MINI-REVIEW

Transcriptional regulation of the caspofungin-induced cell wall damage response in *Candida albicans*

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Abstract

The human fungal pathogen *Candida albicans* maintains pathogenic and commensal states primarily through cell wall functions. The echinocandin antifungal drug caspofungin inhibits cell wall synthesis and is widely used in treating disseminated candidiasis. Signaling pathways are critical in coordinating the adaptive response to cell wall damage (CWD). *C. albicans* executes a robust transcriptional program following caspofungin-induced CWD. A comprehensive analysis of signaling pathways at the transcriptional level facilitates the identification of prospective genes for functional characterization and propels the development of novel antifungal interventions. This review article focuses on the molecular functions and signaling crosstalk of the *C. albicans* transcription factors Sko1, Rlm1, and Cas5 in caspofungin-induced CWD signaling.

Keywords Fungi · Cell wall · Antifungals · Stress signaling · Transcription circuits · Transcription networks

Epidemiology of *Candida* infections

Invasive fungal infections are a major global health concern, and *Candida* species remain the major causative agent of these mycoses (Pfaller and Diekema 2007). In fact, *Candida* species were reported as the fourth most common cause of nosocomial infections in the United States, with high incidences of chronic infections in adult and neonatal ICUs (Bajpai et al. 2019). Additionally, approximately 138 million women worldwide suffer annually from recurrent vulvovaginal candidiasis caused by *Candida albicans* (Sobel 2016). Finally, dissemination of *Candida* infections into the bloodstream can be lethal, with mortality rates up to 40% even after administration of antifungal drug therapy (Kullberg and Arendrup 2015). Over 90% of reported cases of invasive

candidiasis, in which *Candida* spreads to the blood or other organs in the body, are attributed to the five most common *Candida* species: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida tropicalis* (Dadar et al. 2018). Moreover, other non-*albicans* (NAC)-associated diseases have been increasingly reported globally with low rates of therapeutic success, such as infection by the emerging multidrug-resistant species *Candida auris* (Cortegiani et al. 2018; Pfaller et al. 2014). Despite this, *C. albicans* remains the most commonly isolated fungal pathogen in clinical settings.

Overview of the antifungal activity of caspofungin

The cell wall coordinates functions essential for *C. albicans* pathogenicity, such as biofilm formation, resistance to turgor pressure, adhesion to host surfaces, and morphogenesis (Ene et al. 2015; Gow et al. 2017). The polysaccharide (β-1–3 glucan, β-1–6 glucan, and chitin) and glycoprotein matrix that comprises the cell wall is specific to fungi, rendering it an ideal target for the development of novel antifungal interventions (Gow et al. 2017). Echinocandins, a class of antifungal drugs, are highly effective in treating candidiasis, as they inhibit β-1–3 glucan synthesis (Sucher et al. 2009). To date, most of the knowledge regarding the cellular, molecular, and

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genetic responses associated with echinocandin treatment in *C. albicans* is based on studies using the echinocandin caspofungin.

Cellular and biophysical analyses of *C. albicans* cells exposed to sub-MIC concentrations of caspofungin show extensive cell wall remodeling, including increased chitin content to compensate for the loss of β-1–3 glucan, promoting drug tolerance (Munro et al. 2007; Walker et al. 2008), reduced cell wall mechanical strength, causing cell swelling and osmosensitivity (Letscher-Bru and Herbrecht 2003), and increased cell surface hydrophobicity, causing extensive cell–cell aggregation (El-Kirat-Chatel et al. 2013). These changes typically lead to eventual fungal cell death via apoptosis and/or necrosis (Hao et al. 2013). Exposure to caspofungin also causes redistribution of septin and phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P$_2$] into plasma membrane patches, which are characterized by aberrant chitin and cell wall protein deposition (Badrane et al. 2012, 2016). Noteworthy, *C. albicans* cells exposed to caspofungin concentrations exceeding the standard MIC were found to display paradoxical growth, where cell death becomes attenuated (Rueda et al. 2014; Wiederhold 2009). This paradoxical growth phenomenon is associated with altered glucan synthase function, septin mislocalization, and increased levels of cell wall chitin (Badrane et al. 2016; Rueda et al. 2014). However, the molecular mechanisms and clinical impacts of this phenomenon are not well understood. Nevertheless, the cellular mechanisms of action for caspofungin-induced cell death in *C. albicans* are mainly due to aberrant cell wall remodeling.

Transcriptional regulators of caspofungin-induced cell wall damage signaling

Over the past 2 decades, advancements in gene annotation, gene mutagenesis, genome-wide transcriptional profiling, and genome-wide chromatin immunoprecipitation techniques have revealed a highly complex genetic network of regulators (i.e., transcription factors) and their downstream effector (i.e., "target") proteins governing the response to caspofungin-induced CWD in *C. albicans* (Blankenship et al. 2010; Bruno et al. 2006; Heredia et al. 2020; Rauceo et al. 2008; Xie et al. 2017). One prominent feature of signaling in *C. albicans* is circuitry rewiring, where conserved transcription factors demonstrate novel functions between different organisms by controlling distinct sets of downstream target genes (Blankenship et al. 2010; Polvi et al. 2019). In addition, a common physiological condition such as an environmental stressor could generate a diverse transcriptional response between different organisms by activating distinct transcription factors (Brown et al. 2014), which

is observed for caspofungin-induced CWD signaling in *C. albicans* (Bruno et al. 2006; Rauceo et al. 2008; Xie et al. 2017). Caspofungin-induced CWD signaling also demonstrates pathway redundancy, where multiple transcription factors control overlapping sets of downstream target genes to fine-tune the adaptive response of the cell (Bruno et al. 2006; Heredia et al. 2020; Rauceo et al. 2008; Xie et al. 2017). In the subsequent sections of this review, we focus our discussion on the regulators of caspofungin-induced CWD signaling, namely the transcription factors Cas5, Sko1, and Rlm1, that were identified in genetic screens for caspofungin hypersensitivity from a library of 150 putative and known transcription factor mutant strains (Bruno et al. 2006; Rauceo et al. 2008).

Cas5

The transcription factor Cas5 contains a C-terminal zinc-finger DNA-binding domain, and orthologs have been identified in *C. glabrata*, *C. dubliniensis*, *C. parapsilosis*, and *C. auris* but not in non-pathogenic yeasts (Skrzypek et al. 2017). Indirect immunofluorescence imaging using a *C. albicans* wild-type strain containing a Cas5-HA tagged protein showed that Cas5 is diffusely localized to the cytoplasm under basal growth conditions; however, caspofungin exposure significantly increased *CAS5* transcription and led to translocation of Cas5 to the nucleus (Xie et al. 2017).

