Zap1 Control of Cell-Cell Signaling in *Candida albicans* Biofilms

Shantanu Ganguly,¹ Andrew C. Bishop,² Wenjie Xu,¹ Suman Ghosh,³ Kenneth W. Nickerson,³ Frederick Lanni,¹ Jana Patton-Vogt,² and Aaron P. Mitchell¹*

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213; Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282; and School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0666

Received 4 August 2011/Accepted 25 August 2011

Biofilms of *Candida albicans* include both yeast cells and hyphae. Prior studies indicated that a *zap1ΔΔ* mutant, defective in zinc regulator Zap1, has increased accumulation of yeast cells in biofilms. This altered yeast-hypha balance may arise from internal regulatory alterations or from an effect on the production of diffusible quorum-sensing (QS) molecules. Here, we develop biosensor reporter strains that express yeast-specific *YWP1-RFP* or hypha-specific *HWP1-RFP*, along with a constitutive *TDH3-GFP* normalization standard. Seeding these biosensor strains into biofilms allows a biological activity assay of the surrounding biofilm milieu. A *zap1ΔΔ* biofilm induces the yeast-specific *YWP1-RFP* reporter in a wild-type biofilm strain, as determined by both quantitative reverse transcription-PCR (qRT-PCR) gene expression measurements and confocal microscopy. Remediation of the *zap1ΔΔ* zinc uptake defect through zinc transporter gene *ZRT2* overexpression reverses induction of the yeast-specific *YWP1-RFP* reporter. Gas chromatography-mass spectrometry (GC-MS) measurements of known organic QS molecules show that the *zap1ΔΔ* mutant accumulates significantly less farnesol than wild-type or complemented strains and that *ZRT2* overexpression does not affect farnesol accumulation. Farnesol is a well-characterized inhibitor of hypha formation; hence, a reduction in farnesol levels in *zap1ΔΔ* biofilms is unexpected. Our findings argue that a Zap1- and zinc-dependent signal affects the yeast-hypha balance and that it is operative in the low-farnesol environment of the *zap1ΔΔ* biofilm.

In addition, our results indicate that Zap1 is a positive regulator of farnesol accumulation.

Biofilms are complex microbial communities embedded in a matrix layer (10). Biofilm cells exhibit unique phenotypes compared to free-living planktonic cells (10). Microbial biofilms are common in nature and are a leading cause of human infection (11, 15). Therefore, it is important to identify the mechanisms that govern biofilm formation to better understand microbial ecology and disease.

Quorum-sensing (QS) molecules are extracellular metabolites that relay information about cell density and affect cell physiology (18, 25). These molecules assume vital importance in multicellular structures like biofilms. QS molecules were first identified in bacteria but are also found in eukaryotic microbes, such as *Candida albicans*, a major fungal pathogen and the focus of our study. Several QS molecules have been shown to affect cell physiology in *C. albicans* (18, 25). The roles of these molecules have been studied both with planktonic cells (4, 5, 13, 20) and in the context of biofilms (23, 28).

The accumulation and distribution of QS molecules in *C. albicans* biofilms play important roles in biofilm development. For example, the first discovered *C. albicans* QS molecule, farnesol, functions as an inhibitor of the transition from ovoid yeast cells to filamentous hyphal cells (4, 5, 13, 20). As yeast cells are less adherent than hyphal cells, it is believed that production of yeast cells in a mature biofilm, promoted by farnesol accumulation, leads to dispersal of the biofilm. Ultimately, dispersal *in vivo* leads to disseminated infection. Yeast cells released from a biofilm have novel properties, including increased virulence and drug tolerance, that augment the severity of biofilm-based *C. albicans* infections (10, 32).

