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A role for *Candida albicans* superoxide dismutase enzymes in glucose signaling

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Abstract

The *Saccharomyces cerevisiae* and *Candida albicans* yeasts have evolved to differentially use glucose for fermentation versus respiration. *S. cerevisiae* is Crabtree positive, where glucose represses respiration and promotes fermentation, while the opportunistic fungal pathogen *C. albicans* is Crabtree negative and does not repress respiration with glucose. We have previously shown that glucose control in *S. cerevisiae* involves the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1), where H₂O₂ generated by SOD1 stabilizes the casein kinase YCK1 for glucose sensing. We now demonstrate that *C. albicans* SODs also participate in glucose regulation. *C. albicans* expresses two cytosolic SODs, Cu/Zn SOD1 and Mn containing SOD3, and both complemented a *S. cerevisiae* *sod1* mutant in stabilizing YCK1. Moreover, in *C. albicans* cells, both SODs functioned to repress glucose transporter genes in response to glucose. However, the action of SODs in glucose control has diverged in the two yeasts. In *S. cerevisiae*, SOD1 specifically functions in the glucose sensing pathway involving YCK1 and the RGT1 repressor, but the analogous YCK/RGT1 pathway in *C. albicans* shows no control by SOD enzymes. Instead *C. albicans* SODs work in the glucose repression pathway involving the MIG1 transcriptional repressor. In *C. albicans*, the SODs repress glucose uptake, while in *S. cerevisiae*, SOD1 activates glucose uptake, in accordance with the divergent modes for glucose utilization in these two distantly related yeasts.

Keywords

Superoxide dismutase; yeast; *Candida albicans*; glucose

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INTRODUCTION

The nutrient glucose generates metabolic energy through the processes of glycolysis, fermentation and oxidative phosphorylation. In the bakers yeast *Saccharomyces cerevisiae*, glucose is preferentially metabolized through fermentation, and mitochondrial respiration is repressed by glucose through a process known as the Crabtree effect [1, 2]. Such control by glucose involves two transcriptional repressor pathways: (i) the sugar receptor repressor (SRR) pathway where glucose activates a signaling cascade involving the casein kinases YCK1/YCK2 and the transcriptional repressor RGT1; and (ii) the glucose repression pathway where energy sensing kinases regulate the transcriptional repressors MIG1 and MIG2 [3–5]. Together these pathways maximize glucose assimilation and repress respiration and the usage of alternative carbon sources.

The Crabtree effect does not apply to all yeasts. For example, the opportunistic fungal pathogen *Candida albicans* is defined as Crabtree negative, since glucose does not repress respiration in this yeast [6–8]. Even so, *C. albicans* has evolved with the same catabolite repression pathways as *S. cerevisiae* including the MIG1 and RGT1 transcriptional repressors [7–10]. *C. albicans* exists in glucose poor niches of the animal host where alternative carbon sources are prevalent, and the organism can sense very low glucose and simultaneously assimilate multiple carbon sources. By comparison, the environmental yeast *S. cerevisiae* thrives under glucose “feast or famine” and prefers to use glucose to drive fermentation. Alternative carbon sources are only assimilated when glucose becomes scarce [3, 6–8, 11, 12].

Curiously, we found that in *S. cerevisiae*, catabolite repression involves the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) [13]. SOD1 catalyzes the disproportionation of the free radical superoxide to oxygen and H₂O₂ and is well regarded for its role in oxidative stress protection [14]. However, H₂O₂ can be a signaling molecule [15, 16] and we observed that SOD1 generation of H₂O₂ at the site of *S. cerevisiae* YCK1 stabilizes this casein kinase, thereby helping to activate the SRR pathway and de-repress RGT1 targets [13]. This process requires oxygen to drive formation of the superoxide substrate for SOD1 and C-terminal sequences of YCK1 that we identified as a peroxide sensitive degron [13].

