Published in final edited form as:
Trends Microbiol. 2014 December ; 22(12): 707–714. doi:10.1016/j.tim.2014.09.001.

Candida albicans hyphal initiation and elongation

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Abstract

The fungus *Candida albicans* is a benign member of the mucosal microbiota, but can cause mucosal infections and life-threatening disseminated invasive infections in susceptible individuals. The ability to switch between yeast, pseudohyphal, and hyphal growth forms (polymorphism) is one of the most investigated virulence attributes of *C. albicans*. Recent studies suggest that hyphal development in *C. albicans* requires two temporally linked regulations for initiation and maintenance of the hyphal transcriptional program. Hyphal initiation requires a rapid but temporary disappearance of the Nrg1 transcriptional repressor of hyphal morphogenesis. Hyphal maintenance requires active sensing of the surrounding environment, leading to exclusion of Nrg1 binding to promoters of hypha-specific genes or reduced *NRG1* expression. We discuss recent advances in understanding the complex transcriptional regulation of hyphal gene expression. These provide molecular mechanisms underpinning phenotypic plasticity of *C. albicans* polymorphism.

Keywords

Candida albicans; hyphal initiation; hyphal elongation; removing Nrg1 repression

Yeast and hyphal forms of *Candida albicans*

Candida albicans is a common opportunistic fungal pathogen of humans. It asymptomatically colonizes the skin and mucosal surfaces of most healthy individuals [1, 2]. However, alterations in host immunity, physiology, and/or microbiota can lead to the inability to control *C. albicans* colonization on mucosal surfaces and the development of disease [3]. Disseminated invasive candidiasis has an estimated mortality rate of 40%, even with the use of antifungal drugs[4, 5]. With the limited types of antifungal drugs available and rising populations of susceptible patients, there is a pressing need for understanding mechanisms of *Candida* pathogenesis in order to develop new approaches for treating invasive candidiasis.

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A defining feature of *C. albicans* is its ability to grow either as a unicellular budding yeast or in filamentous forms [2]. Unlike dimorphic fungal pathogens of humans (e.g. *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* and *Penicillium marneffei*) that normally grow in filamentous forms outside the human body but convert to yeast form in human tissues [6], *C. albicans* is able to switch reversibly between yeast, pseudohyphae, and hyphal growth forms, and is found in both yeast and filamentous forms in the host [7]. The morphological plasticity of *C. albicans* is a critical virulence determinant. The hyphal form plays key roles in the infection process, and can promote tissue penetration and escape from immune cells [8, 9]. Hyphal morphogenesis is coupled with virulence, as genes that control hyphal morphology are co-regulated with genes encoding virulence factors. Hypha-specific genes *UME6* and *HGC1* are regulators of hyphal transcription and morphogenesis. Levels of the transcription factor Ume6 control the levels and duration of hypha-specific transcription [10–12]. Dosage studies of Ume6 suggest that pseudohyphae are an intermediate state between yeast cells and hyphae, rather than a distinct fate [12]. Hyphal G_1-type cyclin1 (Hgc1)-Cdc28 is responsible for olarized growth at the hyphal tips and cell chain formation [13–20]. How polarized growth is initiated and maintained during *C. albicans* hyphal development is comprehensively reviewed [21]. Hypha-specific genes *HWP1*, *ALS3*, and *RBT5* encode cell wall proteins that are important for adhesion to host cells and iron acquisition from the host [22–25].

The yeast-to-hypha transition is triggered by many nutritional and environmental cues, including serum [26], *N*-acetylglucosamine (GlcNAc) [27], neutral pH [28], high temperature, nutrient starvation [29], hypoxia, CO_2 [30], and adherence [31]. Many of the strong hyphal inducing signals are sensed and integrated at the adenylate cyclase Cyr1, which is essential for hyphal development under all hyphal induction conditions [32–35]. The target of Cyr1 is cAMP-dependent protein kinase A (PKA). The cAMP-PKA pathway and additional signaling pathways that operate to promote the yeast-to-hypha transition, and the transcription factors that are targeted by these pathways have been thoroughly reviewed [35–39]. This review concentrates on recent findings that provide molecular mechanisms for phenotypic plasticity and signal integration in the transcriptional regulation of hyphal development in *C. albicans*. We emphasize the finding that hyphal development involves two temporally linked regulations: initiation and maintenance. Key signaling pathways and transcription factors important in hyphal initiation and maintenance will also be discussed.

Hyphal initiation and maintenance: two phases of removing Nrg1 inhibition

Hypha-specific gene expression is negatively regulated by a complex consisting of the general transcriptional corepressor Tup1 in association with the transcriptional repressor Nrg1 [40–43]. Cells lacking either of the two repressors constitutively grow as long pseudohyphae, and the expression of hypha-specific genes is derepressed. Ectopic expression of *NRG1* inhibits hyphal filamentation in all *in vitro* growth conditions, and also during invasive infection, leading to attenuated virulence in a systemic infection model [44, 45]. The significance of Nrg1 as the key transcriptional repressor of the hyphal transcriptional program is underscored by phenotypic profiling of 143 transcriptional regulator knockout mutants, where only *nrg1* and *tup1* mutants are filamentous under all conditions examined [46]. Therefore, removing the transcriptional repression by Nrg1

should lead to the yeast-to-hypha transition in *C. albicans*. Indeed, Nrg1 is at the promoters of hypha-specific genes to repress their expression during yeast growth. Upon hyphal induction, Nrg1 dissociates rapidly from the promoters and remains at low levels during hyphal elongation [29]. Nrg1 protein levels decrease sharply during the first 30 min upon hyphal induction at 37°C, coinciding with germ tube formation and disappearance of the Nrg1 protein from the promoter of hypha-specific genes [29]. Interestingly, the Nrg1 protein level recovers after 1 h of hyphal induction, but the level of Nrg1 protein at the promoters of hypha-specific genes remain low during hyphal development [29]. The temporary disappearance of Nrg1 is essential for hyphal induction, as ectopically expressing Nrg1 blocks germ tube formation even under robust hyphal induction conditions [29]. A shift in temperature to 37°C and inoculation of a small amount of cells from a saturated culture into fresh medium is sufficient for the rapid clearance of Nrg1. Other hyphal induction conditions, such as serum and starvation, are not essential for the disappearance of Nrg1 during hyphal initiation. Instead, they are critical for excluding Nrg1 from promoters when Nrg1 protein levels recover during hyphal elongation [29]. Therefore, hyphal development involves two phases of removing Nrg1 repression: (i) for initiation and (ii) for maintenance. Initiation requires a transient downregulation of the Nrg1 protein level, whereas maintenance requires a regulation that prevents Nrg1 from binding at the promoters of hypha-specific genes.