Depletion of Cas5 is associated with a plethora of cellular phenotypes that underscore the importance of Cas5 as a regulator of key biological processes. Pleiotropic effects for growth, morphology, and virulence have been reported with insertion, missense, and homozygous deletion *cas5* mutants (Bruno et al. 2006; Chamilos et al. 2009; Vasicek et al. 2014; Xie et al. 2017). In addition to caspofungin hypersensitivity, a *cas5Δ/Δ* deletion mutant strain is also hypersensitive to other cell wall damaging agents, such as Congo red, Calcofluor white, and SDS (Bruno et al. 2006). In addition, a strain containing a missense mutation in the Cas5 DNA-binding domain (*cas5Δ/CAS5*^{S769E}) is also hypersensitive to caspofungin (Xie et al. 2017). While these observations suggest that *CAS5* deletion may lead to global cell wall defects, high-resolution transmission or scanning electron microscopy images of the cell wall of the *cas5Δ/Δ* mutant strain have not yet been performed. Other *cas5Δ/Δ* mutant strain phenotypes include hypersensitivity to the antifungal drug fluconazole, hyperfilamentous growth on Spider medium at 30 °C, multinucleated yeast-form cells, and attenuated virulence in murine and invertebrate infection models (Chamilos et al. 2009; Vasicek et al. 2014; Xie et al. 2017).

Genome-wide microarray analyses of a *C. albicans* wild-type strain showed that caspofungin treatment significantly altered the expression of 216 genes (Bruno et al. 2006). Major significantly upregulated gene classes include cell

wall biogenesis, cell wall integrity, vesicular transport, and secretion (Bruno et al. 2006). Microarray results in a *cas5Δ/Δ* mutant strain showed that Cas5 regulated 50% (15/30) of the most highly expressed caspofungin-responsive genes, including several cell wall integrity genes (Bruno et al. 2006). Recent RNA polymerase II (RNA Pol II) chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiments demonstrated that the number of caspofungin-responsive genes is significantly higher, with 546 genes showing altered expression in a wild-type strain (Xie et al. 2017). As expected, genes with cell wall-related functions were significantly overrepresented in this gene set. In this study, Cas5 was found to regulate over 60% of caspofungin-responsive genes, including cell wall integrity genes and cell cycle regulatory genes (Xie et al. 2017). Moreover, RNA Pol II ChIP-seq analyses showed that Cas5 modulates 604 genes under basal growth conditions. Gene ontology (GO) analyses of this dataset revealed biological roles for Cas5 as a regulator of gene classes whose functions include metabolism and cell cycle regulation (Xie et al. 2017). Interestingly, the transcriptional profile of a *cas5Δ/Δ* mutant strain under basal growth conditions was vastly different when compared to its caspofungin-induced profile, highlighting distinct regulatory roles for Cas5 depending on the environmental condition (Xie et al. 2017).

Because Cas5 is unique to the *Candida* species, information regarding upstream regulators and/or potential protein–protein physical interactions underlying Cas5 function is limited. Nevertheless, based on recent biochemical analyses performed in *C. albicans*, Cas5 was shown to be dephosphorylated by the phosphatase Glc7 following caspofungin-induced CWD, while the kinase that phosphorylates Cas5 is unknown (Xie et al. 2017). In addition, co-immunoprecipitation coupled with mass spectrometry data demonstrated that Cas5 physically interacts with the cell cycle box-binding factor (SBF) transcriptional complex proteins, Swi4 and Swi6 (Xie et al. 2017). See Fig. 1 for a summary of caspofungin-induced CWD regulation by Cas5.
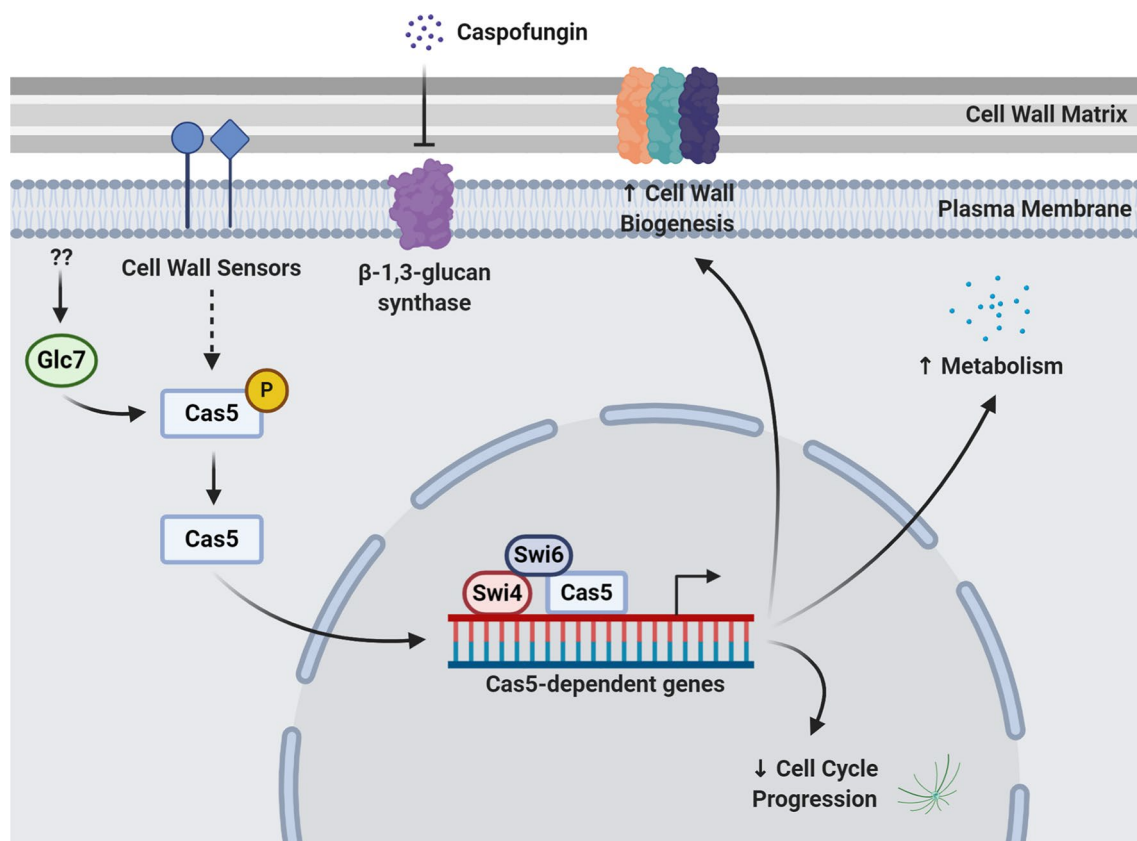
Fig. 1 Caspofungin-induced CWD regulation by Cas5. Following caspofungin-induced CWD, Cas5 is activated via an unknown regulatory pathway involving dephosphorylation by Glc7. Cytoplasmic Cas5 then translocates into the nucleus to activate transcription. In the nucleus, Cas5 interacts with the SBF complex proteins Swi4 and Swi6 to activate transcription of Cas5-dependent genes. Note that the Cas5 DNA-binding motif is currently unknown. Cas5-dependent gene activation leads to the upregulation of genes involved in cell wall biogenesis/integrity and cellular metabolism, and the downregulation of genes involved in cell cycle progression. Dotted arrows represent unknown or hypothetical processes, while solid arrows indicate confirmed relationships and processes. This figure was adapted from Bruno et al. (2006) and Xie et al. (2017) and created with BioRender. com