Our study addresses the role of a *C. albicans* transcription factor, Zap1 (zinc-responsive activator protein; also called Csr1), which governs the balance of yeast and hyphal cells in biofilms. Zap1 has been identified as a regulator that is required for efficient hypha formation (22). It has considerable homology to the *Saccharomyces cerevisiae* zinc regulator ScZap1 (9, 34), and indeed, it also controls the expression of zinc transporters and other zinc-regulated genes (22, 27). Our interest in Zap1 is based on its role in biofilm structure: *zap1ΔΔ* mutant biofilms produce excess β-glucan, a component of the extracellular matrix, and also have increased accumulation of yeast cells (27). Direct Zap1 target genes, identified by chromatin immunoprecipitation, do not include known yeast- or hypha-specific genes (27), so the relationship between Zap1 and cellular morphogenesis seems indirect. Here, we provide evidence that Zap1 governs the accumulation of farnesol in biofilms. In addition, findings point to uptake of zinc itself as a determinant of cell-cell signaling.

**MATERIALS AND METHODS**

*Media*. *C. albicans* strains were grown at 30°C in YPD (2% glucose, 2% Bacto-peptone, 1% Bacto-yeast extract) for Ura* strains or YPD plus Ura (2% glucose, 2% Bacto-peptone, 1% Bacto-yeast extract, 80 μg/ml of uridine) for Ura* strains. Transformants were selected on complete supplemental medium (CSM) (MP Biomedicals, LLC) plates containing 2% glucose, 0.67% yeast nitrogen base (without amino acids), 2% Bacto-agar, and one of the following dropout media: CSM-URA, CSM-ARG-URA, or CSM-HIS (MP Biomedicals, LLC). Biofilms were grown in spider medium (10 g α-mannitol [Sigma], 10 g...
nutrient broth [BD Difco], 2 g K2HPO4 [Sigma] in 1 liter of distilled water) at 37°C.

**Plasmid and strain construction.** The reference (DAY185), zap1::ΔΔ (CJN1201), and zap1::ΔΔ+ (CIN193) strains used in the study have been previously described (7, 27). In addition, the ZRT1- and ZRT2-overexpressing strains CIN1651 and CIN1655 have been previously described (27). Newly constructed *C. albicans* reporter strains (Table 1) were derived from BWP17 (33). All primer sequences are listed in Table S5 in the supplemental material. The strain BWP17 was made Arg+ by addition of ARG4 at the native locus by transformation with the PCR product of primers SG272 and SG273. The strain SGH275, which contained RFP downstream of the YWP1 promoter, was designed by amplifying an RFP-URA3 cassette from plasmid pMG2169 (12), using primers SG228 and SG239, and transforming the PCR product into the Arg+ BWP17 derivative to target the cassette at the YWP1 locus. Similarly, strain SGH278, which contained RFP downstream of the YWP1 promoter, was designed by amplifying an RFP-URA3 cassette from plasmid pMG2169, using primers SG226 and SG257, and transforming the PCR product into BWP17 to target the cassette at the YWP1 locus. For construction of the normalization construct, plasmid pG636 was used. The plasmid was created by in vivo recombination in *S. cerevisiae* strain BY4741 [Arg+ (CJN1193)] strains used in the study have been previously described (13). Briefly, 50 ml of 48-hour biofilm medium depleted of cells was extracted with 20 ml of ethyl acetate (E196-4; Fisher Scientific) by vigorous mixing in a separatory funnel. The ethyl acetate (top) phase was concentrated using rotary evaporation. Samples were suspended in 2 ml of ethyl acetate and filtered through 0.2-µm nylon membrane filters (Whatman 7402-001) into GC vials. One microliter of each sample was injected into a Varian CP-3800 gas chromatograph in tandem with a Varian Saturn 2000 mass spectrometer (GC-MS). Varian Factor Four VF-5ms 30-m (CP8944) or 60-m (CP8960) capillary columns were used. The MS was in electron ionization (EI) mode, scanning from a range of 40 to 350 mass-to-charge ratio. The transfer line temperature was set to 280°C, and the trap temperature was set to 200°C. The following standards were used for identification and quantification of peaks: farnesol, Alfa Aesar 526-55-6; tyrosol, Maybridge SB01196DE; and phenethyl alcohol, Acros Organics 130180050. For the 30-m column, the flow rate was 1.0 ml/min, and the inlet temperature was set to 280°C. The temperature program was 80°C held for 2 min followed by a 60°C/min increase to 160°C, which was held for another 2 min. Finally, there was a 15°C/min increase in temperature until 300°C was reached, followed by a 5-min hold. The total run time was 24.33 min, and a 5-min solvent delay was used for the MS.

