



**HAL**  
open science

## Glucose promotes stress resistance in the fungal pathogen, *Candida albicans*

Alexandra Rodaki, Brice Enjalbert, Tim Young, Frank C Odds, Neil A.R. Gow, Alistair J.P Brown

### ► To cite this version:

Alexandra Rodaki, Brice Enjalbert, Tim Young, Frank C Odds, Neil A.R. Gow, et al.. Glucose promotes stress resistance in the fungal pathogen, *Candida albicans*. *Molecular Biology of the Cell*, 2009, 20 (22), pp.4845-4855. 10.1091/mbc.e09-01-0002 . hal-02146789v1

**HAL Id: hal-02146789**

**<https://hal.science/hal-02146789v1>**

Submitted on 4 Jun 2019 (v1), last revised 12 Jun 2019 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Glucose promotes stress resistance in the fungal pathogen,  
*Candida albicans*

Alexandra Rodaki<sup>1</sup>, Brice Enjalbert<sup>1§</sup>, Tim Young<sup>2</sup>  
Frank C. Odds<sup>1</sup>, Neil A.R. Gow<sup>1</sup>, Alistair J.P. Brown<sup>1\*</sup>

1. *Aberdeen Fungal Group*, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK.
  2. Discovery Biology, Pfizer Ltd, Sandwich, Kent CT13 9NJ, UK
- § Current address:  
UMR5504, UMR792 CNRS, INRA, INSA.  
ISBP/INSA, 31077 Toulouse, Cedex 4, France

\* Corresponding Author:  
Professor Alistair J P Brown  
Aberdeen Fungal Group  
School of Medical Sciences  
Institute of Medical Sciences  
University of Aberdeen  
Aberdeen AB25 2ZD, UK  
E: [al.brown@abdn.ac.uk](mailto:al.brown@abdn.ac.uk)  
T: 44-1224-555883  
F: 44-1224-555844

Database Accession Numbers: ArrayExpress accession number E-MEXP-1151

Running Title: Glucose promotes stress resistance in *C. albicans*

Key Words: *Candida albicans*; glucose responses; stress resistance; transcript profiling; oxidative stress; osmotic stress

## ABSTRACT

Metabolic adaptation, and in particular the modulation of carbon assimilatory pathways during disease progression, is thought to contribute to the pathogenicity of *Candida albicans*. Therefore, we have examined the global impact of glucose upon the *C. albicans* transcriptome, testing the sensitivity of this pathogen to wide-ranging glucose levels (0.01, 0.1 and 1.0%). We show that, like *S. cerevisiae*, *C. albicans* is exquisitely sensitive to glucose, regulating central metabolic genes even in response to 0.01% glucose. This indicates that glucose concentrations in the bloodstream (approximate range 0.05–0.1%) have a significant impact upon *C. albicans* gene regulation. However, in contrast to *S. cerevisiae* where glucose down-regulates stress responses, some stress genes were induced by glucose in *C. albicans*. This was reflected in elevated resistance to oxidative and cationic stresses and resistance to an azole antifungal agent. Cap1 and Hog1 probably mediate glucose-enhanced resistance to oxidative stress, but neither is essential for this effect. However, Hog1 is phosphorylated in response to glucose and is essential for glucose-enhanced resistance to cationic stress. The data suggest that, upon entering the bloodstream, *C. albicans* cells respond to glucose increasing their resistance to the oxidative and cationic stresses central to the armoury of immuno-protective phagocytic cells.

200 words

## INTRODUCTION

Effective responses to environmental change are fundamentally important for the survival of microbes. Environmental adaptation is particularly relevant for pathogenic microbes, which must counteract the defence systems of their host as well as tune their metabolism and stress homeostatic mechanisms to the complex microenvironments they encounter in the host.

*Candida albicans* is a major fungal pathogen of humans (Odds, 1988; Calderone, 2002). It exists as a commensal organism in the urogenital and gastrointestinal tracts and on the skin. It causes mucosal infections such as oral candidiasis and vaginitis in otherwise healthy individuals. Furthermore, it causes potentially fatal infections of the bloodstream and internal organs in severely immunocompromised patients. Therefore *C. albicans* can thrive in diverse and complex niches within its human host.

The ability of this yeast to respond effectively to its microenvironment must contribute to its success as a pathogen. Two main observations reinforce this view. Firstly, the disruption of certain metabolic or stress genes attenuates the virulence of *C. albicans*. For example, mutations that inactivate the glyoxylate cycle, gluconeogenesis, glycolysis or fatty acid beta-oxidation reduce virulence, albeit slightly in some cases (Lorenz and Fink, 2001; Barelle *et al.*, 2006; Piekarska, *et al.* 2006; Ramirez and Lorenz, 2007; Zhou and Lorenz, 2008). Therefore this yeast requires metabolic flexibility, particularly in its pathways of carbon assimilation if it is to display normal levels of virulence even in the classical mouse model of disseminated candidiasis. Similarly, the inactivation of catalase, superoxide dismutase or the Hog1 stress activated protein kinase (SAPK) attenuates the virulence of *C. albicans* (Wysong *et al.* 1998; Alonso-Monge *et al.* 1999; Hwang *et al.*, 2002; Martchenko *et al.* 2004; Fradin *et*

*al.* 2005). Therefore both stress signalling and stress protective functions also contribute to the virulence of *C. albicans*.

The second main observation that revealed the environmental flexibility of *C. albicans* arose through studies of gene regulation. Genome-wide expression profiling has revealed that *C. albicans* modulates its metabolism and activates specific stress responses upon host contact. For example, glycolytic genes are down-regulated and glyoxylate cycle and fatty acid  $\beta$ -oxidation genes are up-regulated when *C. albicans* cells are exposed to blood, macrophages or granulocytes (Fradin *et al.* 2003; 2005; Rubin-Berejano *et al.*, 2003; Lorenz *et al.*, 2004). Furthermore oxidative stress functions are up-regulated following phagocytosis by granulocytes (Fradin *et al.* 2005). Similar observations were made using more complex *ex vivo* and *in vivo* infection models (Thewes *et al.*, 2007; Zakikhany *et al.*, 2007). The metabolic and stress adaptation of *C. albicans* during the infection process has been confirmed by profiling the molecular behaviour of single yeast cells using diagnostic GFP fusions (Barelle *et al.*, 2006; Enjalbert *et al.*, 2007). However, this single cell approach has revealed that *C. albicans* populations infecting the kidney are highly heterogeneous with respect to their molecular behaviour. For example, only a small proportion of *C. albicans* cells infecting renal tissue activate the oxidative stress response (Enjalbert *et al.*, 2007). Also, some *C. albicans* cells infecting kidney appear to assimilate carbon via glycolysis whilst others appear to exploit the anabolic pathways of carbon metabolism (Barelle *et al.*, 2006).

One must define the molecular responses of *C. albicans* to specific environmental stimuli if one is to understand the heterogeneous behaviour of this pathogen within the complex microenvironments *in vivo*. We reasoned that the differential exposure of individual *C. albicans* cells to glucose might contribute to their heterogeneous metabolic behaviour *in vivo*. Therefore in this study we have examined the responses of *C. albicans* to glucose by genome-wide expression profiling. We have compared the responses of this pathogen to those

of the relatively benign yeast *Saccharomyces cerevisiae* because glucose responses are well characterised in this species.

Glucose has profound effects upon the metabolism and physiology of *S. cerevisiae*. In particular, metabolic pathways for the assimilation of alternative carbon sources are repressed in response to glucose. In addition fermentative metabolism is up-regulated, and respiratory metabolism is down-regulated. Ribosome biogenesis and other growth related functions are up-regulated in cells exposed to glucose, whereas stress responses are down-regulated. *S. cerevisiae* has evolved complex and partially redundant signalling mechanisms to regulate its glucose responses (for reviews see Gancedo, 1998; 2008; Johnston, 1999; Carlson, 1999; Thevelein and de Winde, 1999; Rolland *et al.*, 2001). These include the Ras-cAMP-protein kinase A, the Snf3-Rgt2 and the Snf1-Glc7-Reg1 signalling pathways (Gancedo, 2008). In particular, the Ras-cAMP-protein kinase A pathway plays a key role in the activation of ribosome biogenesis as well as in the down-regulation of stress responses (Gounalaki and Thireos, 1994; Gorner *et al.*, 1998; Stanhill *et al.*, 1999; Garreaux *et al.*, 2000).

