	References
HMS1	Hms1, a basic helix-loop-helix (HLH) transcription factor that is stimulated by high temperatures and the inhibition of Hsp90. When Hsp90 is inhibited, Hms1p binds to *UME6* and *RBT5*, which are known to be DNA elements that play a role in hyphal formation. It functions downstream of the cyclin-dependent-kinase Pho85p and the cyclin Pcl1p, which ultimately leads to hyphal formation.	*HMS1* deletion mutants showed reduced colonization in the gut.	(Shapiro *et al.* 2012; Perez, Kumamoto and Johnson 2013)
HOT1	Increases expression of Pho81p. Hot1p binds to the *PHO81* promoter site. Hot1p homozygous null mutants displayed hyperfilamentation without any response to farnesoic acid, regardless of the chemical's effect on other pathways.		(Ahn *et al.* 2017)
HSF1	Depletion of Hsf1p compromises the function of Hsp90 and induces filamentation. Overexpression of Hsf1p enables filamentation. *HSF1* gene mutation causes defects in hyphal development.		(Nair *et al.* 2017; Veri *et al.* 2018)
MED7	A subunit of the mediator complex required for filamentation in response to a plethora of cues.	Required for intestinal colonization in mice.	(Tebbji *et al.* 2014)
MSS11	Overexpression of Mss11p induces filamentous growth. Deletion inhibits hyphal growth.		(Su *et al.* 2009).
NDT80	Important for yeast-to-hyphal transition and for nitric oxide inactivation. It activates *HWP1*, *ECE1*, *RBT4*, *ASL3*, *ALS10*, *HYR1*, *SOD5*, *SAP4* and *SAP5* genes. It is also important for repressing several genes including *YWP1*, *CAX4*, *MNN22*, *RHD1*, *RHD3*, *ALD5* and *NRG1*.	Ndt80p is required for hyphal formation. Mutants are avirulent in a mouse model of systemic candidiasis.	(Sellam *et al.* 2010; Yang *et al.* 2012)
NGS1	Works with *REP1* by indirectly activating GlcNAc signaling pathways for hyphal morphogenesis.		(Naseem *et al.* 2017)
NOT3	Heterozygous and homozygous mutant *NOT3*, with *URA3* at its native locus, formed hyphae. Homozygous mutant with ectopic *URA3* expression did not form hyphae.		(Cheng *et al.* 2003a; Staab and Sundstrom 2003)
NOT5	Heterozygous mutant *NOT5*, with *URA3* at its native locus, formed hyphae. Homozygous mutant *NOT5*, with *URA3* at its native locus, was not able to form hyphae on solid medium. Heterozygous mutant *NOT5*, with ectopic *URA3* expression, formed hyphae in growth supplemented with uridine.	Disruption of *NOT5* decreased the adherence to human buccal epithelial cells and reduced mortality in mice with disseminated candidiasis.	(Cheng *et al.* 2003a; Cheng *et al.* 2003b)
NRG1	*NRG1* deletion leads to hyphae formation. Overexpression of *NRG1* blocks filamentation in *C. albicans*.	*NRG1* deletion strain avirulent in a mouse model of infection.	(Braun, Kadosh and Johnson 2001; Saville *et al.* 2003; Cleary and Saville 2010)
OFI1	Overexpression of OfI1pFI1, a zinc-finger containing protein, increased filamentation and invasive growth in *C. albicans*. However, deletion of OfI1pOFI1 did not affect filamentation.		(Du *et al.* 2015)
OPI1	Homozygous mutant exhibits hyperfilamentation at low temperatures (30åC).	Required for virulence in a rat model of vaginitis.	(Chen *et al.* 2015)
PHO4	Loss-of-function mutations in Pho4p exhibited extensive filamentation in conditions with low phosphate concentration.		(Romanowski *et al.* 2012)
PPR1	Zn(II)-Cys6 transcription factor. *PPR1* mutant was found to have decreased hyphae formation.	*PPR1* mutants showed decreased fungal load in a *G. mellonella* infection model.	(Vandeputte *et al.* 2011; Amorim-Vaz *et al.* 2015)
RBF1	RPG-box-binding factor 1 (RBF1) is a *C. albicans* transcription factor that binds to a segment of DNA and has been reported to bind to the *C. albicans* chromosomal telomere. This transcription regulator has glutamine rich regions and is located in the nuclei. The RBF1 factor and its corresponding genes are involved in the regulation of *C. albicans* hyphal growth. Disruption of these genes or deletion of this transcription factor from the genome of *C. albicans* induces filamentous growth.		(Ishii *et al.* 1997)
RCA1	The regulator of carbonic anhydrase (RCA1) controls CO_2 sensing by regulating the expression of the enzyme carbonic anhydrase. This RCA1 factor is known to be an inducer of hyphal growth and acts through cAMP/PKA signaling pathways and also possibly through the interaction with the negative regulator Tup1p.		(Vandeputte *et al.* 2012)

