Cellular Microbiology (2016) **18**(6), 800–806
doi:10.1111/cmi.12593
First published online 14 April 2016

Microreview

Post-transcriptional gene regulation in the biology and virulence of *Candida albicans*

Jiyoti Verma-Gaur and Ana Traven*
Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia.

Summary

In the human fungal pathogen *Candida albicans*, remodelling of gene expression drives host adaptation and virulence. Recent studies revealed that in addition to transcription, post-transcriptional mRNA control plays important roles in virulence-related pathways. Hyphal morphogenesis, biofilm formation, stress responses, antifungal drug susceptibility and virulence in animal models require post-transcriptional regulators. This includes RNA binding proteins that control mRNA localization, decay and translation, as well as the cytoplasmic mRNA decay pathway. Comprehensive understanding of how modulation of gene expression networks drives *C. albicans* virulence will necessitate integration of our knowledge on transcriptional and post-transcriptional mRNA control.

Introduction

The human commensal yeast *Candida albicans* causes oral and vaginal infections, and disseminated disease in severely ill hosts (Brown *et al.*, 2012). Screens of transcription factor mutant libraries have revealed functions for gene expression networks in pathogenesis-related biology of *C. albicans* (Nobile and Mitchell, 2005; Homann *et al.*, 2009; Pukkila-Worley *et al.*, 2009; Finkel *et al.*, 2012; Nobile *et al.*, 2012; Perez *et al.*, 2013). Further to transcription, proper spatio-temporal expression of genes necessitates regulation of the subcellular localization, translation and turnover of mRNAs by RNA binding proteins and post-transcriptional mechanisms. Studies in baker's yeast *Saccharomyces cerevisiae* and other eukaryotic models have illuminated the functions of multiple RNA binding proteins, and the importance of post-transcriptional mRNA networks in dictating cellular physiology, for example (Hogan *et al.*, 2008; Freeberg *et al.*, 2013), reviewed in (Keene, 2007; Quenault *et al.*, 2011; Blackinton and Keene, 2014). How post-transcriptional regulation controls *C. albicans* biology and pathogenicity is a major knowledge gap. Excitingly, recent publications have focussed on this question, laying the foundation for understanding post-transcriptional mRNA networks in this pathogen.

Received 27 January, 2016; revised 28 February, 2016; accepted 16 March, 2016. *For correspondence. E-mail ana.traven@monash.edu; Tel. + 61 3 99029219; Fax + 61 3 99053726.

Post-transcriptional regulation of hyphal morphogenesis

The yeast to hyphae transition is central to *C. albicans* virulence through functions including tissue invasion, cell adhesion, evasion of macrophages and development of clinically relevant biofilm communities. In response to stimuli such as temperature, nutrients or serum, signal transduction pathways and transcription factors induce the hyphal gene expression programme and filamentous growth (Sudbery, 2011). Significant evidence for post-transcriptional mRNA regulation being important in hyphal growth of *C. albicans* is (Fig. 1).

Mutations in the cytoplasmic mRNA decay pathway impair hyphal morphogenesis

The major eukaryotic mRNA decay pathway consists of poly(A) tail degradation by the mRNA deadenylase Ccr4-NOT, hydrolysis of the 5′ cap (decapping) and mRNA digestion by Xrn1/Kem1, reviewed in Goldstrohm and Wickens (2008) (Fig. 1a). RNA binding proteins modulate these processes by recruiting deadenylation and decapping factors, reviewed in Goldstrohm *et al.* (2008) and Quenault *et al.* (2011). *C. albicans* mutants in the deadenylase subunits *CCR4* and *POP2*, the decapping activators *DHH1* and *EDC3* and the exonuclease *XRN1*/*KEM1* are defective in hyphal morphogenesis (Richard *et al.*, 2005; Dagley *et al.*, 2011; Jung and Kim, 2014; Shively *et al.*, 2015). Hyphal defects are not seen in all conditions. For example, *ccr4* and *pop2* can filament in