Hyphal initiation requires two independent mechanisms of downregulating Nrg1

Hyphal initiation requires the temperature of 37°C and inoculation of a small amount of cells from saturated cultures into a fresh medium under most *in vitro* conditions. Under the induction condition of 37°C and inoculation, Nrg1 disappears rapidly through transcriptional downregulation of *NRG1* and degradation of Nrg1 (Figure 1). The decrease in *NRG1* expression is dependent on the cAMP-PKA pathway because the adenylyl cyclase Cyr1 or Tpk2 (a catalytic subunit of the PKA) is required for reduced *NRG1* expression during hyphal initiation [47]. *CYR1* in *C. albicans* is essential for hyphal formation but not yeast-form growth [32, 48]. Cyr1 stimulates cAMP production, which then activates protein kinase A (PKA) [35]. There are two catalytic subunits of PKA, Tpk1 and Tpk2 [49, 50]. Deletion of *TPK2* impairs hyphal development in liquid media. The requirement of Cyr1 and Tpk2 for hyphal development in all media conditions is consistent with their necessity for downregulation of *NRG1* transcription during hyphal initiation. In fact, the major function of Tpk2 in hyphal development is to downregulate Nrg1, as the *tpk2 nrg1* double mutant is constitutively filamentous similar to the *nrg1* single mutant [47]. The transcription factors Efg1 and Flo8, believed to function downstream of the cAMP-PKA pathway in hyphal development [51, 52], are required for the downregulation of *NRG1* expression [29]. The temperature of 37°C is a requirement of the observed transcriptional downregulation of *NRG1* during hyphal initiation. Elevated temperature seems to be sensed by heat shock protein 90 (Hsp90), which inhibits hyphal development, as pharmacological inhibition of Hsp90 by geldanamycin leads to hyphal growth [53]. Hsp90 signaling requires an intact cAMP pathway, as a mutation in any of the cAMP-PKA pathway components blocks the

hypha-inducing effects of Hsp90 inhibition. But this data does not exclude the possibility that Hsp90 functions in parallel with the cAMP-PKA pathway [53, 54].

Nutrients and other conditions affect the robustness of hyphal initiation and Nrg1 downregulation [29]. For example, the timing of hyphal initiation and Nrg1 disappearance is slower in medium with mannitol than that with glucose, consistent with the activation of the cAMP pathway by glucose. In addition, Cyr1 is known to integrate signals that induce hyphal development, such as *N*-acetylglucosamine, CO_2, or the bacterial peptidoglycan found in serum [30, 55, 56]. Although these signals are not essential for the downregulation of Nrg1 during hyphal initiation induced by 37°C and inoculation, they can increase the robustness of hyphal initiation and may even bypass the need for inoculation or temperature upshift. Molecular mechanisms for the downregulation of *NRG1* transcription are not known and likely complex. A recent publication suggests the involvement of an antisense *NRG1* transcript in the downregulation of *NRG1* transcript levels during hyphal development [57]. Further experiments are needed to elucidate how the cAMP-PKA pathway and its downstream transcriptional regulators control the downregulation of *NRG1* transcription during hyphal initiation. It is also necessary to determine if and how other signals, such as temperature and pH, control hyphal initiation by downregulating *NRG1* transcript levels.

In addition to 37°C growth temperature or nutrient signals, the inoculation procedure is another requirement for hyphal initiation *in vitro*. It releases cells from inhibition by farnesol, a quorum-sensing molecule in *C. albicans* that can inhibit germ-tube formation [58]. Farnesol is thought to block hyphal initiation by inhibiting the Ras1-Cyr1 pathway [39, 59]. However, the expression level of *NRG1* is still dramatically reduced in the presence of farnesol [47]. The major function of farnesol is to inhibit Nrg1 degradation and this regulation is independent of the cAMP-PKA pathway. During inoculation, when cells are released from farnesol inhibition, the Cup9 transcriptional repressor is degraded [47]. Cup9 is a homeodomain-containing transcriptional repressor, and is degraded by the N-end rule E3 ligase Ubr1 [47, 60]. It is not clear how Ubr1 senses farnesol to regulate Cup9 degradation. In *Saccharomyces cerevisiae*, Cup9 degradation is regulated by a conformational change of Ubr1, triggered by binding with dipeptides [61]. It is possible that farnesol adopts a similar mechanism to inhibit the binding of Ubr1 to Cup9 in *C. albicans*. Additional experiments are needed to uncover the molecular mechanism of the Ubr1-mediated Cup9 degradation by farnesol. The rapid degradation of Cup9 transiently derepresses the expression of Sok1 to promote Nrg1 protein degradation. Deletion of *SOK1* inhibits hyphal initiation and Nrg1 degradation upon inoculation, and overexpression of *SOK1* can overcome farnesol-mediated inhibition of germ-tube formation. Therefore, release from farnesol inhibition triggers Nrg1 degradation through transient expression of *SOK1* [47]. The major function of Sok1 is to downregulate Nrg1, as the *sok1 nrg1* double mutant is similar in phenotype to the *nrg1* single mutant [47]. In addition to farnesol, other quorum-sensing molecules may also regulate hyphal development of *C. albicans*. For example, the quorum-sensing molecule 3-oxo-C_{12}-homoserine lactone, which is secreted by *Pseudomonas aeruginosa*, can also inhibit the yeast-to-hyphal transition [58, 62, 63]. Altogether, these results demonstrate that *NRG1* transcriptional downregulation requires the cAMP-PKA pathway, whereas release from farnesol inhibition during inoculation triggers

Nrg1 degradation. The two pathways are both required for rapid clearing of Nrg1 to initiate hyphal development.

In addition to the Nrg1-controlled hyphal transcriptional program, post-transcriptional regulations during hyphal initiation have been found important for the initiation of polarized growth [16, 20]. Future research should identify additional post-transcriptional regulations that are necessary for hyphal initiation, but are independent of the hyphal transcriptional program.