Sko1

The transcription factor Sko1 is widely conserved across fungi. Sko1 orthologs share homology mainly in the C-terminal basic leucine zipper (bZIP) DNA-binding domain (Krantz et al. 2006). In the model yeast, *Saccharomyces cerevisiae*, Sko1 is part of the mitogen-activated protein kinase (MAPK) high osmolarity glycerol (HOG) signaling pathway, and its function has been extensively characterized in the osmotic and oxidative stress responses [see Saito et al. for a comprehensive review of the *S. cerevisiae* HOG and Sko1 stress response signaling pathways (Saito and Posas 2012)].

In *C. albicans*, Sko1 is conserved as a regulator of the osmotic stress response. Sko1 is phosphorylated by the MAP kinase Hog1 following hyperosmotic shock (Rauceo et al. 2008). Microarray analysis coupled with gene sequence enrichment mapping demonstrated that Sko1 regulates several gene classes that are conserved in the *S. cerevisiae* osmotic stress response, such as redox metabolism and carbohydrate transport (Marotta et al. 2013). However, Sko1 also regulates *C. albicans*-specific gene classes, such as those involved in mitochondrial ATP synthesis and ribosome synthesis. Also, Sko1 regulates genes whose *S. cerevisiae* orthologs are not required in the *S. cerevisiae* osmotic stress response, thus exemplifying *C. albicans* circuitry rewiring (Marotta et al. 2013). Furthermore, a *C. albicans sko1Δ/Δ* mutant strain displays a mild growth defect under hyperosmotic growth conditions (Marotta et al. 2013). Lastly, microarray analysis in *C. albicans* revealed that Sko1 function is also conserved in regulating the oxidative stress response (Alonso-Monge et al. 2010).

Despite the fact that *SKO1* transcription is significantly elevated in response to the specific environmental stressors discussed above (Marotta et al. 2013; Rauceo et al. 2008), we were unable to visualize localization of a Sko1-GFP fusion protein using fluorescence microscopy under basal conditions, following osmotic shock, or caspofungin treatment (unpublished findings). This observation suggests that *C. albicans* Sko1 is produced at low levels compared to the single allele expression of Sko1-GST in *S. cerevisiae*, where Sko1 was visualized in the cytoplasm and nucleus (Pascual-Ahuir et al. 2001). However, *C. albicans* Sko1-GFP showed exclusive nuclear localization when Sko1 was overproduced using a doxycycline expression system (Alonso-Monge et al. 2010). Thus, the cellular mechanisms underlying *C. albicans* Sko1 localization remain unclear.

Besides caspofungin-induced CWD signaling, other less characterized known Sko1 functions includes regulation of the yeast to hyphal transition and regulation of the hypoxic response (Alonso-Monge et al. 2010; Sellam et al. 2014). Interestingly, homozygous deletion of *SKO1* confers hypersensitivity to caspofungin, but not to Congo red, Calcofluor white, or SDS (Alonso-Monge et al. 2010; Rauceo et al.

2008). This observation suggests that the cell wall of the *sko1Δ/Δ* mutant strain may not be as compromised relative to the cell wall of the *cas5Δ/Δ* mutant strain. Microarray and RT-qPCR results of a *sko1Δ/Δ* mutant strain following caspofungin treatment showed that Sko1 regulates 81 caspofungin-responsive genes, 26 of which are upregulated by Sko1 (Rauceo et al. 2008). Although the majority of Sko1 caspofungin gene targets are uncharacterized, several Sko1-dependent significantly upregulated genes have functions in cell wall integrity, carbohydrate transport, and metabolism (Rauceo et al. 2008). Of note are the cell wall biogenesis genes, *PGA13*, *CRH11*, *SKN1*, and *MNN2*.

Our most recent ChIP-seq analyses on *C. albicans* Sko1 greatly expanded the Sko1 caspofungin-induced CWD circuit, identified new biological roles for Sko1 in CWD signaling, and identified a Sko1 DNA-binding consensus motif (Heredia et al. 2020). Our ChIP-seq results using a Sko1-V5-tagged protein showed that Sko1 was enriched at 85 upstream intergenic regions in caspofungin-treated cells compared to untreated cells (Heredia et al. 2020). A comparison of the Sko1 ChIP-seq data against Sko1 caspofungin microarray data showed that approximately 22% (19/85) of the Sko1 target genes identified by ChIP-seq were previously identified by microarrays performed on *sko1Δ/Δ* mutant strains (Delgado-Silva et al. 2014; Heredia et al. 2020). This finding indicates that Sko1 indirectly regulates several caspofungin-responsive genes. In silico analyses of the Sko1 ChIP-seq data identified a DNA-binding consensus motif similar to the canonical Sko1 ATF/CREB motif previously identified in *S. cerevisiae* (Heredia et al. 2020; Ni et al. 2009; Proft and Serrano 1999).

Strikingly, GO and KEGG analyses of the Sko1 direct target genes identified by ChIP-seq revealed that a major biological role for Sko1 is osmotic stress adaptation, highlighting the functional conservation of Sko1 in the CWD response (Heredia et al. 2020). Among several Sko1 direct target genes with functions in osmotic stress adaptation is *RHR2*, which encodes a glycerol phosphatase that catalyzes the synthesis of glycerol (Fan et al. 2005). Overexpression of *RHR2* in a *sko1Δ/Δ* mutant strain partially suppressed the caspofungin growth defect of the *sko1Δ/Δ* mutant strain (Heredia et al. 2020). This finding suggests that one aspect underlying the caspofungin hypersensitivity of the *sko1Δ/Δ* mutant strain is its inability to counteract the osmotic stress caused by CWD.