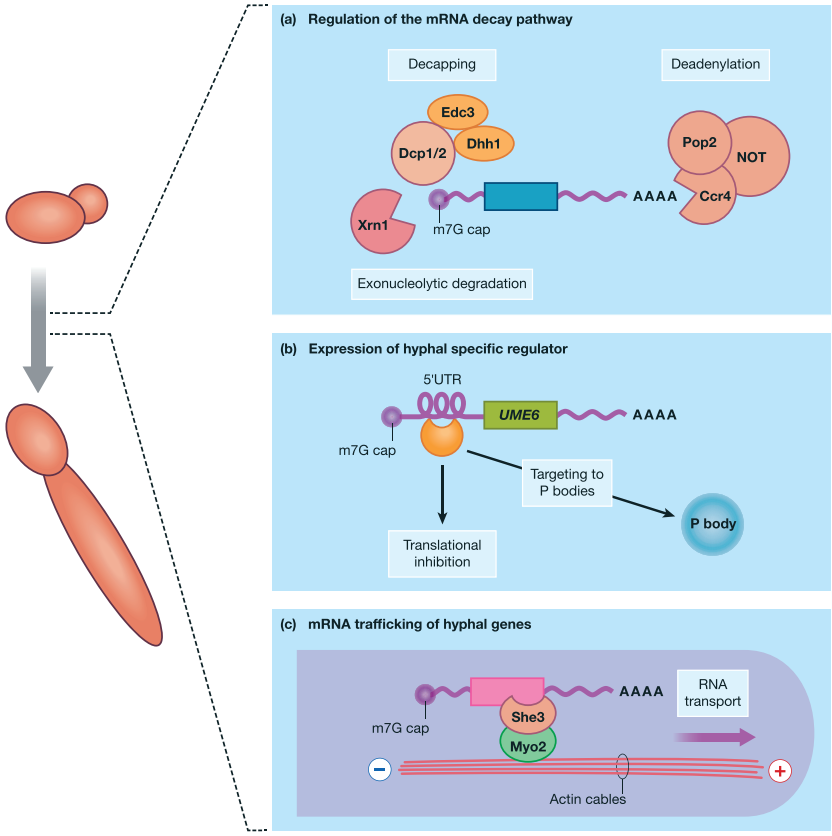

Fig. 1. Post-transcriptional regulation in hyphal morphogenesis.
(a) mRNA decay factors required for hyphal morphogenesis. The pathway is shown as understood in *S. cerevisiae*. For simplicity, not all known protein–protein interactions are depicted, and the mRNA is shown linear instead of circular with 5′ and 3′ ends in proximity. The roles of the Dcp1/Dcp2 decapping enzyme in filamentation are yet to be studied.
(b) Possible mechanisms of 5′ UTR-dependent repression of *UME6* through secondary structure formation, or binding of RNA binding protein(s) (orange circle) to inhibit ribosome association or target to P-bodies, as proposed by (Childers *et al.*, 2014).
(c) mRNA trafficking to the hyphal tip by She3. The precise architecture of the *C. albicans* She3 complex is not known. The homolog of She2 is lacking, and the myosin is likely Myo2 (Elson *et al.*, 2009). Whether *C. albicans* She3 interacts directly with mRNAs is unknown, but RNA binding activity can be predicted based on *S. cerevisiae* (Muller *et al.*, 2011). Based on studies in *S. cerevisiae*, particularly *ASH1* trafficking, mRNA localization elements can be in the open reading frame and 3′ UTR, and the She3 complex is multimeric (Shi *et al.*, 2014). Therefore, our cartoon is a greatly simplified version of the likely protein-RNA complex. *En route*, mRNAs is repressed and activated at the destined location. The mechanism of translational repression–derepression has not been studied in *C. albicans*.

liquid serum at 37°C, and *xrn1* in the *Galleria mellonella* infection model (Fuchs *et al.*, 2010; Dagley *et al.*, 2011). This suggests the mRNA decay pathway is not necessary for hyphal growth *per se*, but rather acts in the response to hyphal signals. Consistent with this, in *S. cerevisiae* Dhh1, Xrn1 and Edc3, together with other post-transcriptional regulators, are substrates of the kinases that orchestrate filamentous growth (Shively *et al.*, 2015). It will be interesting to determine if in *C. albicans* mRNA decay factors are regulated post-translationally by filamentous growth kinases.

It is unclear why the mRNA decay mutants display filamentation defects. Functions important for hyphal growth, such as cell wall biogenesis, nutrient responses and mitochondrial activity, are compromised in mRNA decay mutants in *C. albicans* and/or *S. cerevisiae* (Moriya and Isono, 1999; Kaeberlein and Guarente, 2002; Dagley *et al.*, 2011; Braun *et al.*, 2014), reviewed in Panepinto *et al.* (2013). Consistent with a metabolic reason, in the *C. albicans xrn1* mutant protein levels for several metabolic enzymes are distinct from wild type cells, and overexpression of the pyruvate dehydrogenase subunit Lpd1 rescued hyphal defects (Lee *et al.*, 2010). *S. cerevisiae* Dhh1 has been implicated in translational regulation of Ste12, a transcriptional activator of filamentation (Park *et al.*, 2006). Whether this is conserved in *C. albicans* is unknown. It should be noted that a picture has emerged whereby regulators of mRNA decay play complex, cytoplasmic and nuclear roles in gene expression, and control and coordinate multiple steps such as transcription, post-transcriptional mRNA regulation and translational control (Panasenko *et al.*, 2006; Kruk *et al.*, 2011; Haimovich *et al.*, 2013; Sun *et al.*, 2013). Therefore, future studies will need to decipher the precise molecular mechanisms by which mRNA decay factors regulate hyphal morphogenesis.

The expression of key transcriptional regulators of the yeast-hyphae transition is regulated post-transcriptionally

Evidence for this comes from studies of the hyphal transcriptional activator *UME6* and the transcriptional repressor of the hyphal programme *NRG1* (Cleary *et al.*, 2012; Childers *et al.*, 2014; Lee *et al.*, 2015b).

The 5′ UTR of *UME6* dictates Ume6 protein levels, without impacting on mRNA level or induction by hyphal signals (Childers *et al.*, 2014). Polysome association data are consistent with the effect being on mRNA translation (Childers *et al.*, 2014). The mechanism(s) are not defined, but the authors proposed that secondary structure formation by the 5′ UTR could inhibit translation, or alternatively binding of RNA binding proteins could impair

ribosome association or target the *UME6* mRNA to a translationally silent location, such as P-bodies (Childers *et al*., 2014). Given abundance of Ume6 alone determines whether *C. albicans* exists as yeast or hyphal form (Carlisle *et al*., 2009), translational regulation might enable fast, reversible yeast–hyphae–yeast morphogenesis that could be important for disease.