Table 1. Continued

TF Genes	Description	*In vivo* relevance	References
RFG1	Rfg1p is a high mobility group domain (HMG) protein that is involved in DNA binding and functions as a transcriptional repressor of *C. albicans* filamentous growth. Rfg1p acts through Tup1p dependent and independent pathways.		(Khalaf and Zitomer 2001)
RFX2	A transcriptional repressor that attenuates hyphal morphogenesis. It is activated in response to DNA damage and is known to be regulated by Nrg1p. *C. albicans* mutants lacking Rfx2p regulator demonstrate a decrease in the expression of DNA damage genes and express hyperfilamentous growth.		(Hao *et al.* 2009)
RGT1	Zn(II)2Cys6 transcriptional repressor that is involved in regulating the expression of glucose transporter genes and suppressing filamentous growth.	Required for colonization in a mouse model of disseminated candidiasis.	(Sexton, Brown and Johnston 2007; Vandeputte *et al.* 2011)
RIM101	The expression of *RIM101* is stimulated under alkaline pH and is regulated by Rim8p. Alkaline induced hyphal growth and the pathogenesis of *C. albicans* is controlled by *RIM101* induced pathways. However, *RIM101* has no significant role in the growth of *C. albicans* cells under both acidic and alkaline environments. *C. albicans* mutants lacking *RIM101* show hyphal defects.	Homozygous mutants are avirulent in the mouse models of infection.	(Davis, Wilson and Mitchell 2000).
RLM1	A transcription factor that is essential for regulating and directing carbohydrates into biosynthetic pathways as well as mediating critical pathways involved in cell wall integrity. Rlm1p is a positive regulator and thus plays a stimulatory role in *C. albicans* hyphal growth. Furthermore, Rlm1p induces *C. albicans* resistance to cell wall perturbation by antifungal agents. This data suggests that Rlm1p is essential for remodeling *C. albicans* cell wall, carbon adaptation and stimulating a vital interaction with immune cells.	Mutants are less virulent in the murine model of disseminated candidiasis.	(Amorim-Vaz *et al.* 2015; Oliveira-Pacheco *et al.* 2018)
ROB1	Zn(II)Cys6 transcription factor that has been shown to be a positive regulator in *C. albicans* hyphal and biofilm formation. *C. albicans* mutants that lack the *ROB1* regulator demonstrate abnormal growth and morphology.	*ROB1* mutants did not showed significant difference in fungal load in a *G. mellonella* infection model.	(Amorim-Vaz *et al.* 2015; Glazier *et al.* 2017)
RON1	NDT80-like transcription factor, specific to growth on hexamine sugars. It is not required for hyphal growth, but diploid mutants had delayed hyphal growth. Homozygous null mutants grown on dextrose media with GlcNAc showed significantly decreased expression of *HGC1*, *ALS3*, *UME6*, *FAV2*, *HWP1*, *ECE1*, *RBT4*, *SAP5* and *SAP6*.		(Naseem *et al.* 2017)
RTG3	Leucine zipper transcription factor activated during mitochondrial dysfunction. It is important for calcium regulation. Deletion leads to an increase in calcium/calcineurin signaling activity as well as an increased sensitivity to extracellular calcium. Deletion delays serum induced filamentous growth.		(Yan, Zhao and Jiang 2014)
SEF1	Zinc cluster DNA-binding transcription factor. Promoted by Tbf1p, repressed by Sfu1p. *SEF1* mutant demonstrates increased sensitivity to a rise in pH and the iron chelator bathophenanthroline disulfonate (BPS), as well as decreased hyphal growth.	*SEF1* mutants have decreased colonization in a systemic mice model of infection.	(Vandeputte *et al.* 2011)
SFL1	Suppresses the expression of *FLO11*, *STA1* and *SUC1*. It is inactivated by cAMP-dependent PKA Tpk2p. Sfl1p represses filamentation and antagonistically interacts with Flo8p.	Deletion or overexpression of *SFL1* attenuates virulence in a systemic mouse model of infection.	(Bauer and Wendland 2007; Li *et al.* 2007)
SFL2	HSF-like binding domain. Overexpression lowers the temperature threshold for hyphal growth. Homozygous null mutants exhibited increased hyphal growth. It is incapable of forming hyphae in microaerophilic conditions.	Required for virulence in a murine gastrointestinal infection model.	(Spiering *et al.* 2010; Song, Wang and Chen 2011)
SIN3	Part of a specific histone deacetylase complex. Heterozygous diploid showed decreased filamentous growth. Sin3 mutants were able to grow as pseudohyphae, but they were not able to form as true hyphae.	*SIN3* mutants showed increased fungal load in a *G. mellonella* infection model.	(Tebarth *et al.* 2003; Uhl *et al.* 2003; Amorim-Vaz *et al.* 2015)
SKN7	Responds to oxidative stress specifically H_2O_2 and t-butyl hydroperoxide. *SKN7* mutant formed smooth colonies on Spider agar and M-199. Reduced growth was seen on 10% serum agar.		(Singh *et al.* 2004)