The *S. cerevisiae* cell is exquisitely sensitive to low concentrations of glucose, up-regulating glycolysis and down-regulating alternative pathways of carbon assimilation in response to glucose concentrations as low as 0.01% (Yin *et al.*, 1996; 2003). This exquisite glucose sensitivity may have evolved because this yeast has adapted to cope with sudden transitions from famine to feast in the wild (Johnston, 1999). In contrast, the fungal pathogen *C. albicans* is thought to have evolved on warm-blooded animals (Odds, 1988). In the human host, blood glucose concentrations are maintained within homeostatic limits (0.05–0.1% glucose, equivalent to about 3-5 mM glucose) that are well below the concentrations that are often applied in experimental yeast cultures (1–2% glucose). Furthermore, in contrast to *S. cerevisiae* (Postma *et al.*, 1989), *C. albicans* has been classified as a Crabtree-negative yeast on the basis that it retains respiratory activity during growth at high glucose concentrations

(Aoki and Ito-Kuwa, 1982; Niimi *et al.*, 1988). Therefore, we reasoned that glucose responses might have diverged significantly in these pathogenic and benign yeasts. In this paper, we show that there are broad similarities between *C. albicans* and *S. cerevisiae* with regard to the impact of glucose on their transcriptomes, but that there are fundamental differences with respect to the impact of glucose upon stress responses in these yeasts.

## **MATERIALS AND METHODS**

### ***Strains and Growth Conditions.***

Strains used in this study are in Table 1. Strains were grown at 30°C in either YPD or YPLactate (2% Bacto-peptone and 1% yeast extract containing either 2% D-glucose or 2% lactate).

### ***Transcript Profiling.***

Transcript profiling was performed with the *C. albicans* strain, THE1 (Table 1) using procedures described for *S. cerevisiae* by Yin *et al.* (2003). *C. albicans* cells were grown to mid-log phase ( $OD_{600} = 0.5$ ) overnight in YPLactate at 30°C, re-inoculated into 200 ml fresh YPLactate at  $OD_{600} = 0.05$ , and re-grown at 30°C to mid-log phase ( $OD_{600} = 0.5$ ). Cultures were divided into four 50 ml cultures, and glucose added to a final concentration of 0%, 0.01% (= 0.56 mM), 0.1% (= 5.6 mM) or 1% (= 56 mM). Cells were harvested 30 min later and frozen rapidly in liquid N<sub>2</sub>. RNA was prepared as described before (Hauser *et al.*, 1998), and Cy3- and Cy5- labelled cDNA probes were prepared and hybridised against *C. albicans* microarrays (Eurogentec, Seraing, Belgium) as described previously (Enjalbert *et al.*, 2006). Slides were scanned using a ScanArray Lite scanner (PerkinElmer Life Sciences,

Beaconsfield, United Kingdom) and quantified using QuantArray software (version 2.0). Data analysis and normalisation were performed using GeneSpring (Silicon Genetics, Redwood City, CA), and statistical analyses were performed using SAM (Significance Analysis of Microarrays: Tusher *et al.*, 2001) with a false discovery rate of less than 10%. Expression ratios were calculated relative to control cells exposed to 0% glucose. Data from at least three independent biological replicates were used for analysis.

*C. albicans* gene annotations were obtained from CandidaDB (<http://genolistpasteur.fr/CandidaDB>; d'Enfert *et al.*, 2005) and the *Candida* Genome Database (CGD, <http://candidagenome.org>; Braun *et al.*, 2005). Functional categories for *C. albicans* genes were assigned using gene ontology resources at the *Saccharomyces* Genome Database (SGD, [www.yeastgenome.org/GOcontents.shtml](http://www.yeastgenome.org/GOcontents.shtml)).

The complete dataset is available in the Supplementary Data and in ArrayExpress ([www.ebi.ac.uk/microarray/](http://www.ebi.ac.uk/microarray/): experiment accession number E-MEXP-1151).

### ***Quantitative northern analysis.***

Quantitative northern blotting was performed to validate the transcript profiling data. RNA was isolated from *C. albicans* THE1 cells, fractionated on 1.5% agarose/formaldehyde gels and subjected to northern blotting as described previously (Brown *et al.*, 2001). Gene-specific probes were PCR amplified from genomic DNA (the primers are specified in Supplementary Data) and radiolabelled using the Ready-to-go<sup>TM</sup> dCTP labelling kit (GE Healthcare, Buckinghamshire, UK). Signals were quantified by phosphoimaging relative to *TEF3* mRNA levels, as described previously (Brown *et al.*, 2001).

### ***Western Blotting.***

Protein extracts were prepared from *C. albicans* and subjected to western blotting, as described before (Smith *et al.*, 2004). Hog1 activation was detected using phosphospecific phospho-p38 MAPK antibody (New England Biolabs, Hitchin, Hertfordshire, UK), followed by an HRP-labelled anti-rabbit IgG secondary antibody (Bethyl Laboratories Inc, Texas, USA) using ECL Plus<sup>TM</sup> Western blotting reagents (GE Healthcare). Membranes were stripped and reprobed with an anti-mammalian p38 antibody to control for loading (New England Biolabs).

### ***Stress Phenotypes.***

For all stress assays *C. albicans* strains were first grown overnight at 30°C in YPLactate to mid-log phase ( $OD_{600} = 0.5$ ). Cells were then subcultured into fresh YPLactate and re-grown to mid-log phase ( $OD_{600} = 0.5$ ). For oxidative stress, cultures were then split: one half receiving 1% glucose and control cells receiving 0% glucose. Cells were grown for a further 60 min, then exposed to 0, 0.4, 5, 10, 25 or 50 mM H<sub>2</sub>O<sub>2</sub> for 60 min, and then cell viability assayed (cfu's) relative to unstressed controls. Data represent means from three independent experiments.

For osmotic stress, YPLactate cultures were split, some cells being exposed to 1% glucose and control cells being exposed to 0% glucose. Cells were grown for a further 60 min, and then exposed for 60 min to 0.5 or 1 M NaCl, 0.6 M KCl, or 0.6 or 1.2 M sorbitol. Cell viability was then assayed (cfu's). Data represent means from three independent experiments.

Methods for miconazole treatment were adapted from Abbott and Odds (1989). Mid-log *C. albicans* cells grown on YPLactate were harvested by centrifugation, washed twice in dH<sub>2</sub>O and re-suspended in dH<sub>2</sub>O. Four ml of suspension (about  $8 \times 10^6$  cells/ml) were added to 4 ml of 0.2M citric acid, 0.2M sodium citrate, pH 6.2. The cells were treated for 10 min

with either 10 µg/ml miconazole (Sigma) or carrier (DMSO). Then either no carbon source, 1% lactate, or 1% glucose were added, and the cells incubated for a further 10 min at 30°C, whereupon viable cell numbers were determined as cfu's. The data represent means from three independent experiments.

## RESULTS

### ***C. albicans is exquisitely sensitive to glucose.***

Previously we examined the responses of *S. cerevisiae* to low (0.01%), medium (0.1%) and high (1%) levels of glucose (Yin *et al.* 2003). These genome-wide analyses revealed that *S. cerevisiae* is exquisitely sensitive to glucose, modulating metabolic gene expression even in response to low glucose concentrations. Glycolytic genes were up-regulated, and gluconeogenic and TCA cycle genes were down-regulated even when only 0.01% glucose was added to the growth medium. Ribosome biogenesis, on the other hand, was activated only in response to medium or high levels of glucose.

The first objective in our current study was to test the working hypothesis that *C. albicans* and *S. cerevisiae* respond differently to glucose. Therefore, to examine glucose responses in *C. albicans* we used an analogous experimental approach to our previous study in *S. cerevisiae*. Briefly, RNA was prepared from *C. albicans* cells 30 min after the addition of 0, 0.01%, 0.1% or 1% glucose to cultures growing exponentially on lactate. We chose this timepoint to replicate the conditions used successfully in our genome-wide analysis of glucose responses in *S. cerevisiae* (Yin *et al.* 2003). The *C. albicans* RNA was subjected to microarray analysis, measuring the effects of the zero, low, medium and high glucose

concentrations relative to the control containing zero glucose. Transcripts were analysed further if they displayed a statistically significant and reproducible change of  $\geq 1.5$ -fold in three independent replicate experiments as well as passing the statistical filter imposed by SAM (Statistical Analysis of Microarrays, with a false discovery rate of  $<10\%$ ) (Tuscher *et al.*, 2001). A total of 347 *C. albicans* genes were up-regulated, and 344 genes were down-regulated in response to at least one of the glucose concentrations examined (about 5% of the genome) (Supplementary Data). Of these, 170 genes were up-regulated and 180 genes were down-regulated by 0.01% glucose, indicating that about half of glucose-regulated genes are responsive to low glucose. These transcript profiling data were validated by northern analyses of selected *C. albicans* transcripts (not shown). Therefore, like *S. cerevisiae*, the *C. albicans* transcriptome is exquisitely sensitive to glucose, responding to concentrations as low as 0.01%.

***C. albicans and S. cerevisiae display significant differences in their transcriptomic responses to glucose.***

To compare the glucose responses of *C. albicans* and *S. cerevisiae* more directly, we selected genes that have orthologues in both yeasts (Supplementary Data; Enjalbert *et al.*, 2006). We then examined the behaviour of orthologues involved in central carbon metabolism, listing the responses of individual genes to each condition (Supplementary Data) and then calculating the mean response of a particular pathway to each glucose concentration (Figure 1A). The behaviour of specific metabolic pathways is presented in Figure 1B. In *C. albicans*, glycolytic genes were up-regulated, and gluconeogenic, glyoxylate cycle, TCA cycle and fatty acid  $\beta$ -oxidation genes were down-regulated following exposure to low, medium or high concentrations of glucose. Therefore, the expression of central metabolic pathways in *C. albicans* was regulated even in response to low glucose (0.01%).

