Regulation of Candida glabrata oxidative stress resistance is adapted to host environment

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\textbf{A B S T R A C T}

The human fungal pathogen Candida glabrata is related to Saccharomyces cerevisiae but has developed high resistance against reactive oxygen species. We find that induction of conserved genes encoding antioxidant functions is dependent on the transcription factors CgYap1 and CgSkn7 which cooperate for promoter recognition. Superoxide stress resistance of C. glabrata is provided by superoxide dismutase CgSod1, which is not dependent on CgYap1/Skn7. Only double mutants lacking both CgSod1 and CgYap1 were efficiently killed by primary mouse macrophages. Our results suggest that in C. glabrata the regulation of key genes providing stress protection is adopted to meet a host-pathogen situation.

\textbf{1. Introduction}

The human fungal pathogen Candida glabrata is a common commensal in gastrointestinal and genitourinary tracts, but can turn into an opportunistic fungal pathogen in immunocompromised patients and elderly people [1–4]. C. glabrata lives mostly on mucosal surfaces and does not penetrate tissue efficiently. It is much more related to Saccharomyces cerevisiae than to Candida albicans [5,6]. C. glabrata is obviously adapted to a mammalian environment. In contrast to S. cerevisiae, its optimal growth temperature is near the human body temperature and a number of adhesins allow the organism to avidly adhere to various surfaces and to mammalian cells. These cells have adapted to withstand host defense and the competing microbes on mucosal surfaces. Here we investigate the regulatory basis for the oxidative stress resistance of C. glabrata.

In the mammalian host, cell-mediated immunity, based on phagocytic cells is crucial to counteract fungal infections [7]. In the phagolysosome of phagocytic cells, one of the key defense mechanisms is destruction of the engulfed microorganisms by reactive oxygen species produced by the NADPH oxidase complex [8,9]. The complex catalyzes the production of superoxide anions ($O_2^-$), serving as initial source for reactive oxygen species (ROS). For successful dissemination, pathogenic fungi have to counteract a broad spectrum of reactive oxidants during the oxidative burst. Therefore, commensal and pathogenic microbial fungal organisms carry a number of antioxidant systems (reviewed in [8,10]), such as catalases, superoxide dismutases, thioredoxins and glutathione-dependent peroxidases and reductases. Several of these enzymes are highly relevant for fungal pathogen virulence. The loss of thioredoxin proteins and superoxide dismutases decreased virulence of Cryptococcus neoformans in mice [11,12]. Unique cell surface superoxide dismutases CaSod4 and CaSod5 are essential for survival of C. albicans in macrophages [13].

Oxidative stress causes rapid changes of transcription of many genes. In S. cerevisiae, the induction of the oxidative stress regulon is largely under control of the conserved transcription factors Yap1 and Skn7 [14–17]. In contrast, the transcription factor Sfp1 and the TORC1 complex are repressed by superoxide anions leading to down-regulation of genes encoding components required for protein biosynthesis [18,19]. Activation of Yap1 involves the inhibition...
of a nuclear export signal (NES). Cysteine residues become oxidized by the thiol peroxidase Htr1/Gpx3 which leads to the formation of disulfide bonds, causing Yap1 to accumulate in the nucleus and activate its target genes [20–22]. The role of Yap1 in other stress responses has been reported in several other fungi, such as Kluyveromyces lactis, Ustilago maydis, C. albicans and Aspergillus fumigatus [23–25]. In S. cerevisiae, the presence of both Yap1 and Skn7 is necessary for efficient induction of many oxidative stress response genes [15,26,27]. Skn7 is a nuclear response regulator and part of a two-component system. Mutations in the receiver domain of Skn7 reduce its oxidative stress induced phosphorylation and in the in vitro formation of a ternary complex comprising promoter sequences and Yap1 [27]. However, the exact interaction mechanism between Yap1 and Skn7 is not fully understood.

