Cell. Mol. Life Sci. (2016) 73:4265–4278
DOI 10.1007/s00018-016-2294-y

Cellular and Molecular Life Sciences

REVIEW

Control of *Candida albicans* morphology and pathogenicity by post-transcriptional mechanisms

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Received: 11 March 2016 / Revised: 23 May 2016 / Accepted: 10 June 2016 / Published online: 16 June 2016

Abstract *Candida albicans* is a major human fungal pathogen responsible for both systemic and mucosal infections in a wide variety of immunocompromised individuals. Because the ability of *C. albicans* to undergo a reversible morphological transition from yeast to filaments is important for virulence, significant research efforts have focused on mechanisms that control this transition. While transcriptional and post-translational mechanisms have been well-studied, considerably less is known about the role of post-transcriptional mechanisms. However, in recent years several discoveries have begun to shed light on this important, but understudied, area. Here, I will review a variety of post-transcriptional mechanisms that have recently been shown to control *C. albicans* morphology, virulence and/or virulence-related processes, including those involving alternative transcript localization, mRNA stability and translation. I will also discuss the role that these mechanisms play in other pathogens as well as the potential they may hold to serve as targets for new antifungal strategies. Ultimately, gaining a better understanding of *C. albicans* post-transcriptional mechanisms will significantly improve our knowledge of how morphogenesis and virulence are controlled in fungal pathogens and open new avenues for the development of novel and more effective antifungals.

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Keywords *C. albicans* · Morphogenesis · Virulence · Translational control · mRNA stability · Alternative transcript localization · Antifungal strategies

Introduction

Candida albicans, normally found as a commensal in the human gastrointestinal tract, oral and vaginal cavities, is also a major human fungal pathogen responsible for a wide variety of mucosal and systemic infections [1–5]. About 70 % of women experience a vaginal *Candida* infection over their lifetime [6]. In addition, with a mortality rate approaching 35–60 %, candidiasis represents the fourth-leading cause of hospital-acquired bloodstream infections in the U.S. [7, 8]. Individuals in an immunocompromised state, including organ transplant recipients, cancer patients on chemotherapy, neonates and HIV/AIDS patients, are particularly vulnerable [4, 5, 9]. While about $1 billion per year is spent on treatment for these infections, only three major classes of antifungals are currently available and there is a significant demand for the development of new and more effective therapies [10–12].

Candida albicans possesses a number of virulence properties that contribute significantly to pathogenicity, including the ability to tightly adhere to host cells, secrete degradative enzymes (e.g.: phospholipases, secreted aspartyl proteases), form biofilms, evade the immune system and switch phenotypes [1, 13–24]. One of the most important virulence traits is the ability to undergo a reversible morphological transition from yeast (single oval budding cells) to pseudohyphal and hyphal filaments (elongated cells attached end-to-end). This transition is known to occur in response to a wide variety of conditions which mimic those encountered in the host environment,

including growth in the presence of serum, body temperature (37 °C), high CO_2/low O_2, neutral pH, certain carbon sources (e.g., GlcNAc) and amino acids (e.g., proline) as well as in an embedded matrix [14, 17, 20, 25, 26]. *C. albicans* hyphal filament extension is important for a variety of virulence-related properties, including biofilm formation, lysis of macrophages, breaching of endothelial cells, invasion of epithelial cell layers and thigmotropism (contact sensing) [19, 21, 27–29]. Consistent with these observations, multiple studies, based on mutants locked in the yeast or filamentous form and strains that are engineered to transition between morphologies during infection, have suggested that the *C. albicans* yeast-filament transition is important for virulence [19, 21, 30–32]. However, mutants defective for filamentation, but not infectivity (based on relative abundance in mouse kidneys during infection), have also been identified [33], suggesting that this relationship is more complex.

Given the importance of the yeast-filament transition for *C. albicans* virulence and virulence-related processes, intense research efforts have focused on determining mechanisms that drive this transition in response to host environmental cues. Post-translational mechanisms that control *C. albicans* morphology have been relatively well-characterized. A mitogen activated protein (MAP) kinase pathway promotes *C. albicans* filamentation under nitrogen starvation conditions, a Ras-cAMP-Protein Kinase A (PKA) pathway is important for the response to serum, 37 °C and high CO_2, and a Cbk1 kinase/RAM signaling pathway has been shown to mediate the response to serum, temperature and starvation conditions [26, 34, 35]. The Hgc1/Cdc28 cyclin/Cdk complex directs septin phosphorylation important for hyphal development and controls activation of the Cdc42 master regulator of polarized growth [36]. Additional post-translational modifications, including sumoylation, histone acetylation/deacetylation and ubiquitination have been shown to control morphology, survival in macrophages, stress response/adaptation, phenotypic switching and/or cell cycle progression [37–42]. A significant number of transcriptional regulators that control the *C. albicans* yeast-filament transition in response to multiple environmental cues have also been identified. Several of these regulators (e.g., Efg1, Cph1, Nrg1) are downstream targets of the signal transduction cascades described above [26, 43, 44]. Importantly, many transcription factors that control *C. albicans* filamentous growth have also been shown to regulate other virulence-related processes including adhesion, biofilm formation and secretion of degradative enzymes [45–52].