To lift repression of the hyphal programme, the mRNA and protein levels of the repressor Nrg1 drop rapidly upon hyphal induction, and *NRG1* is regulated transcriptionally (Braun *et al*., 2001; Murad *et al*., 2001; Sellam *et al*., 2010; Lassak *et al*., 2011; Lu *et al*., 2011; Childers and Kadosh, 2015). Further to this, the stability of the *NRG1* mRNA is controlled by the transcription factor Brg1, via production of an anti-sense transcript located in the *NRG1* open reading frame (Cleary *et al*., 2012). The exact mechanism of this regulation remains to be elucidated. Nrg1 protein levels are further controlled by the RNA binding protein Ssd1 (Lee *et al*., 2015b). In *S. cerevisiae* Ssd1 interacts with, and regulates the translation and subcellular localization of, a suite of mRNAs encoding cell wall remodelling factors (Hogan *et al*., 2008; Jansen *et al*., 2009; Kurischko *et al*., 2011). The translational repressor activity of Ssd1 is negatively regulated through phosphorylation by the RAM (regulation of Ace2 and morphogenesis) network kinase Cbk1, and this process is important for cell wall remodelling during cell division (Jansen *et al*., 2009). In *C. albicans* the RAM network is required for hyphal morphogenesis (Song *et al*., 2008). The *cbk1* mutant does not reduce Nrg1 protein levels upon hyphal signalling, but this can be rescued by deletion of *SSD1* (Lee *et al*., 2015b). This indicates that in the absence of Cbk1, Ssd1 promotes *NRG1* translation even when hyphal signalling is 'on'. This, together with the *C. albicans ssd1* mutant not being compromised for hyphal morphogenesis (Song *et al*., 2008), suggests that Ssd1 is a repressor of filamentation.

mRNA trafficking via She3 impacts on hyphal morphogenesis

In *S. cerevisiae*, the She3-system transports mRNAs to the site of bud growth and repress their translation *en route*, reviewed in Haag *et al*. (2015). The RNA binding proteins She2 and She3 interact together and with localization sites in the mRNA, and form a complex with the myosin V motor Myo4 that enables mRNA trafficking along actin cables (Haag *et al*., 2015). As shown by the prototypical example of the *ASH1* mRNA, two further RNA binding proteins, Puf6 and Khd1, bind mRNA and are required for translational repression (Paquin *et al*., 2007; Deng *et al*., 2008). Three of the RNA binding proteins (She3, Puf6 and Khd1) have orthologues in *C. albicans*, but only She3 has been characterized. *C. albicans* She3 interacts with 31 and 38 mRNAs during yeast and hyphal growth respectively (Elson *et al*., 2009). The targets represent several functions, including cell wall, hyphal-induced genes and transcription factors, and She3 is required for asymmetric mRNA localization (Elson *et al*., 2009). The *she3* mutant is defective in invasive filamentation on solid media and is further defective in causing damage to epithelial cells (Elson *et al*., 2009). However, *she3* could initiate hyphal morphogenesis in liquid media (Elson *et al*., 2009), suggesting that mRNA targeting by She3 is dispensable for hyphal initiation, but important for long-term filamentous growth and invasion.

Further to the regulators mentioned earlier, the serine-arginine RNA binding protein of *C. albicans* Slr1 is also required for hyphal morphogenesis (Ariyachet *et al*., 2013). Slr1 could play multiple roles in mRNA physiology, including splicing and translation, but its RNA targets for hyphal morphogenesis remain to be identified.

Post-transcriptional regulation of biofilm formation

Candida albicans grows drug-resistant biofilms on various substrates and medical devices, seeding life-threatening infections. Given that hyphae are important structural components of biofilms, it can be predicted that post-transcriptional regulators described earlier will play roles in biofilm formation. This is exemplified by the *xrn1* mutant, which was identified as biofilm-defective because of impaired hyphal morphogenesis (Richard *et al*., 2005).

Transcription factors have been extensively studied for biofilm phenotypes (Nobile *et al*., 2005, Nobile *et al*., 2012). How post-transcriptional regulators control the biofilm transcriptome is poorly defined. The first indication that post-transcriptional mRNA regulation might play a role came from a bioinformatics approach performed by our colleagues and us (Verma-Gaur *et al*., 2015). Thirty six genes related to mitochondria and down-regulated in biofilms (Nobile *et al*., 2012) are putative targets of the RNA binding protein Puf3 (Verma-Gaur *et al*., 2015). Puf3 is a PUF family member that in *S. cerevisiae* regulates a network of mRNAs necessary for mitochondrial biogenesis (Gerber *et al*., 2004). It does so by binding to sequence elements in 3′ UTRs to control mRNA decay, as well as transcript localization to mitochondria (Olivas and Parker, 2000; Gerber *et al*., 2004; Saint-Georges *et al*., 2008). Bioinformatics and functional data in *C. albicans* are consistent with regulation of mitochondrial biogenesis being a conserved role, as is the function of Puf3 in promoting mRNA decay (Verma-Gaur *et al*., 2015). We proposed therefore that Puf3 is involved in controlling mitochondria-related genes, as part of metabolic changes characteristic of *C. albicans* biofilms (Verma-Gaur *et al*., 2015). However, the *puf3* mutant displayed a normal biofilm phenotype. This could result from redundancy, as studies in *S. cerevisiae* have shown transcripts tend to