It is not known whether this role of SOD1 in SRR can be extended to Crabtree negative yeasts such as *C. albicans* where glucose does not repress respiration. Additionally, *C. albicans* does not always express a Cu/Zn containing SOD1. *C. albicans* and closely related fungi have uniquely evolved with two SODs in the cytosol whereby Cu/Zn SOD1 is repressed under Cu starvation conditions and is replaced by an alternative SOD3 enzyme that uses a Mn co-factor [17, 18]. Mn and Cu containing SODs are unrelated polypeptides and it is not known whether a Mn SOD can function in cytosolic signaling.

Here we report the surprising finding that the Crabtree negative *C. albicans* does in fact exploit its SODs in glucose regulation. Both the Cu/Zn SOD1 and Mn SOD3 of *C. albicans* were able to complement a *sod1* mutant of *S. cerevisiae* and stabilize the YCK1 kinase. Moreover, in *C. albicans*, the SODs contribute to repression of glucose uptake when glucose is abundant. However, unlike *S. cerevisiae* where SOD1 acts in the SRR pathway involving

YCK1 and RGT1, *C. albicans* SOD1 operates in the glucose repression pathway involving MIG1. The role of SOD enzymes in glucose control has been re-wired in *C. albicans* to accommodate the metabolism of this opportunistic pathogen and Crabtree negative yeast.

MATERIALS AND METHODS

Yeast strains, plasmids and growth conditions

The parental *S. cerevisiae* strain was AR203 (MATa, *met15*⁰, *ura3*⁰, *his3*¹ *sod1*⁺ ::LEU2), gift of Amit Reddi. This *sod1*⁺ strain was transformed with one of several SOD expressing plasmids: pLJ175 (*CEN URA3*) harboring *S. cerevisiae* SOD1 sequences –674 to +584, pJG102 (*CEN LEU2*) expressing *C. albicans* P144L SOD1 under the *S. cerevisiae* SOD1 promoter [19], and pLJ486, pJL111, pVTSOD3 or pCB002 (*URA3* 2 micron) expressing *S. cerevisiae* SOD1, the P144S derivative, *C. albicans* SOD3 and *S. cerevisiae* SOD2 respectively all under *S. cerevisiae* ADH1 [17, 20]. pCB002 represents *S. cerevisiae* SOD2 sequences +265 to +889 (no mitochondrial pre-sequence) inserted into the BamHI site of pVTSOD3. All strains co-expressed either *TEF1* driven GFP fused to full length YCK1 (pAR113) or *MET25* driven GFP fused to C-terminal YCK1 residues 368–538 (pAR119) [13].

C. albicans strains used in this study include CA-IF100 (*arg4*⁺ /*arg4*⁺, *leu2*⁺ /*leu2*⁺ ::cmLEU2, *his1*⁺ /*his1*⁺ ::cdHIS1, *URA3/ura3*⁺) and isogenic *sod1*⁺ ::cmLEU2/*sod1*⁺ ::cdHIS1 and *sod3*⁺ ::cmLEU2/*sod3*⁺ ::cdHIS1 strains provided by K. Kuchler [21]. SN250 (*ura3*⁺ -*iro1*⁺ ::imm⁴³⁴/URA3-IRO1, *his1*⁺ /*his1*⁺, *arg4*⁺ /*arg4*⁺, *leu2*⁺ ::C.m.LEU2/*leu2*⁺ ::C.d.HIS1), the isogenic *rgt1*⁺ / and *mig1*⁺ / strains, DAY286 (*ura3*⁺ ::imm434/*ura3*⁺ ::imm434, *his1*⁺ ::hisG/*his1*⁺ ::hisG, *pARG4*⁺ ::URA3::arg4::hisG/*arg4*⁺ ::hisG) and the isogenic *yck22*⁺ / strain were obtained from the fungal genetics stock center. The *yck2*⁺ / strain JJH34H (*yck2*⁺ -*Tn7*⁺ ::UAU1/*yck2*⁺ -*Tn7*⁺ ::URA3::pHIS1 *ura3*⁺ ::imm434/*ura3*⁺ ::imm434, *his1*⁺ ::hisG/*his1*⁺ ::hisG, *arg4*⁺ ::hisG/*arg4*⁺ ::hisG) and isogenic YCK2 re-integrand strain JJH34C were provided by Hyunsook Park [22].