**Quantification of excreted alcohols in biofilm supernatants.** For quantification of alcohols in biofilm supernatants, cells were grown as biofilms in 50 ml of spider medium in 150-cm2 tissue culture flasks (Corning flask, tissue culture treated; catalog number 430823) in order to maximize the surface area of biofilm growth for gas chromatography–mass spectrometry (GC-MS). The cell debris was suspended in distilled water and vacuum filtered to be used for dry-weight estimation. Filters with vacuum-filtered cell pellets for each sample were allowed to bench dry in petri dishes and weighed regularly to calculate the biofilm dry weight. The final biofilm dry weights were determined by subtracting the weight of the filter before from the weight after collection of the biofilm cell pellet for each sample.

**Extraction and GC-MS analysis of secreted farnesol, phenethyl alcohol, tyrosol, and tryptophol** were performed by modification of a previously described method (13). Briefly, 50 ml of 48-hour biofilm medium depleted of cells was extracted with 20 ml of ethyl acetate (E196-4; Fisher Scientific) by vigorous mixing in a separatory funnel. The ethyl acetate (top) phase was concentrated using rotary evaporation. Samples were suspended in 2 ml of ethyl acetate and filtered through 0.2-µm nylon membrane filters (Whatman 7402-001) into GC vials. One microliter of each sample was injected into a Varian CP-3800 gas chromatograph in tandem with a Varian Saturn 2000 mass spectrometer (GC-MS). Varian Factor Four VF-5ms 30-m (CP8944) or 60-m (CP8960) capillary columns were used. The MS was in electron ionization (EI) mode, scanning from a range of 40 to 350 mass-to-charge ratio. The transfer line temperature was set to 280°C, and the trap temperature was set to 200°C. The following standards were used for identification and quantification of peaks: farnesol, Alfa Aesar A19316; tryptophol, Alfa Aesar 526-55-6; tyrosol, Maybridge SB01196DE; and phenethyl alcohol, Acros Organics 130180050. For the 30-m column, the flow rate was 1.0 ml/min, and the inlet temperature was set to 280°C. The temperature program was 80°C held for 2 min, followed by a 60°C/min increase to 160°C, which was held for another 2 min. Finally, there was a 15°C/min increase in temperature until 300°C was reached, followed by a 5-min hold. The total run time was 24.33 min, and a 5-min solvent delay was used for the MS.

**For the 60-m column, the flow rate was 1.5 ml/min, and the inlet temperature was set to 280°C.** The temperature program was 80°C held for 2 min, followed by a 60°C/min increase to 160°C, which was held for another 2 min. Finally, there was a 15°C/min increase in temperature until 300°C was reached, followed by a 5-min hold. The total run time was 19.67 min, and a 6-min solvent delay was used for the MS.

**For the 100-m column, the flow rate was 1.0 ml/min, and the inlet temperature was set to 280°C.** The temperature program was 80°C held for 2 min, followed by a 60°C/min increase to 160°C, which was held for another 2 min. Finally, there was a 15°C/min increase in temperature until 300°C was reached, followed by a 5-min hold. The total run time was 19.67 min, and a 6-min solvent delay was used for the MS.