interact with several RNA binding proteins (Hogan *et al*., 2008). For example, although an earlier study reported minimal correspondence between Puf3 targets and those of other PUFs in yeast (Gerber *et al*., 2004), more recent works found that close to one third (30.5%) of mRNAs bound by Puf3 are also bound by Puf5 (Wilinski *et al*., 2015). An alternative explanation is that although the mRNA targets of Puf3 are differentially expressed in *C. albicans* biofilms, their proper regulation is not required for biofilm maturation. It could also be that alternative mechanisms compensate when Puf3 targets are dis-regulated. In contrast to the *puf3* mutant, deadenylase mutants *ccr4* and *pop2* displayed altered biofilms, with the most intriguing phenotype being overproduction of extracellular matrix (Verma-Gaur *et al*., 2015). Ccr4 likely regulates matrix production by two mechanisms, through the expression of genes involved in matrix carbohydrate regulation (specifically mannan), as well as via metabolic effects (Verma-Gaur *et al*., 2015). Extracellular matrix production is related to biofilm drug resistance (Nett *et al*., 2010a; Nett *et al*., 2010b), but its control remains to be fully understood. In addition to Ccr4 the transcription factor Zap1 is a further negative regulator of biofilm matrix production (Nobile *et al*., 2009). Understanding the interface between transcriptional and post-transcriptional regulation of this important biofilm phenotype is a topic for future studies.

Post-transcriptional regulators in stress responses and virulence

Mutants in several mRNA regulators display reduced virulence in the mouse systemic candidiasis model: *ccr4* (Dagley *et al*., 2011), *slr1* (Ariyachet *et al*., 2013) and *ssd1* (Gank *et al*., 2008). In addition, the *xrn1* mutant is less virulent in *Galleria mellonella* (Fuchs *et al*., 2010). The reasons for reduced virulence are likely multifactorial, including crippled fitness, hyphal defects and altered cell wall integrity. The cell wall is particularly relevant, because its biogenesis is targeted by the echinocandin drugs used to treat *Candida* infections. Given that the *C. albicans ccr4* and *pop2* mutants display changes to cell wall composition and increased susceptibility to the echinocandin drug caspofungin (Dagley *et al*., 2011), we proposed that inactivation of the deadenylase could be considered for combinatorial therapy (Panepinto *et al*., 2013). Several cell wall-related genes are dis-regulated in the *ccr4* mutant, but their levels are mostly up-regulated compared with controls suggestive of compensatory activation (Dagley *et al*., 2011; Verma-Gaur *et al*., 2015). Ccr4 has further been implicated in regulating cell wall genes in hypoxia (Sellam *et al*., 2014).

Ssd1 is also required for cell wall integrity in *C. albicans* (Gank *et al*., 2008; Song *et al*., 2008), consistent with roles in *S. cerevisiae* (Kaeberlein *et al*., 2002, Hogan *et al*., 2008). In *S. cerevisiae*, deletion of *SSD1* and *CCR4* results in a negative genetic interaction, suggesting that they act in parallel pathways for wall integrity (Kaeberlein *et al*., 2002). Ssd1 has an additional role in regulating the susceptibility of *C. albicans* to antimicrobial peptides (Gank *et al*., 2008; Jung *et al*., 2013). This might stem from functions in cell surface biogenesis, and a role in the expression of *BCR1*, a transcription factor necessary for antimicrobial peptide resistance (Jung *et al*., 2013). The *C. albicans* decapping factor Edc3 also has a stress responsive role in oxidative stress and translational regulation of superoxide dismutase Sod1 and the catalase Cat1 (Jung *et al*., 2014). Collectively, these observations suggest that post-transcriptional regulators could be considered as drug targets, because of requirements for virulence and potential for combinatorial treatment with current antifungal drugs or antimicrobial peptides.

Evolution of post-transcriptional gene regulation in fungi

Changes to gene expression control are a known mechanism of evolutionary divergence between species. Evolutionary rewiring of transcriptional regulation has been extensively studied in fungi and other eukaryotes. For this, *C. albicans* has served as a useful comparison to *S. cerevisiae* because it is a related, but biologically divergent yeast (e.g. Tsong *et al*., 2003; Ihmels *et al*., 2005; Hogues *et al*., 2008; Brown *et al*., 2009). As with transcription factors, RNA binding proteins interact with functionally related genes, thereby establishing post-transcriptional RNA networks, reviewed in Keene (2007) and Blackinton *et al*. (2014). Understanding the evolution of post-transcriptional regulatory networks in fungi is still in its infancy.