The upstream mechanisms underlying Sko1 function in *C. albicans* are more complex than *S. cerevisiae*, in which the HOG pathway exclusively governs Sko1 transcriptional activity. While salt-dependent Hog1 phosphorylation of Sko1 demonstrates functional conservation in *C. albicans*, caspofungin-induced Sko1 activity is independent of HOG pathway function (Rauceo et al. 2008). RT-qPCR findings showed that caspofungin exposure significantly increases

SKO1 transcription and requires the glucose-partitioning PAS kinase, Psk1 (Rauceo et al. 2008); however, Psk1 does not directly regulate transcription. Microarray results showed that Rlm1 regulates *SKO1* expression under basal growth conditions (Delgado-Silva et al. 2014). Additionally, RT-qPCR results showed that caspofungin-induced *SKO1* expression was significantly reduced in an *rlm1Δ/Δ* mutant strain but not in a *cas5Δ/Δ* mutant strain (Heredia et al. 2020). It is unknown whether Psk1 directly regulates Rlm1 to activate *SKO1* transcription. Remarkably, ChIP-seq analyses revealed that Sko1 binds to its own upstream intergenic region following caspofungin exposure, demonstrating that *SKO1* expression is, in part, dependent on autoactivation (Heredia et al. 2020).

We identified the two-component phosphotransferase regulator-encoding gene, *YPD1,* and the phosphatase-encoding gene, *PTP2,* as Sko1 direct target genes following caspofungin treatment (Heredia et al. 2020). Both Ypd1 and Ptp2 inhibit HOG pathway signaling, suggesting that Sko1 suppresses HOG pathway function following

caspofungin-induced cell wall stress (Day et al. 2017; Mavrianos et al. 2014). Thus, the ability of Sko1 to regulate the CWD response independently of HOG pathway regulation is a new paradigm in *C. albicans* cell wall stress signaling. See Fig. 2 for a summary of caspofungin-induced CWD regulation by Sko1.

Rlm1

The transcription factor Rlm1 is conserved across fungi, and orthologs possess the N-terminal MADS box DNA-binding domain (Dodou and Treisman 1997). Rlm1 function has been extensively characterized in *S. cerevisiae*, in which it is a principal transcriptional regulator of the MAPK Protein Kinase C (PKC) cell wall integrity signaling pathway (Dodou and Treisman 1997; Jung and Levin 1999). See (Levin 2011) for an excellent review on cell wall integrity signaling in *S. cerevisiae*.

Rlm1 function is conserved in *C. albicans* in terms of CWD signaling; however, our understanding of the roles of
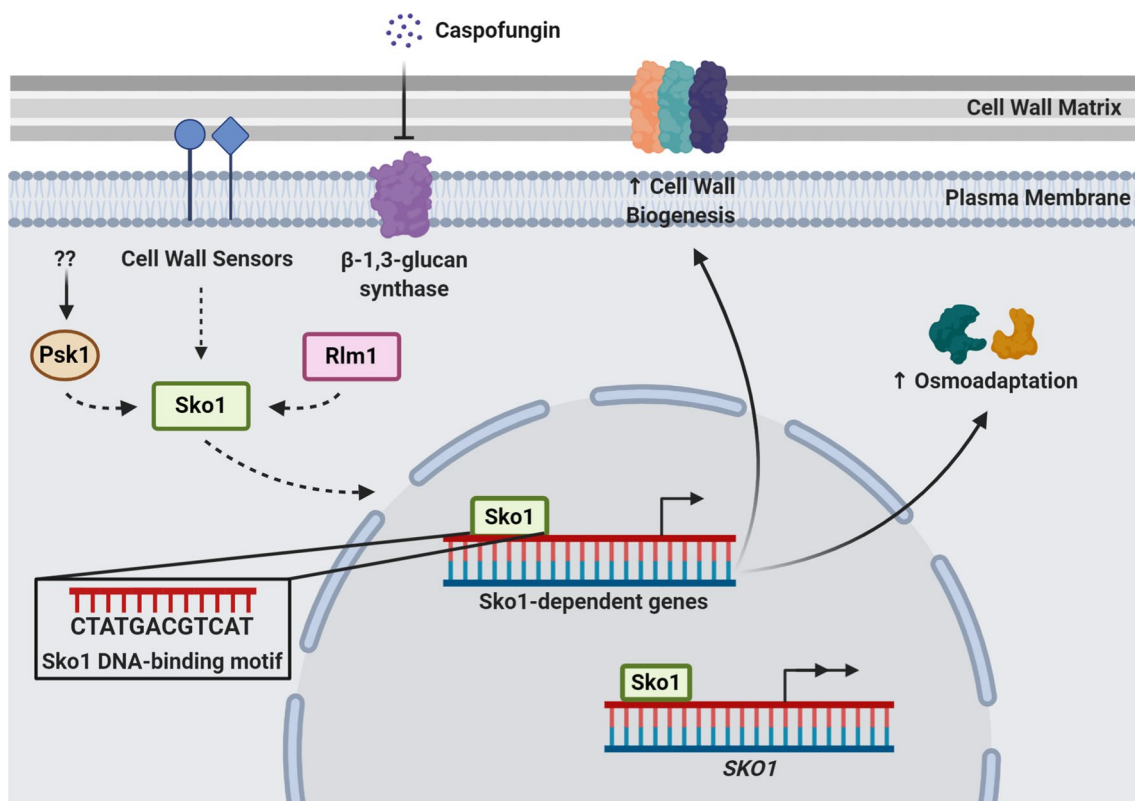
Fig. 2 Caspofungin-induced CWD regulation by Sko1**.** Following caspofungin-induced CWD, Sko1 is indirectly activated by Psk1 and Rlm1, whose upstream regulators are unknown. Cytoplasmic Sko1 then translocates into the nucleus to activate transcription of Sko1-dependent genes. In addition, Sko1 binds to its own upstream intergenic region to promote self-activation. The *C. albicans* Sko1 DNA-binding consensus motif is depicted in the inset. Sko1-dependent gene activation leads to the upregulation of genes involved in cell wall biogenesis/integrity and osmoadaptation. Dotted arrows represent unknown or hypothetical processes, while solid arrows indicate confirmed relationships and processes. This figure was adapted from Rauceo et al. (2008) and Heredia et al. (2020) and created with BioRender.com