**Individual peak areas** were identified from the GC-MS spectra based on the positions of the standards in the spectra. The peak area and known standard concentration value for each alcohol on the mass spectra were used to calculate the amounts of alcohol extracted from the biofilm supernatant. The efficiency of this ethyl acetate extraction was measured by performing the extraction on known concentrations of the alcohols in spider medium. The approximate percent recoveries for the alcohols were as follows: tyrosol, 33% ± 7%; farnesol,
CJN1201, CJN1193, CJN1651, and CJN1655 were added to each well at a final concentration of 75 μM in water, and equivalent amounts of DMSO were used for mock purging with argon, and a fresh stock solution was used each time before an experiment. The effects of farnesol under planktonic and biofilm conditions. The effects of farnesol on yeast and hyphal cell reporters under planktonic condition was carried by using a previously described method with the following modifications (8). Dimethyl sulfoxide (DMSO) (Fisher Scientific; D128-500) was used as a solvent (19) for making 2 M stock solutions of farnesol (Alfa Aesar A19316 and Sigma F203-25G). No difference in the biological activities of the farnesols from the two manufacturers was observed. These stocks were stored at −20°C after deoxygenation for 3 h in a UV-transparent plastic box under flowing argon gas, after which a standard long-wave UV light box was used to initiate GMA polymerization for 30 min with continued argon flow and with air cooling to prevent heat transfer from the light box to the specimens. After 30 min, polymerization was allowed to continue for several hours under argon at ambient temperature. GMA polymer formed in this way is a transparent glassy solid with a refractive index close to 1.5, which greatly reduces the opacity of the fixed biofilms. Specimens were imaged through the coverglass in an apical-to-basal direction by serial-focus optical-sectioning fluorescence microscopy carried out using a slit-scan confocal optical unit on a Zeiss Axiovert 200 microscope. A 40× 0.85-numerical-aperture (NA) oil immersion objective (Zeiss 461707 with ICS adapter 444907) provided sufficient working distance to image the base of all mounted biofilms. “Three-dimensional” (3D) image stacks corresponding to a 100- to 450-μm focus range in the Z plane were collected at 1.00-μm focus steps and 0.10-μm spatial resolution. Image processing was carried out using ImageJ software (http://rsbweb.nih.gov/ij/). Separate confocal image stacks obtained for each field of view using fluorescent/green fluorescent protein (GFP) and rhodamine/Cy3/red fluorescent protein (RFP) filter sets were registered axially and transversely. The images were then converted to 32-bit format for floating-point operations. Out-of-focus fluorescence was removed from each image by subtraction of a smoothed background, and a threshold of 0.1 was set in both the green and red stacks to exclude background pixels. Even with the index-matching afforded by poly-GMA embedding, attenuation of fluorescence with focus depth was evident in both the green and red stacks. Therefore, to quantify RFP reporter gene expression, the ratio image stack was computed through pixel-by-pixel division of the processed “red-channel” image stack by the processed “green-channel” image stack. The resulting red/green ratio image stack was then resliced to produce a side view stack showing the axial profile from apex to base. The side view stack was then projected onto a single plane and displayed in a 16-color spectral scale in which a low red/green ratio (low reporter expression) was coded blue and a high red/green ratio (high reporter expression) was coded red/white.

RESULTS
Yeast cell and hyphal biosensor reporter strain development. Microarray data had indicated that zap1ΔΔ mutant biofilms have altered levels of yeast- and hypha-specific gene RNA accumulation, in keeping with their increased numbers.
of yeast cells (27). The role of Zap1 in the yeast-hypha balance might be direct; previously identified Zap1 targets may include an uncharacterized regulator of cell morphology. A second model is that Zap1 governs the yeast-hypha balance indirectly through production of QS molecules. We sought to test this model by including wild-type biosensor reporter cell populations in zap1ΔΔ mutant biofilms. The reporter cells would allow an assay of the response of wild-type cells to the zap1ΔΔ mutant biofilm environment.

We developed the biosensor reporter strains as follows. The yeast cell reporter strain had an RFP coding region replacing one allele of the yeast-specific YWPI (yeast form wall protein) gene (14, 17, 29), so that RFP was fused to the YWPI promoter and 5′ region. The hyphal reporter strain had an RFP coding region replacing one allele of the hypha-specific HWPI (hyphal wall protein) gene (17, 29, 31), so that RFP was fused to the HWP1 promoter and 5′ region. Each strain also contained a copy of GFP fused to the constitutive TDH3 promoter as a normalization control. Thus, we could measure the RFP/GFP ratio, through gene expression or microscopy assays, to determine relative YWPI or HWP1 expression among the biosensor reporter cells. To verify the behavior of the reporter strains, we grew them under well-characterized planktonic conditions that favor yeast or hypha formation and analyzed gene expression by microscopy and quantitative RT-PCR. We observed (see Fig. S1 in the supplemental material) that the yeast cell reporter strain expressed RFP under yeast growth conditions and not hyphal growth conditions; it expressed the GFP control under both conditions. In addition, the hyphal reporter strain expressed RFP under hyphal growth conditions and not yeast growth conditions; again, GFP was expressed under both conditions. Quantitative RT-PCR assays (see Fig. S1 in the supplemental material), in which RFP RNA was normalized to GFP RNA for each strain, verified that the yeast cell reporter strain expressed RFP RNA at higher levels under yeast growth conditions than under hyphal growth conditions; the hyphal reporter strain did the opposite. The reporter strains thus behave as expected from prior studies of YWPI and HWP1 expression and of C. albicans morphogenesis.