C. glabrata contains genes encoding putative orthologues of Yap1 and Skn7 [28]. CgYap1 has undergone a recent mutation causing a shift of its preferred recognition site [29]. Similar to other fungi, CgYap1 and CgSkn7 are required for full resistance to hydrogen peroxide stress [28,30,39]. The role of Yap1 and Skn7 for virulence in pathogenic fungi varies between species. In C. albicans, the lack of CaSkn7 caused a slight attenuation of virulence [31]. In the fungal pathogen U. maydis CaSkn7 caused a slight attenuation of virulence [31]. In S. cerevisiae, the presence of both Yap1 and Skn7 is necessary for efficient induction of many oxidative stress response genes [20–22]. The role of Yap1 in other stress response is also mediated by the thiol peroxidase Hyr1/Gpx3 which leads to the formation of hydrogen peroxide [30].

Table 1

| Yeast strains used in this study. | Genotype | Source |
|----------------------------------|----------|--------|
| C. glabrata strain               |          |        |
| AHTU                             | his3A trp1A ura3A                           | [33]   |
| AHT6                             | his3A trp1A                               | [33]   |
| ARGc skn7A                       | his3A trp1A ura3A skn7A::ScHIS3             | This study |
| ARGc yap1A                       | his3A trp1A ura3A yap1A::ScHIS3             | This study |
| ARGc skn7 yap1A                  | his3A trp1A ura3A skn7A::ScHIS3             | This study |
| ARGc yap1A                       | his3A trp1A ura3A yap1A::ScHIS3             | This study |
| ARGc sod1A                       | his3A trp1A ura3A sod1A::ScHIS3             | This study |
| ARGc sod1 yap1A                  | his3A trp1A ura3A sod1A::ScHIS3             | This study |
| S. cerevisiae strain             |          |        |
| BY4741                           | MATa; his3A1; leu2A0; met15A0; ura3A0       | Euroscarf |
| BY4741 sod1A                     | MATa; his3A1; leu2A0; met15A0; ura3A0       | Euroscarf |
| BY4741 yap1A                     | MATa; his3A1; leu2A0; met15A0; ura3A0       | Euroscarf |

Table 2

| Plasmids used in this study. | Genotype | Source |
|-------------------------------|----------|--------|
| pRS316                        | CEN6, ARSH4, ScURA3                           | [35]   |
| pRS313                        | CEN6, ARSH4, ScHIS3                           | [35]   |
| pGEM-ACT                      |ARS, CEN and TRP1 marker from C. glabrata      | [40]   |
| pCGADH1-CgMSN2-CFP            |CgADH1-CgMSN2-CFP (Sphl/SacII and SalI/NotI); | [40]   |
| pCGADH1-CgSN7-CFP             |CgADH1-CgSN7-CFP (Sphl/SacII and NotI);       | This study |
| pCGCgskn7                    |CgSKN7-CgSN7 (native promoter Sphl and SacII);| This study |
| pGEM-ACT-CgYAP1               | Native promoter inserted via Sphl/NotI;       | This study |
| pCGYSYAP1                     |CgYAP1-CgYAP1 (NotI and NotII); CgTRP1         | This study |
| pCGADH1-GFP-CgYAP1            |CgADH1-GFP-CgYAP1 (NotI/NotII); GFP inserted  | [40]   |
| pCGSKN7-HA                    |CgSKN7-CgSN7-HA (HA tag inserted with NcoI);  | This study |
| pHA-CgYAP1                    |CgYAP1-A-HA-CgYAP1 (HA tag inserted with NotI);| This study |
| pCGYAP1-CgSKN7                |CgSKN7-CgSN7 inserted with NotI into pCgYAP1-CgYAP1; | This study |

2. Materials and methods

2.1. Yeast strains and plasmids

Strains and plasmids are listed in Tables 1 and 2. Oligonucleotides used are listed in Table S1. Additional information is available as Supplementary data. C. glabrata strains ARGc skn7A, ARGc yap1A, ARGc sod1A, ARGc yap1 skn7A, and ARGc yap1 sod1A were obtained by replacing the ORFs in strain AHTU [34] with the S. cerevisiae URA3 or HIS3 genes using fusion PCR [35] from the plasmids pRS316 and pRS313 [36] with the oligonucleotides SKN7-1 to 6, YAP1-1 to 6, and SOD1-1 to 6 and tested by southern analysis (Fig. S1). Gradient plates were prepared as described [37]. All PCR fragments were sequenced. Cells were grown for four generations in YPD at 30°C to OD600 of 1 before menadione or H2O2 was added for 20 min. The Microarray dataset has been deposited at array express (http://www.ebi.ac.uk/arrayexpress/; E-MEXP-2915). GFP was visualized in live cells without fixation as described [38].