Unlike post-translational and transcriptional mechanisms, considerably less is known about post-transcriptional mechanisms that control *C. albicans* morphology and virulence. For the purposes of this review, I will define post-transcriptional mechanisms as those that occur following synthesis of the primary transcript but before the gene is translated to protein. In recent years new studies are beginning to provide novel and valuable information about this highly important, but understudied area [53]. More specifically, a number of advances have been made towards our understanding of how *C. albicans* morphology and/or pathogenicity are controlled at the level of alternative transcript localization, mRNA transcript stability and translation (specific genes associated with these mechanisms are shown in Table 1 and discussed below). In this review we will not only summarize these important findings, but also provide new insights and perspectives as well as highlight promising avenues for future research in this area.

Contribution of alternative transcript localization to *C. albicans* morphogenesis and pathogenicity

RNA transport mechanisms have been shown to play an important role in a variety of biological processes in higher eukaryotes [54–57]. During mitosis in the model yeast *Saccharomyces cerevisiae* the She complex transports a specific set of mRNAs via actomyosin from the mother cell to the newly forming bud cell [58–61]. While the She2 component of the complex binds directly to mRNAs, She3 functions as an adaptor to link She2 to the myosin motor protein Myo4 [62–64]. Transport of the mRNA encoding for Ash1, a key transcriptional repressor, by the She complex is critical for the establishment of *S. cerevisiae* cell fate [65, 66].

Similar to *S. cerevisiae*, *C. albicans* has also been shown to possess a She-dependent mRNA transport system [67] (Fig. 1). While there is no obvious *SHE2* ortholog in *C. albicans*, a *SHE3* ortholog is present. *C. albicans* strains deleted for *SHE3* initially form normal germ tubes when grown in liquid medium in the presence of serum at 37 °C (one of the strongest filament-inducing conditions) but eventually display abnormal hyphae with uneven filament width and constrictions at septa [67]. More pronounced defects in both filamentous and invasive growth were observed on solid filament-inducing media. While *C. albicans she3*Δ/Δ mutants were not attenuated for virulence in a mouse model of systemic candidiasis, they did exhibit a significant defect in epithelial cell damage. Largely overlapping sets of 31 and 38 transcripts were shown to associate with She3 in *C. albicans* yeast and hyphal cells, respectively [67]. These mRNAs are involved in a diverse array of cellular processes, including cell polarity, cell wall structure and function, transcription, virulence, mitosis/cytoskeletal dynamics as well as small molecule transport and regulation. In addition to *ASH1*, transcripts

Table 1 Genes associated with post-transcriptional regulation of *C. albicans* morphology and/or pathogenicity

Post-transcriptional regulation	Gene	Description	References
Alternative transcript localization	*SHE3*	Transports mRNA transcripts to hyphal tip via actomyosin	[67]
	SEC2	Guanine nucleotide exchange factor for the Sec4 Rab-GTPase	[69, 70]
mRNA stability	*CCR4*	Component of Ccr4–Pop2 mRNA deadenylase complex	[75]
	POP2	Component of Ccr4–Pop2 mRNA deadenylase complex	[75]
	KEM1	5′–3′ exoribonuclease	[76, 77]
	EDC3	P-body scaffolding protein	[78]
	PUF3	RNA-binding protein important for recruitment of Ccr4–Pop2 mRNA deadenylase complex	[80]
	CDR1	ABC cassette multi-drug transporter	[81, 82]
	PAP1	Poly(A) polymerase	[81]
	ZFS1	TTP family Zn-finger protein	[84]
	NRG1	Transcriptional regulator of morphology and virulence	[92–94]
Translation	*GCN4*	Transcriptional regulator of amino acid starvation and oxidative stress response, filamentation and biofilm formation	[98–101]
	UME6	Transcriptional regulator of morphology and virulence	[102–105]
	WOR1	Master transcriptional regulator of white-opaque switching	[106–109]
	NRG1	Transcriptional regulator of morphology and virulence	[93, 94, 113]
	SSD1	Putative RNA-binding protein important for virulence and host antimicrobial peptide resistance	[113, 117]
Unknown	*SLR1*	SR-family RNA-binding protein important for filamentous growth, virulence and host cell damage	[88]

implicated in virulence including *SAP5*, encoding a secreted aspartyl protease, and *RBT4*, encoding a secreted factor, were identified by this analysis. Many She3-associated transcripts localized to the bud site of yeast cells or filament tip of hyphae in a She3-dependent manner and deletion of about one-third of these transcripts resulted in filamentation defects. Interestingly, She3 cargo transcripts in *C. albicans* were distinctly different when compared to those of *S. cerevisiae* [67]. Overall, the She3 mRNA localization system appears to make important contributions to *C. albicans* filamentous growth, invasion and virulence-related processes. A wide variety of biological processes, including protein synthesis, are known to occur at the tips of fungal hyphal cells [68] and it is likely that transport of certain key mRNAs to this region for rapid site-specific translation would greatly facilitate polarized growth, secretion of degradative enzymes and other virulence-related processes.