Hyphal maintenance in air requires Brg1- and Hda1-mediated chromatin remodeling

Unlike hyphal initiation, hyphal maintenance requires active sensing of the surrounding environment. After hyphal initiation, Nrg1 protein levels increase gradually, and return to the levels similar to that in yeast cells. However, Nrg1 is excluded from hyphal promoters to sustain hyphal development (Figure 2A, I). Cells deleted of Hda1, a class II histone deacetylase (HDAC) [64], are unable to maintain hyphal growth [29]. Hda1 is recruited to the hyphal promoters during hyphal elongation in response to environmental signals known to sustain hyphal development. With the exception of serum in YPD, media that favor sustained hyphal development are often nutrient-poor, such as Lee's medium [65], Spider medium (with mannitol as a carbon source) [66], and mammalian tissue culture media M199. Treatment of *C. abicans* cells with a sub-lethal level of rapamycin in a rich medium mimics a nutrient-poor medium and induces robust hyphal elongation [29]. Rapamycin inhibits Tor1 kinase, a central regulator of cell growth in response to nitrogen and amino acid availability in yeast [67] and is conserved in *C. albicans* [68].

The major function of Hda1 in hyphal development is to deacetylate Yng2. Yng2 is a subunit of NuA4 histone acetyltransferase (HAT) module [69]. Hda1 deacetylates Yng2 at K175, leading to Yng2 degradation. This regulation of Yng2 is critical for blocking Nrg1 binding to the promoters and sustaining hyphal elongation *in vitro*. Substituting K175 with glutamine (K175Q, a mutation mimicking constitutive acetylation) results in defective hyphal maintenance under all media known to support prolonged hyphal development [29]. Conversely, the $yng2^{K175R}$ mutant (a mutant mimicking the constitutive deacetylation state of Yng2) completely bypasses the requirement of Hda1 in hyphal elongation [29]. In addition to Hda1, the Set3/Hos2 histone deacetylase complex has been shown to inhibit the yeast-to-filament transition and modulate transient expression changes of key transcription factors that influence morphogenesis [70, 71]. Therefore, not just DNA-binding transcription factors, but also chromatin-modifying enzymes, play critical roles in the regulation of hyphal transcriptional program in *C. albicans*.

Brg1, a GATA family transcription factor, is required for both biofilm formation and hyphal elongation in *C. albicans* [57, 72–74]. Through a forward genetic screen, Brg1 was identified as the transcription factor that recruits Hda1 to promoters of hypha-specific genes for chromatin remodeling, leading to occlusion of Nrg1 binding during hyphal elongation [72]. *BRG1* expression requires both the removal of Nrg1 and a sub-growth inhibitory level of rapamycin; therefore, it is a sensitive readout of Tor1 signaling [72, 75]. Overexpression

of Brg1 sustains hyphal development at 37°C in the absence of environmental signals for hyphal elongation [72], indicating that hyphal development is maintained through activation of Brg1 expression. Brg1 expression is activated by several hypha-inducing conditions, including rapamycin [72]. Reduced Tor1 signaling lowers the basal activity of the HOG (high osmolarity) mitogen-activated protein kinase (MAPK) to activate *BRG1* expression. Hog1 is activated by osmotic stress, oxidative stress, and heavy metal stress, and is required for the survival of *C. albicans* cells when they encounter these stresses [76–79]. In contrast to stress-induced rapid Hog1 activation, rapamycin treatment leads to a downregulation of Hog1 basal activity for a prolonged period of time through the functions of the two Hog1 tyrosine phosphatases, Ptp2 and Ptp3, leading to the activation of *BRG1* expression [75]. In addition, the Set3/Hos2 complex also modulates the transcription kinetics of *BRG1* during hyphal development [71]. Brg1 sustains hyphal elongation by prolonging Ume6 expression. *UME6* expression is dependent on Brg1 and Hda1. Ectopically expressing Ume6 rescues the hyphal growth defect of the *brg1* and *hda1* mutants [72]. Therefore, hyphal elongation in response to nutrient limitation is maintained through the activation of *BRG1* expression, which in turn activates *UME6* expression. Transcriptional regulation of *BRG1* or *UME6* expression is critical for sustained hyphal development. Their regulations are likely complex considering that both genes have long upstream sequences. Future research should determine if and how different signaling pathways and transcriptional regulators, such as Eed1 [80, 81], Sfl2 [82, 83], Cph2 [84], and Rim101 [85, 86], converge to regulate their expression. In addition to transcriptional regulation, both *BRG1* and *UME6* transcripts contain a long 5′ untranslated region (UTR). The 5′ UTR of *UME6* has recently been found to regulate Ume6 translational efficiency [87]. Considering that transcripts of many hyphal regulators have a long 5′ UTR, translational regulation may be another level of regulation important for polymorphism that awaits further investigation.

Hyphal elongation in hypoxia and high CO_2 is maintained by stabilizing Ume6

Hda1-mediated deacetylation of Yng2 at K175 is essential for hyphal extension *in vitro*. However, the $yng2^{K175Q}$ mutant is not defective in virulence and hyphal elongation during disseminated infection in mice [88]. Thus, conditions to which *C. albicans* is exposed within the host must activate a signaling pathway that is independent of the Hda1-mediated hyphal elongation pathway. In the foci of infection, fungal cells are exposed to both hypoxia and hypercarbia relative to standard *in vitro* growth conditions. Indeed, hypoxia together with 5% CO_2, but neither condition alone, maintains hyphal development. This condition bypasses the *brg1* or $yng2^{K175Q}$, but not *ume6* mutant for hyphal elongation [88]. Ume6 is continuously degraded in air, partially stabilized in either low oxygen or high CO_2, but is completely stable under low oxygen combined with 5% CO_2 [88]. Similar to Ume6, Hgc1 is also stabilized only in both hypoxia and 5% CO_2, suggesting that *C. albicans* uses a common pathway to stabilize hyphal regulators in hypoxia plus high CO_2. Stable Ume6 can activate its own expression and repress *NRG1*, thus bypassing the requirement for Brg1 and Hda1 in hyphal maintenance (Figure 2A,II).