S. cerevisiae and *C. albicans* cells were cultured at 30°C in enriched YP media containing 1% yeast extract, 2% peptone and 0.2% or 2% glucose or 5% glycerol (w/v). Anaerobic cultures of *S. cerevisiae* (95% nitrogen, 5% hydrogen gas) were supplemented with 15 mg/L ergosterol and 0.5% Tween-80.

Biochemical analyses

To analyze *S. cerevisiae* GFP-YCK, 100 µg lysate protein was subjected to immunoblot analysis using 12% Tris-Glycine gels and anti-GFP and anti-PGK1 antibodies [13]. Analysis of SOD enzymatic activity used 30 µg lysate protein subjected to native gel electrophoresis and nitroblue tetrazolium staining [19].

For RNA analyses, *C. albicans* strains were either grown to an optical density at 600 nm (OD₆₀₀) of 1.0–2.0 in YP + 2% glucose (Figs. 2, 3A,B) or grown first under either low (.2% glucose) or no glucose (5% glycerol) conditions to OD₆₀₀ 1.0–3.0, followed by dilution and re-growth in 2% glucose or 5% glycerol to OD₆₀₀ ≈ 2.0. RNA for both qRT-PCR and RT-PCR was isolated using RNeasy (Qiagen). For qRT-PCR, RNA was converted to cDNA

using Superscript IV reverse transcriptase; cDNA was amplified using iQ SYBR Green Supermix [18] and the following primers: *TUB2*, as described [18]; *HGT17*, TGGACTATGGGTTTCAATTC and CATGACTCCGACAAAGAAAT; *HGT12*, CTGTCACTGCTTCATACCAA and CTTGGAAACCCAGTATCTTG; *HGT2*, ACTTGACAACAAATCTGCCT and ATACATGGCAATAGCGAAAT; *QDR1*, CATCCAACTTGGCCGATACA and TATCACCAGCAACACCAGAAC; *HGT7*, CCATCCACTTCTACTACGGTTTC and CCCGTAGAGGTTGCCATTT. Values were normalized to *TUB2*. For RT-PCR, cDNA was amplified using Taq DNA Polymerase and primers as previously described [23].

To measure glucose consumption, *C. albicans* cells were seeded at $OD_{600} \approx 0.2$ in YP+2% glucose. At designated OD_{600} , 200 μ L aliquots were centrifuged to remove cells and assayed for glucose at 520 nm using the Glucose Colorimetric Assay Kit (Cayman Chemical Company).

RESULTS

C. albicans SOD1 and SOD3 can stabilize the *S. cerevisiae* casein kinase, YCK1

We tested whether *C. albicans* SOD1 and SOD3 can complement a *S. cerevisiae* *sod1* mutant and stabilize the YCK1 casein kinase of the SRR pathway. For *C. albicans* SOD1, we expressed a P144L derivative. Wild type *C. albicans* SOD1 cannot be activated by the *S. cerevisiae* Cu chaperone CCS1 [19], while the P144L derivative is activated independent of CCS1 and exhibits abundant activity in *S. cerevisiae* [19]. *S. cerevisiae* *sod1* cells expressing *S. cerevisiae* SOD1 or *C. albicans* P144L SOD1 were transformed with our previously published reporter for monitoring YCK1 stability. In this reporter, YCK1 sequences are fused to the C-terminus of GFP, and GFP expression levels provide a read-out of YCK1 stability [13]. As seen in Fig. 1A, the *sod1* defect in GFP-YCK1 stability was complemented by *C. albicans* SOD1, as has been reported for human and *C. elegans* SOD1 expressed in this heterologous system [13].