Do additional mechanisms, besides the She system, play a role in localizing mRNA transcripts to the *C. albicans* hyphal tip during filamentous growth? A recent report suggests that such a mechanism is in place for the *SEC2* transcript [69]. *SEC2* encodes a guanine nucleotide exchange factor for Sec4, a Rab-GTPase required for secretory vesicle transport from the Golgi apparatus to the filament tip [70]. Phosphorylation by Cdk1 is critical for hyphal, but not yeast, growth as well as localization of Sec2 to the Spitzenkörper at the hyphal tip. Interestingly, the Sec2 protein was shown to be physically associated with its own transcript on secretory vesicles [69]. This association appeared to be significantly stronger in hyphal vs. yeast cells and also occurred in a manner that was independent of the She3-based system. In addition, phosphorylation of Sec2 promoted dissociation of this protein from its own transcript and mutations which prevented this phosphorylation event and led to a significant impairment of hyphal development [69, 70]. The phosphorylation event appears to be a regulatory or autoregulatory step that could possibly promote translation of *SEC2* and a subsequent increase in Sec2 protein levels, specifically at the hyphal tip. However, at this point, the exact significance of Sec2 dissociation from its own transcript remains unclear. In addition, mechanisms that may control this dissociation event in response to host environmental cues that promote filamentation have not yet been established. The mechanism described above appears to be highly specialized, since *SEC2* was the only transcript confirmed to physically associate with Sec2 protein [69]. It is interesting to speculate whether this is a more general phenomenon by which other key regulatory factors might also be transported to the hyphal tip in association with their own transcript to facilitate hyphal development.

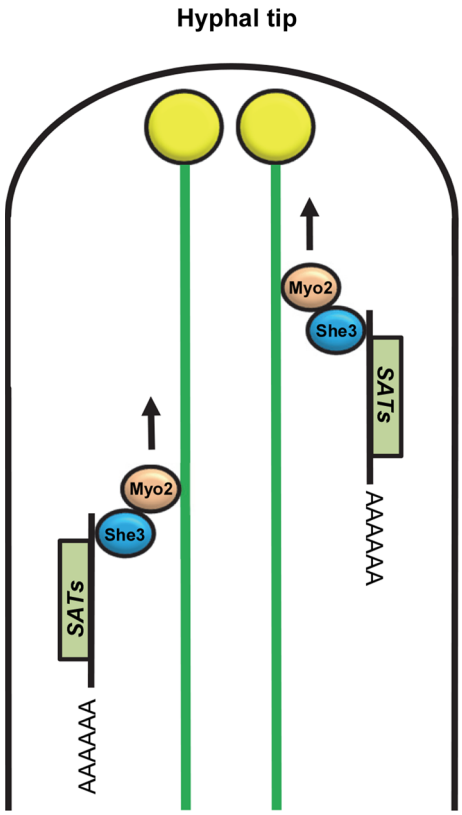

Fig. 1 She3-dependent mRNA localization at the *C. albicans* hyphal tip. Actin cables (*green lines*) are anchored to the hyphal tip by formin proteins (*yellow*). She3 associates with both a myosin motor protein (Myo2) as well cargo transcripts (She3-associated transcripts, *SATs*). Myo2 moves the complex along the actin cables towards the hyphal tip. A variety of cargo transcripts have been identified, several of which are important for *C. albicans* morphology and/or pathogenicity [67]

Regulation of *C. albicans* morphology and pathogenicity by mRNA stability mechanisms

A wide variety of processes in diverse eukaryotes are known to be controlled at the level of mRNA stability [71–74] and several recent studies have suggested that *C. albicans* morphology, virulence and virulence-related properties are no exception. More specifically, the Ccr4–Pop2 mRNA deadenylase complex, which contains exonuclease activity important for shortening mRNA poly(A) tails to control stability and translation, was demonstrated to play a role in *C. albicans* cell wall integrity, antifungal drug resistance, filamentation and virulence [53, 75] (Fig. 2a). Both cell wall integrity defects and resistance to the echinocandin antifungal caspofungin were linked to defects in mitochondrial function and phospholipid homeostasis. An enzyme important for maintaining mRNA stability, the Kem1 putative exonuclease, has also been shown to play a role in the *C. albicans* morphological transition [76, 77] (Fig. 2a). Kem1, as well as the Dcp1 putative decapping enzyme, Dhh1 putative RNA helicase/decapping activator and Edc3 processing body (P-body) scaffolding protein have been shown to localize to P-bodies (translationally silenced cytoplasmic mRNA-containing granules) and both the *C. albicans edc3*Δ/Δ and *dhh1*Δ/+ mutants are defective for filamentation [78, 79]. Interestingly, *C. albicans* P-bodies were shown to accumulate during hyphal growth as well as during growth in a number of stress conditions, including glucose deprivation, heat shock, osmotic and oxidative stress, suggesting that certain transcripts may be targeted for degradation and/or translational silencing under these conditions. While the molecular basis for the *kem1*Δ/Δ, *edc3*Δ/Δ and *dhh1*Δ/+ morphological defects is unclear, at least in the case of *ccr4*Δ/Δ and *pop2*Δ/Δ mutants, these phenotypes may result as a consequence of primary defects in cell wall integrity and mitochondrial function [75]. A recent study has demonstrated that the *C. albicans* Puf3 RNA-binding protein, important for recruitment of the Ccr4–Pop2 deadenylase complex to mRNAs, is also involved in mRNA decay [80]. In this study Ccr4 was shown to respond to both nutrient levels and hypoxia to regulate biofilm maturation and extracellular matrix production. This regulation is believed to occur via the control of the expression of genes involved in both cell wall integrity as well as mitochondrial activity. Importantly, this study was the first to demonstrate that biofilm maturation, a key virulence property (since biofilms are highly resistant to current antifungal therapies), is under post-transcriptional regulation by an mRNA stability mechanism.