Ofd1, a prolyl 4-hydroxylase-like 2-oxoglutarate-Fe(II) dioxygenase, is an oxygen sensor [88, 89]. Deletion of Ofd1 in *C. albicans* results in stabilization of Ume6 in 5% CO_2, but not in air [88]. This indicates that Ofd1 senses oxygen concentration to regulate Ume6 stability; but in parallel to Ofd1, an additional regulator(s) that senses high CO_2 may exist and further stabilize Ume6. CO_2 has been shown to regulate hyphal morphogenesis through the activation of the adenylyl cyclase Cyr1, resulting in activation of the cAMP-PKA pathway [30]. Stabilization of Ume6 by CO_2 is likely mediated through a Cyr1-independent pathway, as CO_2 and hypoxia promote hyphal elongation, not initiation. Ofd1 has two functionally distinct domains: the N-terminal dioxygenase domain is required for oxygen sensing, and inhibits the activity of the C-terminal degradation domain in an O_2-dependent manner. Removal of the N-terminal dioxygenase domain creates a constitutively active Ofd1 (designated *OFD1–1*) that is no longer inhibited by hypoxia. Ectopically expressing *OFD1–1* leads to Ume6 degradation and impaired hyphal elongation even in hypoxia with 5% CO_2. However, *OFD1–1* has no effect in hyphal elongation in rapamycin-containing media in air. Conversely, $yng2^{K175Q}$ mutants are defective in hyphal elongation in air, but not in hypoxia plus 5% CO_2 [88]. Therefore, *C. albicans* employs two different strategies to maintain hyphal elongation in air versus hypoxia. Disrupting one pathway blocks hyphal elongation only in response to its corresponding inducing conditions.

Two parallel pathways control hyphal elongation and virulence during disseminated infection

Ofd1-mediated regulation also functions in parallel to the Brg1-Hda1 pathway in controlling hyphal elongation and virulence *in vivo* (Figure 2B). The *OFD1–1* single mutant does not show a defect in hyphal maintenance and virulence compared to wild-type *OFD1* during disseminated infection; but the $yng2^{K175Q}OFD1–1$ double mutant displays a profound defect in hyphal elongation and attenuated virulence in comparison to the $yng2^{K175Q}$ single mutant [88]. Therefore, hyphal elongation *in vivo* is regulated by two parallel pathways that share overlapping functions in hyphal elongation and pathogenesis. Virulence and hyphal elongation *in vivo* are attenuated only when both pathways are blocked. This synergy between two pathways of hyphal elongation for virulence indicates that nutrient limitation, as well as hypoxia and high CO_2, must all exist at the same time during the disseminated infection. The multitude of host signals and the redundancy for hyphal regulation may explain why some *C. albicans* mutants have profound defects in hyphal formation and elongation *in vitro*, yet have normal virulence in mice [90]. These findings suggest that *C. albicans* can sense multiple host conditions through parallel pathways to promote hyphal elongation and pathogenicity during systemic infections.

Temporal connection between hyphal initiation and maintenance

Temporal regulation of cell fate by different signaling pathways is common in development of organisms. For hyphal development in *C. albicans*, the two phases of regulation for initiation and maintenance are temporally linked. Nrg1 removal during hyphal initiation is a prerequisite for the subsequent Brg1-Hda1 mediated hyphal maintenance. Moreover, adding serum or rapamycin after 2 h of hyphal induction showed no effect on hyphal elongation [29]. Therefore, the time period of reduced Nrg1 during hyphal initiation can be viewed as a

window of opportunity for establishing the sustained hyphal transcription program (Figure 3). The dynamic change of nucleosome positions during yeast-to-hypha transition determines promoter accessibility to Nrg1 and Brg1 in yeast and hyphal states, which establishes the temporal connection between hyphal initiation and maintenance. In yeast cells, the Nrg1 binding site is located in the nucleosome free region in the middle of the UAS region on the *HWP1* promoter, whereas the Brg1 binding site is occupied by a nucleosome [72]. Removal of Nrg1 during hyphal initiation leads to rapid nucleosome disassembly and repositioning so both Brg1 and Nrg1 binding sites are accessible. Nrg1 also represses *BRG1* expression. Removal of Nrg1 during initiation allows the activation of *BRG1* expression in response to environmental signals that promote hyphal elongation. Therefore, during this time window, accumulated Brg1 can recruit Hda1 to promoters of hypha-specific genes to reposition nucleosomes, leading to obstruction of Nrg1 binding sites and sustained hyphal development. The removal of Nrg1 repression during hyphal initiation also allows transient expression of hypha-specific genes, including *UME6*. If cells are under hypoxia and high CO_2 condition during this time window, Ume6 is then stabilized and further activates its own transcription and represses *NRG1* expression. These positive feedback loops sustain cells in the hyphal form.

The temporal link between hyphal initiation and elongation provides underlying mechanisms for the plasticity of polymorphism observed in *C. albicans*, and how cells can simultaneously grow in both yeast and hyphal forms in the same culture or at the same site in the host. In order to initiate hyphal transcription, Nrg1 must be temporarily removed. Under *in vitro* conditions, the timing, duration, and extent of Nrg1 downregulation correlates with the timing and efficiency of hyphal initiation, and are sensitive to multiple factors, including the growth state and inoculum size, media, and temperature. The combination of temperature of 37°C and releasing from farnesol inhibition is sufficient to induce robust and synchronous hyphal initiation and temporary disappearance of Nrg1. Because the sustained hyphal transcriptional program can only be established during the absence of Nrg1, duration of the low Nrg1 period in some cells may not be long enough to accumulate enough Brg1 for the Hda1-mediated chromatin remodeling, or accumulate enough Ume6 under hypoxia and high CO_2 condition. These cells will grow as yeast. Other cells that have a window of opportunity sufficient to establish the hyphal transcription program will develop into hyphae. Therefore, the different length of window of opportunity in each cell can lead to cell-to-cell variation in hyphal development in a given culture, and quality of the initial hyphal induction can affect the fate of hyphal development. Furthermore, duration of hyphal development is determined by growth environments. Hyphal cells continue to grow as hyphae under hypoxia and high CO_2 or nutrient starvation, but convert to yeast when nutrients are replete. These regulations provide underlying mechanisms for the plasticity of polymorphism.

Concluding remarks and future directions

The yeast-to-hyphal transition of *C. albicans* is linked to a number of properties important for its interactions with the host: adhesion to epithelial and endothelial cells; primary and intercellular invasion via induced endocytosis and active penetration; and escape from phagocytes and immune evasion. The capacity of *C. albicans* to reversibly switch between

yeast, pseudophal and hyphal morphologies is widely believed to be essential for pathogenicity at both superficial and systemic levels. Recent findings reviewed here provide molecular mechanisms for plasticity of polymorphism in *C. albicans*. Despite the recent advances in our understanding of *C. albicans* polymorphism *in vitro*, little is known about temporal-dynamic regulation of *C. albicans* polymorphism in the host. Future studies are needed to determine morphologies of *C. albicans* during colonization and infection, and identify host signals that control hyphal initiation and elongation in different host niches (Box 1). Future experiments should also address how these host signals are sensed by *C. albicans*. Additional studies are also needed to integrate the new and known signaling pathways into the recently identified pathways that repress Nrg1 for hyphal initiation and elongation. In addition, *C. albicans* may also employ Nrg1-independent regulations to control polymorphism, and this remains to be addressed. Besides studying the regulation of polymorphism and understanding how host environment influences *C. albicans* growth forms, it is also important to learn more about the roles of the yeast, pseudohyphal and hyphal growth forms in pathogenesis and commensal colonization. We predict that studies along these lines will provide insights on mechanisms that control the yeast-to-hypha transition in the host. Targeted inhibition of this morphological switch should provide an alternative approach to current antifungals for controlling *C. albicans* infections.