Although Cu/Zn SODs can readily stabilize YCK1, this may not be the case for Mn SOD enzymes. For example, the mitochondrial Mn SOD2 of *S. cerevisiae* cannot stabilize YCK1 when this SOD is targeted to the cytosol [13], even when expressed under the strong *ADH1* promoter (Fig. 1B top). By comparison, Mn SOD3 from *C. albicans* complements the *sod1* mutation and stabilizes YCK1 (Fig. 1B top). Both Mn SODs are enzymatically active (Fig. 1B bottom) but only *C. albicans* SOD3 stabilizes YCK1.

Protection of YCK1 by *S. cerevisiae* SOD1 requires oxygen to generate the superoxide substrate for SOD and C-terminal sequences of YCK1 we identified as a peroxide-sensitive decon [13]. The same appears true for Mn SOD3. Under anaerobic conditions, SOD3 cannot stabilize GFP-YCK1 even though active SOD3 enzyme is produced without oxygen (Fig. 1C lane 5), similar to findings with enzymatically active SOD1 (Fig. 1C lane 4; [13]). Furthermore, SOD3 stabilizes a GFP fusion to the peroxide-sensitive decon of YCK1, residues 367–538 (Fig. 1D). Despite being a Mn SOD, *C. albicans* SOD3 can stabilize *S. cerevisiae* YCK1 in a manner similar to Cu/Zn SOD1.

A role for SOD1 and SOD3 in regulation of glucose transporter genes in *Candida albicans*

Although *C. albicans* SOD1 and SOD3 can affect a glucose signaling pathway in *S. cerevisiae*, it was not clear whether the same holds true in *C. albicans*. Like *S. cerevisiae*, glucose control in *C. albicans* involves transcriptional re-programming and we tested whether specific markers of glucose control were altered in *sod1* / strains. In *C. albicans*, glucose represses specific HGT glucose transporter genes [3, 8, 11, 12], and we find that several of these are de-repressed in *sod1* / mutants. As seen in Fig. 2, *HGT17*, *HGT12* and *HGT2* were all strongly induced in *C. albicans sod1* / mutants (Fig. 2).

As mentioned above, *C. albicans* will switch from expressing SOD1 to Mn SOD3 when cells are starved for Cu [18]. We therefore tested whether SOD3 can also repress glucose transporter genes under Cu starvation conditions. Cells can be limited for Cu by treatment with the bathocuproinedisulfonate (BCS) Cu(I) chelator that effectively induces *SOD3* [18] (Fig. 3A, right). We observed that *HGT17* expression in BCS treated cells was significantly increased in *sod3* / mutants compared to WT cells, similar to effects of *sod1* / mutations under Cu-replete (no BCS) conditions (Fig. 3A, left). Moreover, the induction of *HGT17* in *sod1* / cells was totally reversed upon inducing *SOD3* through BCS (Fig. 3A left). Thus, like SOD1, SOD3 can contribute to repression of specific glucose transporters genes. Since SOD3 is only operative under extreme cases of Cu starvation, the remainder of our studies focused on SOD1.

C. albicans SOD1 helps repress HGT genes targets of the MIG1 repressor

At least two explanations may account for the induction of glucose transporter genes in *C. albicans sod1* / mutants: the yeast could either be defective in glucose consumption or in glucose signaling. We find that *sod1* / mutants have no obvious defect in consumption of extracellular glucose (Fig. 3B) suggesting these mutants may be defective in glucose signaling.

As mentioned above, glucose signaling in *C. albicans* involves the RGT1 and MIG1 repressors, whereby MIG1 represses glucose transport genes when glucose is abundant, and RGT1 represses genes with no glucose (i.e., glycerol) [11, 12]. We observe that all three HGT genes impacted by *sod1* / mutations are normally repressed in high (“H”) glucose and de-repressed in low (“L”) glucose (Fig. 3C left panels). *HGT2* is an established MIG1 target while *HGT12* falls under both MIG1 and RGT1 control [12], and accordingly, both genes are de-repressed in high glucose by *mig1* / mutations and *HGT12* is also de-repressed in glycerol (“G”) by *rgt1* / mutations (Fig 3C). The regulation of *HGT17* was previously unknown and we find its expression mirrors that of *HGT2*. *HGT17* repression by high glucose was reversed by *mig1* / mutations but there was no repression by glycerol and no effect of *rgt1* / mutations (Fig. 3C). Thus, *HGT17* is a target of MIG1, not RGT1. All three genes affected by *sod1* / mutations are targets of the MIG1 pathway for glucose repression.