CDR1, encoding an ABC cassette multi-drug transporter important for antifungal resistance [81], also appears to be controlled at the level of mRNA stability. Manoharlal, et al., observed that the poly(A) tail of *CDR1* transcripts is hyperadenylated in azole-resistant (AR) vs. azole-susceptible (AS) isolates [82] (Fig. 2b). Interestingly, mutation of a single allele of the poly(A) polymerase (*PAP1-a*) led to increased polyadenylation and transcript stability of *CDR1*, as well as enhanced resistance to the drugs fluconazole, terbinafine and cycloheximide [83]. Mutation of *PAP1-a* is believed to lead to de-repression of *PAP1*-α, resulting in *CDR1* hyperadenylation and increased transcript stability. Thus, loss of heterozygosity at the *PAP1* locus is directly linked to antifungal resistance via an mRNA stability mechanism. It is unclear at this point whether the stability of transcripts important for other *C. albicans* virulence-related properties is controlled by a similar mechanism.

A recent study has shown that Zfs1, a tristetraprolin (TTP) family zinc-finger protein conserved in multiple fungal species, plays an important role in controlling the stability of many *C. albicans* transcripts [84]. TTP proteins are known to interact with AU-rich sequences in the 3′ untranslated regions (UTRs) of target transcripts to

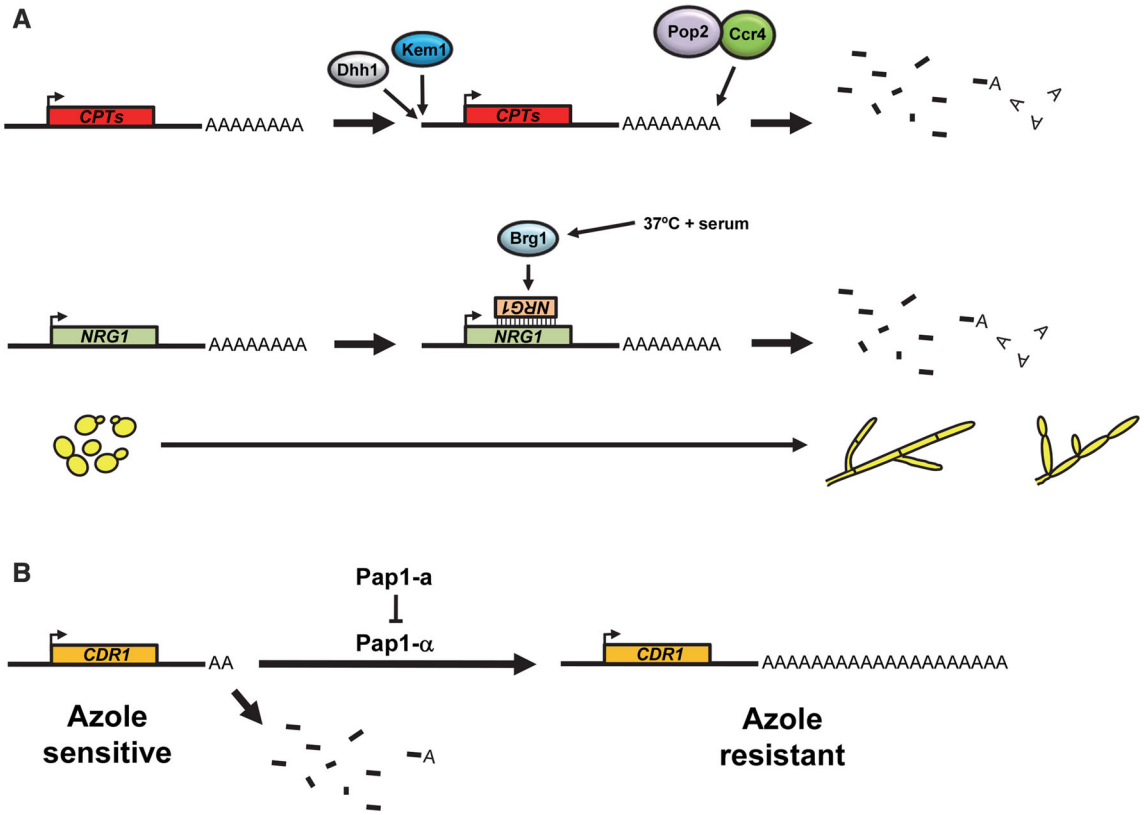

Fig. 2 mRNA stability mechanisms that control *C. albicans* morphology and virulence-related processes. **a** Destabilization of target mRNAs (*CPTs*) by the Ccr4–Pop2 mRNA deadenylase complex as well as the Kem1 5′–3′ exoribonuclease and Dhh1 putative helicase/decapping activator is important for the *C. albicans* transition from yeast to filaments (Kem1 plays a greater role in hyphal vs. pseudohyphal growth [77]). The Dcp1 putative decapping enzyme is also most likely involved in this process, although a direct role for Dcp1 in *C. albicans* filamentation has not yet been demonstrated. In addition, the transcript for *NRG1*, encoding a key transcriptional repressor of *C. albicans* filamentation, is destabilized by association with an antisense transcript. The antisense transcript, in turn, is generated by Brg1, a transcriptional regulator, following filament induction by growth in serum at 37 °C. Destabilization of *NRG1* by this mechanism promotes the transition from yeast to filamentous growth. **b** *CDR1*, encoding an ABC cassette multi-drug transporter, is also controlled by an mRNA stability mechanism. When the poly(A) polymerase-α (*PAP1*-α) allele is expressed at low levels, the *CDR1* transcript is believed to be destabilized and strains are sensitive to azole antifungal drugs. However, in a strain in which they *PAP1-a* allele is absent, the *PAP1*-α allele is thought to be derepressed. As a consequence, the *CDR1* transcript is hyperadenylated and stabilized, resulting in azole resistance