Box 1

Outstanding questions

- What are the morphologies of *C. albicans* and the signaling pathways that control the morphologies during colonization and infection?
- What signals control hyphal initiation and elongation in different host niches?
- How are host signals sensed by *C. albicans*?
- How are the signaling pathways integrated to downregulate or repress Nrg1 for hyphal initiation and elongation?
- Are there Nrg1-independent regulations that control hyphal development?
- What are the roles of the yeast and hyphal growth forms in pathogenesis and commensal colonization?

Acknowledgments

Research in the authors' laboratory is supported by the National Institutes of Health grants R01GM/AI55155 and R01AI099190 to H.L.

References

1. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol. 2001; 9:327–335. [PubMed: 11435107]
2. Odds, FC. Candida and candidosis. Bailliere Tindall; 1988.
3. Kirkpatrick CH. Chronic mucocutaneous candidiasis. J Am Acad Dermatol. 1994; 31:S14–17. [PubMed: 8077500]

4. Kullberg, BJ.; Filler, SG. Candidemia. In: Calderone, RA., editor. Candida and Candidiasis. ASM Press; 2002. p. 327-340.
5. Filler, SG.; Kullberg, BJ. Deep-seated candidal infections. In: Calderone, RA., editor. Candida and candidiasis. ASM Press; 2002. p. 341-348.
6. Klein BS, Tebbets B. Dimorphism and virulence in fungi. Curr Opin Microbiol. 2007; 10:314–319. [PubMed: 17719267]
7. Romani L, et al. Adaptation of Candida albicans to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. Curr Opin Microbiol. 2003; 6:338–343. [PubMed: 12941401]
8. Dalle F, et al. Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. Cell Microbiol. 2010; 12:248–271. [PubMed: 19863559]
9. Lorenz MC, et al. Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryot Cell. 2004; 3:1076–1087. [PubMed: 15470236]
10. Banerjee M, et al. UME6, a novel filament-specific regulator of *Candida albicans* hyphal extension and virulence. Mol Biol Cell. 2008; 19:1354–1365. [PubMed: 18216277]
11. Zeidler U, et al. UME6 is a crucial downstream target of other transcriptional regulators of true hyphal development in *Candida albicans*. FEMS Yeast Res. 2009; 9:126–142. [PubMed: 19054126]
12. Carlisle PL, et al. Expression levels of a filament-specific transcriptional regulator are sufficient to determine *Candida albicans* morphology and virulence. Proc Natl Acad Sci U S A. 2009; 106:599–604. [PubMed: 19116272]
13. Zheng X, et al. Hgc1, a novel hypha-specific G1 cyclin-related protein regulates *Candida albicans* hyphal morphogenesis. Embo J. 2004; 23:1845–1856. [PubMed: 15071502]
14. Zheng XD, et al. Phosphorylation of Rga2, a Cdc42 GAP, by CDK/Hgc1 is crucial for *Candida albicans* hyphal growth. EMBO J. 2007; 26:3760–3769. [PubMed: 17673907]
15. Wang A, et al. Hyphal chain formation in *Candida albicans*: Cdc28-Hgc1 phosphorylation of Efg1 represses cell separation genes. Mol Cell Biol. 2009; 29:4406–4416. [PubMed: 19528234]
16. Bishop A, et al. Hyphal growth in *Candida albicans* requires the phosphorylation of Sec2 by the Cdc28-Ccn1/Hgc1 kinase. EMBO J. 2010; 29:2930–2942. [PubMed: 20639857]
17. Gutierrez-Escribano P, et al. CDK-dependent phosphorylation of Mob2 is essential for hyphal development in *Candida albicans*. Mol Biol Cell. 2011; 22:2458–2469. [PubMed: 21593210]
18. Gonzalez-Novo A, et al. Sep7 is essential to modify septin ring dynamics and inhibit cell separation during *Candida albicans* hyphal growth. Mol Biol Cell. 2008; 19:1509–1518. [PubMed: 18234840]
19. Caballero-Lima D, Sudbery PE. In *Candida albicans*, phosphorylation of Exo84 by Cdk1-Hgc1 is necessary for efficient hyphal extension. Mol Biol Cell. 2014; 25:1097–1110. [PubMed: 24501427]
20. Sinha I, et al. Cyclin-dependent kinases control septin phosphorylation in *Candida albicans* hyphal development. Dev Cell. 2007; 13:421–432. [PubMed: 17765684]
21. Arkowitz RA, Bassilana M. Polarized growth in fungi: symmetry breaking and hyphal formation. Seminars in cell & developmental biology. 2011; 22:806–815. [PubMed: 21906692]
22. Staab JF, et al. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. Science. 1999; 283:1535–1538. [PubMed: 10066176]
23. Almeida RS, et al. the hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. PLoS Pathog. 2008; 4:e1000217. [PubMed: 19023418]
24. Phan QT, et al. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol. 2007; 5:e64. [PubMed: 17311474]
25. Weissman Z, Kornitzer D. A family of *Candida* cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. Mol Microbiol. 2004; 53:1209–1220. [PubMed: 15306022]
26. Taschdjian CL, et al. Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. AMA journal of diseases of children. 1960; 99:212–215.