We characterized the response of the reporter strains to farnesol under planktonic growth conditions. Prior studies had shown that exogenous farnesol inhibits hypha formation and hypha-specific gene expression (8, 20, 25). Exogenous farnesol, at concentrations reported to suppress hypha formation (75 μM and 200 μM farnesol [8]), repressed RFP fluorescence and RNA accumulation in the hyphal reporter strain and promoted RFP fluorescence and RNA accumulation in the yeast cell reporter strain (see Fig. S2 in the supplemental material). These observations confirm that the reporter strains manifest the expected responses to farnesol.

Control of intercellular signaling in biofilms by Zap1 and Zrt2. In order to assay the role of Zap1 in intercellular biofilm signaling, we seeded reporter strains into biofilms of the zap1ΔΔ mutant or the wild-type and complemented strains. The reporter strain comprised 20% of the population, a level that permitted reliable detection of reporter strain RNA. When the yeast cell reporter strain was seeded into biofilms, we observed approximately 2-fold-higher levels of YWPI-RFP RNA in biofilms dominated by the zap1ΔΔ mutant than in the zap1ΔΔ/+ complemented strain and the wild-type strain (Fig. 1). Confocal microscopy verified increased RFP expression by the reporter cells in zap1ΔΔ mutant biofilm compared to the wild-type biofilm (Fig. 2; see Video S6 in the supplemental material). Phase-contrast images indicated that yeast cells were found primarily in the basal layer of the biofilm (data not shown), as described previously for this in vitro model (10, 16). (Fixed embedded biofilms were used for microscopy because fixed samples have a nearly uniform refractive index, reducing light scattering and thus improving light penetration through thick biofilms.) We did not observe a significant difference in HWP1-RFP RNA levels when the hyphal reporter strain was seeded into such biofilms (see Fig. S3 in the supplemental material), probably because of the preponderance of hyphal cells in mature biofilms. These results indicate that the zap1ΔΔ biofilm environment includes signals that promote YWPI expression in wild-type cells.

We envisioned that the loss of Zap1 has phenotypic impact for two general reasons. First, there are the direct consequences of altered Zap1-regulated gene expression. Second, there are the consequences of zinc limitation. The latter point reflects the fact that Zap1 activates the expression of two genes specifying zinc transporter homologs, ZRT1 and ZRT2 (zinc-regulated transporter), and thus, a zap1ΔΔ mutant grows poorly on media with low zinc levels (22). We observed previously that overexpression of ZRT2, but not ZRT1, improves the growth of the zap1ΔΔ mutant on low-zinc medium (27). To test whether biofilm zinc limitation leads to an altered yeast-hypha balance, we assayed YWPI-RFP expression in the yeast cell reporter strain seeded into biofilms of zap1ΔΔ strains that overexpress ZRT1 or ZRT2. Overexpression of ZRT2, but not ZRT1, restored YWPI-RFP expression to levels observed in wild-type biofilms (Fig. 3). These results indicate that zinc limitation contributes to the altered yeast-hypha ratio that is induced in zap1ΔΔ biofilms.