promote their deadenylation and destruction [85, 86]. The *ZFS1* transcript is induced both in response to biofilm formation as well as during the *C. albicans* yeast-hyphal transition [84]. While the *zfs1*Δ/Δ mutant does not appear to show any defects in morphogenesis or infectivity, a mild, but reproducible, increase in biofilm formation was observed. Although a clear role for Zfs1 in other *C. albicans* virulence-related processes has not been firmly established, an RNA-seq analysis has demonstrated that the *zfs1*Δ/Δ mutant shows an increased abundance in transcripts important for the tricarboxylic acid (TCA) cycle, cellular respiration, oxidation–reduction and numerous metabolic processes which may be important for survival in the host [84]. In addition, this mutant showed increased abundance of *ERG6*, which encodes a delta(24)-sterol C-methyltransferase involved in ergosterol biosynthesis and antifungal resistance [87]. Zfs1 targets appear to be highly conserved in many CTG-clade fungal species, including several less pathogenic non-*albicans Candida* species [84].

Another RNA-binding protein, Slr1, has been shown to play an important role in *C. albicans* filamentous growth and virulence as well as the ability to damage both epithelial and endothelial cell layers in vitro [88]. Slr1 is a member of the serine-arginine (SR) family of RNA-binding proteins, which have previously been shown to affect the yeast-pseudohyphal transition and polarized growth in *Saccharomyces cerevisiae* and *Aspergillus nidulans*, respectively [89–91]. However, the precise post-transcriptional mechanism by which Slr1 functions to control *C. albicans* virulence and virulence-related properties has not yet been determined.

Finally, an antisense transcript mechanism has been demonstrated to control the stability of *NRG1*, which encodes a key transcriptional repressor of *C. albicans* filamentous growth [92–94] (Fig. 2a). *NRG1* is known to function in a negative feedback loop with *BRG1*, which encodes a strong transcriptional activator of the yeast-hyphal transition. Interestingly, Brg1 was also shown to induce an antisense transcript corresponding to part of the *NRG1* open reading frame [92]. Induction of this transcript was important for reducing *NRG1* mRNA stability. While it is unclear at this point exactly how *NRG1* mRNA stability is reduced, the available evidence favors a mechanism in which both sense and antisense transcripts are destroyed. This mechanism appears to play an important role in controlling *C. albicans* morphology, invasion, biofilm formation and virulence in a mouse model of systemic candidiasis [92].

Translational control of *C. albicans* morphology and pathogenicity

Translational control of gene expression in eukaryotes is typically mediated by the 5′ UTR of transcripts [95, 96]. This region can contain uORFs, short open reading frames upstream of the main ORF. The start codons of these uORFs can serve as alternative translation initiation sites to that of the main ORF. In the case of these transcripts, most translation occurs at the uORF and only low level translation occurs in the main gene due to re-initiation or leaky ribosome scanning. 5′ UTRs can also form complex secondary structures that inhibit ribosome accessibility and/or promote ribosome stalling. Regulatory factors that bind to the 5′ UTR region can control secondary structure formation and/or compete with ribosomes to access the transcript. Finally, 5′ UTRs can contain a zip code sequence that specifies alternative subcellular localization to a location where the transcript is not actively translated [95, 96]. In *S. cerevisiae*, translation of the transcription factor encoded by *GCN4*, important for the response to amino acid starvation, is known to be controlled by a well-characterized uORF-dependent mechanism [97]. In *C. albicans*, *GCN4* not only controls the amino acid starvation response but also has a role in both filamentation and biofilm formation [98, 99]. A recent study has shown that, as in *S. cerevisiae*, translation of *C. albicans GCN4* is controlled by a uORF-dependent mechanism [100] (Fig. 3a). *C. albicans GCN4* is also required for hydrogen peroxide resistance and several oxidants have been shown to inhibit *C. albicans* translational initiation by mechanisms that are both dependent and independent of phosphorylation of the translation initiation factor eIF2α [101].

UME6, which encodes a key filament-specific transcriptional regulator of *C. albicans* morphology determination, biofilm formation, invasion and virulence, is under translational control (Fig. 3b) and contains one of the longest 5′ UTRs reported in fungi to date [102–105]. Deletion of the 5′ UTR resulted in enhanced filamentous growth under multiple inducing conditions [105]. A polysome profiling analysis demonstrated that the *UME6* 5′ UTR specifically functions to inhibit translational efficiency. Interestingly, the level of translational inhibition directed by the 5′ UTR was differentially modulated by various host filament-inducing conditions. While a potential uORF-dependent translational inhibition mechanism has been excluded, an analysis of the predicted structure of the *UME6* 5′ UTR suggests that it is highly complex, extremely stable and could inhibit translation by ribosomes [105]. Modulation of *UME6* translational efficiency in response to host filament-inducing conditions, in turn, could potentially occur via RNA-binding proteins (Fig. 3b). Efforts are currently underway in our laboratory to determine the precise mechanism by which the *UME6* 5′ UTR inhibits translational efficiency.