27. Simonetti N, et al. Yeast-mycelial conversion induced by N-acetyl-D-glucosamine in *Candida albicans*. Nature. 1974; 250:344–346. [PubMed: 4605454]
28. Buffo J, et al. A characterization of pH-regulated dimorphism in *Candida albicans*. Mycopathologia. 1984; 85:21–30. [PubMed: 6374461]
29. Lu Y, et al. Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. PLoS Biol. 2011; 9:e1001105. [PubMed: 21811397]
30. Klengel T, et al. Fungal adenylyl cyclase integrates CO2 sensing with cAMP signaling and virulence. Curr Biol. 2005; 15:2021–2026. [PubMed: 16303561]
31. Brown DH Jr, et al. Filamentous growth of *Candida albicans* in response to physical environmental cues and its regulation by the unique CZF1 gene. Mol Microbiol. 1999; 34:651–662. [PubMed: 10564506]
32. Rocha CR, et al. Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus *Candida albicans*. Mol Biol Cell. 2001; 12:3631–3643. [PubMed: 11694594]
33. Bahn YS, Sundstrom P. CAP1, an adenylate cyclase-associated protein gene, regulates bud-hypha transitions, filamentous growth, and cyclic AMP levels and is required for virulence of *Candida albicans*. J Bacteriol. 2001; 183:3211–3223. [PubMed: 11325951]
34. Zou H, et al. *Candida albicans* Cyr1, Cap1 and G-actin form a sensor/effector apparatus for activating cAMP synthesis in hyphal growth. Mol Microbiol. 2009; 75:579–591. [PubMed: 19943905]
35. Hogan DA, Sundstrom P. The Ras/cAMP/PKA signaling pathway and virulence in *Candida albicans*. Future microbiology. 2009; 4:1263–1270. [PubMed: 19995187]
36. Sudbery PE. Growth of *Candida albicans* hyphae. Nature reviews Microbiology. 2011; 9:737–748.
37. Huang G. Regulation of phenotypic transitions in the fungal pathogen *Candida albicans*. Virulence. 2012; 3:251–261. [PubMed: 22546903]
38. Biswas S, et al. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. Microbiology and molecular biology reviews : MMBR. 2007; 71:348–376. [PubMed: 17554048]
39. Hogan DA, Muhlschlegel FA. *Candida albicans* developmental regulation: adenylyl cyclase as a coincidence detector of parallel signals. Curr Opin Microbiol. 2011; 14:682–686. [PubMed: 22014725]
40. Braun BR, Johnson AD. Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. Science. 1997; 277:105–109. [PubMed: 9204892]
41. Kadosh D, Johnson AD. Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. Mol Biol Cell. 2005; 16:2903–2912. [PubMed: 15814840]
42. Braun BR, et al. NRG1, a repressor of filamentous growth in *C.albicans*, is down-regulated during filament induction. EMBO J. 2001; 20:4753–4761. [PubMed: 11532939]
43. Murad AM, et al. NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. Embo J. 2001; 20:4742–4752. [PubMed: 11532938]
44. Park YN, Morschhauser J. Tetracycline-inducible gene expression and gene deletion in *Candida albicans*. Eukaryot Cell. 2005; 4:1328–1342. [PubMed: 16087738]
45. Saville SP, et al. Inhibition of filamentation can be used to treat disseminated candidiasis. Antimicrobial agents and chemotherapy. 2006; 50:3312–3316. [PubMed: 17005810]
46. Homann OR, et al. A phenotypic profile of the *Candida albicans* regulatory network. PLoS Genet. 2009; 5:e1000783. [PubMed: 20041210]
47. Lu Y, et al. Quorum sensing controls hyphal initiation in *Candida albicans* through Ubr1-mediated protein degradation. Proc Natl Acad Sci U S A. 2014; 111:1975–1980. [PubMed: 24449897]
48. Harcus D, et al. Transcription profiling of cyclic AMP signaling in *Candida albicans*. Mol Biol Cell. 2004; 15:4490–4499. [PubMed: 15269278]

49. Bockmuhl DP, et al. Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of *Candida albicans*. Mol Microbiol. 2001; 42:1243–1257. [PubMed: 11886556]
50. Cloutier M, et al. The two isoforms of the cAMP-dependent protein kinase catalytic subunit are involved in the control of dimorphism in the human fungal pathogen *Candida albicans*. Fungal genetics and biology : FG & B. 2003; 38:133–141. [PubMed: 12553943]
51. Bockmuhl DP, Ernst JF. A potential phosphorylation site for an A-type kinase in the Efg1 regulator protein contributes to hyphal morphogenesis of *Candida albicans*. Genetics. 2001; 157:1523–1530. [PubMed: 11290709]
52. Cao F, et al. The Flo8 transcription factor is essential for hyphal development and virulence in *Candida albicans*. Mol Biol Cell. 2006; 17:295–307. [PubMed: 16267276]
53. Shapiro RS, et al. Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. Curr Biol. 2009; 19:621–629. [PubMed: 19327993]
54. Shapiro RS, Cowen L. Coupling temperature sensing and development: Hsp90 regulates morphogenetic signalling in *Candida albicans*. Virulence. 2010; 1:45–48. [PubMed: 21178413]
55. Xu XL, et al. Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating the adenylyl cyclase Cyr1p. Cell Host Microbe. 2008; 4:28–39. [PubMed: 18621008]
56. Huang G, et al. N-acetylglucosamine induces white to opaque switching, a mating prerequisite in *Candida albicans*. PLoS pathogens. 2010; 6:e1000806. [PubMed: 20300604]
57. Cleary IA, et al. BRG1 and NRG1 form a novel feedback circuit regulating *Candida albicans* hypha formation and virulence. Mol Microbiol. 2012; 85:557–573. [PubMed: 22757963]
58. Hornby JM, et al. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol. 2001; 67:2982–2992. [PubMed: 11425711]
59. Davis-Hanna A, et al. Farnesol and dodecanol effects on the *Candida albicans* Ras1-cAMP signalling pathway and the regulation of morphogenesis. Mol Microbiol. 2008; 67:47–62. [PubMed: 18078440]
60. Turner GC, et al. Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway. Nature. 2000; 405:579–583. [PubMed: 10850718]
61. Hu RG, et al. The N-end rule pathway is a sensor of heme. Proc Natl Acad Sci U S A. 2008; 105:76–81. [PubMed: 18162538]
62. Hall RA, et al. The quorum-sensing molecules farnesol/homoserine lactone and dodecanol operate via distinct modes of action in *Candida albicans*. Eukaryot Cell. 10:1034–1042. [PubMed: 21666074]
63. Hogan DA, et al. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol. 2004; 54:1212–1223. [PubMed: 15554963]
64. Srikantha T, et al. The histone deacetylase genes HDA1 and RPD3 play distinct roles in regulation of high-frequency phenotypic switching in *Candida albicans*. J Bacteriol. 2001; 183:4614–4625. [PubMed: 11443097]
65. Lee KL, et al. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia. 1975; 13:148–153. [PubMed: 808868]
66. Liu H, et al. Suppression of hyphal formation in *Candida albicans* by mutation of a STE12 homolog. Science. 1994; 266:1723–1726. [PubMed: 7992058]
67. Wullschleger S, et al. TOR signaling in growth and metabolism. Cell. 2006; 124:471–484. [PubMed: 16469695]
68. Bastidas RJ, et al. The protein kinase Tor1 regulates adhesin gene expression in *Candida albicans*. PLoS Pathog. 2009; 5:e1000294. [PubMed: 19197361]
69. Doyon Y, Cote J. The highly conserved and multifunctional NuA4 HAT complex. Current opinion in genetics & development. 2004; 14:147–154. [PubMed: 15196461]
70. Hnisz D, et al. The Set3/Hos2 histone deacetylase complex attenuates cAMP/PKA signaling to regulate morphogenesis and virulence of *Candida albicans*. PLoS Pathog. 2010; 6:e1000889. [PubMed: 20485517]
71. Hnisz D, et al. A histone deacetylase adjusts transcription kinetics at coding sequences during *Candida albicans* morphogenesis. PLoS Genet. 2012; 8:e1003118. [PubMed: 23236295]