Examples of differences in post-transcriptional mRNA control have been uncovered between *S. cerevisiae* and *C. albicans*, with orthologous RNA binding proteins displaying distinct regulation of the same mRNA, or having distinct sets of targets in the two species. Within fungi, the PUF family of RNA binding proteins is best studied for evolutionary aspects (Jiang *et al*., 2012; Hogan *et al*., 2015; Verma-Gaur *et al*., 2015). There are five PUF family members in *C. albicans*, all with orthologues in *S. cerevisiae*. Bioinformatics suggests that several *C. albicans* PUFs predominantly share their mRNA targets with *S. cerevisiae* (Jiang *et al*., 2010; Jiang *et al*., 2012; Hogan *et al*., 2015; Verma-Gaur *et al*., 2015). However, in one case uncovered so far, regulation by Puf3 appears to be distinct in the two yeasts (Verma-Gaur *et al*., 2015). In *C. albicans* Puf3 facilitates mRNA decay of the mitochondrial ribosomal subunit *MRPL25* in two carbon sources important in human body environments, fermentable glucose and non-fermentable lactate

(Verma-Gaur *et al*., 2015). In *S. cerevisiae* this is the case in glucose, but not lactate (Verma-Gaur *et al*., 2015). This recent work from our laboratory fits with previous studies showing that *S. cerevisiae* Puf3 is an mRNA decay factor only in glucose (Miller *et al*., 2014), and moreover, it can mediate translation of mitochondrial biogenesis factors in non-fermentable carbon, with the change from repressor to activator regulated by phosphorylation of Puf3 via glucose-regulated signalling (Lee and Tu, 2015a). Importantly, transcription of mitochondrial ribosomal genes is a well-known example of distinct control in *C. albicans* and *S. cerevisiae*, likely reflecting their differences in fermentative growth. Of note, although numerous mitochondrial ribosomal subunits are Puf3 targets in both *S. cerevisiae* and *C. albicans*, for two other transcripts tested a much smaller degree of difference was seen in their regulation between the two species (Verma-Gaur *et al*., 2015). It will be important to comprehensively address similarities and differences in Puf3-dependent regulation of mitochondrial biogenesis factors in *C. albicans* and *S. cerevisiae* by transcriptome-wide approaches, as well as determine how *C. albicans* Puf3 regulates mRNA translation and localization to mitochondria in response to environmental and nutritional signals. This will lead to more complete understanding of how evolutionary changes shaped distinct regulation of mitochondrial biogenesis on multiple levels of control.

In contrast to the conservation of PUF targets between *S. cerevisiae* and *C. albicans*, the mRNAs localized by the She3-system differ substantially, with only two shared targets (Elson *et al*., 2009). The authors proposed that this reflects the two yeasts being subject to divergent environmental conditions in the human body (*C. albicans*) versus habitats such as fruit (*S. cerevisiae*) (Elson *et al*., 2009). Similarly, the targets of the *C. albicans* tristetraprolin family RNA binding protein Zfs1 have diverged from its *S. cerevisiae* orthologues Cth1 and Cth2 (Wells *et al*., 2015). Moreover, bioinformatic searches for Zfs1 binding sites in very closely related *Candida* species from the CTG clade suggested rapid evolution of Zfs1 functions (Wells *et al*., 2015). In *S. cerevisiae* Zfs1 orthologues regulate the response to iron deficiency through decay of mRNAs with functions in iron-dependent processes (Puig *et al*., 2005; Puig *et al*., 2008). The ability to respond to iron is important for commensalism and pathogenicity of *C. albicans* (Chen *et al*., 2011). It will be interesting to determine whether an RNA binding protein different to Zfs1 is involved in iron homeostasis.

Outlook

With studies showing that post-transcriptional mRNA regulators are important for *C. albicans* biology and virulence, the challenge now is to understand the functions and identify the mRNA targets of many more RNA binding proteins in this pathogen and decipher how regulation of post-transcriptional mRNA networks mediates the response of *C. albicans* to its environment. Constructing libraries of RNA binding protein mutants, coupled with systems-biology approaches that have been developed in *S. cerevisiae*, will achieve these goals. Ultimately, integrating this knowledge with transcriptional circuits will provide a more accurate picture of how *C. albicans* adapts to host and antifungal drug stresses and might offer new avenues for therapy.

Acknowledgements

We apologize to colleagues whose work could not be cited because of space limitations. Our work on post-transcriptional regulation has been supported by the Australian Research Council: Discovery Projects DP1092850 and DP130100578 to A.T., and DECRA Fellowship to J.V-G.

References

Ariyachet, C., Solis, N.V., Liu, Y., Prasadarao, N.V., Filler, S. G., and McBride, A.E. (2013) SR-like RNA-binding protein Slr1 affects *Candida albicans* filamentation and virulence. *Infect Immun* **81**: 1267–1276.

Blackinton, J.G., and Keene, J.D. (2014) Post-transcriptional RNA regulons affecting cell cycle and proliferation. *Semin Cell Dev Biol* **34**: 44–54.

Braun, B.R., Kadosh, D., and Johnson, A.D. (2001) NRG1, a repressor of filamentous growth in *C. albicans*, is down-regulated during filament induction. *EMBO J* **20**: 4753–4761.

Braun, K.A., Vaga, S., Dombek, K.M., Fang, F., Palmisano, S., Aebersold, R., and Young, E.T. (2014) Phosphoproteomic analysis identifies proteins involved in transcription-coupled mRNA decay as targets of Snf1 signaling. *Sci Signal* **7**: ra64.

Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., and White, T.C. (2012) Hidden killers: human fungal infections. *Sci Transl Med* **4**: 165rv113.

Brown, V., Sabina, J., and Johnston, M. (2009) Specialized sugar sensing in diverse fungi. *Current biology : CB* **19**: 436–441.