Similar to *UME6*, *WOR1*, which encodes a master transcriptional regulator of white-opaque phenotypic switching in *C. albicans*, also possesses an unusually long 5′ UTR [106–110]. This element was shown to control both *WOR1* translational efficiency as well as *C. albicans* white-opaque phenotypic switching frequency, which plays an important physiological role in mating [109, 111]. Interestingly, the *WOR1* 5′ UTR appeared to direct a greater level of translational repression in white vs. opaque cells. An RNA-seq analysis has demonstrated that, like *UME6* and *WOR1*, transcripts of many important regulators of *C. albicans* morphology, virulence and virulence-related processes, including biofilm formation, antifungal drug resistance, adhesion, resistance to oxidative stress and degradation of host cell membranes/membrane proteins also possess unusually long (>500 nucleotides) 5′ UTRs [112]. Four of these transcripts, like *UME6* and *WOR1*, have also been shown to accumulate in monosome vs. polysome fractions in a polysome profiling experiment, suggesting that their translational efficiency is reduced [109]. While not all *C. albicans* genes with long 5′ UTRs may be inhibited by translational efficiency mechanisms, the presence of these regions upstream of so many genes involved in virulence and virulence-related processes strongly suggests that they play important roles in *C. albicans* pathogenicity, perhaps by tightly modulating and fine-tuning the expression of their targets in response to host environmental cues.

Recent evidence suggests that *NRG1* may also be controlled by a translation-based mechanism. Both *NRG1* transcript and protein levels show a significant reduction

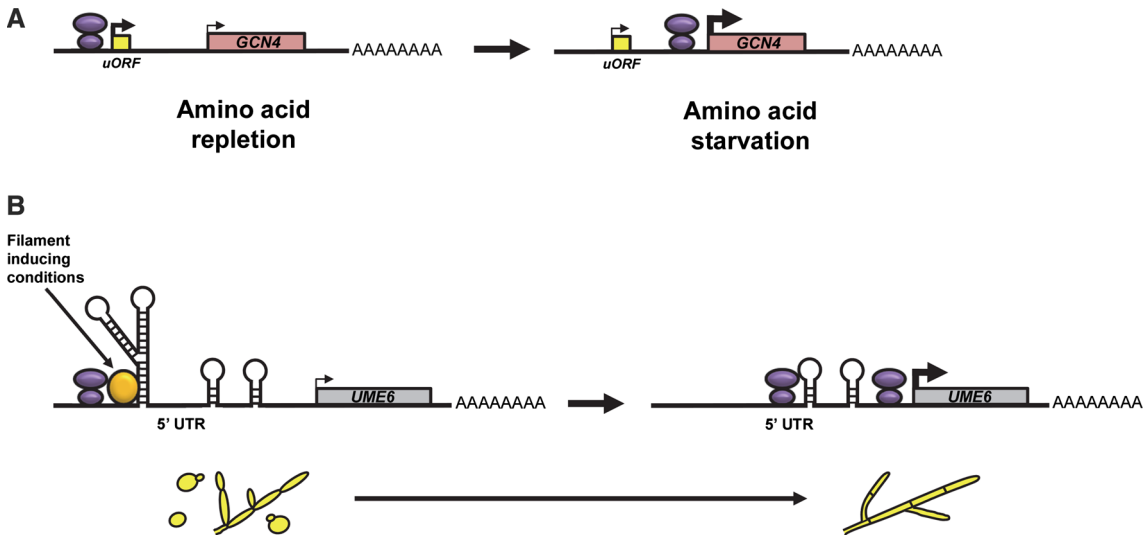

Fig. 3 Translational control mechanisms in *C. albicans*. **a** Translation of *GCN4*, encoding a transcriptional regulator of morphology, oxidative stress resistance and the amino acid starvation response, is inhibited by a uORF-dependent mechanism when cells are grown under amino acid replete conditions. Under these conditions, the large majority of ribosomes (shown in *purple*) are engaged in translation of the uORF rather than *GCN4*. However, when cells are grown under amino acid starvation conditions, eIF2α is phosphorylated by Gcn2, promoting leaky ribosome scanning and *GCN4* translation. **b** A 5′ UTR-mediated translational efficiency mechanism controls expression of *UME6*, a key transcriptional regulator of *C. albicans* morphology and virulence. The 5′ UTR is predicted to form a complex and highly stable secondary structure which, along with RNA-binding protein(s), inhibits translational efficiency of *UME6*, causing reduced filamentation. The level of translational inhibition resulting from complex secondary structure formation and/or blocking of ribosome accessibility, in turn, is believed to be modulated by RNA-binding protein(s) in response to certain filament-inducing conditions so as to favor enhanced filamentous growth. A less likely alternative model in which a zip code sequence in the 5′ UTR directs the *UME6* transcript to translationally inactive P-bodies, has not yet been excluded. *Arrow size* = translation level