Table 1. Continued

TF Genes	Description	*In vivo* relevance	References
SKO1	Represses yeast-to-hyphae transition by inhibiting the expression of *ECE1* and *HWP1*. Filamentation occurred in homozygous null mutants regardless of serum, pH, or temperature.		(Alonso-Monge *et al.* 2010)
SNF4	*SNF4* mutants were found to have severe filamentation defects under different conditions of liquid and solid media. Mutations in *SNF4* have severe filamentation defects.		(Azadmanesh *et al.* 2017)
SNF5	A subunit of SWI/SNF chromatin remodeling complex required for filamentation in response to different cues.	Required for gut colonization in mice and for systemic infection in *Galleria* larvae.	(Finkel *et al.* 2012; Burgain *et al.* 2019)
SNF6	Snf6p is a subunit of the Swi/Snf complex essential for differentiation of invasive hyphae. Snf6p is required for carbon utilization, hyphal and invasive growth.		(Tebbji *et al.* 2017)
STD1	Mediates the sugar sensing pathways. Std1p is a negative transcription factor and a repressor of filamentous growth.		(Brown, Sabina and Johnston 2009)
STP2	Stp2p is a positive transcription factor that functions in regulating the gene expression of extracellular amino acids. Upon activation, Stp2p will translocate to the nucleus and induce gene expression of essential genes involved in the SPS system, and it also stimulates the morphologic transition to the filamentous hyphal form.	Required for virulence in a mouse model of disseminated candidiasis.	(Martinez and Ljungdahl 2005)
SPT3	Homozygous mutants are hyperfilamentous.	Required for virulence in a systemic mouse model of infection.	(Laprade *et al.* 2002)
SPT6	Deletion of *SPT6* causes impairment in hyphae growth.		(Al-Rawi, Laforce-Nesbitt and Bliss 2010)
SPT20	Mutation of *SPT20* results in decreased hyphae formation.		(Tan *et al.* 2014)
SSN6	*SSN6* mutants did do not form true hyphae or extensive filamentation. Overexpression of *SSN6* increased filamentation.	Deletion or overexpression of *SSN6* attenuates virulence in a systemic mouse model of infection.	(Hwang *et al.* 2003)
SWI1/SNF2	Deletion of either *SWI1* or *SNF2* fails to form true hyphae. Mutants of both *SNF2* and *SWI1* were unable to promote filamentation under hyphal inducing environments in liquid, solid or embedded conditions. The Swi/Snf complex is recruited by hyphae-specific genes to recruit activators and promote expression of other hyphae-specific genes.		(Mao *et al.* 2006)
SWI4/SWI6	Both Swi4p and Swi6p play a significant role in the G1/S progression in the cell cycle of *C. albicans* and influence cell proliferation. Moreover, both of these transcription factors are positive regulators that stimulate hyphal growth and contribute to the virulence of *C. albicans*.	*SWI4* mutants showed increased fungal load in *G. mellonella* and mouse infection models.	(Hussein *et al.* 2011; Amorim-Vaz *et al.* 2015)
TAC1	Tac1p, or transcriptional activator of CDR genes, is involved in the regulation of *C. albicans* ABC transporters CDR1 and CDR2. Tac1p has demonstrated its role in allowing azole drug resistance as well as stimulating virulence and pathogenesis. *TAC1* (orf19.3188) mutants show decreased hyphae formation.	Gain of function mutation in *TAC1* gene exhibit neutral effect on virulence in a mouse model of intravenous infection.	(Coste *et al.* 2004; Vandeputte *et al.* 2011; Lohberger, Coste and Sanglard 2014)
TCC1	Tcc1p factor contains 4 tetratricopeptide repeat (TPR) motifs and interacts with Tup1p to form a complex that inhibits filamentous growth.	Null mutants are less virulent in a mouse model of systemic infection.	(Kaneko *et al.* 2006)
TEA1	Tea1p is a negative transcription factor and contains zinc cluster DNA binding motifs. Tea1p suppresses the genes involved in hyphal growth and limits virulence in *C. albicans*.		(Vandeputte *et al.* 2011)
TEC1	Pheromone receptors induce MAPK cascade leading to *TEC1* expression, which induces filamentation.	Required for virulence in a mouse model of systemic candidiasis.	(Schweizer *et al.* 2000; Lane *et al.* 2001; Staib *et al.* 2004; Sahni *et al.* 2010)
TFG1	Upstream transposon insertion led to hyperfilamentation in solid YEPD plus serum.		(Uhl *et al.* 2003)
TUP1	Regulatory transcription factor induced by a variety of environmental factors that represses morphogenesis, specifically *WH11*, *HWP1* and *RBT1*. Works with Mig1p, Nrg1p and Rfg1p to repress specific genes based on environmental stimuli.	Mutants are virulent in a gastrointestinal infection model.	(Braun and Johnson 1997; Murad *et al.* 2001; Zhao *et al.* 2002; Kebaara *et al.* 2008; Homann *et al.* 2009; Song, Wang and Chen 2011)

Table 1. Continued

TF Genes	Description	*In vivo* relevance	References
TYE7	Regulates glycolytic genes and represses hyphal formation in hypoxic environments. *TYE7* homozygous knockout mutant exhibited hyphae formation on solid medium under hypoxic conditions.	Attenuated virulence in both *Galleria* and murine infection models.	(Askew *et al.* 2009; Bonhomme *et al.* 2011)
UGA3	Zn(II)-Cys6 transcription factor. Homozygous null led to hyperfilamentation.	Required for colonization in a mouse model of disseminated candidiasis.	(Vandeputte *et al.* 2011)
UME6	Zn(II)-Cys6 transcription factor. Gene expression induced in serum, 37°C, pH 6.8, and repressed by Nrg1p-Tup1p and Rfg1p-Tup1p pathways. Specifically, *UME6* is important for hyphal extension. Homozygous null mutants expressed significantly less *SAP4*, *SAP5*, *HYR1* and *RBT4*. Ume6p plays a role in the expression of *HWP1*, *ECE1*, *ALS3* and *HGC1*. It is directly associated with increased virulence and is stabilized by Ofd1p and high CO_2 signaling pathways.	*UME6* mutants unable to filament and are attenuated for virulence in a mouse model of systemic candidiasis.	(Banerjee *et al.* 2008; Carlisle *et al.* 2009; Zeidler *et al.* 2009)
ZCF3	Zn(II)-Cys6 transcription factor. *ZCF3* (orf19.1168) mutants have increased hyphae formation.		(Vandeputte *et al.* 2011)
ZCF7	Zn(II)-Cys6 transcription factor. *ZCF7* (orf19.1685) mutants have decreased hyphae formation.	Mutants showed increased colonization in a mouse model of disseminated candidiasis.	(Vandeputte *et al.* 2011)
ZCF11	Zn(II)-Cys6 transcription factor. Abnormal filamentous growth is observed in homozygous null mutants.		(Uhl *et al.* 2003; Elson *et al.* 2009; Vandeputte *et al.* 2011)
ZCF14	Zn(II)-Cys6 transcription factor. *ZCF14* (orf19.2647) mutants have decreased hyphae formation.	Mutants showed decreased fungal load in a *Galleria* infection model.	(Vandeputte *et al.* 2011; Amorim-Vaz *et al.* 2015)
ZCF17	Zn(II)-Cys6 transcription factor. *ZCF1 7*(orf19.3305) mutants have increased hyphae formation.		(Uppuluri and Chaffin 2007; Vandeputte *et al.* 2011)
ZCF18	Zn(II)-Cys6 transcription factor. *ZCF18* (orf19.3405) mutants have increased hyphae formation.	Required for colonization in a mouse model of disseminated candidiasis.	(Vandeputte *et al.* 2011)
ZCF29	Zn(II)-Cys6 transcription factor. *ZCF29* (orf19.5133) mutants have decreased hyphae formation.	Required for colonization in a mouse model of disseminated candidiasis.	(Vandeputte *et al.* 2011)
ZCF32	Novel ZN(II)2-Cys6 binuclear cluster transcription factor, negatively regulates biofilm formation by repressing adhesion and yeast to hyphae transition and dispersion.		(Kakade *et al.* 2016)