Rlm1 in *C. albicans* is rudimentary compared to *S. cerevisiae*. A *C. albicans rlm1Δ/Δ* mutant strain is hypersensitive to Congo red and Calcofluor white, suggesting that *RLM1* deletion could lead to a global defect in cell wall architecture (Bruno et al. 2006). In support of this hypothesis, HPLC analysis of the cell wall of an *rlm1Δ/Δ* mutant strain grown under basal conditions showed a significant reduction in mannan levels and an increase in chitin levels compared to a wild-type strain grown under the same conditions (Delgado-Silva et al. 2014). These cell wall abnormalities of the *C. albicans rlm1Δ/Δ* mutant strain were not observed in the *S. cerevisiae rlm1Δ* mutant strain treated with Congo red or Calcofluor white, highlighting the divergence of these orthologs (Delgado-Silva et al. 2014). Additional *C. albicans* Rlm1-specific functions include regulation of lactate-dependent filamentation (Oliveira-Pacheco et al. 2018) and virulence (Delgado-Silva et al. 2014). Notably, an *rlm1Δ/Δ* mutant strain demonstrated attenuated virulence in a murine infection model. Despite the numerous cellular and genetic studies on the *rlm1Δ/Δ* mutant strain in *C. albicans*, it is unknown whether *C. albicans* Rlm1 localizes exclusively to the nucleus, as is observed with Rlm1 in *S. cerevisiae* (Jung et al. 2002).

The caspofungin hypersensitivity phenotype of the *rlm1Δ/Δ* mutant strain suggests that Rlm1 plays a major role in *C. albicans* caspofungin-induced CWD signaling. However, genome-wide microarray analyses revealed that Rlm1 regulates only five genes under basal conditions, with only two genes being induced by caspofungin (Bruno et al. 2006). On the other hand, results from a separate genome-wide microarray study showed that Rlm1 regulates the expression of 773 genes under basal conditions (Delgado-Silva et al. 2014). Among some of the most highly upregulated Rlm1-dependent genes were genes associated with cell wall functions and carbohydrate metabolism. These findings suggest that Rlm1 may play additional roles in cell wall remodeling associated with general physiological activities, such as vegetative growth and budding.

Our genome-wide ChIP-seq findings using an Rlm1-V5-tagged protein revealed that Rlm1 binds to the upstream intergenic regions of 25 genes, with 18 genes showing increased binding in the presence of caspofungin (Heredia et al. 2020). Several Rlm1 direct target genes encode proteins with cell wall-related functions, although the majority of Rlm1 direct target genes are uncharacterized (Heredia et al. 2020). Of the uncharacterized genes we identified in our ChIP-seq data, only two matched Rlm1-dependent genes identified in prior microarray studies (Delgado-Silva et al. 2014; Heredia et al. 2020). GO analyses of Rlm1 direct target genes identified novel *C. albicans* functions for Rlm1 in regulating secretion and organelle localization (Heredia et al. 2020). Notably, in silico analyses of our Rlm1 ChIP-seq data identified a novel *C. albicans*-specific DNA-binding

consensus motif for Rlm1, CACCACCACAACC (Heredia et al. 2020). Orthologs of the PKC signaling pathway are conserved in *C. albicans* (Monge et al. 2006); however, it is unknown whether the MAP kinase, Mkc1, activates Rlm1. Overall, similar to Sko1, cellular, biochemical, and genomic findings highlight circuitry rewiring in Rlm1 CWD signaling. See Fig. 3 for a summary of caspofungin-induced CWD regulation by Rlm1.

A transcription factor circuit in CWD signaling

Genome wide transcriptional profiling results showed that numerous caspofungin-responsive genes are directly or indirectly regulated by Sko1, Rlm1, and Cas5 (Bruno et al. 2006; Heredia et al. 2020; Xie et al. 2017). While such redundancy is not uncommon in gene regulation, it can reveal a broader regulatory network where signaling pathways operate independently and in concert with one another depending on environmental inputs to fine-tune the adaptive response of the cell.

Further evidence supporting the interconnected nature of Cas5, Sko1, and Rlm1 CWD signaling is demonstrated in our gene expression and growth experiments. We found that, although Rlm1 does not bind to the upstream intergenic region of Sko1, Rlm1 indirectly regulates *SKO1* caspofungin-induced expression along with several Sko1-dependent caspofungin-induced cell wall integrity genes (Heredia et al. 2020). Furthermore, overexpression of *SKO1* in an *rlm1Δ/Δ* mutant strain partially alleviates its caspofungin hypersensitivity (Heredia et al. 2020). Thus, Sko1 and Rlm1 caspofungin-induced CWD signaling pathways are indirectly linked. In addition, *RLM1* expression is significantly elevated in a *cas5Δ/Δ* mutant strain in the absence of CWD, and expression of PKC pathway kinase genes are all elevated in a *sko1Δ/Δ* mutant strain (Rauceo et al. 2008; Xie et al. 2017). Taken together, these observations suggest that a cell wall compensatory signaling mechanism between Sko1, Cas5, and Rlm1 is likely involved in maintaining cell wall integrity in *C. albicans*.