Carlisle, P.L., Banerjee, M., Lazzell, A., Monteagudo, C., Lopez-Ribot, J.L., and Kadosh, D. (2009) Expression levels of a filament-specific transcriptional regulator are sufficient to determine *Candida albicans* morphology and virulence. *Proc Natl Acad Sci U S A* **106**: 599–604.

Chen, C., Pande, K., French, S.D., Tuch, B.B., and Noble, S. M. (2011) An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. *Cell Host Microbe* **10**: 118–135.

Childers, D.S., and Kadosh, D. (2015) Filament condition-specific response elements control the expression of NRG1 and UME6, key transcriptional regulators of morphology and virulence in *Candida albicans*. *PLoS One* **10**: e0122775.

Childers, D.S., Mundodi, V., Banerjee, M., and Kadosh, D. (2014) A 5' UTR-mediated translational efficiency mechanism inhibits the *Candida albicans* morphological transition. *Mol Microbiol* **92**: 570–585.

Cleary, I.A., Lazzell, A.L., Monteagudo, C., Thomas, D.P., and Saville, S.P. (2012) BRG1 and NRG1 form a novel feedback circuit regulating *Candida albicans* hypha formation and virulence. *Mol Microbiol* **85**: 557–573.

Dagley, M.J., Gentle, I.E., Beilharz, T.H., Pettolino, F.A., Djordjevic, J.T., Lo, T.L., *et al.* (2011) Cell wall integrity is linked to mitochondria and phospholipid homeostasis in *Candida albicans* through the activity of the post-transcriptional regulator Ccr4-Pop2. *Mol Microbiol* **79**: 968–989.

Deng, Y., Singer, R.H., and Gu, W. (2008) Translation of ASH1 mRNA is repressed by Puf6p-Fun12p/eIF5B interaction and released by CK2 phosphorylation. *Genes Dev* **22**: 1037–1050.

Elson, S.L., Noble, S.M., Solis, N.V., Filler, S.G., and Johnson, A.D. (2009) An RNA transport system in *Candida albicans* regulates hyphal morphology and invasive growth. *PLoS Genet* **5**: e1000664.

Finkel, J.S., Xu, W., Huang, D., Hill, E.M., Desai, J.V., Woolford, C.A., *et al.* (2012) Portrait of *Candida albicans* adherence regulators. *PLoS Pathog* **8**: e1002525.

Freeberg, M.A., Han, T., Moresco, J.J., Kong, A., Yang, Y.C., Lu, Z.J., *et al.* (2013) Pervasive and dynamic protein binding sites of the mRNA transcriptome in *Saccharomyces cerevisiae*. *Genome Biol* **14**: R13.

Fuchs, B.B., Eby, J., Nobile, C.J., El Khoury, J.B., Mitchell, A.P., and Mylonakis, E. (2010) Role of filamentation in Galleria mellonella killing by *Candida albicans*. *Microbes and infection / Institut Pasteur* **12**: 488–496.

Gank, K.D., Yeaman, M.R., Kojima, S., Yount, N.Y., Park, H., Edwards, J.E., Jr., *et al.* (2008) SSD1 is integral to host defense peptide resistance in *Candida albicans*. *Eukaryot Cell* **7**: 1318–1327.

Gerber, A.P., Herschlag, D., and Brown, P.O. (2004) Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. *PLoS Biol* **2**: E79.

Goldstrohm, A.C., and Wickens, M. (2008) Multifunctional deadenylase complexes diversify mRNA control. *Nat Rev Mol Cell Biol* **9**: 337–344.

Haag, C., Steuten, B., and Feldbrugge, M. (2015) Membrane-coupled mRNA trafficking in fungi. *Annu Rev Microbiol* **69**: 265–281.

Haimovich, G., Medina, D.A., Causse, S.Z., Garber, M., Millan-Zambrano, G., Barkai, O., *et al.* (2013) Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell* **153**: 1000–1011.

Hogan, D.J., Riordan, D.P., Gerber, A.P., Herschlag, D., and Brown, P.O. (2008) Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. *PLoS Biol* **6**: e255.

Hogan, G.J., Brown, P.O., and Herschlag, D. (2015) Evolutionary conservation and diversification of Puf RNA binding proteins and their mRNA targets. *PLoS Biol* **13**: e1002307.

Hogues, H., Lavoie, H., Sellam, A., Mangos, M., Roemer, T., Purisima, E., *et al.* (2008) Transcription factor substitution during the evolution of fungal ribosome regulation. *Mol Cell* **29**: 552–562.

Homann, O.R., Dea, J., Noble, S.M., and Johnson, A.D. (2009) A phenotypic profile of the *Candida albicans* regulatory network. *PLoS Genet* **5**: e1000783.

Ihmels, J., Bergmann, S., Gerami-Nejad, M., Yanai, I., McClellan, M., Berman, J., and Barkai, N. (2005) Rewiring of the yeast transcriptional network through the evolution of motif usage. *Science (New York, NY)* **309**: 938–940.

Jansen, J.M., Wanless, A.G., Seidel, C.W., and Weiss, E.L. (2009) Cbk1 regulation of the RNA-binding protein Ssd1 integrates cell fate with translational control. *Current biology : CB* **19**: 2114–2120.