While carbon metabolism genes behaved similarly in *C. albicans* and *S. cerevisiae*, ribosomal protein genes responded differently in these species. In *S. cerevisiae* ribosomal protein gene expression is up-regulated following glucose addition (Mager and Planta, 1991) and this was confirmed by the transcript profiling study of Yin *et al.* (2003). However, no significant increase in ribosomal protein gene expression was observed in *C. albicans* under equivalent conditions (Figure 1B). Almost certainly, differences in the growth of these species on YPLactate account for this contrasting behaviour. *S. cerevisiae* grew relatively slowly on YPLactate, the doubling time halving following addition of glucose to a concentration of 1%. As expected, ribosomal protein gene expression accelerated with growth in this yeast (Yin *et al.*, 2003). In contrast, *C. albicans* THE1 cells grew equally well on YP medium containing lactate or glucose ( $T_d = \sim 110$  min). Therefore there was no increase in ribosomal protein gene expression in this pathogen under these conditions (Figure 1B).

We then examined the glucose responses of *C. albicans* and *S. cerevisiae* more broadly by categorising glucose-regulated genes on the basis of their gene ontology (*Saccharomyces* Genome Database; <http://db.yeastgenome.org/cgi-bin/SGD/GO/goTermFinder>) and asking which functional categories were significantly up- or down-regulated by glucose (Figure 2). The functional categories ‘glycolysis’, ‘fermentation’ and ‘hexose transport’ were up-regulated in *C. albicans* and *S. cerevisiae*. Also ‘gluconeogenesis’, ‘tricarboxylic acid cycle’, ‘aerobic metabolism’ and ‘fatty acid metabolism’ were down-regulated in both species. In contrast, the functional categories ‘ribosome biogenesis’ and ‘protein synthesis’ behaved differently in *C. albicans* and *S. cerevisiae*, displaying no significant regulation or being down-regulated in the pathogen, but up-regulated in the benign yeast (Figure 2). Not surprisingly the behaviour of these functional categories reflected the behaviour of the individual genes in these categories (Figure 1).

The analysis of functional categories revealed some other interesting differences between *C. albicans* and *S. cerevisiae* (Figure 2). In response to glucose, ‘energy reserve metabolism’ was significantly up-regulated in *C. albicans*, but not *S. cerevisiae*. Further analysis of this functional category revealed that trehalose metabolism genes were up-regulated in *C. albicans* (eg *TPS1*, *TPS2*, *TPS3*), unlike *S. cerevisiae* (Figure 3). Trehalose is a well-known stress protectant. Therefore, this observation was consistent with the up-regulation of ‘response to oxidative stress’, and ‘response to drug’ in *C. albicans*, but not in *S. cerevisiae* (Figure 2). To look at this in more detail we examined behaviour of individual genes in these functional categories.

With respect to ‘response to drug’, three *C. albicans* genes belonging to the ATP binding cassette superfamily of transporters (eg *CDR1*, *CDR2*, *SNQ2*) and a member of the major facilitator family (*QDR1*) were up-regulated in response to all of the glucose concentrations tested. All of these genes are involved in responses to antifungal drugs or mutagens (Servos *et al.*, 1993; Decontignies *et al.*, 1995; Sanglard, *et al.*, 1995; 1997; Nunes *et al.*, 2001). With the exception of *PDR5* (the *S. cerevisiae* homologue of *CDR1*), which was slightly up-regulated in response to 0.1 and 1% glucose, the expression of the corresponding *S. cerevisiae* genes was unchanged in response to glucose (Yin *et al.*, 2003).

Three *C. albicans* genes important for the ‘response to oxidative stress’ were induced by glucose. *TRX1* and *TTR1* encode thio- and glutaredoxins involved in oxidative stress protection. The up-regulation of *CAP1* by glucose (Figure 3) was particularly noteworthy because this gene encodes a transcriptional activator required for resistance to oxidative stress as well as multidrug resistance (Alarco & Raymond, 1999). *YAP1* (the *S. cerevisiae* homologue of *C. albicans* *CAP1*) was down-regulated two-fold by glucose (Figure 3). The contrasting behaviour of these key transcription factors provided a mechanistic explanation

for the up-regulation of oxidative stress functions by glucose in *C. albicans* but not in *S. cerevisiae*.

‘Response to osmotic stress’ was not represented in our GO output because insufficient genes from this functional category were regulated by glucose. This was partly because this functional category contains a relatively large proportion of signalling functions that are regulated at post-transcriptional, rather than transcriptional levels. Therefore, upon further examination we realised that genes critical for the osmotic stress response in *C. albicans* were up-regulated by glucose. These included the *ENA22* (which encodes a sodium cation transporter) and the *GPD1* and *GPD2* (which encode glycerol-3-phosphate dehydrogenase isoenzymes required for synthesis of the osmolyte, glycerol) (Supplementary Data).

The above observations provided the first clue that *C. albicans* might differ significantly from *S. cerevisiae* with regard to the impact of glucose upon stress responses. Stress responses are down-regulated by glucose in *S. cerevisiae* (Gorner *et al.*, 1998; Garreaux *et al.*, 2000). In contrast, our transcript profiling data suggested that in *C. albicans* some stress responses might be up-regulated by glucose. We tested this by examining the impact of glucose upon the resistance of *C. albicans* to various stresses.

### ***Glucose increases the resistance of C. albicans to an azole antifungal.***

Our microarray experiments revealed a rapid increase in the levels of key transcripts involved in antifungal drug resistance in cells exposed to glucose. Therefore we wanted to test whether glucose causes a corresponding increase the resistance of *C. albicans* to azole antifungals. We selected miconazole because, unlike most azole antifungals, this drug exerts cidal effects upon *C. albicans* (Abbott and Odds, 1989). This allowed us to test whether exposure to glucose protects *C. albicans* from miconazole-induced killing. Cells were grown under equivalent conditions to the transcript profiling experiments (exponential phase in YPLactate), treated

with glucose for an hour, then exposed to 10 µg/ml miconazole for an hour, and cell viability assayed (Figure 4). Compared with control cells that were treated with water or fresh lactate, cells exposed to glucose displayed highly significant protection against miconazole-induced killing. Therefore, as well as inducing the expression of genes involved in drug resistance, glucose increases the resistance of *C. albicans* to miconazole.

***Glucose increases the resistance of C. albicans to oxidative stress.***

We then tested whether the effects of glucose upon the expression of oxidative stress genes have an impact upon the resistance of *C. albicans* to this type of environmental insult. Again, *C. albicans* cells were grown to exponential phase in YPLactate, exposed with glucose for an hour, and then the cells treated with various doses of hydrogen peroxide for an hour. The viability of these cells was compared to equivalent cells that were not exposed to glucose (Figure 5). Glucose significantly increased the resistance of *C. albicans* to high doses of oxidative stress (>10 mM hydrogen peroxide).

***Glucose increases the cation resistance of C. albicans.***

Our microarray analyses revealed that some osmotic stress genes are induced by glucose in *C. albicans*. Therefore, to test whether glucose affects osmotic stress tolerance, *C. albicans* was grown on lactate, exposed to glucose and then treated with various concentrations of sorbitol, NaCl or KCl. Once again, the viability of these cells was compared with control cells that were not exposed to glucose (Figure 6). Glucose did not affect the resistance of *C. albicans* to sorbitol. However, resistance to both NaCl and KCl was increased following glucose exposure. Therefore, glucose affects cationic stress resistance, rather than osmotic stress resistance, in this pathogenic yeast.

This observation was consistent with our finding that a small set of osmotic stress genes was induced by glucose in our microarray experiments. This set included *ENA22*, a homologue of the *S. cerevisiae* *ENA2* gene which encodes a sodium transporter that contributes to salt tolerance (Rodriguez-Navarro *et al.*, 1994).

### ***Roles of Cap1, Hog1, Ras1 and alternative oxidase in glucose-enhanced peroxide stress resistance.***

The bZIP transcription factor, Cap1, activates oxidative stress genes via YRE elements in their promoters and is required for oxidative stress resistance in *C. albicans* (Alarco & Raymond, 1999; Nicholls *et al.*, 2004). Also, *CAP1* transcript levels are elevated in glucose-treated *C. albicans* cells (Figure 3), suggesting that Cap1 contributes to glucose-enhanced oxidative stress resistance (Figure 5). Therefore we tested whether Cap1 is required for this phenomenon. Congenic *C. albicans* wild type (CAI4 and RM1000) and *cap1* $\Delta$  strains were pre-treated with glucose and then exposed to a high dose of peroxide (25 mM). The inactivation of Cap1 did not affect the ability of glucose to increase peroxide resistance (Figure 7). Therefore, while Cap1 might contribute to this effect, it is not required for glucose-enhanced peroxide resistance.