Based on the extensive genetic, genomic, and cellular evidence we present here, we propose that Sko1, Cas5, and Rlm1 comprise a core transcriptional circuit for caspofungin-induced CWD signaling in *C. albicans*, which is summarized in Fig. 4. Given that Sko1 indirectly regulates numerous caspofungin-responsive genes, it is likely that other transcription factors are also involved in regulating caspofungin-induced CWD signaling. Indeed, we found that the transcription factor-encoding genes, *NRG1*, *EFG1*, *AHR1*, and *TCC1* are Sko1 direct targets and may also contribute to the CWD response (Heredia et al. 2020). Interestingly, Efg1 regulates the function of the cell wall
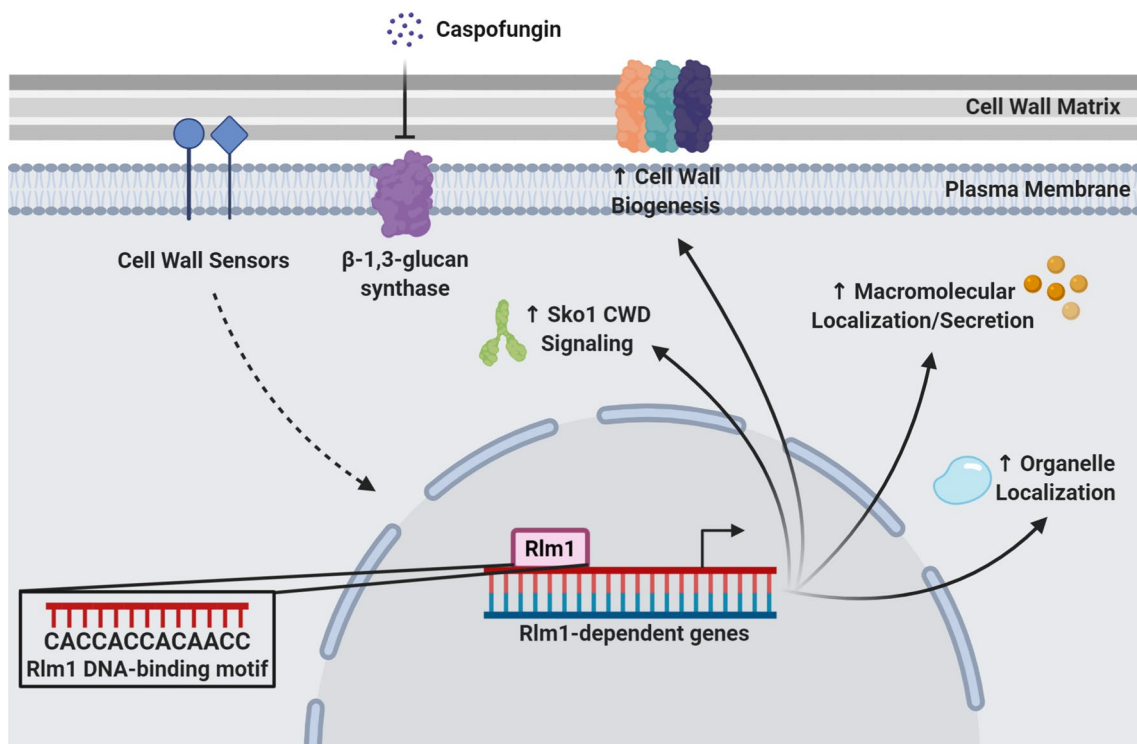
Fig. 3 Caspofungin-induced CWD regulation by Rlm1. Following caspofungin-induced CWD, Rlm1 is activated via an unknown regulatory pathway. In the nucleus, Rlm1 activates transcription of Rlm1-dependent genes. The *C. albicans* Rlm1 DNA-binding consensus motif is depicted in the inset. Rlm1-dependent gene activation leads to the upregulation of genes involved in cell wall biogenesis/integrity, macromolecular localization and secretion, and organelle localization. Rlm1 also indirectly activates Sko1 signaling. Dotted arrows represent unknown or hypothetical processes, while solid arrows indicate confirmed relationships and processes. This figure was adapted from Heredia et al. (2020) and created with BioRender.com
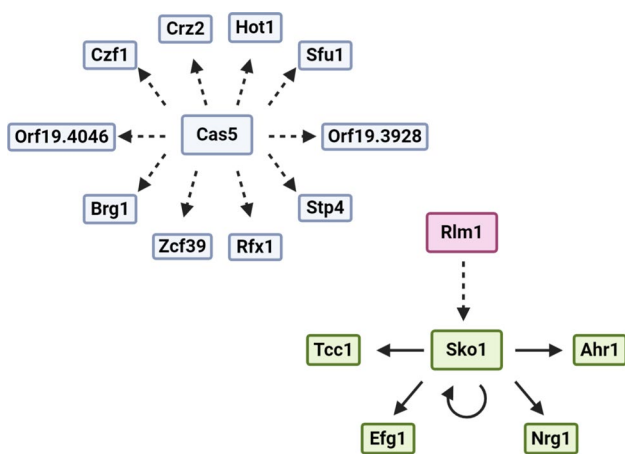
Fig. 4 A caspofungin-induced transcriptional circuit. Sko1, Cas5, and Rlm1 form the core of the caspofungin-induced transcriptional circuit. Transcription factors directly regulated (solid lines) and indirectly regulated (dashed lines) by Sko1 or Cas5 are included. The curved arrow indicates Sko1 self-activation. This figure was adapted from Heredia et al. (2020) and Xie et al. (2017) and created with BioRender.com

adhesin, Als1, to mediate caspofungin-induced cellular aggregation (Gregori et al. 2011). Moreover, genome-wide transcriptional profiling results showed that Cas5 regulates ten transcription factor-encoding genes, including the adhesion regulators, Czf1 and Crz2 (Finkel et al. 2012; Xie et al. 2017). *SKO1* is the only transcription factor-encoding gene indirectly regulated by Rlm1 following caspofungin-induced CWD; however, we did not identify any Rlm1 direct target genes that encode transcription factors (Heredia et al. 2020).

Perspectives and future directions

The elucidation of the transcription factors and their downstream target genes underlying caspofungin-induced CWD signaling in *C. albicans* has revealed new roles for uncharacterized genes and provided mechanistic insight into cell wall repair. Caspofungin-resistant *C. albicans* strains have been identified in the clinic, underscoring the need to develop novel antifungal interventions (Pfaller 2012; Walker et al. 2010). In addition, CWD signaling mechanisms identified in *C. albicans* could be conserved in related clinically relevant *Candida* species, such as *C.*

tropicalis, C. parapsilosis, and *C. auris*. Targeting the transcription factor, Cas5, or its downstream target proteins is one example of a pathway that may have pharmacological value, especially given that Cas5 itself lacks a mammalian ortholog. Notably, recent studies are beginning to explore the development of transcription factor inhibitors to combat fungal infections (Bahn 2015; Nishikawa et al. 2016).

Despite the extensive progress made in revealing the caspofungin-induced transcriptome, the signaling mechanisms regulating Sko1, Cas5, and Rlm1 remain elusive, and identifying the upstream activators of the CWD response should be a priority for future studies in the field. Approximately 25 kinase mutants display caspofungin hypersensitivities, including MAP kinases of the PKC cell wall integrity pathway (Blankenship et al. 2010; Rauceo et al. 2008). Given the conservation of the PKC signaling components, Rlm1 is likely to be a downstream target of the PKC cell wall integrity pathway. The identification of kinases that are not involved in MAPK signaling suggests that there may be non-traditional signaling mechanisms involved in Cas5 regulation, which was similarly observed for Psk1 and Sko1 (Rauceo et al. 2008; Xie et al. 2017).

Additionally, the identities of the molecular components that relay stress signals from the cell wall to Sko1, Rlm1, and Cas5 are unknown. Deletion of the cell wall stress sensor, *WSC1*, confers caspofungin hypersensitivity (Norice et al. 2007) and thus may be a player in relaying stress signals to Sko1, Rlm1, and Cas5. PI(4,5)P$_2$ is known to activate the PKC CWD signaling pathway (Badrane et al. 2016). Thus, exploring the functions of Sko1, Rlm1, and Cas5 in strains containing mutations to cell wall sensor genes and PI(4,5)P$_2$ synthetic genes would provide valuable insight into the physiological cues that initiate CWD signaling cascades.