The role of *C. albicans* SOD1 in repressing MIG1 target genes was surprising because in *S. cerevisiae*, SOD1 acts in the RGT1, not MIG1, pathway [13]. In *S. cerevisiae*, *sod1* mutations prevent the de-repression of RGT1 targets with glucose, an example being the

glucose transporter gene *HXT1* [13]. An analogous glucose transport gene regulated by *C. albicans* RGT1 is *HGT7*. Like *S. cerevisiae* *HXT1*, *C. albicans* *HGT7* is normally repressed without glucose (e.g., glycerol) and de-repressed with glucose (Fig. 4A) [11]. We observed that de-repression of *C. albicans* *HGT7* by glucose was unaffected by *sod1* / mutations (Fig. 4A) in stark contrast to the effects of *S. cerevisiae* *sod1* mutations on *HXT1* de-repression [13]. We also examined the *QDR1* target of RGT1, encoding a multidrug resistance transporter [10, 12]. *QDR1* is strongly de-repressed by glucose, but again, there was no effect of *sod1* / mutations on this RGT1 target (Fig. 4B).

As mentioned above, *S. cerevisiae* SOD1 acts to regulate stability of YCK1 and YCK2 casein kinases [13]. The most closely related casein kinases in *C. albicans* are YCK2 and YCK22 [8, 10, 22]. We observed that neither *yck2* / nor *yck22* / mutations phenocopy *sod1* / in de-repressing the MIG1 target *HGT17* (Fig. 4C), indicating that these kinases may not operate in the same glucose regulatory pathway as SOD1. *C. albicans* YCK2 is believed to act in the RGT1, not MIG1 pathway, analogous to *S. cerevisiae* YCK1 [8, 10, 22]. However, single mutations in *C. albicans* *yck2* or *yck22* / did not prevent the de-repression of RGT1 targets *HGT7* or *QDR1* (Fig. 4D, E). While the role of *C. albicans* YCK2 and YCK22 in glucose regulation is uncertain (see Discussion), the cytosolic SODs of *C. albicans* clearly contribute to glucose control by helping repress glucose transporter targets of MIG1.

DISCUSSION

Since their divergence 200 million years ago, *C. albicans* and *S. cerevisiae* have evolved to maximize utilization of nutrients in their disparate environments, including carbon sources such as glucose. Although many aspects of glucose regulation are similar in the two yeasts (e.g., the RGT1 and MIG1 repressors), the regulatory pathways have been finely-tuned to accommodate the lifestyles of a fermentative yeast versus an opportunistic fungal pathogen [6–8]. Here we provide another example of how glucose sensing and utilization has diverged in these two organisms. The cytosolic SODs of *C. albicans* have chosen to participate in repression of glucose transporter targets of the MIG1 repressor, while SOD1 of *S. cerevisiae* acts to control RGT1 gene targets by stabilizing the YCK1 casein kinase. This differential usage of SOD enzymes correlates well with respiratory control in the two yeasts. *S. cerevisiae* prefers fermentation over respiration, and with high glucose, SOD1 helps to repress respiration [13]. In *C. albicans*, glucose does not repress respiration that is essential for pathogenesis, and the SODs have been re-purposed to repress glucose uptake rather than respiration.