The Hog1 SAPK also contributes to oxidative stress resistance in *C. albicans* (Alonso-Monge *et al.*, 2003; Smith *et al.*, 2004). Therefore we tested whether Hog1 is required for glucose-enhanced peroxide resistance. The peroxide resistance of congenic wild type and *hog1* $\Delta$  strains was compared, revealing that, Hog1 is not essential glucose-enhanced peroxide resistance, although *hog1* $\Delta$  cells displayed reduced resistance to this oxidative stress.

In *S. cerevisiae*, the Ras-cAMP signalling pathway is activated by glucose leading to the down-regulation of oxidative stress resistance through down-regulation of the transcription factors, Yap1, Msn2 and Msn4 (Gounalaki and Thireos, 1994; Stanhill *et al.*, 1999). The

functions of Msn2/4 homologues have diverged in *C. albicans* and are not involved in oxidative stress responses in this pathogen (Nicholls *et al.*, 2004; Ramsdale *et al.*, 2008). Also the *C. albicans* homologue of *S. cerevisiae* Yap1 was not required for glucose-enhanced peroxide resistance (Figure 7). Nevertheless, Ras-cAMP signalling has been reported to affect the expression of stress genes and influence stress sensitivity in *C. albicans* (Harcus *et al.*, 2004; Bahn *et al.*, 2007; Wilson *et al.*, 2007). Therefore, it was conceivable that Ras-cAMP signalling might mediate the effects of glucose on peroxide resistance in *C. albicans*. Therefore we tested whether this effect was dependent upon Ras1 (Figure 7). *Ras1Δ* cells were more sensitive to this oxidative stress. However, glucose still increased the resistance of these *ras1Δ* cells to peroxide, indicating that this effect was not dependent upon Ras-cAMP signalling.

The alternative oxidases encoded by *AOX1a* and *AOX1b* contribute to a cyanide-resistant respiratory pathway in *C. albicans* (Huh and Kang, 2001). It has been suggested that this pathway helps to protect *C. albicans* against endogenous reactive oxygen species generated by mitochondrial respiratory activity (Huh and Kang, 2001). We reasoned that the addition of glucose might cause a respiratory burst that would lead to the production of reactive oxygen species via the alternative oxidases Aox1a and Aox1b. According to this model, this would trigger an oxidative stress response that would protect against a subsequent exposure to high levels of peroxide. However, we were unable to detect an increase in the levels of intracellular reactive oxygen species following the addition of glucose to lactate-grown *C. albicans* cells (not shown). Furthermore, the inactivation of Aox1a and Aox1b did not block glucose-enhanced peroxide resistance (Figure 7). Therefore the generation of reactive oxygen species via a respiratory burst does not appear to account for the observed increase in peroxide resistance following glucose exposure.

***Hog1 is required for glucose-enhanced cationic stress resistance.***

Exposure to glucose increases the resistance of *C. albicans* to cationic stress (Figure 6). In *S. cerevisiae* and *C. albicans*, adaptation to cationic stress occurs through the evolutionarily conserved Hog1 SAPK pathway (Brewster *et al.*, 1993; San-Jose *et al.*, 1996; Smith *et al.*, 2004). Therefore we tested whether glucose stimulates osmotic stress resistance via this signalling pathway. *C. albicans*  $\Delta$ *hog1* cells did not display the glucose-simulated cationic stress resistance that was observed for their isogenic parental strain RM1000 (Figure 8). Therefore, Hog1 is required for this effect.

If Hog1 mediates the effects of glucose upon cationic stress resistance, then one would expect this MAP kinase to become activated following glucose exposure. Therefore we examined the phosphorylation status of Hog1 in glucose-treated *C. albicans* cells by western blotting with a phospho-specific anti-Hog1 antibody (Figure 9). Two control strains were examined in these experiments: RM1000 was the isogenic parent of the  $\Delta$ *hog1* mutant, and THE1 was the *C. albicans* strain used for the transcript profiling experiments. Hog1 phosphorylation was observed in both of these strains in response to a control cationic stimulus. As expected, Hog1 phosphorylation was not observed in control  $\Delta$ *hog1* cells. Significantly, Hog1 phosphorylation was observed in glucose-treated cells (Figure 9), indicating that Hog1 is activated in response to glucose. These observations are consistent with the idea that the Hog1 pathway is activated in response to glucose, thereby protecting *C. albicans* cells against subsequent exposure to a cationic stress.

## DISCUSSION

*C. albicans* and *S. cerevisiae* inhabit contrasting niches, and therefore in the wild these pathogenic and relatively benign yeasts are likely to experience different patterns of glucose exposure. Hence we predicted that these yeasts might have evolved different responses to glucose. We tested this prediction by examining the global responses of *C. albicans* to low (0.01%), medium (0.1%) and high concentrations of glucose (1%) and comparing these responses to those of *S. cerevisiae* under equivalent conditions (Yin *et al.*, 2003). A number of notable conclusions may be drawn from our observations.

Firstly, we conclude that, like *S. cerevisiae*, *C. albicans* is exquisitely sensitive to low concentrations of glucose in the environment. Dramatic changes in the *C. albicans* transcriptome were observed within 30 min of cells being exposed to 0.01% glucose. This concentration is significantly lower than the levels that are homeostatically maintained in human blood (3–5mM; equivalent to about 0.06 – 0.1% glucose) (Figures 1 and 2). Therefore, *C. albicans* is able to detect and respond to the levels of glucose present in the blood during disseminated haematological infections. Not surprisingly, diabetic patients have an increased risk of systemic *Candida* infections (Odds, 1988), and dietary glucose enhances *C. albicans* colonization and invasion (Vargas *et al.*, 1993).

Secondly, we found that *C. albicans* cells modulate the expression of metabolic genes even when they are exposed only to 0.01% glucose. Like *S. cerevisiae* (Yin *et al.*, 2003), *C. albicans* down-regulates genes involved in gluconeogenesis, the TCA and glyoxylate cycles, and alternative pathways of carbon assimilation, whilst glycolytic and fermentation genes are up-regulated (Figure 1). Therefore *C. albicans* regulates the metabolic genes involved in carbon assimilation even in response to low glucose signals.

These observations appear to contradict earlier reports that *C. albicans* is a glucose-insensitive Crabtree-negative yeast. This definition was based on the observation that *C. albicans* continues to respire in the presence of glucose (Aoki and Ito-Kuwa, 1982; Niimi *et al.*, 1988). However it is clear that *C. albicans* is glucose-sensitive in that even low levels of glucose trigger major changes in the transcriptome (Figures 1 and 2: Supplementary Data). Our conclusion is supported by analyses of specific glucose-regulated genes (*eg* Leuker *et al.*, 1997; Munro *et al.* 2001; Barelle *et al.* 2006; Ramirez and Lorenz, 2007) and by a microarray study of carbon starvation in *C. albicans* (Lorenz *et al.*, 2004). Furthermore, *C. albicans* morphogenesis can be stimulated by glucose concentrations of between 0.025 and 0.25% (Hudson *et al.*, 2004; Maidan *et al.*, 2005). Although these morphogenetic changes are not relevant to our current study (because our cultures were grown at 30°C, not 37°C), these other studies reinforce the view that *C. albicans* is a glucose-sensitive yeast.

Thirdly, *C. albicans* and *S. cerevisiae* display fundamental differences with regard to the impact of glucose upon their stress responses. Glucose down-regulates stress responses in *S. cerevisiae*. In this yeast glucose activates Ras-cAMP signalling, leading to protein kinase A-mediated phosphorylation of the transcription factors Msn2 and Msn4, thereby inhibiting their nuclear accumulation and preventing the activation of stress genes that carry the cognate general stress response element (STRE) in their promoters (Garreaux *et al.*, 2000; Gorner *et al.*, 2002). Ras-cAMP signalling also regulates *YAPI* (Gounalaki and Thireos, 1994; Stanhill *et al.*, 1999) which encodes an AP1-like transcription factor that contributes to the global transcriptional response to oxidative stress and is essential for resistance to such stresses (Stephen *et al.*, 1995; Cohen *et al.*, 2002). Glucose also represses the expression of some stress genes such as *ENA1* (Alepez *et al.*, 1997). Ena1 is a P-type ATPase Na<sup>+</sup> pump, the inactivation of which increases the sensitivity of *S. cerevisiae* cells to cationic stresses (Haro *et al.*, 1991). Therefore in *S. cerevisiae*, stress responses are down-regulated by glucose via

several signalling mechanisms (Figure 5). In contrast we found that some *C. albicans* stress genes are up-regulated by glucose (Figures 2 and 3), and that these transcriptomic changes are reflected in an increased resistance to cationic stress, oxidative stress and an azole antifungal drug (Figures 4-6).