Lastly, uncovering the crosstalk between signaling pathways will further expand our understanding of CWD signaling. One area of interest is in determining the molecular mechanism underlying Rlm1 activation of Sko1 signaling. Based on our Rlm1 and Sko1 ChIP-seq findings, we would hypothesize that post-translational regulation of carbohydrate accumulation may be the interface between these signaling pathways (Heredia et al. 2020).

Overall, further genome-wide transcriptional profiling and chromatin immunoprecipitation approaches are needed to comprehensively understand the caspofungin-induced CWD transcriptional network. For example, genome-wide chromatin immunoprecipitation coupled with high-throughput sequencing performed on the transcription factors directly regulated by Sko1, Rlm1, and Cas5, combined with existing genome-wide data for Sko1, Rlm1, and Cas5, would allow us to get a complete overview of the likely complex regulatory network governing caspofungin-induced CWD signaling.

Compliance with ethical standards

Conflicts of interest Not applicable.

References

Alonso-Monge R, Roman E, Arana DM, Prieto D, Urrialde V, Nombela C, Pla J (2010) The Sko1 protein represses the yeast-to-hypha transition and regulates the oxidative stress response in Candida albicans. Fungal Genet Biol 47:587–601. https://doi.org/10.1016/j.fgb.2010.03.009

Badrane H, Nguyen MH, Blankenship JR, Cheng S, Hao B, Mitchell AP, Clancy CJ (2012) Rapid redistribution of phosphatidylinositol-(4,5)-bisphosphate and septins during the Candida albicans response to caspofungin. Antimicrob Agents Chemother 56:4614–4624. https://doi.org/10.1128/AAC.00112-12

Badrane H, Nguyen MH, Clancy CJ (2016) Highly dynamic and specific phosphatidylinositol 4,5-bisphosphate, septin, and cell wall integrity pathway responses correlate with caspofungin activity against candida albicans. Antimicrob Agents Chemother 60:3591–3600. https://doi.org/10.1128/AAC.02711-15

Bahn YS (2015) Exploiting fungal virulence-regulating transcription factors as novel antifungal drug targets. PLoS Pathog 11:e1004936. https://doi.org/10.1371/journal.ppat.1004936

Bajpai VK et al (2019) Invasive fungal infections and their epidemiology: measures in the clinical scenario biotechnology and bioprocess. Engineering. https://doi.org/10.1007/s12257-018-0477-0

Blankenship JR, Fanning S, Hamaker JJ, Mitchell AP (2010) An extensive circuitry for cell wall regulation in Candida albicans. PLoS Pathog 6:e1000752. https://doi.org/10.1371/journal.ppat.1000752

Brown AJ et al (2014) Stress adaptation in a pathogenic fungus. J Exp Biol 217:144–155. https://doi.org/10.1242/jeb.088930

Bruno VM, Kalachikov S, Subaran R, Nobile CJ, Kyratsous C, Mitchell AP (2006) Control of the C. albicans cell wall damage response by transcriptional regulator Cas5. PLoS Pathog 2:e21

Chamilos G, Nobile CJ, Bruno VM, Lewis RE, Mitchell AP, Kontoyiannis DP (2009) Candida albicans Cas5, a regulator of cell wall integrity, is required for virulence in murine and toll mutant fly models. J Infect Dis 200:152–157. https://doi.org/10.1086/599363

Cortegiani A, Misseri G, Fasciana T, Giammanco A, Giarratano A, Chowdhary A (2018) Epidemiology, clinical characteristics, resistance, and treatment of infections by Candida auris. J Intensive Care 6:69. https://doi.org/10.1186/s40560-018-0342-4

Dadar M, Tiwari R, Karthik K, Chakraborty S, Shahali Y, Dhama K (2018) Candida albicans: biology, molecular characterization, pathogenicity, and advances in diagnosis and control: an update. Microb Pathog 117:128–138. https://doi.org/10.1016/j.micpath.2018.02.028

Day AM et al (2017) Blocking two-component signalling enhances Candida albicans virulence and reveals adaptive mechanisms that counteract sustained SAPK activation. PLoS Pathog 13:e1006131. https://doi.org/10.1371/journal.ppat.1006131

Delgado-Silva Y et al (2014) Participation of Candida albicans transcription factor RLM1 in cell wall biogenesis and virulence. PLoS ONE 9:e86270. https://doi.org/10.1371/journal.pone.0086270

Dodou E, Treisman R (1997) The Saccharomyces cerevisiae MADS-box transcription factor Rlm1 is a target for the Mpk1 mitogen-activated protein kinase pathway. Mol Cell Biol 17:1848–1859. https://doi.org/10.1128/mcb.17.4.1848

El-Kirat-Chatel S, Beaussart A, Alsteens D, Jackson DN, Lipke PN, Dufrene YF (2013) Nanoscale analysis of caspofungin-induced cell surface remodelling in Candida albicans. Nanoscale 5:1105–1115. https://doi.org/10.1039/c2nr33215a

Ene IV et al (2015) Cell wall remodeling enzymes modulate fungal cell wall elasticity and osmotic stress resistance. mBio 6:e00986. https://doi.org/10.1128/mBio.00986-15

Fan J, Whiteway M, Shen SH (2005) Disruption of a gene encoding glycerol 3-phosphatase from Candida albicans impairs intracellular glycerol accumulation-mediated salt-tolerance FEMS. Microbiol Lett 245:107–116

Finkel JS et al (2012) Portrait of Candida albicans adherence regulators. PLoS Pathog 8:e1002525. https://doi.org/10.1371/journal.ppat.1002525

Gow NAR, Latge JP, Munro CA (2017) The fungal cell wall: structure biosynthesis, and function. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.FUNK-0035-2016

Gregori C, Glaser W, Frohner IE, Reinoso-Martin C, Rupp S, Schuller C, Kuchler K (2011) Efg1 controls caspofungin-induced cell aggregation of Candida albicans through the adhesin Als1. Eukaryot Cell 10:1694–1704. https://doi.org/10.1128/EC.05187-11

Hao B, Cheng S, Clancy CJ, Nguyen MH (2013) Caspofungin kills Candida albicans by causing both cellular apoptosis and necrosis. Antimicrob Agents Chemother 57:326–332. https://doi.org/10.1128/AAC.01366-12

Heredia MY et al (2020) An expanded cell wall damage signaling network is comprised of the transcription factors Rlm1 and Sko1 in Candida albicans. PLoS Genet 16:e1008908. https://doi.org/10.1371/journal.pgen.1008908

Jung US, Levin DE (1999) Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. Mol Microbiol 34:1049–1057. https://doi.org/10.1046/j.1365-2958.1999.01667.x

Jung US, Sobering AK, Romeo MJ, Levin DE (2002) Regulation of the yeast Rlm1 transcription factor by the Mpk1 cell wall integrity MAP kinase. Mol Microbiol 46:781–789. https://doi.org/10.1046/j.1365-2958.2002.03198.x