S. cerevisiae expresses just one cytosolic SOD that controls glucose signaling, while *C. albicans* has evolved two cytosolic SODs (SOD1 and SOD3) that can help repress glucose transporter genes. The occurrence of a Mn SOD in the cytosol is highly irregular and most eukaryotes typically restrict Mn SODs to the mitochondrial matrix. *C. albicans* SOD3 is very similar to mitochondrial Mn SOD2 in terms of amino acid sequence and catalytic efficiency [24], yet only SOD3, and not SOD2, can stabilize the peroxide-sensitive degron of *S. cerevisiae* YCK1. SOD3 must harbor unique structural or biophysical attributes that empower its activity in the cytosol, including a remarkable ability to capture Mn in this

compartment [25] and an ability to act in glucose signaling. SOD3 is part of an elegant system in *C. albicans* for sparing intracellular Cu when extracellular Cu becomes low as is seen during fungal invasion of the kidney [18, 26]. The interchangeable roles of SOD3 and SOD1 in glucose signaling would allow for uninterrupted control of carbon source utilization regardless of environmental Cu conditions.

How do *C. albicans* SODs repress glucose transporter genes? The SODs are likely to affect a component(s) of the MIG1 pathway, although the precise molecular target is difficult to predict because MIG1 regulation in *C. albicans* is poorly understood. *C. albicans* MIG1 does not contain the same phosphorylation sites for regulation by the SNF1 kinase as does *S. cerevisiae* MIG1, and how glucose activates *C. albicans* MIG1 is not clear [8, 9]. Regardless of the target, *C. albicans* SODs most likely function in a manner analogous to *S. cerevisiae* SOD1, where the SOD stabilizes proteins with peroxide-sensitive degrons. We favor this model because both *C. albicans* SOD1 and SOD3 have the capacity to stabilize the peroxide-sensitive degron of *S. cerevisiae* YCK1. In *C. albicans*, the closely related YCK2 casein kinase is proposed to work in the RGT1 pathway [8, 10, 22]. Although we saw no effects of *yck2* / mutations on glucose induction of RGT1 targets or glucose repression of MIG1 targets (Fig. 4), it is possible that the second casein kinase YCK22 is compensating for loss of YCK2. Finally, we cannot exclude the possibility that glucose control by SOD1 and SOD3 occurs independent of stabilizing peroxide-sensitive degrons. The SODs might act directly in the nucleus to repress MIG1 target genes, as has been shown for SOD1 regulation of Cu starvation stress and DNA damage response genes in *S. cerevisiae* [27, 28]. In any case, it is clear that *C. albicans* has evolved to use its SODs to minimize glucose uptake when glucose is abundant, perfectly in line with the lifestyle of this Crabtree negative yeast.

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Abbreviations

SRR	sugar receptor repressor
SOD	superoxide dismutase
OD₆₀₀	optical density at 600 nM
BCS	bathocuproinedisulfonate
qRT-PCR	quantitative real time PCR
RT-PCR	reverse transcriptase PCR

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- Superoxide dismutase (SOD) enzymes act in glucose signaling in yeast species.
- In *Saccharomyces cerevisiae*, SODs induce glucose uptake and repress respiration.
- In the human fungal pathogen *Candida albicans*, SODs repress glucose uptake genes.
- *C. albicans* SODs affect gene targets of the MIG1 transcriptional repressor.
- *C. albicans* has re-purposed its SOD enzymes to accommodate life in an animal host.