and *CZF1* (Cao *et al.* 2006). Serum activates the cAMP-PKA pathway through Ras1p, which leads to the activation of Cyr1p and subsequently Tpk1p and Tpk2p dissociation from Bcy1p. This signaling cascade results in the phosphorylation of Efg1p. Once phosphorylated, Efg1p activates hyphae-inducing genes and induce filamentation (Stoldt *et al.* 1997) (Fig. 2). Mutants that lack *EFG1* are unable to form hyphae in response to serum or GlcNAc under standard laboratory conditions (Lo *et al.* 1997; Stoldt *et al.* 1997; Whiteway and Bachewich 2007), and these mutants are also less virulent *in vivo* (Lo *et al.* 1997). Interestingly, under microaerophilic and embedded conditions, mutants lacking *EFG1* can form hyphae, as Efg1p acts as a repressor of filamentation under embedded growth conditions (Brown *et al.* 1999; Riggle *et al.* 1999; Sonneborn, Bockmuhl and Ernst 1999).

Ume6p is a positive regulator of hyphal formation and works antagonistically with Nrg1p, a hyphal repressor, through a negative feedback loop to control filamentation (Banerjee *et al.* 2008). When *NRG1* expression levels are high, the transition from yeast to hyphae is suppressed and the virulence of *C. albicans* is reduced (Braun, Kadosh and Johnson 2001; Saville *et al.* 2003). Nrg1p has been shown to repress *UME6* under non-hyphal inducing conditions (Kadosh and Johnson 2005), however *NRG1* expression is downregulated under hyphal inducing conditions, such as serum and 37°C (Braun, Kadosh and Johnson 2001), which in turn leads to hyphal formation. Moreover, *UME6* expression has been shown to be induced by serum and 37°C temperature (Kadosh and Johnson 2005). Banerjee *et al* further elucidated the regulatory relationship between *NRG1* and *UME6* by demonstrating that Ume6p downregulates *NRG1* in the presence of serum and 37°C temperature, which ultimately regulates hyphal formation (Banerjee *et al.* 2008). Importantly, *in vivo* results from this study showed that the mutants lacking *UME6* were unable to undergo the morphological transition from yeast to hyphae, which also led to decreased virulence (Banerjee *et al.* 2008).

Temperature

C. albicans is sensitive to temperature with elevated temperatures (37–39°C) typically inducing filamentation. Temperature-dependent regulation of hyphal morphogenesis is primarily mediated by Hsp90p (heat shock protein 90) which inhibits filamentation under non-inducing conditions through cAMP-PKA