How does glucose mediate these changes in stress sensitivity in *C. albicans*? Ras1 is not essential for these effects (Figure 7), reinforcing the view that glucose signalling in this pathogen differs significantly from its distant benign relative, *S. cerevisiae*. Furthermore the effects of glucose upon stress resistance are not dependent upon an alternative oxidase-mediated respiratory burst (Figure 7). Instead Cap1 and Hog1 probably mediate the impact of glucose upon oxidative stress and drug resistance. The expression of *CAP1*, which is required for oxidative stress and drug resistance (Alarco and Raymond, 1999), was up-regulated by glucose (Figure 3). Hog1, which also contributes to oxidative stress resistance (Alonso-Monge *et al.*, 2003; Smith *et al.*, 2004), was activated by glucose (Figure 9). We favour the idea that Cap1 and Hog1 fulfil partially redundant roles in mediating glucose-enhanced oxidative stress resistance (Figure 10), as this would explain why neither Cap1 nor Hog1 is essential for this effect (Figure 7).

The impact of glucose upon cationic stress resistance appears to be mediated through Hog1. Glucose stimulates the phosphorylation and activation of *C. albicans* Hog1 (Figure 9). Furthermore, the inactivation of Hog1 blocks glucose-enhanced cationic stress resistance (Figure 8). Previously we showed that in *C. albicans*, the *ENA21*, *GPD2*, *TPS2* and *TPS3* genes are up-regulated in response to osmotic stress, and that Hog1 is required for their activation (Enjalbert *et al.*, 2006). Therefore it is already known that Hog1 mediates the up-regulation of genes encoding cation transporters and enzymes involved in glycerol and trehalose biosynthesis in response to an osmotic stress. We now suggest that cation transporters and glycerol and trehalose biosynthetic enzymes are up-regulated in response to

glucose through Hog1, thereby protecting *C. albicans* from subsequent exposure to cationic stress.

It is well known that Cap1 and Hog1 are required for osmotic and oxidative stress resistance (San Jose *et al.*, 1996; Alarco and Raymond, 1999; Alonso-Monge *et al.*, 2003; Smith *et al.*, 2004). However we noted that *cap1* and *hog1* cells did not display decreased resistance to the osmotic and oxidative stresses examined in this study compared with their isogenic controls (Figures 7 and 8). Differences in the stress resistance assays almost certainly account for this apparent contradiction. Previous studies assayed resistance by monitoring growth on plates over days in the presence of stress. In contrast, to investigate the immediate effects of glucose, we assayed cell viability after one hour of exposure to each stress. We suggest that while Cap1 and Hog1 are required for the adaptive responses that allow *C. albicans* cells to recover and grow in the presence of osmotic and oxidative stresses, these regulators are not essential for the immediate responses of *C. albicans* to these stresses.

Our findings indicate that *C. albicans* has evolved molecular mechanisms that link glucose responses to oxidative and cationic stress resistance. We suggest that this might be of relevance to the infection process. For example, when invasive *C. albicans* cells enter the bloodstream they will become prone to attack by blood-borne phagocytes. These phagocytes generate an oxidative burst that is a primary line of defence against *Candida* infections (Sasada and Johnston, 1980; Murphy, 1991; Vasquez-Torres and Balish, 1997). Neutrophil killing also depends upon the influx of cations into the phagocytic vacuole (Reeves *et al.*, 2002). However upon entry into the bloodstream, *C. albicans* cells will also become exposed to glucose. Our data indicate that this glucose exposure will increase the resistance of these *C. albicans* cells to oxidative and cationic stresses, and hence may protect them against immediate attack from phagocytic leukocytes.

Our findings also help to account for the high degree of metabolic heterogeneity of *C. albicans* populations growing in infected tissue (Barelle *et al.*, 2006). The majority of *C. albicans* cells infecting the mouse kidney express glycolytic genes, suggesting that they assimilate carbon primarily via glycolysis. However a significant proportion of these cells exhibit gluconeogenic growth (Barelle *et al.*, 2006). Our data suggest that the differential exposure of *C. albicans* cells to glucose within these complex microenvironments probably contributes to this metabolic heterogeneity.

## **ACKNOWLEDGEMENTS**

We are grateful for the financial support for this work from the UK Biotechnology and Biological Sciences Research Council (BBS/B/06670; BBS/S/B/2003/10842), the Wellcome Trust (063204; 080088), the European Commission (MRTN-CT-2003-504148) and Pfizer.

## **REFERENCES**

- Abbott, A.B., and Odds, F.C. (1989). Abrogation by glucose of the ATP suppression induced by miconazole in *Candida albicans*. *J. Antimicrobial Chemotherapy* 24, 905-919.
- Alarco, A.M., and Raymond, M. (1999). The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. *J. Bacteriol.* 181, 700-708.

Alepuz, P.M., Cunningham, K.W., and Estruch, F. (1997). Glucose repression affects ion homeostasis in yeast through the regulation of the stress-activated *ENAI* gene. *Molec. Microbiol.* *26*, 91-98.

Alonso-Monge, R., Navarro-Garcia, F., Molero, G., Diez-Orejas, R., Gustin, M., Pla, J., Sanchez, M., and Nombela, C. (1999). Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. *J. Bacteriol.* *181*, 3058-3068.

Alonso-Monge, R., Navarro-Garcia, F., Roman, E., Negredo, A.I., Eisman, B., Nombela, C., and Pla, J. (2003). The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamyospore formation in *Candida albicans*. *Eukaryotic Cell* *2*, 351-361.

Aoki, S., and Ito-Kuwa, S. (1982) Respiration of *Candida albicans* in relation to its morphogenesis. *Plant Cell Physiol.* *23*, 721-726.

Bahn, Y.-S., Molenda, M., Staab, J.F., Lyman, C.A., Gordon, L.J., and Sundstrom, P. (2007). Genome-Wide Transcriptional Profiling of the Cyclic AMP-Dependent Signaling Pathway during Morphogenic Transitions of *Candida albicans*. *Eukaryotic Cell* *6*, 2376–2390.

Barelle, C.J., Priest, C.L., MacCallum, D.M. Gow, N.A.R., Odds, F.C., and Brown, A.J.P. (2006). Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cellular Microbiol.* *8*, 961-971.

Braun, B.R., van het Hoog, M., d'Enfert, C., Martchenko, M., Dungan, J., Kuo, A., Inglis, D.O., Uhl, A.M., Hogues, H., Berriman, M., Lorenz, M., Levitin, A., Oberholzer, U., Bachewich, C., Harcus, D., Marcil, A., Dignard, D., Iouk, T., Zito, R., Frangeul, L., Tekaia, F., Rutherford, K., Wang, E., Munro, C.A., Bates, S., Gow, N.A., Hoyer, L.L., Kohler, G., Morschhauser, J., Newport, G., Znaidi, S., Raymond, M., Turcotte, B., Sherlock, G.,

- Constanzo, M., Ihmels, J., Berman, J., Sanglard, D., Agabian, N., Mitchell, A.P., Johnson, A.D., Whiteway, M., and Natel, A. (2005) A human-curated annotation of the *Candida albicans* genome. *PLoS Genetics* 1, 36-57.
- Brewster, J.L., de Valoir, T., Dwyer N.D., Winter E., and Gustin, M.C. (1993). An osmosensing signal transduction pathway in yeast. *Science* 259, 1760–1763.
- Brown, A.J.P., Planta, R.J., Restuhadi, F., Bailey, D.A., Butler, P.R., Cadahia, J.L., Cerdan, M.E., De Jonge, M., Gardner, D.C.J., Gent, M.E., Hayes, A., Kolen, C.P.A.M., Lombardia, L.J., Murad, A.M.A., Oliver, R.A., Sefton, M., Thevelein, J., Tournu, H., van Delft, Y.J., Verbart, D.J., Winderickx, J., and Oliver, S.G. (2001). Transcript analysis of 1003 novel yeast genes using high-throughput northern hybridisations. *EMBO. J.* 20, 3177-3186.
- Calderone, R.A. (2002). *Candida and Candidiasis*. ASM Press, Washington, D.C.
- Carlson, M. (1999). Glucose repression in yeast. *Curr. Opin. Microbiol.* 2, 202-207.
- Cohen, B.A., Pilpel, Y., Mitra, R.D., and Church, G.M. (2002). Discrimination between paralogs using microarray analysis: application to the Yap1p and Yap2p transcriptional networks. *Mol. Biol. Cell* 13, 1608-1614.
- Decottignies, A., Lambert, L., Catty, P., Degand, H., Eppings, E.A., Moye-Rowley, S.W., Balzi, E., and Goffeau, A. (1995). Identification and characterisation of SNQ2, a new multidrug ATP binding cassette transporter of yeast plasma membrane. *J. Biol. Chem.* 270, 18150-18157.
- Enjalbert, B., Smith, D.A., Cornell, M.J., Alam, I., Nicholls, S., Brown, A.J.P., and Quinn, J. (2006). Role of the Hog1 Stress-Activated Protein Kinase in the Global Transcriptional

Response to Stress in the Fungal Pathogen *Candida albicans*. *Molec. Biol. Cell* 17, 1018-1032.