Zap1 control of farnesol accumulation. Our above observations could be explained if Zap1 is required for normal accumulation of QS molecules, such as farnesol, tyrosol, tryptophol, and phenethyl alcohol (4, 5, 13, 20). We used GC-MS analysis of biofilm supernatants (13) to test this idea. Biofilms
were grown for 48 h to yield comparable biomasses (see Table S4 in the supplemental material). We observed that biofilms of the zap1ΔΔ mutant strain produced less farnesol per gram of biofilm biomass than the zap1ΔΔ+ complemented strain or the wild-type strain (Fig. 4A). Similar results were obtained under planktonic growth conditions (data not shown). No significant differences in the levels of tyrosol, tryptophol, and phenethyl alcohol were observed among the mutant, complemented, and wild-type strains (Fig. 4A), though there was a trend toward lower tyrosol levels in the mutant that was not significant. These results indicate that Zap1 is a positive regulator of farnesol production.

We then tested the hypothesis that ZRT2 overexpression in the zap1ΔΔ strain may impact the yeast reporter strain response through an effect on farnesol production. GC-MS analysis of biofilm supernatants indicated that ZRT2 overexpression did not alter the production of known QS molecules in the zap1ΔΔ background (Fig. 4B). Our results indicate that diminished farnesol production results from a Zap1 regulatory defect that is independent of the ZRT2 expression defect.

DISCUSSION

Biofilms are a significant context in which QS molecules function. In C. albicans, the yeast-hypha transition is a major target of QS control. Here, we have studied cell-cell signaling in biofilms formed by a zap1ΔΔ mutant, which have elevated yeast form cell contents. We implemented a biosensor approach, the use of wild-type reporter cells, which revealed that the increased propensity of the zap1ΔΔ mutant to form yeast cells is mediated by cell-cell signaling, as opposed to an internal cell-delimited regulatory circuit. GC-MS measurements of all known organic QS molecules yielded a surprising result: that the zap1ΔΔ mutant has greatly reduced levels of farnesol, a situation that would be expected to decrease the proportion of yeast cells. Interestingly, while Zap1 is required for full production of farnesol, it appears that zinc uptake has an additional effect on cell-cell signaling, one that is mediated by an unidentified molecule (Fig. 5).

Previously published microarray analysis (27) of the zap1ΔΔ mutant provides a simple explanation for its reduced levels of farnesol. The mutant strain CN1201 (zap1ΔΔ); the mutant strains overexpressing ZRT1, CJN1651 (TDH3-ZRT1 in zap1ΔΔ), and ZRT2, CJN1655 (TDH3-ZRT2 in zap1ΔΔ), and the reference wild-type strain, DAY185 (ZAP1/ZAP1), were grown with SGH281 (yeast cell reporter) at a ratio of 4 to 1. Quantitative RT-PCR expression analysis of the yeast reporter was carried out on total biofilm RNA extracted from 48-h mixed biofilms. The asterisk indicates that expression levels of the yeast cell reporter were significantly lower in the mixed biofilm with zap1ΔΔ overexpressing ZRT2 (P = 0.003) than in the zap1ΔΔ mutant.
It seems counterintuitive that the zap1ΔΔ mutant biofilm would accumulate excess yeast cells, given that it has low levels of farnesol. However, a similar paradox exists for the hyperfilamentous tup1ΔΔ and nrg1ΔΔ mutants (1). Specifically, we note that tup1ΔΔ and nrg1ΔΔ mutants grow almost exclusively as filamentous cells, yet they overproduce farnesol (21). The fact that cellular morphology does not always correlate with QS molecule production in mutant strains emphasizes the utility of our biosensor strain approach. The biosensor permits sampling of a biofilm environment independently of mutant cell morphology. Our re-
sults suggest that a potentially significant mechanism of \textit{C. albicans} cell-cell signaling has yet to be defined. A detailed comparison of the extracellular metabolome of wild-type, \textit{zap1}Δ/Δ, and \textit{zap1}Δ/Δ \textit{TDH3-ZRT2} strains should define relevant candidate molecules. It will be interesting to determine whether the molecule’s activity is manifested only in a low-farnesol environment.