Figure 1. *C. albicans* Cu/Zn SOD1 and Mn SOD3 can stabilize *S. cerevisiae* casein kinase, YCK1
 The *S. cerevisiae* *sod1* strain expressing GFP-YCK1 or GFP fused to YCK1 residues 367–538 (D, bottom) were transformed with SOD-expressing plasmids or empty vector (EV). Lysates were subjected to immunoblot analyses using anti-GFP or anti-PGK1 as a loading control (A, B and C top and middle panels and D) or to SOD activity analysis by native gel electrophoresis and nitroblue tetrazolium staining as described in *Materials and Methods* (B bottom, C bottom). Indicated on left are molecular weight markers. (A) Strains expressed *S. cerevisiae* SOD1 (Sc SOD1) or *C. albicans* P144L SOD1 (Ca SOD1) from the *S. cerevisiae* *SOD1* promoter. (B) Strains expressed *S. cerevisiae* SOD1, *C. albicans* SOD3 (Ca SOD3) or *S. cerevisiae* cytosolic SOD2 (Sc SOD2) all under the *ADH1* promoter. With Sc SOD2, cultures contained 0.75 mM MnCl₂ as required to activate cytosolic SOD2 [13, 25]. (C) Strains were grown either in atmospheric oxygen (“+ oxygen”) or under nitrogen (“– oxygen”). SODs expressed include *C. albicans* SOD3 under *ADH1* (Ca SOD3), *S. cerevisiae* SOD1 under its native promoter (Sc SOD1) and *S. cerevisiae* P144S SOD1 under *ADH1* (Sc SOD1*). *S. cerevisiae* SOD1 is normally inactive without oxygen (lane 3) due to the oxygen dependence of CCS1, but P144S SOD1 is CCS1-independent and is activated without oxygen [20] (lane 4). Results are representative of 2–6 experimental trials.

Figure 2. Effect of *C. albicans* *sod1* / mutations on the expression of glucose transporters
 Shown is qRT-PCR analysis of *HGT17*, *HGT12* and *HGT2* expression in *C. albicans* *sod1* / mutants compared to the CA-IF100 “WT” strain grown in 2% glucose. Results are averages of biological duplicates where error bars are standard deviation and are representative of 2–4 experimental trials. Significance was assessed using paired t-test, * $p < 0.05$.

Figure 3. Glucose repression in *C. albicans* involves SOD1 and SOD3 and genes that are targets of the MIG1 repressor

(A) *C. albicans* CA-IF100 “WT” and isogenic *sod3* / and *sod1* / mutants were grown in 2% glucose medium supplemented where indicated with 800 μM of the Cu(I) chelator BCS to induce a Cu starvation state. As designated, qRT-PCR analysis was conducted on *HGT17* or *SOD3* and results normalized to WT cells without BCS. Results represent the averages of three biological replicates over two experimental trials; error bars are standard deviation. Significance was assessed using paired t-test, *p<0.05, **p 0.01. There is no statistical difference between *sod3* / + BCS and *sod1* / without BCS. (B) Extracellular glucose was measured as described in *Materials and Methods* in the indicated cultures as they reached OD₆₀₀ of 0.2, 3.0, 10.0 and 13.0. Shown are averages of two biological replicates representative of 2 experimental trials; error bars are standard deviation. (C) The SN250 “WT” and isogenic *mig1* / and *rgt1* / mutant strains were grown in either YP+ 2% glucose (H), YP+ .2% glucose (L), or YP + 5% glycerol (G). RT-PCR analysis of the indicated genes was carried out as described in *Materials and Methods*. cDNA reactions with and without reverse transcriptase are indicated as +RT and –RT.

Figure 4. RGT1 target genes are not affected by *sod1* / mutations

The designated strains were grown in YP +2% glucose (C) or as indicated, in YP + 5% glycerol or 2% glucose (A, B, D, E), and expression of *HGT7*, *QDR1* and *HGT17* was analyzed by qRT-PCR and normalized to *TUB2*. Shown are the fold changes compared to the corresponding “WT” strain grown in glucose. Results represent the averages of 4–6 biological replicates over 2–3 experimental trials and significance determined by paired t-test; ***p=0.0002; **p 0.01; *p 0.05. ns, not significant. The change in *HGT7* expression in glycerol grown cells was significant with *yck22* / cells, p=0.01, but not with *yck2* / cells. Strains used: (A, B) CA-IF100 “WT” and the isogenic *sod1* / strain; (D) JJH34C “WT” strain and the isogenic *yck2* / strain; (E) DAY286 “WT” and the isogenic *yck22* mutant; (E) all six aforementioned strains in groups of isogenic pairs separated by dotted lines. Each mutant was compared to its corresponding “WT” = 1.0.