under strong filament-inducing conditions, such as growth at 37 °C in the presence of serum [93, 94, 113]. Interestingly, however, levels of an Nrg1-Myc protein remained unchanged during filament induction in a strain deleted for *CBK1* [113], which encodes a terminal Ser/Thr kinase of the RAM signaling pathway that plays an important role in cell wall integrity as well as filamentous/polarized growth [35, 114]. However, deletion of both *CBK1* and *SSD1*, encoding the ortholog of a *S. cerevisiae* RNA-binding protein involved in translational control, restored down-regulation of the Nrg1-Myc protein [113, 115, 116]. Cbk1 specifically phosphorylates Ssd1 and the RAM-Cbk1-Ssd1 pathway plays an important role in controlling *C. albicans* filamentation, particularly filamentation of the *nrg1*Δ/Δ mutant [113]. One model suggests that phosphorylation of Ssd1 by Cbk1 allows for translation of one or more target transcripts, which, in turn, encode factor(s) important for down-regulation of Nrg1 during filamentation. However, in the unphosphorylated form, Ssd1 is believed to inhibit translation of this target(s), resulting in increased expression of Nrg1 and subsequent growth in the yeast form. While the precise mechanism by which Ssd1 controls Nrg1 down-regulation remains to be fully elucidated, these results establish an important link between an RNA-binding protein associated with translational regulation and *C. albicans* morphogenesis. Ssd1 also plays an important role in controlling resistance to antimicrobial peptides and virulence [117], most likely by a translational mechanism.

A role for alternative splicing in *C. albicans* morphogenesis and virulence?

Following the publication of a comprehensive annotation of the *C. albicans* genome [118], both computational and experimental approaches were used to identify a total of 415 introns [119]. A subsequent RNA-seq analysis added 41 new introns to the total [112]. Only about 6 % of *C. albicans* genes possess introns, the large majority of which typically contain only a single intron [119]. Several introns are located in 5′ UTR regions, suggesting the possibility that translational regulation of certain genes is controlled by alternative splicing. A gene ontology (GO) analysis indicated that genes associated with two categories, ribosomes and meiosis, showed a significantly high proportion of introns in both *C. albicans* and *S. cerevisiae* [119]. Interestingly, genes in other categories possessed a high proportion of introns only in *C. albicans*. These classes included microtubules, splicing, mitochondrial respiration and protein degradation. In addition, several *C. albicans* transcripts have been documented to undergo alternative splicing, including *RPL30*, encoding a ribosomal protein,

SPR28, encoding a septin, and *GND1*, which encodes 6-phosphogluconate dehydrogenase [119–121]. Alternative splicing of *SPR28* was shown to be controlled by the α-factor mating pheromone [119]. Alternative splicing of *GND1* yields two isoforms, one of which is targeted to peroxisomes and the second of which lacks the peroxisomal targeting sequence and is localized to the cytosol [121]. While a role for alternative splicing in *C. albicans* morphology and/or pathogenicity has not yet been firmly established, several genes involved in filamentation, virulence and/or virulence-related processes are known to possess introns [119]. These genes include *SOD1* and *SOD2*, encoding superoxide dismutases important for the ability to tolerate oxidative stress [122–124], *ECM33*, encoding a GPI-anchored cell wall protein important for adhesion and host cell damage [125, 126], *INT1*(*BUD4*), which encodes a protein structurally similar to a subunit of human leukocyte integrin [127], and the previously discussed *KEM1,* encoding a putative 5′–3′ exonuclease that controls mRNA stability [77]. In addition, previously described intron-containing genes associated with microtubule formation and mitochondrial respiration could be associated with hyphal development and/or virulence. Given that few genes in *C. albicans* possess introns, it is tempting to speculate that these introns may have been adapted to play important regulatory functions; the few known examples described above generally support this hypothesis. If so, it is entirely possible that many alternative splicing mechanisms which play important roles in controlling *C. albicans* morphogenesis and pathogenicity have yet to be discovered. An alternative, and perhaps, more likely explanation for the low number of introns in *C. albicans*, is that the process of intron loss in yeast evolution is not complete. Other post-transcriptional mechanisms, including RNA editing, micro-RNA-mediated regulation and control of mRNA 5′ CAPing have not yet been reported in *C. albicans* and it is unclear at this point whether they play any significant role in *C. albicans* morphogenesis and/or pathogenicity.

Post-transcriptional control of virulence and virulence-related properties in other pathogens

Do post-transcriptional mechanisms also play an important role in controlling virulence and/or virulence-related processes in other pathogens, including fungal pathogens? The large majority of evidence available appears to suggest that this is the case. In the plant fungal pathogen *Ustilago maydis*, an RNA-binding protein that is transported along microtubules, Rrm4, has been associated with both polarized growth and virulence [128, 129]. In the human fungal pathogen *Cryptococcus neoformans*, a long non-coding RNA (lncRNA), *RZE1*, has recently been shown to control morphogenesis by changing the ratio of *ZNF2* transcript in the nucleus vs. cytoplasm [130]. *ZNF2* encodes a key transcriptional regulator of *C. neoformans* mating, filamentation and virulence [131]. mRNA stability mechanisms also appear to play a role in controlling *C. neoformans* pathogenesis. When this pathogen transitions from 30 °C to host body temperature (37 °C), mRNA decay mediated by the Ccr4 deadenylase is accelerated in a manner that is influenced by Rpb4, a subunit of RNA polymerase II [132]. Coupling of *C. neoformans* mRNA synthesis and decay via Rpb4 was shown to play an important role in host temperature adaptation and virulence. In *Mycobacterium tuberculosis*, RNase AS, an exonuclease that degrades polyadenylated RNA, functions as an important virulence factor [133]. Both Hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV) have 5′ UTRs with regions that stall and repress the activity of the Xrn1 exoribonuclease [134]. As a consequence, there is a global increase in cellular mRNA stability that most likely promotes cell growth and pathogenesis. Finally, an antisense RNA mechanism has recently been shown to control pilin antigenic variation, important for immune escape, in the bacterial pathogen *Neisseria meningitidis* [135].