72. Lu Y, et al. A GATA transcription factor recruits Hda1 in response to reduced Tor1 signaling to establish a hyphal chromatin state in*Candida albicans*. PLoS Pathog. 8:e1002663. [PubMed: 22536157]
73. Du H, et al. Roles of *Candida albicans* Gat2, a GATA-type zinc finger transcription factor, in biofilm formation, filamentous growth and virulence. PloS one. 2012; 7:e29707. [PubMed: 22276126]
74. Nobile CJ, et al. A recently evolved transcriptional network controls biofilm development in *Candida albicans*. Cell. 2012; 148:126–138. [PubMed: 22265407]
75. Su C, et al. Reduced TOR signaling sustains hyphal development in *Candida albicans* by lowering Hog1 basal activity. Mol Biol Cell. 2013; 24:385–397. [PubMed: 23171549]
76. Alonso-Monge R, et al. Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. J Bacteriol. 1999; 181:3058–3068. [PubMed: 10322006]
77. Smith DA, et al. A conserved stress-activated protein kinase regulates a core stress response in the human pathogen *Candida albicans*. Mol Biol Cell. 2004; 15:4179–4190. [PubMed: 15229284]
78. Arana DM, et al. The Pbs2 MAP kinase kinase is essential for the oxidative-stress response in the fungal pathogen *Candida albicans*. Microbiology. 2005; 151:1033–1049. [PubMed: 15817773]
79. Enjalbert B, et al. Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. Mol Biol Cell. 2006; 17:1018–1032. [PubMed: 16339080]
80. Martin R, et al. The *Candida albicans*-specific gene EED1 encodes a key regulator of hyphal extension. PloS one. 2011; 6:e18394. [PubMed: 21512583]
81. Wheeler RT, et al. Dynamic, morphotype-specific *Candida albicans* beta-glucan exposure during infection and drug treatment. PLoS Pathog. 2008; 4:e1000227. [PubMed: 19057660]
82. Spiering MJ, et al. Comparative transcript profiling of *Candida albicans* and *Candida dubliniensis* identifies SFL2, a *C. albicans* gene required for virulence in a reconstituted epithelial infection model. Eukaryot Cell. 2010; 9:251–265. [PubMed: 20023067]
83. Song W, et al. *Candida albicans* Sfl2, a temperature-induced transcriptional regulator, is required for virulence in a murine gastrointestinal infection model. FEMS Yeast Res. 2011; 11:209–222. [PubMed: 21205158]
84. Lane S, et al. The basic helix-loop-helix transcription factor Cph2 regulates hyphal development in *Candida albicans* partly via TEC1. Mol Cell Biol. 2001; 21:6418–6428. [PubMed: 11533231]
85. Davis D, et al. RIM101-dependent and-independent pathways govern pH responses in *Candida albicans*. Mol Cell Biol. 2000; 20:971–978. [PubMed: 10629054]
86. El Barkani A, et al. Dominant active alleles of RIM101 (PRR2) bypass the pH restriction on filamentation of *Candida albicans*. Mol Cell Biol. 2000; 20:4635–4647. [PubMed: 10848590]
87. Childers DS, et al. A 5′ UTR-mediated translational efficiency mechanism inhibits the *Candida albicans* morphological transition. Mol Microbiol. 2014; 92:570–585. [PubMed: 24601998]
88. Lu Y, et al. Synergistic regulation of hyphal elongation by hypoxia, CO(2), and nutrient conditions controls the virulence of *Candida albicans*. Cell Host Microbe. 2013; 14:499–509. [PubMed: 24237696]
89. Hughes BT, Espenshade PJ. Oxygen-regulated degradation of fission yeast SREBP by Ofd1, a prolyl hydroxylase family member. Embo J. 2008; 27:1491–1501. [PubMed: 18418381]
90. Noble SM, et al. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet. 42:590–598. [PubMed: 20543849]

Highlights

- Hyphal development requires two phases of regulation to remove Nrg1 inhibition.
- Nrg1 removal upon activation of cAMP and release from farnesol initiate hyphal growth.
- Chromatin regulation and Ume6 stability act in parallel for hyphal elongation *in vivo*.
- Hyphal initiation and maintenance are temporally linked.