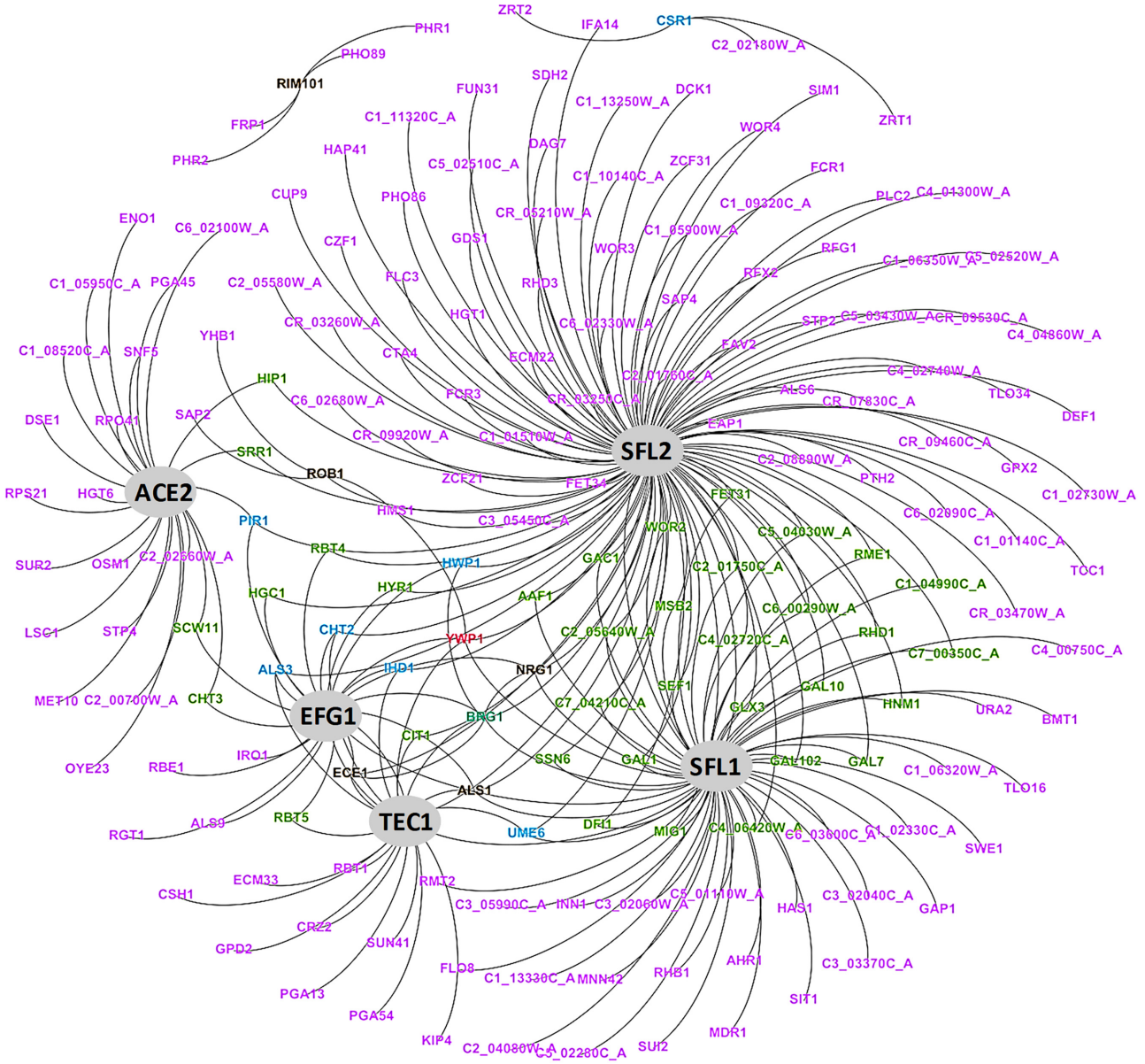

Figure 1. Network map of TFs from Table 1 showing the documented regulations based on simultaneous DNA binding and expression evidence where the TF can act as an activator or inhibitor. The environmental conditions were filtered for pseudohyphal/hyphal growth. Target genes that are targeted by only one TF are in violet and genes targeted by multiple TFs have non-violet colors.

signaling pathway dependent and independent mechanisms (Shapiro *et al.* 2009; Shapiro *et al.* 2012; Leach *et al.* 2016; Noble, Gianetti and Witchley 2017; Veri *et al.* 2018). In response to elevated temperatures, Hsp90p mediated repression of Ras1p is alleviated (Shapiro *et al.* 2009), resulting in increased Ras1 GTPase activity. Ras1p then stimulates cAMP production by Cyr1p, ultimately activating the cAMP-PKA pathway for hyphal induction (Fig. 2). Hsp90p appears to repress filamentation primarily through the cAMP-PKA signaling pathway, as any perturbations in the upstream components of the cAMP-PKA pathway that block PKA-dependent signaling prevents the induction of hyphal growth (Shapiro *et al.* 2009). Importantly, Shapiro *et al* also showed that genetic depletion of Hsp90p attenuates virulence in a murine model of systemic disease.

At elevated temperatures, Hsp90p also regulates hyphal morphogenesis through transcriptional regulators Hms1p and Hsf1p, a mechanism that is independent of the cAMP-PKA pathway (Shapiro *et al.* 2012; Veri *et al.* 2018). In response to elevated temperatures and inhibition of Hsp90p, Hms1p is recruited to the DNA elements of *UME6* and *RBT5* genes via cyclin-dependent kinase Pho85p and cyclin Pcl1p-dependent manner (Shapiro and Cowen 2012; Shapiro *et al.* 2012; Diezmann, Leach and Cowen 2015). Pho85p and PcI1p in turn regulate the expression levels of *UME6*, a key activator of hyphal growth, and *RBT5*, a cell wall protein-encoding gene that is activated in response to hyphal inducing cues (Shapiro and Cowen 2012; Shapiro *et al.* 2012; Diezmann, Leach and Cowen 2015). Furthermore, *HMS1* deletion results in both temperature-dependent filamen-