2.2. Macrophage cell culture

Primary bone marrow derived macrophages (BMDMs) were obtained from the femur bone marrow of 6–10 weeks old C57Bl/6 mice. Cells were cultivated in DMEM supplemented with 10% FCS in the presence of L cell-derived CSF-1 as described C. glabrata macrophage infection assays were done as described previously [38]. For infection assays, BMDMs were seeded at 5 × 10⁵ cells/dish in 3.5-cm dishes containing medium without antibiotics.

2.3. Chromatin immunoprecipitation assay

Primer pairs: TRR2 (639/787) for CgTRR2 (CAGL0I01166g) and GPX2 (787/617) for CgGPX2 (CAGL0C01705g). A centromeric region of Chromosome B was used as a negative control.

3. Results

3.1. C. glabrata reacts differently to various oxidative stress causing agents

To explore the high oxidative stress resistance of C. glabrata [16], we investigated the role of the transcription factors CgYap1 and CgSkn7. We generated strains lacking either CgYap1 and CgSkn7. Cells were cultivated in DMEM supplemented with 10% FCS.
(CAGL0H04631g), CgSkn7 (CAGL0F09097g). To test the susceptibility spectrum of the mutants, we used plates with gradients of hypochlorite, peroxynitrite, hydrogen peroxide, and the superoxide generating compound menadione (Fig. 1A). On plates containing menadione, we observed a small growth difference. In contrast, growth of Cgyap1Δ and Cgyap1Δskn7Δ mutants was severely diminished in the presence of peroxynitrite. Both Cgyap1Δ and Cgyap1Δskn7Δ mutants had reduced survival on plates containing hydrogen peroxide (Fig. 1A, lower left panel). The Cgskn7Δ mutant was sensitive only to high level of hydrogen peroxide consistent with an earlier report [39]. Similar to S. cerevisiae [40], we observed that C. glabrata and Cgyap1Δskn7Δ mutant cells from over-night grown cultures which had switched to fermentative metabolism were highly resistant to hydrogen peroxide (Fig. 1A, lower panel). This is consistent with an earlier report on high level resistance of stationary phase C. glabrata cells [28]. Cells retained this high resistance also in the presence of glucose (Fig. 1A, lower right panel). The Cgsod1Δ mutant lacking the cytosolic copper–zinc superoxide dismutase CgSOD1 (CAGLOC04741g) was highly sensitive to superoxide and additional deletion of CgyAP1 did not enhance its sensitivity. Carbon source starvation slightly elevated the resistance against menadione-caused oxidative stress (Fig. 1B).

3.2. The core oxidative stress response of C. glabrata is similar to S. cerevisiae

To define the oxidative stress regulon of C. glabrata, the transcriptional response to 0.4 mM hydrogen peroxide was determined by microarray analysis (Fig. 2A). cDNAs from stressed Cgyap1Δ and Cgskn7Δ mutants were co-hybridized with the stressed wild type. Genes dependent on Cgyap1, CgSkn7 or both were classified into three groups. Group 1 comprised genes dependent on both CgSkn7 and Cgyap1 and included many generic oxidative stress response genes similarly regulated in S. cerevisiae (CgTRR1/2, CgTRX2, CgTSA1/2, CgGPX2, and CgCTA1) [14,41]. Based on this congruence, we designate this group of genes as “core oxidative stress response” (COR; Table 3a). Furthermore, a group of genes was largely dependent on Cgyap1 (Fig. 2A, Group 3). We identified enrichment of Yap1 and Skn7 consensus sites in the promoters of the C. glabrata induced genes of the different groups (Fig. 2E). A group of 18 induced genes was not dependent on either Cgyap1 or Cgskn7 (Fig. 2A, Group 2). Group 2 contained genes associated to mitochondrial processes (CgACP1, CgPOP3, CgHSP10 and CgMRP10). Transcription of these genes might be redundantly regulated by Cgyap1 or CgSkn7. However, most genes of Group 2 were highly induced in the Cgyap1Δskn7Δ double mutant (Fig. 2A), suggesting the involvement of additional oxidative stress responsive factors. We found an overlap of 26 genes induced by oxidative stress to glucose starvation [42] (Fig. 2A, right panel). The majority of these belonged to Group 2, but also included core oxidative stress genes CgCTA1, CgGPX2 and CgTRX2.