Jiang, H., Guan, W., and Gu, Z. (2010) Tinkering evolution of post-transcriptional RNA regulons: puf3p in fungi as an example. *PLoS Genet* **6**: e1001030.

Jiang, H., Guo, X., Xu, L., and Gu, Z. (2012) Rewiring of posttranscriptional RNA regulons: Puf4p in fungi as an example. *Mol Biol Evol* **29**: 2169–2176.

Jung, J.H., and Kim, J. (2014) Roles of Edc3 in the oxidative stress response and CaMCA1-encoded metacaspase expression in *Candida albicans*. *FEBS J* **281**: 4841–4851.

Jung, S.I., Finkel, J.S., Solis, N.V., Chaili, S., Mitchell, A.P., Yeaman, M.R., and Filler, S.G. (2013) Bcr1 functions downstream of Ssd1 to mediate antimicrobial peptide resistance in *Candida albicans*. *Eukaryot Cell* **12**: 411–419.

Kaeberlein, M., and Guarente, L. (2002) *Saccharomyces cerevisiae* MPT5 and SSD1 function in parallel pathways to promote cell wall integrity. *Genetics* **160**: 83–95.

Keene, J.D. (2007) RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet* **8**: 533–543.

Kruk, J.A., Dutta, A., Fu, J., Gilmour, D.S., and Reese, J.C. (2011) The multifunctional Ccr4-Not complex directly promotes transcription elongation. *Genes Dev* **25**: 581–593.

Kurischko, C., Kim, H.K., Kuravi, V.K., Pratzka, J., and Luca, F.C. (2011) The yeast Cbk1 kinase regulates mRNA localization via the mRNA-binding protein Ssd1. *J Cell Biol* **192**: 583–598.

Lassak, T., Schneider, E., Bussmann, M., Kurtz, D., Manak, J.R., Srikantha, T., *et al.* (2011) Target specificity of the *Candida albicans* Efg1 regulator. *Mol Microbiol* **82**: 602–618.

Lee, C.D., and Tu, B.P. (2015a) Glucose-regulated phosphorylation of the PUF protein Puf3 regulates the translational fate of its bound mRNAs and association with RNA granules. *Cell reports* **11**: 1638–1650.

Lee, H.J., Kim, J.M., Kang, W.K., Yang, H., and Kim, J.Y. (2015b) The NDR kinase Cbk1 downregulates the transcriptional repressor Nrg1 through the mRNA-binding protein Ssd1 in *Candida albicans*. *Eukaryot Cell* **14**: 671–683.

Lee, K.H., Kim, S.Y., Jung, J.H., and Kim, J. (2010) Proteomic analysis of hyphae-specific proteins that are expressed differentially in cakem1/cakem1 mutant strains of *Candida albicans*. *Journal of microbiology (Seoul, Korea)* **48**: 365–371.

Lu, Y., Su, C., Wang, A., and Liu, H. (2011) Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. *PLoS Biol* **9**: e1001105.

Miller, M.A., Russo, J., Fischer, A.D., Lopez Leban, F.A., and Olivas, W.M. (2014) Carbon source-dependent alteration of Puf3p activity mediates rapid changes in the stabilities of mRNAs involved in mitochondrial function. *Nucleic Acids Res* **42**: 3954–3970.

Moriya, H., and Isono, K. (1999) Analysis of genetic interactions between DHH1, SSD1 and ELM1 indicates their involvement in cellular morphology determination in *Saccharomyces cerevisiae*. *Yeast (Chichester, England)* **15**: 481–496.

Muller, M., Heym, R.G., Mayer, A., Kramer, K., Schmid, M., Cramer, P., *et al.* (2011) A cytoplasmic complex mediates specific mRNA recognition and localization in yeast. *PLoS Biol* **9**: e1000611.

Murad, A.M., Leng, P., Straffon, M., Wishart, J., Macaskill, S., MacCallum, D., *et al.* (2001) NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. *EMBO J* **20**: 4742–4752.

Nett, J.E., Crawford, K., Marchillo, K., and Andes, D.R. (2010a) Role of Fks1p and matrix glucan in *Candida albicans* biofilm resistance to an echinocandin, pyrimidine, and polyene. *Antimicrob Agents Chemother* **54**: 3505–3508.

Nett, J.E., Sanchez, H., Cain, M.T., and Andes, D.R. (2010b) Genetic basis of *Candida* biofilm resistance due to drug-sequestering matrix glucan. *J Infect Dis* **202**: 171–175.

Nobile, C.J., Fox, E.P., Nett, J.E., Sorrells, T.R., Mitrovich, Q. M., Hernday, A.D., *et al.* (2012) A recently evolved transcriptional network controls biofilm development in *Candida albicans*. *Cell* **148**: 126–138.

Nobile, C.J., and Mitchell, A.P. (2005) Regulation of cell-surface genes and biofilm formation by the *C. albicans* transcription factor Bcr1p. *Current biology : CB* **15**: 1150–1155.

Nobile, C.J., Nett, J.E., Hernday, A.D., Homann, O.R., Deneault, J.S., Nantel, A., *et al.* (2009) Biofilm matrix regulation by *Candida albicans* Zap1. *PLoS Biol* **7**: e1000133.

Olivas, W., and Parker, R. (2000) The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J* **19**: 6602–6611.