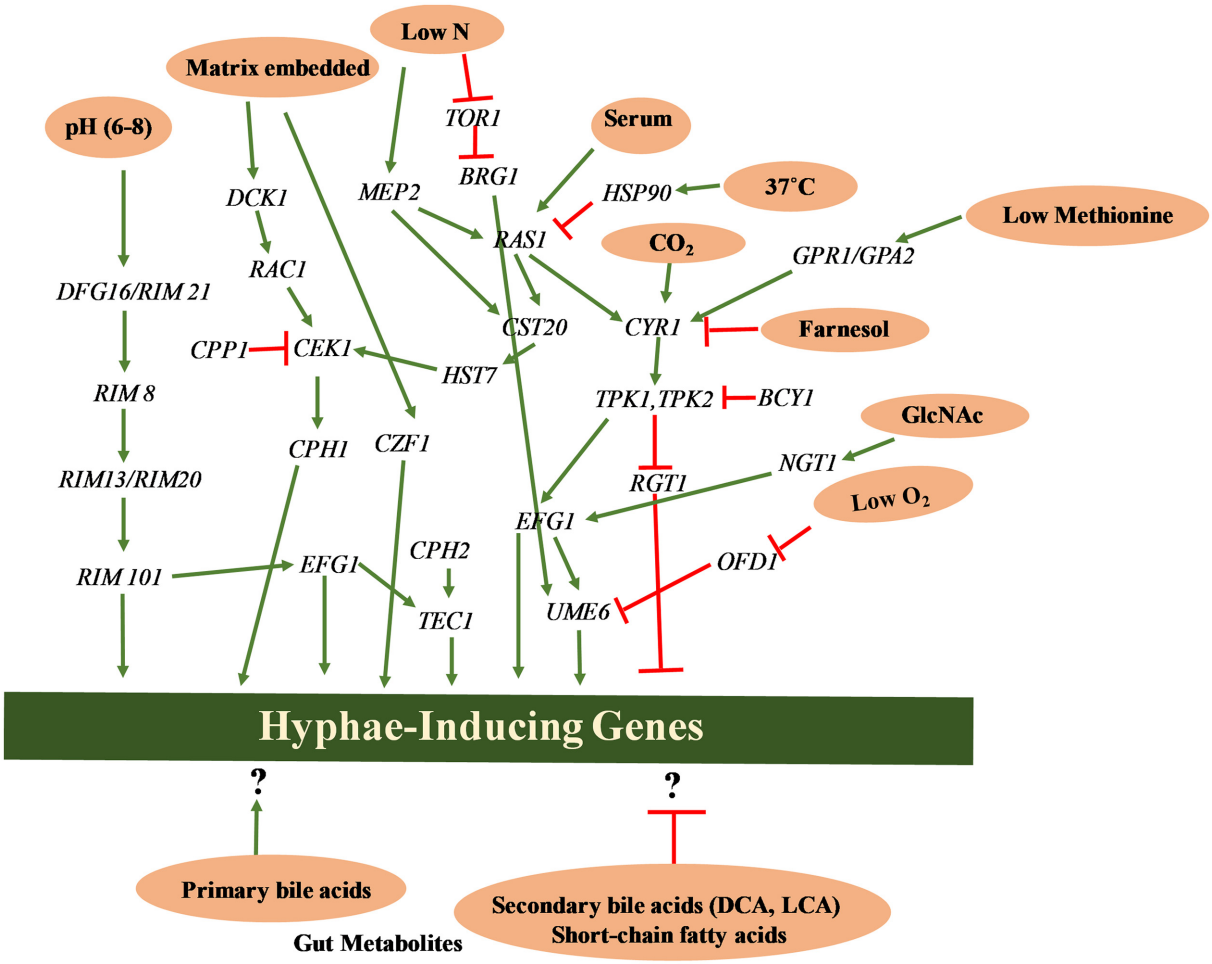

Figure 2. Environmental signals regulate TF genes in *C. albicans* hyphal morphogenesis.

tation defects and attenuation of virulence in a *Galleria mellonella* model of infection (Shapiro *et al.* 2012). Hsf1p and Hsp90p collaboratively regulate temperature-induced hyphal morphogenesis through an entirely different mechanism. Although Hsp90p has been shown to inhibit Hsf1p activity under non-heat shock conditions, a subset of Hsf1p-dependent heat shock-induced genes are dependent upon Hsp90p activity for upregulation in response to temperature stress (Leach *et al.* 2016). This positive effect of Hsp90p on specific Hsf1p target genes is mediated through Hsp90p-dependent depletion of nucleosomes which would otherwise occlude Hsf1p binding motifs and prevent Hsf1p-dependent transcriptional activation under heat shock. This work highlights the complex roles of Hsp90p in temperature-dependent gene expression in *C. albicans*.

pH

Hyphal induction is responsive to environmental pH, with neutral-to-alkaline pH (≥6.5) inducing, and acidic pH (< 6.5) repressing, the transition from yeast to hyphal morphologies (Davis, Wilson and Mitchell 2000; Ernst 2000; Sudbery, Gow and Berman 2004; Kornitzer 2019). pH-mediated hyphal morphogenesis is primarily regulated though a signaling pathway that converges upon the transcriptional regulator Rim101p, which is activated via C-terminal proteolytic processing in response to elevated pH (Dorn 1965; Orejas *et al.* 1995; Li and Mitchell 1997; Davis, Wilson and Mitchell 2000; Hollomon *et al.* 2016). Under acidic environments, Rim101p found in the full-length unprocessed form, and has no known function (Orejas *et al.* 1995; Li and Mitchell 1997). However, under alkaline environments, proteolytic cleavage of the C-terminus yields a truncated active form of Rim101p (Davis, Wilson and Mitchell 2000). This proteolytic activation of Rim101p is controlled by a number of gene products, with *RIM101* itself being an alkaline induced gene that depends on Rim8p, Rim13p and Rim20p for its induction (Denison, Orejas and Arst 1995; Li and Mitchell 1997; Maccheroni *et al.* 1997; Denison *et al.* 1998; Davis, Wilson and Mitchell 2000). The Rim101p signaling cascade is initiated by two transmembrane proteins, Dfg16p and Rim21p, which act as the pH sensors for this pathway (Barwell *et al.* 2005). Although Rim101p directly mediates the repression of acid-induced genes, and activation of alkaline-induced genes (*PHR1/2*) (Davis, Wilson and Mitchell 2000), Rim101-mediated activation of filamentation is dependent upon *EFG1* (El Barkani *et al.* 2000; Lane *et al.* 2001). The activation of *EFG1* via Rim101p also leads to the activation of *TEC1*, which subsequently activates the expression of filament genes (Lane *et al.* 2001) (Fig. 2).