Enjalbert, B., MacCallum, D., Odds, F.C., and Brown, A.J.P. (2007). Niche-specific activation of the oxidative stress response by the pathogenic fungus *Candida albicans*. *Infection & Immunity* 75, 2143-2151.

d'Enfert, C., Goyard, S., Rodriguez-Arnaveilhe, S., Frangeul, L., Jones, L., Tekaiia, F., Bader, O., Castillo, L., Dominguez, A., Ernst, J., Fradin, C., Gaillardin, C., Garcia-Sanchez, S., de Groot, P., Hube, B., Klis, F., Krishnamurthy, S., Kunze, D. Lopez, M.-C., Mavor, A., Martin, N., Moszer, I., Onésime, D., Perez Martin, J., Sentandreu, R., and Brown, A.J.P. (2005). CandidaDB : a genome database for *Candida albicans* pathogenomics. *Nucleic Acids Res.* 33, D353-D357.

Feng, Q., Summers, E., Guo, B., and Fink, G.R. (1999). Ras signaling is required for serum-induced hyphal differentiation in *Candida albicans*. *J. Bacteriol.* 181, 6339-6346.

Fonzi, W.A. and Irwin, M.Y. (1993) Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134: 717-728.

Fradin, C., Kretschmar, M., Nichterlein, T., Gaillardin, C., d'Enfert, C., and Hube, B. (2003). Stage-specific gene expression of *Candida albicans* in human blood. *Molec. Microbiol.* 47, 1523-1543.

Fradin, C., De Groot, P., MacCallum, D., Schaller, M., Klis, F., Odds, F.C., and Hube, B. (2005). Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Molec. Microbiol.* 56, 397-415.

Gancedo, J.M. (1998). Yeast carbon catabolite repression. *Microbiol. Molec. Biol. Rev.* 62, 334-361.

Gancedo, J.M. (2008). The early steps of glucose signaling in yeast. *FEMS Microbiol. Rev.* 32, 673–704.

Garreau, H., Hasa, R.N., Renault, G., Estruch, F., Boy-Marcotte, E., and Jacquet, M. (2000). Hyperphosphorylation of Msn2 and Msn4 in response to heat shock and the diauxic shift is inhibited by cAMP in *Saccharomyces cerevisiae*. *Microbiol.* 146, 2113-2120.

Gorner, W., Durchschlag, E., Martinez-Pastor, M.T., Estruch, F., Ammerer, G., Hamilton, B., Ruis, H., and Schuller, C. (1998). Nuclear localisation of the C<sub>2</sub>H<sub>2</sub> zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Devel.* 12, 586-597.

Gorner, W., Durchschlag, E., Wolf, J., Brown, E.L., Ammerer, G., Hamilton, B., Ruis, H., and Schuller, C. (2002). Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *EMBO J.* 21, 135-144.

Gounalaki, N., and Thireos, G. (1994). Yap1p, a yeast transcriptional activator that mediates multidrug resistance, regulates the metabolic stress response. *EMBO J.* 13, 4036–4041.

Harcus, D., Nantel, A., Marcil, A., Rigby, T., and Whiteway, M. (2004). Transcription profiling of cyclic AMP signaling in *Candida albicans*. *Molec. Biol. Cell* 15, 4490-4499.

Haro R., Garciadeblas, B., and Rodriguez-Navarro, R. (1991). A novel P-type ATPase from yeast involved in sodium transport. *FEBS Lett.* 291, 189-191.

Hauser, N.C., Vingron, M., Scheideler, M., Krems, B., Hellmuth, K., Entian, K.-D., and Hoheisel, J.D. (1998). Transcriptional profiling on all open reading frames of *Saccharomyces cerevisiae*. *Yeast* 14, 1209-1221.

- Hudson, D.A., Sciascia, Q.L., Sanders, R.J., Norris, G.E., Edwards, P.J.B., Sullivan, P.A., and Farley, P.C. (2004). Identification of the dialysable serum inducer of germ-tube formation in *Candida albicans*. *Microbiol.* *150*, 3041–3049.
- Huh, W.-K., and Kang, S.-O. (2001). Characterization of the gene family encoding alternative oxidase from *Candida albicans*. *Biochem. J.* *356*, 595-604.
- Hwang, C.S., Rhie, G.E., Oh, J.H., Huh, W.K., Yim, H.S., and Kang, S.O. (2002). Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of *Candida albicans* against oxidative stresses and the expression of its full virulence. *Microbiol.* *148*, 3705-3713
- Johnston, M. (1999). Feasting, fasting and fermenting. *Trends Genet.* *15*, 29-33.
- Leuker, C.E., Sonneborn, A., Delbruck, S., and Ernst, J.F. (1997). Sequence and promoter regulation of the *PCK1* gene encoding phosphoenolpyruvate carboxykinase of the fungal pathogen *Candida albicans*. *Gene* *192*, 235-240.
- Lorenz, M.C., and Fink, G.R. (2001). The glyoxylate cycle is required for fungal virulence. *Nature* *412*, 83-86.
- Lorenz, M.C., Bender, J.A., and Fink, G.R. (2004). Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryotic Cell* *3*, 1076-1087.
- Mager, W.H., and Planta, R.J. (1991). Coordinate expression of ribosomal protein genes in yeast as a function of cellular growth rate. *Molec. Cell. Biochem.* *104*, 181-187.
- Maidan, M.M., Thevelein, J.M., and Van Dijck, P. (2005). Carbon source induced yeast-to-hypha transition in *Candida albicans* is dependent on the presence of amino acids and on the G-protein-coupled receptor Gpr1. *Biochem. Soc. Trans.* *33*, 291-293.

- Martchenko, M., Alarco, A.M., Harcus, D., and Whiteway, M. (2004). Superoxide dismutases in *Candida albicans*: transcriptional regulation and functional characterization of the hyphal-induced SOD5 gene. *Molec. Biol. Cell* 15, 456-467.
- Munro, C.A., Winter, K., Buchan, A., Henry, K., Becker, J.M., Brown, A.J.P., Bulawa, C.E., and Gow, N.A.R. (2001). Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. *Molec. Microbiol.* 39, 1414 – 1426.
- Murphy, J.M. (1991). Mechanisms of natural resistance to human pathogenic fungi. *Ann. Rev. Microbiol.* 45, 509-538.
- Nakayama, H., Mio, T., Nagahashi, S., Kokado, M., Arisawa, M., and Aoki Y. (2000). Tetracycline-regulatable system to tightly control gene expression in the pathogenic fungus *Candida albicans*. *Infect. Immun.* 68, 6712-6719.
- Nicholls, S., Straffon, M., Enjalbert, B., Nantel, A., Macaskill, S., Whiteway, M., and Brown, A.J.P. (2004). Msn2/4-like transcription factors play no obvious roles in the stress responses of the fungal pathogen, *Candida albicans*. *Eukaryotic Cell* 3, 1111-1123.
- Niimi, M., Kamiyama, A., and Tokunaga, M. (1988). Respiration of medically important *Candida* species and *Saccharomyces cerevisiae* in relation to glucose effect. *J. Med. Vet. Mycol.* 26, 195-198.
- Nunes, P.A., Tenreiro, S., and Sa-Correia, I. (2001). Resistance and adaptation to quinidine in *Saccharomyces cerevisiae*: role of *QDR1* (*YIL120w*), encoding a plasma membrane transporter of the major facilitator superfamily required for multidrug resistance. *Antimicrob. Agents Chemother.* 45, 1528-1534.

Odds, F.C. (1988). *Candida and Candidosis*, 2<sup>nd</sup> ed. Bailliere Tindall, London, United Kingdom.

Piekarska, K., Mol, E., van den Berg, M., Hardy, G., van den Burg, J., van Roermund, C., MacCallum, D., Odds, F.C., and Distel, B. (2006). Peroxisomal fatty acid  $\beta$ -oxidation is not essential for virulence of *Candida albicans*. *Eukaryotic Cell* 5, 1847-1856.

Postma, E., Verduyn, C., Scheffers, W.A, and Van Dijken, J.P. (1989). Enzymic analysis of the Crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae*. *Appl. Env. Microbiol.* 55, 468-477.

Ramirez, M.A., and Lorenz, M.C. (2007). Mutations in alternative carbon utilization pathways in *Candida albicans* attenuate virulence and confer pleiotropic phenotypes. *Eukaryotic Cell* 6, 280–290.

Ramsdale, M., Selway, L., Stead, D., Walker, J., Yin, Z., Nicholls, S.M., Shiels, E.M., and Brown, A.J.P. (2008). *MNLI* regulates weak acid induced stress responses of the fungal pathogen *Candida albicans*. *Molec. Biol. Cell* 19, 4393-4403.

Reeves, E. P., Lu, H., Jacobs, H. L., Messina, C.G.M., Bolsover, S., Gabella, G., Potma, E.O., Warley, A., Roes, J., and Segal, A.W. (2002). Killing activity of neutrophils is mediated through activation of proteases by  $K^+$  flux. *Nature Rev. Microbiol.* 416, 291-297.

Rodriguez-Navarro, A., Quintero, F.J., and Garciadablas, B. (1994).  $Na^+$ -ATPases and  $Na^+/H^+$  antiporters in fungi. *Biochim Biophys Acta.* 1187, 203-205.