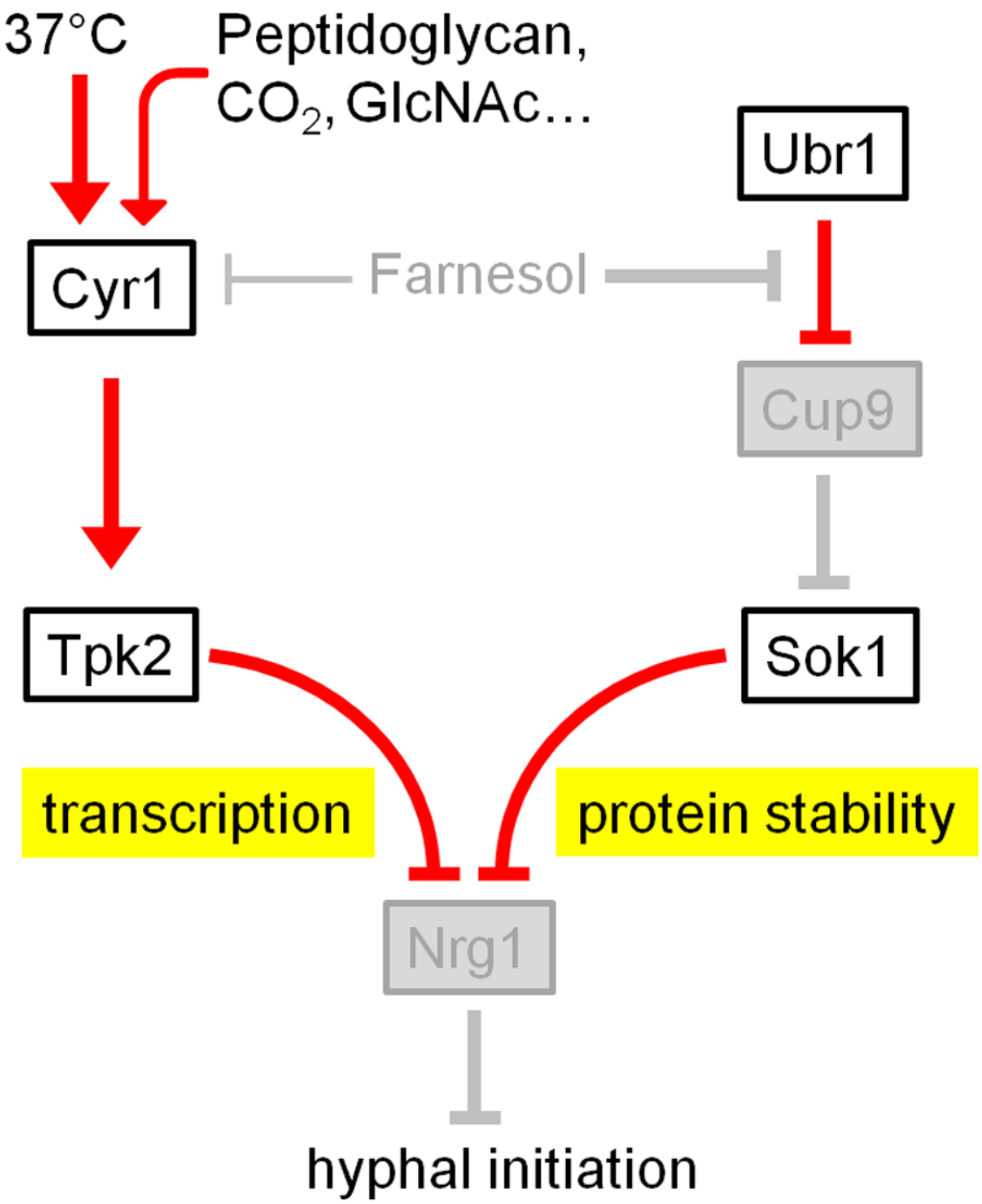

Figure 1.

A schematic diagram depicting the two independent pathways involved in downregulation of Nrg1 protein during hyphal initiation. *NRG1* transcriptional downregulation requires the activation of the cAMP-PKA pathway, whereas Nrg1 protein degradation requires release from farnesol inhibition. The function of genes indicated by white boxes is activated, and the function of genes in gray boxes is repressed. Red lines represent active regulatory relationships; gray lines represent relationships that are inactive.

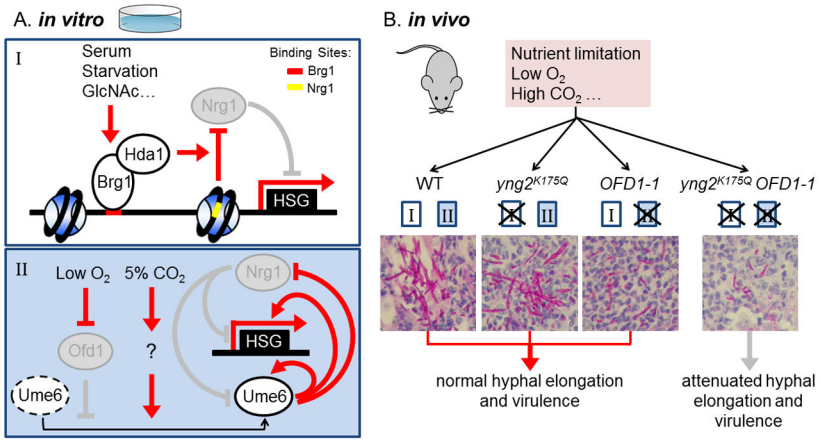

Figure 2.

Two parallel pathways control hyphal elongation and virulence during invasive infection. (A) Hyphal maintenance in vitro is controlled by two pathways: (I) Brg1-mediated chromatin remodeling and (II) Ume6 stabilization in hypoxia plus 5% CO_2. Red lines represent active regulatory relationships; gray lines represent relationships that are inactive. Dashed circles represent degraded proteins. (B) Synergy between two hyphal elongation pathways for *C. albicans* pathogenesis. Virulence is attenuated only when both hyphal elongation pathways are blocked. Kidney tissues from the mice infected with the indicated strains were fixed, sectioned, and stained to visualize fungal cells. The rectangles in (B) represent the two hyphal elongation pathways of corresponding colors in (A). The images from the mouse kidneys in (B) are from [88]. Abbreviations: HSG, hypha-specific genes; WT, wildtype.

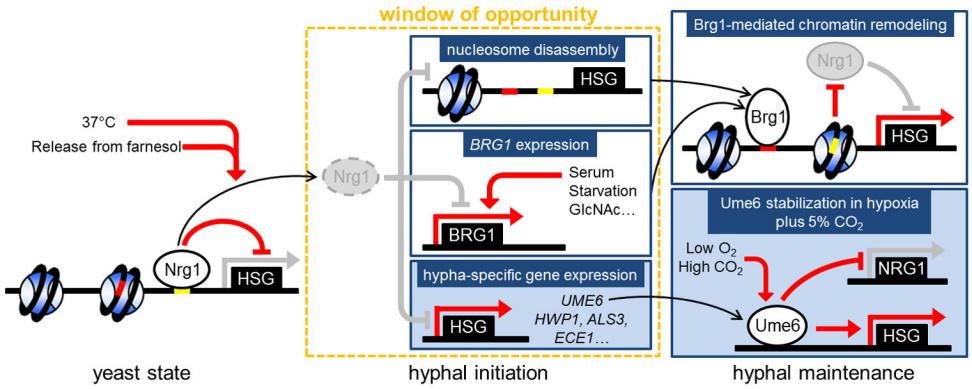

Figure 3.

Temporal connection between hyphal initiation and maintenance. The transient disappearance of Nrg1 during hyphal initiation provides a time window to establish the hyphal maintenance program. The function of proteins in white circles is activated, and the function of proteins in gray circles is repressed. Dashed circles represent degraded proteins. Red lines represent active regulatory relationships; gray lines represent relationships that are inactive. Black arrows represent the connection between each state of hyphal development. Abbreviations: HSG, hypha-specific genes.