pH-dependent regulation of filamentation also plays an important role in the interaction between *C. albicans* and

the host immune system. Upon being phagocytosed by macrophages, *C. albicans* has the ability to induce the morphological switch from yeast to hyphae, allowing it to penetrate and rupture the immune cell (Vylkova and Lorenz 2014). This process is dependent upon *STP2*, which encodes a transcription factor that regulates the expression of amino acid permeases (Vylkova and Lorenz 2014; Vesely *et al.* 2017). Stp2p expression enables *C. albicans* to catabolize amino acids as a carbon source, which generates large amounts of ammonia, raises extracellular pH within the phagolysosome, and ultimately induces hyphal formation and escape from the macrophage (Fernandez-Arenas *et al.* 2009; Vylkova *et al.* 2011; Vylkova and Lorenz 2014; Danhof *et al.* 2016; Miramon and Lorenz 2016; Vesely *et al.* 2017). Mutant strains lacking *STP2* are unable undergo hyphal morphogenesis within macrophages, are defective in macrophage killing, and have a reduced ability to survive subsequent to phagocytosis (Vylkova and Lorenz 2014). Moreover, *stp2* null mutant are attenuated for virulence in a disseminated candidiasis model of infection (Vylkova and Lorenz 2014). Together these results indicate that auto-induction of filamentation via alkalization of the phagosome, and the resulting escape from macrophages, is a key virulence attribute for *C. albicans* (Vylkova *et al.* 2011; Vylkova and Lorenz 2014).

Oxygen

The ability of *C. albicans* to adapt to varying levels of oxygen is critical for hyphal formation and pathogenicity (Lu *et al.* 2013). Hypoxia in combination with 5% CO_2 sustains the elongation of hyphae by stabilizing the Ume6p transcription factor (Lu *et al.* 2013; Lu, Su and Liu 2014). Upon being stabilized, Ume6p binds to its own promoter and activate its own transcription through a positive feedback loop, which in turn leads to the sustained elongation of hyphal growth (Carlisle *et al.* 2009; Lu *et al.* 2013). Thus, sustained expression of *UME6* plays an important role in determining the morphology of *C. albicans* by maintaining hyphal development, while *ume6* mutants are unable to sustain hyphal development (Lu *et al.* 2013). Moreover, the expression of *NRG1*, which encodes a negative regulator of hyphal development, is significantly reduced under hypoxic plus CO_2 conditions. Low Nrg1p levels could be attributed to the high levels of Ume6p, suggesting that the overexpression of *UME6* could possibly repress *NRG1* expression, ultimately leading to hyphal formation (Banerjee *et al.* 2008; Lu *et al.* 2013; Lu, Su and Liu 2014).

The induction of filamentation under hypoxic conditions is also mediated by the 2-OG-Fe (II)-dependent dioxygenase enzyme Ofd1p. Ofd1p functions as an oxygen sensor, and modulates Ume6p stability (Lu *et al.* 2013). Ofd1p possess two functional domains, the Ofd1C domain and the Ofd1N domain (Lu *et al.* 2013). Under relatively high oxygen levels, Ofd1C promotes Ume6p degradation, while under hypoxic conditions, Ofd1N inhibits the function of Ofd1C, leading to the stabilization of Ume6p and hyphal elongation is maintained (Lu *et al.* 2013). Lu *et al.*, demonstrated that strains lacking *OFD1* are able to filament more readily than wild type in nutrient and oxygen rich media, however the *ofd1* mutants are unable to completely sustain hyphal elongation (Lu *et al.* 2013). Furthermore, when these *ofd1* mutants are exposed to 5% CO_2, Ume6p is further stabilized and hyphal elongation is fully maintained. This indicates that hyphal elongation is regulated by two parallel pathways, with Ofd1p regulating the stability of Ume6p in response to oxygen levels, and CO_2 stabilizing Ume6p through an unknown mechanism(s) (Lu *et al.* 2013).

Nutrient starvation

Nitrogen and amino acid starvation can also induce the transition from yeast to hyphae (Csank *et al.* 1998; Biswas and Morschhauser 2005; Flanagan *et al.* 2017). Two ammonium permease genes, *MEP1* and *MEP2*, are expressed under nitrogen starvation conditions and, in addition to enabling growth in nitrogen-poor environments, these permeases trigger a signaling cascade that induces filamentation (Biswas and Morschhauser 2005). Mep2p stimulates a cascade of events ultimately activating Cph1-dependent MAPK and cAMP-dependent signaling pathways (Biswas and Morschhauser 2005) (Fig. 2). This cascade of events includes activation of Cst20p and Ras1p via Mep2p, which then activates Hst7p, Cek1p and finally activates Cph1p to induce hyphal formation (Csank *et al.* 1998; Biswas and Morschhauser 2005). Mutants lacking *MEP2* fail to induce hyphal formation under nitrogen starvation conditions (Dabas and Morschhauser 2007).

The Tor1 (target of rapamycin) pathway also responds to nitrogen starvation by regulating Brg1p and Ume6p TFs (Bastidas, Heitman and Cardenas 2009; Flanagan *et al.* 2017; Noble, Gianetti and Witchley 2017). Tor1p kinase is part of the target of rapamycin complex 1 (TORC1) that negatively regulates filamentation (Flanagan *et al.* 2017). Inhibition of TORC1 activates Brg1p which in turn blocks the Nrg1p-Tup1p transcriptional repressor complex (Bastidas, Heitman and Cardenas 2009; Flanagan *et al.* 2017). *RHB1* another transcription factor also plays a role in nitrogen starvation-induced morphogenesis through the expression of *MEP2* (Tsao, Chen and Lan 2009; Chen *et al.* 2012; Flanagan *et al.* 2017).