Rolland, F., Winderickx, J., and Thevelein, J.M. (2001). Glucose sensing mechanisms in eukaryotic cells. *Trends Biochem. Sci.* 26, 310-317.

Rubin-Bejerano, I., Fraser, I., Grisafi, P., and Fink, G.R. (2003). Phagocytosis by neutrophils induces an amino acid deprivation response in *Saccharomyces cerevisiae* and *Candida albicans*. *Proc. Natl. Acad. Sci. USA* *100*, 11007-11012.

San Jose, C., Monge, R.A., Perez-Diaz, R., Pla, J., and Nombela, C. (1996). The mitogen-activated protein kinase homolog *HOG1* gene controls glycerol accumulation in the pathogenic fungus *Candida albicans*. *J. Bacteriol.* *178*, 5850-5952.

Sanglard, D., Kuchler, K., Ischer, F., Pagani, J.L., Monod, M., and Bille, J. (1995). Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. *Antimicrob. Agents Chemother.* *39*, 2378-2386.

Sanglard, D., Ischer, F., and Monod, M. (1997). Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of *CDR 2*, a new multidrug ABC transporter gene. *Microbiol.*, *143*, 405-416.

Sasada, M., and Johnston, R.B. (1980). Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and the killing of *Candida* by macrophages. *J. Exp. Med.* *152*, 85-98.

Servos, J., Haase, E., and Brendel, M. (1993). Gene *SNQ 2* of *Saccharomyces cerevisiae*, which confers resistance to 4-nitroquinilone-N-oxide and other chemicals, encodes a 169 kDa protein homologous to the ATP-dependent permeases. *Mol. Gen. Genet.* *236*, 214-218.

Smith, D.A., Nicholls, S., Morgan, B.A., Brown, A.J.P., and Quinn, J. (2004). A conserved stress-activated protein kinase regulates a core stress response in the human pathogen *Candida albicans*. *Molec. Biol. Cell* *15*, 4179-4190.

Stanhill, A., Schick, N., and Engelberg, D. (1999). The yeast Ras/cyclic AMP pathway induces invasive growth by suppressing the cellular stress response. *Molec. Cell. Biol.* *19*, 7529-7538

Stephen, D.W., Rivers, S.L., and Jamieson, D.J. (1995). The role of the YAP1 and YAP2 genes in the regulation of the adaptive oxidative stress responses of *Saccharomyces cerevisiae*. *Molec. Microbiol.* *16*, 415-423.

Thevelein, J.M., and de Winde J.H. (1999). Novel sensing mechanisms and targets for cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Molec. Microbiol.* *33*, 904-918.

Thewes, S., Kretschmar, M., Park, H., Schaller, M., Filler, S.G., and Hube, B. (2007). *In vivo* and *ex vivo* comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. *Molec. Microbiol.* *63*, 1606-1628.

Tusher, V.G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* *98*, 5116-5121.

Vargas, S.L., Patrick, C.C., Ayers, G.D., and Hughes, W.T. (1993). Modulating the effects of dietary carbohydrate supplementation on *Candida albicans* colonization and invasion in a neutropenic mouse model. *Infect. Immun.* *61*, 619-626.

Vasquez-Torres, A., and Balish, E. (1997) Macrophages in resistance to candidiasis. *Microbiol. Molec. Biol. Rev.* *61*, 170-192.

Wilson, R.B., Davis, D., and Mitchell, A.P. (1999). Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J. Bacteriol.* *181*, 1868-1874.

Wilson, D., Tutulan-Cunita, A., Jung, W., Hauser, N.C., Hernandez, R., Williamson, T., Piekarska, K., Rupp, S., Young, T., and Stateva, L. (2007). Deletion of the high-affinity cAMP phosphodiesterase encoded by *PDE2* affects stress responses and virulence in *Candida albicans*. *Molec. Microbiol.* *65*, 841–856.

Wysong, D.R., Christin, L., Sugar, A.M., Robbins, P.W., and Diamond, R.D. (1998). Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. *Infect. Immun.* *66*, 1953-1961.

Yin, Z., Smith, R.J., and Brown, A.J.P. (1996). Multiple signalling pathways trigger the exquisite sensitivity of yeast gluconeogenic mRNAs to glucose. *Molec. Microbiol.*, *20*, 751-761.

Yin, Z., Wilson, S., Hauser, N.C., Tournu, H., Hoheisel, J.D., and Brown, A.J.P. (2003). Glucose triggers different global responses in yeast depending on the strength of the signal, and transiently stabilises ribosomal protein mRNAs. *Molec. Microbiol.* *48*, 713-724.

Zakikhany, K., Naglik, J.R., Schmidt-Westhausen, A., Holland, G., Schaller, M., and Hube, B. (2007). *In vivo* transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. *Cell. Microbiol.* *9*, 2938 – 2954.

Zhou, H., and Lorenz, M.C. (2008). Carnitine acetyltransferases are required for growth on non-fermentable carbon sources but not for pathogenesis in *Candida albicans*. *Microbiol.* *154*, 500–509.

Table1. Strains used in this study

Strain	Genotype	Source
CAI4	<i>ura3::λ imm434/ura3:: λ imm434</i>	Fonzi and Irwin, 1993
RM1000	<i>ura3::λ imm434/ura3:: λ imm434, his1::hisG/his1::hisG</i>	Wilson <i>et al.</i> , 1999
THE1	<i>Ade2::hisG/ade2::hisG, ura3:: λ imm434/ ura3:: λ imm434, ENO1/eno1::ENO1-tetR-ScHAP4AD-3XHA-ADE2</i>	Nakayama <i>et al.</i> , 2000
<i>Δhog1</i>	<i>ura3::λ imm434/ura3:: λ imm434, his1::hisG/his1::hisG, hog1:: loxP-URA3-loxP, hog1::loxP-HIS1-loxP</i>	Smith <i>et al.</i> , 2004
<i>Δcap1</i>	<i>ura3::_ imm434/ura3::_ imm434, his1::hisG/his1::hisG, arg4::hisG/arg4::hisG, cap1::hisG/cap1::hisG-URA3-hisG</i>	Enjalbert <i>et al.</i> , 2006
<i>Δcap1 Δhog1</i>	<i>ura3::_ imm434/ura3::_ imm434, his1::hisG/his1::hisG, arg4::hisG/arg4::hisG, hog1::loxP-ARG4-ura3-loxP/hog1::loxP-HIS1-loxP, cap1::hisG/cap1::hisG-URA3-hisG</i>	Enjalbert <i>et al.</i> , 2006
<i>ras1-2/ras1-3</i>	<i>ura3::λ imm434/ura3::λ imm434, ras1Δ::hisG/ras1Δ::hph-URA3-hph</i>	Feng <i>et al.</i> , 1999
WH324	<i>ura3::λ imm434/ura3:: λ imm434, aox1a::hisG/aox1b::hisG</i>	Huh and Kang, 2001

## FIGURE LEGENDS

**Figure 1.** Impact of glucose upon the expression of genes involved in central carbon metabolism in *C. albicans* and *S. cerevisiae*. Cells were grown to mid-exponential phase in YPLactate, exposed to no (0%), low (0.01%), medium (0.1%) or high (1%) glucose for 30 minute, and then subjected to transcript profiling. Data for *S. cerevisiae* are taken from Yin *et al.* (2003). (A) Regulation of glycolytic transcripts. (B) Average responses for transcripts encoding glycolytic, TCA cycle, gluconeogenic and glyoxylate cycle, fatty acid  $\beta$ -oxidation, and ribosomal proteins: filled squares, *C. albicans*; open squares, *S. cerevisiae*.

**Figure 2.** Effect of glucose upon the regulation of various functional categories in *C. albicans* and *S. cerevisiae*. Regulated genes of known function were assigned to functional categories according to information from the *Saccharomyces* and *Candida* Genome Databases. Transcript profiling data for *S. cerevisiae* are taken from Yin *et al.* (2003). (A) Functional categories that showed statistically significant enrichment in up-regulated (red) or down-regulated genes (green) are shown: light orange or green, P-value  $<10^{-2}$ ; mid orange or green, P-value  $<10^{-3}$ ; dark red or green, P-value  $<10^{-4}$ .

**Figure 3.** Effect of glucose upon mRNAs encoding *YAPI*, *CAP1* or trehalose synthetic enzymes. Filled squares, *C. albicans*; open squares, *S. cerevisiae*.

**Figure 4.** Exposure to glucose increases the resistance of *C. albicans* to an azole antifungal agent. Cells were grown to mid-exponential phase in YPLactate, exposed to 1% glucose or lactate for one hour, then subjected to 10  $\mu$ g/ml miconazole and their cell viability assayed. Means and standard deviations from triplicate experiments are shown.

**Figure 5.** Glucose increases the resistance of *C. albicans* to acute doses of hydrogen peroxide. Cells were grown to mid-exponential phase in YPLactate, exposed to 1% glucose or lactate for one hour, then subjected to different concentrations of H<sub>2</sub>O<sub>2</sub>, and their cell viability assayed. Means and standard deviations from triplicate experiments are shown.