Krantz M, Becit E, Hohmann S (2006) Comparative analysis of HOG pathway proteins to generate hypotheses for functional analysis. Curr Genet 49:152–165

Kullberg BJ, Arendrup MC (2015) Invasive Candidiasis. N Engl J Med 373:1445–1456. https://doi.org/10.1056/NEJMra1315399

Letscher-Bru V, Herbrecht R (2003) Caspofungin: the first representative of a new antifungal class. J Antimicrob Chemother 51:513–521

Levin DE (2011) Regulation of cell wall biogenesis in Saccharomyces cerevisiae: the cell wall integrity signaling pathway. Genetics 189:1145–1175. https://doi.org/10.1534/genetics.111.128264

Marotta DH, Nantel A, Sukala L, Teubl JR, Rauceo JM (2013) Genome-wide transcriptional profiling and enrichment mapping reveal divergent and conserved roles of Sko1 in the Candida albicans osmotic stress response. Genomics 102:363–371. https://doi.org/10.1016/j.ygeno.2013.06.002

Mavrianos J, Desai C, Chauhan N (2014) Two-component histidine phosphotransfer protein Ypd1 is not essential for viability in Candida albicans. Eukaryot Cell 13:452–460. https://doi.org/10.1128/EC.00243-13

Monge RA, Roman E, Nombela C, Pla J (2006) The MAP kinase signal transduction network in Candida albicans. Microbiology 152:905–912. https://doi.org/10.1099/mic.0.28616-0

Munro CA et al (2007) The PKC, HOG and Ca2+ signalling pathways co-ordinately regulate chitin synthesis in Candida albicans. Mol Microbiol 63:1399–1413. https://doi.org/10.1111/j.1365-2958.2007.05588.x

Ni L et al (2009) Dynamic and complex transcription factor binding during an inducible response in yeast. Genes Dev 23:1351–1363. https://doi.org/10.1101/gad.1781909(23/11/1351 [pii])

Nishikawa JL et al (2016) Inhibiting fungal multidrug resistance by disrupting an activator-Mediator interaction. Nature 530:485–489. https://doi.org/10.1038/nature16963

Norice CT, Smith FJ Jr, Solis N, Filler SG, Mitchell AP (2007) Requirement for Candida albicans Sun41 in biofilm formation and virulence. Eukaryot Cell 6:2046–2055. https://doi.org/10.1128/EC.00314-07

Oliveira-Pacheco J et al (2018) The Role of Candida albicans transcription factor RLM1 in response to carbon adaptation. Front Microbiol 9:1127. https://doi.org/10.3389/fmicb.2018.01127

Pascual-Ahuir A, Posas F, Serrano R, Proft M (2001) Multiple levels of control regulate the yeast cAMP-response element-binding protein repressor Sko1p in response to stress. J Biol Chem 276:37373–37378. https://doi.org/10.1074/jbc.M105755200

Pfaller MA (2012) Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am J Med 125:S3–13. https://doi.org/10.1016/j.amjmed.2011.11.001

Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20:133–163

Pfaller MA et al (2014) Epidemiology and outcomes of invasive candidiasis due to non-albicans species of Candida in 2,496 patients: data from the prospective antifungal therapy (PATH) registry 2004–2008. PLoS ONE 9:e101510. https://doi.org/10.1371/journal.pone.0101510

Polvi EJ et al (2019) Functional divergence of a global regulatory complex governing fungal filamentation. PLoS Genet 15:e1007901. https://doi.org/10.1371/journal.pgen.1007901

Proft M, Serrano R (1999) Repressors and upstream repressing sequences of the stress-regulated ENA1 gene in Saccharomyces cerevisiae: bZIP protein Sko1p confers HOG-dependent osmotic regulation. Mol Cell Biol 19:537–546. https://doi.org/10.1128/mcb.19.1.537

Rauceo JM et al (2008) Regulation of the Candida albicans cell wall damage response by transcription factor Sko1 and PAS kinase Psk1. Mol Biol Cell 19:2741–2751. https://doi.org/10.1091/mbc.E08-02-0191

Rueda C, Cuenca-Estrella M, Zaragoza O (2014) Paradoxical growth of Candida albicans in the presence of caspofungin is associated with multiple cell wall rearrangements and decreased virulence. Antimicrob Agents Chemother 58:1071–1083. https://doi.org/10.1128/AAC.00946-13

Saito H, Posas F (2012) Response to hyperosmotic stress. Genetics 192:289–318. https://doi.org/10.1534/genetics.112.140863

Sellam A, van het Hoog M, Tebbji F, Beaurepaire C, Whiteway M, Nantel A (2014) Modeling the transcriptional regulatory network that controls the early hypoxic response in Candida albicans. Eukaryot Cell 13:675–690. https://doi.org/10.1128/EC.00292-13

Skrzypek MS, Binkley J, Binkley G, Miyasato SR, Simison M, Sherlock G (2017) The Candida genome database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. Nucleic Acids Res 45:D592–D596. https://doi.org/10.1093/nar/gkw924

Sobel JD (2016) Recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 214:15–21. https://doi.org/10.1016/j.ajog.2015.06.067

Sucher AJ, Chahine EB, Balcer HE (2009) Echinocandins: the newest class of antifungals. Ann Pharmacother 43:1647–1657. https://doi.org/10.1345/aph.1M237

Vasicek EM, Berkow EL, Bruno VM, Mitchell AP, Wiederhold NP, Barker KS, Rogers PD (2014) Disruption of the transcriptional regulator Cas5 results in enhanced killing of Candida albicans by Fluconazole Antimicrob. Agents Chemother 58:6807–6818. https://doi.org/10.1128/AAC.00064-14

Walker LA, Munro CA, de Bruijn I, Lenardon MD, McKinnon A, Gow NA (2008) Stimulation of chitin synthesis rescues Candida albicans from echinocandins. PLoS Pathog 4:e1000040. https://doi.org/10.1371/journal.ppat.1000040

Walker LA, Gow NA, Munro CA (2010) Fungal echinocandin resistance. Fungal Genet Biol 47:117–126. https://doi.org/10.1016/j.fgb.2009.09.003

Wiederhold NP (2009) Paradoxical echinocandin activity: a limited in vitro phenomenon? Med Mycol 47(Suppl 1):S369–375. https://doi.org/10.1080/13693780802428542

Xie JL et al (2017) The Candida albicans transcription factor Cas5 couples stress responses, drug resistance and cell cycle regulation. Nat Commun 8:499. https://doi.org/10.1038/s41467-017-00547-y