Translational efficiency mechanisms have been shown to regulate the expression of virulence factors important for production of *Listeria monocytogenes* listeriolysin O toxin [136–138]. In addition, replication of both poliovirus and Hepatitis C virus is controlled by translational efficiency via Internal Ribosome Entry Sequences (IRESs) in the 5′ UTR region [139]. A recent study has found that a subset of transcripts associated with morphogenesis of the human fungal pathogen *Histoplasma capsulatum*, like *C. albicans UME6*, possess long 5′ UTRs and are tightly controlled at both the transcriptional and translational level [140]. Finally, a recent RNA-seq analysis has indicated that many genes likely to control morphology and/or pathogenicity in *Candida parapsilosis*, a less pathogenic non-*albicans Candida* species, possess long 5′ UTRs (>500 bp) which may be involved in translational regulation [141]. Interestingly, several of these genes encode orthologs of *C. albicans* genes with long 5′ UTRs.

While not yet well-established in *C. albicans*, alternative splicing mechanisms play important roles in controlling the virulence of other pathogens. For example, *Ustilago maydis*, a corn smut fungus, uses alternative splicing to regulate peroxisomal targeting and virulence [142]. A comparative study of 23 fungal genomes indicated that many genes associated with pathogenesis, particularly those involved in stress response and morphogenesis; undergo alternative splicing [143]. Alternative splicing is also known to control

the expression of a major neurovirulence factor by Herpes Simplex Virus-2 (HSV-2), which likely contributes to pathogenesis [144].

While transcriptional, post-translational and post-transcriptional regulatory mechanisms all appear to play highly important roles in *C. albicans* morphogenesis, the relative importance of post-transcriptional vs. transcriptional and post-translational mechanisms in controlling the *C. albicans* yeast-filament transition has yet to be fully elucidated. However, given the role that post-transcriptional regulation plays in controlling virulence traits of viral, bacterial and other fungal pathogens, the discoveries reported thus far in *C. albicans* are likely to represent the tip of the iceberg. How and why did post-transcriptional mechanisms evolve to play important roles in pathogenicity and virulence-related processes of *C. albicans* as well as a wide range of other pathogens? While the answer to this question is not entirely clear, post-transcriptional regulation likely allows pathogens to tightly and rapidly fine-tune gene expression in response to host environmental cues. Given that pathogens are exposed to a wide array of diverse host environments, each with its own unique combination of nutritional resources and stresses, the ability to rapidly modulate gene expression would likely confer a very strong evolutionary advantage. In addition, pathogens may also have evolved post-transcriptional regulation of gene expression to subvert specific host defense mechanisms and/or immune responses. For example, transcripts encoding highly antigenic proteins could be specifically targeted for degradation or translational inhibition when the pathogen is exposed to the host environment. In either case, our knowledge of how host–pathogen interactions may affect post-transcriptional regulatory mechanisms is lacking and it is hoped that future research will shed more light on this area.

Perspective: targeting post-transcriptional mechanisms for the development of new antifungal strategies

While little is known about how post-transcriptional mechanisms control fungal pathogenicity, as we continue to gain more knowledge, an obvious question that arises is whether such mechanisms could eventually serve as targets for the development of new therapeutics. Most current antifungal therapies target various aspects of the cell membrane and cell wall integrity (e.g., azoles affect ergosterol biosynthesis and echinocandins disrupt β-glucan synthesis) [11, 12]. In this respect, fungal-specific post-transcriptional mechanisms that are particularly important for maintaining cell wall or cell membrane integrity may serve as promising targets. The Ccr4–Pop2 deadenylase complex, important for cell wall integrity, antifungal resistance and maturation of pre-formed biofilms [75, 80], may represent such a target. However, this complex is also conserved in higher eukaryotes and could present toxicity problems with regard to drug development. More recently, several promising potential small molecule compounds have been shown to specifically inhibit *C. albicans* filamentation and biofilm development and could serve as potential therapeutics [145, 146]. In this regard, fungal-specific components of post-transcriptional mechanisms that control *C. albicans* morphogenesis and/or biofilm formation, are essential for viability and are well-conserved across pathogenic fungi may represent potential targets for broad-spectrum antifungal drug discovery. Translational mechanisms represent targets of particular interest since potential therapeutics could be used to specifically halt fungal pathogen protein synthesis. A major caveat, however, is that many of these mechanisms are conserved in higher eukaryotes. One possible solution to this problem may be to design therapeutics that target fungal-specific regions and/or structural domains of conserved components of key pathogen translational mechanisms.

The recent advent of deep sequencing technology, as well as the publication of large-scale knockout and conditional knockout collections [33, 147] has greatly facilitated the ability to study post-transcriptional regulatory mechanisms in pathogenic fungi, such as *C. albicans*, on a genome-wide scale. Future studies in this area, particularly those that employ genome-wide approaches, are likely to not only significantly expand our knowledge of post-transcriptional regulation in pathogenic fungi, but also provide new fertile ground for the development of novel antifungal strategies.

Acknowledgments We thanks Brian Wickes for useful comments and suggestions. The author was supported by Grants 5R01AI083344 and 1R21AI117299 from the National Institute of Allergy and Infectious Diseases as well as a Young Investigator Award from the Max and Minnie Tomerlin Voelcker Fund. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

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