**Figure 6.** Glucose increases the resistance of *C. albicans* to cationic stress. Cells were grown to mid-exponential phase in YPLactate, exposed to 1% glucose or lactate for one hour, then subjected to an NaCl, KCl or sorbitol stress, and their cell viability assayed. Means and standard deviations from triplicate experiments are shown.

**Figure 7.** Role of regulators in glucose-enhanced resistance to oxidative stress. Isogenic strains (Table 1) were grown to mid-exponential phase in YPLactate, treated with 1% glucose or lactate for one hour, exposed to 25 mM H<sub>2</sub>O<sub>2</sub> for one hour, and cell viability assayed. Means and standard deviations from triplicate experiments are shown.

**Figure 8.** Role of regulators in glucose-enhanced resistance to cationic stress. Strains (Table 1) were grown in YPLactate, treated with glucose or lactate for one hour, then exposed to 1M NaCl for one hour, and their cell viability assayed. Means and standard deviations from triplicate experiments are shown.

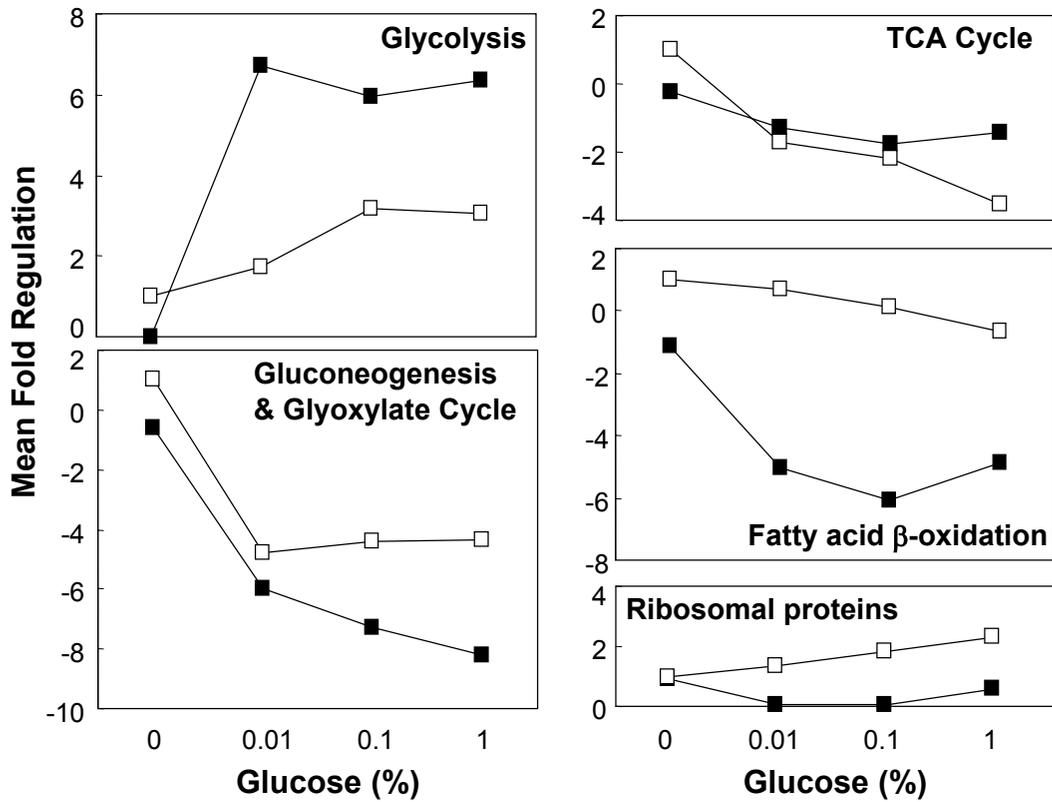
**Figure 9.** Hog1 is phosphorylated in response to glucose. Western blot of Hog1-P with an antibody specific for the phosphorylated version of Hog1, and an anti-p38 antibody to control for loading. The strains are: RM1000, RM; THE-1, T;  $\Delta$ ,  $\Delta$ hog1 (Table 1).

**Figure 10.** Model comparing the effects of glucose upon stress responses in *C. albicans* and *S. cerevisiae*. In *S. cerevisiae*, glucose represses stress responses by down-regulating the general stress response through Msn2 and Msn4, down-regulating the oxidative stress response through Yap1, and by repressing the expression of some stress genes (see text). In *C. albicans*, Cap1 and Hog1 may contribute to glucose-enhanced oxidative stress resistance (see text). Hog1 is phosphorylated in response to glucose and is required for glucose-enhanced cationic stress resistance. The Msn2/4-like proteins, Msn4 and Mnl1, do not contribute to oxidative or osmotic stress resistance in *C. albicans* (Nicholls *et al.*, 2004).

**A**

Function	<i>C. albicans</i> gene		Glucose Concentration (%)				<i>S. cerevisiae</i> gene		Glucose Concentration (%)			
	Systematic	Common	0	0.01	0.1	1	Systematic	Common	0	0.01	0.1	1
Hxt3	CA1067	<i>HXT62</i>	1.3	45.4	34.6	42.5	YDR345C	<i>HXT3</i>	1.0	8.7	11.4	9.1
Hxt6	CA1070	<i>HXT61</i>	1.2	4.6	8.0	3.9	YDR343C	<i>HXT6</i>	1.0	7.1	10.3	3.3
Glk1	CA0263	<i>GLK1</i>	-1.2	1.0	1.0	1.1	YCL040W	<i>GLK1</i>	1.0	1.6	1.0	-1.8
Hxk2	CA0127	<i>HXK2</i>	-1.1	9.5	9.7	7.9	YGL253W	<i>HXK2</i>	1.0	-1.1	1.4	1.4
Pgi1	CA3559	<i>PGI1</i>	1.0	1.7	1.8	1.7	YBR196C	<i>PGI1</i>	1.0	-1.4	1.1	1.7
Pfk1	CA1834	<i>PFK1</i>	-1.2	6.9	6.1	6.6	YGR240C	<i>PFK1</i>	1.0	-1.9	-1.1	1.5
Pfk2	CA3112	<i>PFK2</i>	-1.0	9.6	8.3	8.5	YMR205C	<i>PFK2</i>	1.0	1.2	2.1	2.7
Fba1	CA5180	<i>FBA1</i>	-1.2	2.1	1.6	2.1	YKL060C	<i>FBA1</i>	1.0	2.0	3.7	4.6
Tpi1	CA5950	<i>TPI1</i>	1.1	2.2	2.3	2.2	YDR050C	<i>TPI1</i>	1.0	1.8	2.3	3.7
Tdh3	CA5892	<i>GAP1</i>	1.1	1.9	2.1	2.1	YGR192C	<i>TDH3</i>	1.0	-1.1	1.4	1.9
Pgk1	CA1691	<i>PGK1</i>	1.0	2.5	2.4	2.5	YCR012W	<i>PGK1</i>	1.0	2.0	2.9	3.5
Gpm1	CA4671	<i>GPM1</i>	-1.1	2.2	1.9	2.2	YKL152C	<i>GPM1</i>	1.0	1.5	1.8	2.1
Eno1	CA3874	<i>ENO1</i>	1.6	1.6	1.2	1.5	YGR254W	<i>ENO1</i>	1.0	1.8	2.7	4.2
Pyk1	CA3483	<i>CDC19</i>	-1.1	3.2	2.1	4.2	YAL038W	<i>CDC19</i>	1.0	2.3	3.6	5.4
<b>Mean</b>		<b>Glycolysis</b>	<b>0.0</b>	<b>6.7</b>	<b>5.9</b>	<b>6.4</b>		<b>Glycolysis</b>	<b>1.0</b>	<b>1.7</b>	<b>3.2</b>	<b>3.1</b>

**B**



Rodaki *et al.*: Figure 2

UP	C. albicans			S. cerevisiae			DOWN	C. albicans			S. cerevisiae		
	% [Glucose]			% [Glucose]				% [Glucose]			% [Glucose]		
Functional category	0.01	0.1	1	0.01	0.1	1	Functional category	0.01	0.1	1	0.01	0.1	1
glycolysis	■	■	■	■	■	■	gluconeogenesis	■	■	■	■	■	■
fermentation	■	■	■	■	■	■	aerobic respiration	■	■	■	■	■	■
hexose transport	■		■	■	■	■	tricarboxylic acid cycle	■	■	■	■	■	■
energy reserve metabolism	■	■	■		■		fatty acid biosynthesis	■	■	■	■	■	
cell-cell adhesion	■	■	■				amino acid catabolism	■	■	■			■
cell wall organization and biogenesis		■	■		■		amino acid biosynthesis	■	■	■		■	
protein biosynthesis					■	■	protein import	■	■	■			
ribosome biogenesis and assembly					■	■	ribosome biogenesis and assembly		■	■			
response to osmotic stress				■	■		response to pheromone				■		■
response to oxidative stress	■	■	■				drug transport				■	■	■
response to drug	■	■	■				response to drug				■	■	■