How might Zap1 be related to the other transcription factors known to regulate farnesol production, \textit{NRg1} and \textit{Tup1}? Kebaara et al. have proposed that \textit{NRg1} and \textit{Tup1} may act in a feedback circuit \cite{21}. Specifically, farnesol induces \textit{Tup1} RNA and protein, and that in turn represses filamentous growth and further production of farnesol. We can fit Zap1 into that model with the suggestion that Zap1 may be a negative regulator of \textit{NRg1} or \textit{Tup1}. As expected from the model, \textit{NRg1} and \textit{TUP1} RNAs are upregulated 1.3-fold in biofilms of the \textit{zap1}Δ/Δ mutant compared to wild-type and complemented strains \cite{unpublished}. Indeed, for the yeast \textit{S. cerevisiae}, overexpression of \textit{S. cerevisiae} Zap1 (ScZap1) reduces \textit{ScTUP1} RNA levels, as expected if a negative Zap1-Tup1 relationship were evolutionarily conserved \cite{6}.

\section*{ACKNOWLEDGMENTS}
This work was supported by NIH grant R01 AI067703 to A.P.M. We acknowledge our late friend and colleague Mitchell E. Johnson for expert advice regarding the GC-MS analyses.

\section*{REFERENCES}
1. Biswas, S. P., Van Dijck, and A. Datta. 2007. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of \textit{Candida albicans}. Microbiol. Mol. Biol. Rev. 71:348–376.
2. Blankenship, J. R., S. Fanning, J. J. Hamaker, and A. P. Mitchell. 2010. An extensive circuitry for cell wall regulation in \textit{Candida albicans}. PLoS Pathog. 6:e1000752.
3. Brachmann, C. B., et al. 1998. Designer deletion strains derived from \textit{Saccharomyces cerevisiae} S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14:115–132.
4. Chen, H., and G. R. Fink. 2006. Feedback control of morphogenesis in fungi by aromatic alcohols. Genes Dev. 20:1150–1161.
5. Chen, H., M. Fujita, Q. Feng, J. Clardy, and G. R. Fink. 2004. Tyrosol is a quorum-sensing molecule in \textit{Candida albicans}. Proc. Natl. Acad. Sci. U. S. A. 101:5048–5052.
6. Chua, G., et al. 2006. Identifying transcription factor functions and targets by phagotrophic activation. Proc. Natl. Acad. Sci. U. S. A. 103:12045–12050.
7. Davis, D., J. E. Edwards, Jr., A. P. Mitchell, and A. S. Ibrahim. 2000. \textit{Candida albicans} RIM101 \textit{pH} response pathway is required for host-pathogen interactions. Infect. Immun. 68:3953–3959.
8. Davis-Hanna, A., E. Piispanen, L. I. Stave, and D. A. Hogan. 2008. Farnesol and dodecanol effects on the \textit{Candida albicans} \textit{Ras1-cAMP} signaling pathway and the regulation of morphogenesis. Mol. Microbiol. 67:47–62.
9. Eide, D. J. 2009. Homeostatic and adaptive responses to zinc deficiency in \textit{Saccharomyces cerevisiae}. J. Biol. Chem. 284:18565–18569.
10. Finkel, J. S., and A. P. Mitchell. 2011. Genetic control of \textit{Candida albicans} biofilm development. Nat. Rev. Microbiol. 11:99–118.
11. Fux, C. A., J. W. Costerton, P. S. Stewart, and P. Stoodley. 2005. Survival strategies of infectious biofilms. Trends Microbiol. 13:34–40.
12. Gerami-Nejad, M., K. Dulmage, and J. Berman. 2009. Additional cassettes for epitope and fluorescent fusion proteins in \textit{Candida albicans}. Yeast 26:399–406.
13. Ghosh, S., B. W. Kebaara, A. L. Atkin, and K. W. Nickerson. 2008. Regulation of aromatic alcohol production in \textit{Candida albicans}. Appl. Environ. Microbiol. 74:7211–7218.
14. Granger, B. L., M. L. Flenniken, D. A. Davis, A. P. Mitchell, and J. E. Cutler. 2005. Yeast wall protein 1 of \textit{Candida albicans}. Microbiology 151:1631–1644.