Amino acids including methionine are sensed through GPR1 receptors and regulate hyphal morphogenesis through cAMP pathway. *GPR1* encodes a protein, with seven transmembrane domains that is associated with Gpa2p (Xue, Batlle and Hirsch 1998). *GPR1* signaling is activated by methionine (Maidan *et al.* 2005) and functions upstream of the cAMP pathway (Fig. 2). In the absence of methionine, wild-type and *GPR1* mutants form smooth colonies but fail to induce hyphae (Maidan *et al.* 2005). However, with low concentrations of methionine, wild type but not *GPR1* mutant strain induce filamentation (Maidan *et al.* 2005) (Fig. 2).

Embedded conditions

Growth under embedded conditions is a strong inducer of the yeast-to-hyphal transition in *C. albicans* (Giusani, Vinces and Kumamoto 2002). Although cells grown at 25°C in liquid medium, or on the surface of semi-solid agar medium, fail to produce hyphae, or do so very slowly, the same cells when embedded within agar medium will rapidly transition to the hyphal growth program (Brown *et al.* 1999; Vinces, Haas and Kumamoto 2006; Petrovska and Kumamoto 2012). This induction of hyphal growth within an agar matrix is mediated by three TFs, Czf1p, Efg1p and Cph1p (Brown *et al.* 1999; Vinces, Haas and Kumamoto 2006; Petrovska and Kumamoto 2012). Efg1p, which typically plays a positive role in inducing hyphal formation in response to a wide range of environmental cues, acts as a repressor of filamentation during growth in embedded conditions (Sonneborn, Bockmuhl and Ernst 1999; Giusani, Vinces and Kumamoto 2002; Vinces, Haas and Kumamoto 2006). Induction of filamentation in response to embedded conditions is mediated by Czf1p, which acts to alleviate Efg1p-dependent repression of filamentation. Giusani, Vinces and Kumamoto 2002). *CZF1* transcription is upregulated in response

to embedded conditions, and ectopic expression of *CZF1* results in accelerated filamentation in embedded growth conditions, however *CZF1* expression or deletion has no effect on filamentation of *efg1* null cells grown under the same conditions (Giusani, Vinces and Kumamoto 2002). These results indicate that Czf1p is a key inducer of hyphal growth under embedded growth conditions, via alleviation of Efg1p-mediated repression. It is interesting to note that *CZF1* expression under embedded growth conditions is dependent upon Efg1p, highlighting the complex interplay between these two morphological regulators.

Deletion of *CZF1* showed a defect in filamentous growth under embedded conditions, however a greater defect in filamentation was seen when both *CZF1* and *CPH1* were deleted (Brown *et al*. 1999). Although *CPH1* contributes to the activation of filamentation under embedded growth conditions, it is not required for filamentation in liquid medium (Liu, Kohler and Fink 1994). Under embedded conditions, Cek1p is also stimulated and promotes hyphal growth in white cells (Csank *et al*. 1998; Noble, Gianetti and Witchley 2017). The Cek1p mitogen-activated protein kinase pathway responds to embedded conditions and initiates a signaling cascade that ultimately activates Cph1p via Cek1p, leading to hyphal growth (Lane *et al*. 2001; Noble, Gianetti and Witchley 2017). Furthermore, embedded conditions also stimulate Dck1p leading to the activation of Rac1p and Czf1p (Bassilana and Arkowitz 2006; Hope *et al*. 2008). Rac1p and Dck1p are not required for hyphae growth in liquid media, however they are activated under embedded conditions and stimulate filamentation (Bassilana and Arkowitz 2006; Hope *et al*. 2008). *DCK1* and *RAC1* mutant strains fail to form filaments under embedded conditions (Hope *et al*. 2010). Nrg1p, a negative regulator of hyphal formation, is suppressed in low-oxygen conditions of embedded growth and is also mediated by both the Czf1p and Efg1p TFs (Cleary and Saville 2010).

CONCLUSION

C. albicans, a polymorphic fungus, resides in host niches in both yeast and hyphal forms. Elucidating the mechanisms by which regulatory TFs integrate and respond to the environmental signals that control morphogenesis of *C. albicans* is important for understanding its pathogenesis, and for the potential development of novel treatment strategies. Further investigation of known environmentally-responsive regulatory systems, and the identification of novel host-specific environmental signals involved in morphological switching of *C. albicans,* represent important areas for future research. Recent studies have revealed that gut metabolites differentially regulate the hyphal morphogenesis of *C. albicans*. For example, the primary bile acid taurocholic acid (TCA) promotes hyphal morphogenesis, whereas secondary bile acids including deoxycholic (DCA), lithocholic acid (LCA), short-chain fatty acids and *Bacteroides ovatus*-secreted metabolites inhibit the *C. albicans* hyphal morphogenesis (Garcia *et al*. 2017; Guinan and Thangamani 2018; Guinan, Villa and Thangamani 2018; Guinan *et al*. 2019; Gutierrez *et al*. 2020). While these signals have been identified as environmental cues for morphogenesis, future studies are necessary to dissect the role of the transcription factor(s) and signaling mechanisms that mediate the response of *C. albicans* to these signals. Understanding how these, and other environmental signals, modulate morphogenesis should yield valuable new insights into the control of commensalism versus pathogenicity of *C. albicans*. In addition, targeting hyphal morphogenesis should be considered as an alternative, or complement, to the current antifungal therapies used to control and treat drug resistant *C. albicans* infections.

Conflicts of interest. None declared.

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