Panasenko, O., Landrieux, E., Feuermann, M., Finka, A., Paquet, N., and Collart, M.A. (2006) The yeast Ccr4-Not complex controls ubiquitination of the nascent-associated polypeptide (NAC-EGD) complex. *J Biol Chem* **281**: 31389–31398.

Panepinto, J.C., Heinz, E., and Traven, A. (2013) The cellular roles of Ccr4-NOT in model and pathogenic fungi-implications for fungal virulence. *Frontiers in genetics* **4**: 302.

Paquin, N., Menade, M., Poirier, G., Donato, D., Drouet, E., and Chartrand, P. (2007) Local activation of yeast ASH1 mRNA translation through phosphorylation of Khd1p by the casein kinase Yck1p. *Mol Cell* **26**: 795–809.

Park, Y.U., Hur, H., Ka, M., and Kim, J. (2006) Identification of translational regulation target genes during filamentous growth in *Saccharomyces cerevisiae*: regulatory role of Caf20 and Dhh1. *Eukaryot Cell* **5**: 2120–2127.

Perez, J.C., Kumamoto, C.A., and Johnson, A.D. (2013) Candida albicans commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. *PLoS Biol* **11**: e1001510.

Puig, S., Askeland, E., and Thiele, D.J. (2005) Coordinated remodeling of cellular metabolism during iron deficiency through targeted mRNA degradation. *Cell* **120**: 99–110.

Puig, S., Vergara, S.V., and Thiele, D.J. (2008) Cooperation of two mRNA-binding proteins drives metabolic adaptation to iron deficiency. *Cell Metab* **7**: 555–564.

Pukkila-Worley, R., Peleg, A.Y., Tampakakis, E., and Mylonakis, E. (2009) Candida albicans hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryot Cell* **8**: 1750–1758.

Quenault, T., Lithgow, T., and Traven, A. (2011) PUF proteins: repression, activation and mRNA localization. *Trends Cell Biol* **21**: 104–112.

Richard, M.L., Nobile, C.J., Bruno, V.M., and Mitchell, A.P. (2005) *Candida albicans* biofilm-defective mutants. *Eukaryot Cell* **4**: 1493–1502.

Saint-Georges, Y., Garcia, M., Delaveau, T., Jourdren, L., Le Crom, S., Lemoine, S., *et al.* (2008) Yeast mitochondrial biogenesis: a role for the PUF RNA-binding protein Puf3p in mRNA localization. *PLoS One* **3**: e2293.

Sellam, A., Askew, C., Epp, E., Tebbji, F., Mullick, A., Whiteway, M., and Nantel, A. (2010) Role of transcription factor CaNdt80p in cell separation, hyphal growth, and virulence in *Candida albicans*. *Eukaryot Cell* **9**: 634–644.

Sellam, A., van het Hoog, M., Tebbji, F., Beaurepaire, C., Whiteway, M., and Nantel, A. (2014) Modeling the transcriptional regulatory network that controls the early hypoxic response in *Candida albicans*. *Eukaryot Cell* **13**: 675–690.

Shi, H., Singh, N., Esselborn, F. and Blobel, G. (2014). Structure of a myosin*adaptor complex and pairing by cargo. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E1082-1090.

Shively, C.A., Kweon, H.K., Norman, K.L., Mellacheruvu, D., Xu, T., Sheidy, D.T., *et al.* (2015) Large-scale analysis of kinase signaling in yeast pseudohyphal development identifies regulation of ribonucleoprotein granules. *PLoS Genet* **11**: e1005564.

Song, Y., Cheon, S.A., Lee, K.E., Lee, S.Y., Lee, B.K., Oh, D. B., *et al.* (2008) Role of the RAM network in cell polarity and hyphal morphogenesis in *Candida albicans*. *Mol Biol Cell* **19**: 5456–5477.

Sudbery, P.E. (2011) Growth of *Candida albicans* hyphae. *Nat Rev Microbiol* **9**: 737–748.

Sun, M., Schwalb, B., Pirkl, N., Maier, K.C., Schenk, A., Failmezger, H., *et al.* (2013) Global analysis of eukaryotic mRNA degradation reveals Xrn1-dependent buffering of transcript levels. *Mol Cell* **52**: 52–62.

Tsong, A.E., Miller, M.G., Raisner, R.M., and Johnson, A.D. (2003) Evolution of a combinatorial transcriptional circuit: a case study in yeasts. *Cell* **115**: 389–399.

Verma-Gaur, J., Qu, Y., Harrison, P.F., Lo, T.L., Quenault, T., Dagley, M.J., *et al.* (2015) Integration of posttranscriptional gene networks into metabolic adaptation and biofilm maturation in *Candida albicans*. *PLoS Genet* **11**: e1005590.

Wells, M.L., Washington, O.L., Hicks, S.N., Nobile, C.J., Hartooni, N., Wilson, G.M., *et al.* (2015) Post-transcriptional regulation of transcript abundance by a conserved member of the tristetraprolin family in *Candida albicans*. *Mol Microbiol* **95**: 1036–1053.

Wilinski, D., Qiu, C., Lapointe, C.P., Nevil, M., Campbell, Z.T., Tanaka Hall, T.M., and Wickens, M. (2015) RNA regulatory networks diversified through curvature of the PUF protein scaffold. *Nat Commun* **6**: 8213.