15. Hassan, F., X. L. Xess, X. Wang, N. Jain, and B. G. C. 2019. Biofilm formation in clinical \textit{Candida} isolates and its association with virulence. Microbes Infect. 11:753–761.
16. Hawser, S. P., and L. J. Douglas. 1994. Biofilm formation by \textit{Candida} species on the surface of catheter materials in vitro. Infect. Immun. 62:915–921.
17. Heilman, C. J., et al. 2011. Hyphal induction in the human fungal pathogen \textit{Candida albicans} reveals a characteristic wall protein profile. Microbiology 157:2297–2307.
18. Hogan, D. A. 2006. Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot. Cell 5:613–619.
19. Hogan, D. A., A. Vik, and R. Kolter. 2004. A Pseudomonas aeruginosa quorum-sensing molecule influences \textit{Candida albicans} morphology. Mol. Microbiol. 54:1212–1223.
20. Hornby, J. M., et al. 2001. Quorum sensing in the dimorphic fungus \textit{Candida albicans} is mediated by farnesol. Appl. Environ. Microbiol. 67:2982–2992.
21. Kebaara, B. W., et al. 2008. \textit{Candida albicans} \textit{Tup1} is involved in farnesol-mediated inhibition of filamentous-growth induction. Eukaryot. Cell 7:960–967.
22. Kim, M. J., M. Kil, J. H. Jung, and J. Kim. 2008. Roles of zinc-responsive transcription factor \textit{Csr1} in filamentous growth of the pathogenic yeast \textit{Candida albicans}. J. Microbiol. Biotechnol. 18:242–247.
23. Martins, M., et al. 2007. Morphogenesis control in \textit{Candida albicans} and \textit{Candida dubliniensis} through signaling molecules produced by planktonic and biofilm cells. Eukaryot. Cell 6:2429–2436.
24. Navarathna, D. H., et al. 2007. Effect of farnesol on a mouse model of systemic candidiasis, determined by use of a DPP3 knockout mutant of \textit{Candida albicans}. Infect. Immun. 75:1609–1618.
25. Nickerson, K. W., A. L. Atkin, and J. M. Hornby. 2006. Quorum sensing in dimorphic fungi: farnesol and beyond. Appl. Environ. Microbiol. 72:3805–3812.
26. Noble, C. J., and A. P. Mitchell. 2005. Regulation of cell-surface genes and biofilm formation by the \textit{C. albicans} transcription factor \textit{Bcr1p}. Curr. Biol. 15:1150–1155.
27. Noble, C. J., et al. 2009. Biofilm matrix regulation by \textit{Candida albicans} Zap1. PLoS Biol. 7:e1000133.
28. Ramage, G., S. P. Saville, B. L. Wickes, and J. L. Lopez-Ribot. 2002. Inhibition of \textit{Candida albicans} biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. 68:5459–5463.
29. Sohn, K., C. Urban, H. Brunner, and S. Rupp. 2003. \textit{EFG1} is a major regulator of cell wall dynamics in \textit{Candida albicans} as revealed by DNA microarrays. Mol. Microbiol. 47:89–102.
30. Spreghini, E., D. A. Davis, R. Subaran, M. Kim, and A. P. Mitchell. 2003. Roles of \textit{Candida albicans} \textit{Dfg5p} and \textit{Dew1p} cell surface proteins in growth and hypha formation. Eukaryot. Cell 2:746–755.
31. Staab, J. F., and P. Sundstrom. 1998. Genetic organization and sequence analysis of the hypha-specific cell wall protein gene \textit{HWPl} of \textit{Candida albicans}. Yeast 14:681–686.
32. Uppuluri, P., et al. 2010. Dispersion as an important step in the \textit{Candida albicans} biofilm developmental cycle. PLoS Pathog. 6:e1000828.
33. Wilson, R. B., D. Davis, and A. P. Mitchell. 1999. Rapid hypothesis testing with \textit{Candida albicans} through gene disruption with short homology regions. J. Bacteriol. 181:1868–1874.
34. Zhao, H., and D. J. Eide. 1997. Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in \textit{Saccharomyces cerevisiae}. Mol. Cell. Biol. 17:5044–5052.