3.3. Expression of important key enzymes to overcome oxidative stress is dependent on Cgyap1 and Cgskn7

To confirm the functions of Cgyap1 and CgSkn7 for C. glabrata, we measured the expression levels of exemplary genes of the defined groups (Fig. 3A and B). For genes dependent both on Cgyap1 and CgSkn7, we chose CgCTA1, CgTRR2, and CgTSA1 (Fig. 3A). In C. glabrata wild type cells, expression levels were rapidly and strongly induced upon treatment with 0.4 mM hydrogen peroxide and highly dependent on CgSkn7 and Cgyap1. To confirm the mutant data we included strains complementing the mutations with plasmids carrying the CgyAP1 and CgSKN7 genes regulated by their own promoters. We investigated if the expression of the superoxide dismutases CgSOD1 and CgSOD2 is dependent on the CgSkn7 and Cgyap1. We found that CgSOD1 and was constitutively expressed, and levels of both CgSOD1 and CgSOD2 were not changed during peroxide stress in the Cgyap1 and Cgskn7 mutant strains (Fig. 3A).

Group 2 comprised genes independent from Cgyap1 and Cgskn7. The reason for this could also be a redundant function of Cgyap1 and CgSkn7. Expression of CgHSP78 was not dependent on Cgyap1 and CgSkn7 or both (Fig. 3B). Therefore, other factors are involved in the regulation of Group 2 genes. Finally, Group 3 predicted a group of genes solely dependent on Cgyap1 and we observed a high dependency of CgHSP31 on Cgyap1.

Carbon source starvation induces resistance to oxidative stress. In addition, genes induced by carbon source starvation are up-regulated during phagocytosis [43]. Therefore, we investigated expression levels of oxidative stress genes during glucose depletion (Fig. 3C). After 1 h growth in medium lacking glucose CgCTA1

**Fig. 1.** C. glabrata survival during different chronic oxidative stress types. (A) C. glabrata resistance against oxidative stress causing agents. C. glabrata wild type, CgSOD1Δ, Cgyap1Δ, Cgsod1Δyap1Δ, CgSkn7Δ, and Cgyap1Δskn7Δ mutant cells were grown to an OD600 of 1 in YPD. 5 x 105 cells were dropped on gradient plates containing menadione, hypochlorite, peroxynitrite and hydrogen peroxide. In addition 5 x 105 cells from stationary over-night cultures were dropped on plates containing hydrogen peroxide (lower right panels).
was strongly up-regulated in a CgSkn7 and CgYap1 and also CgMsn2/4 (not shown) independent manner. Furthermore, expression of the superoxide dismutases CgSOD1 and CgSOD2 was similar in Cgyap1Δskn7Δ mutant cells (Fig. 3C).

Fig. 2. Comparison of genome-wide expression levels in response to oxidative stress. (A) Transcript sets represent average inductions and comparison of wild type strain versus Cgyap1Δ and Cgskn7Δ mutant strains, and the Cgyap1Δskn7Δ double mutant strain. All treatments were done at 30°C for 20 min. Genes were clustered after selection (at least once >3-fold induction). Group 1: dependent on CgSkn7 and CgYap1; Group 2: independent of CgSkn7 or CgYap1; Group 3: dependent on CgYap1. Genes found to be upregulated in S. cerevisiae upon oxidative stress are highlighted in red, asterisk indicates those dependent on Skn7/Yap1 or Yap1 alone in S. cerevisiae. Profile of C. glabrata wild type during glucose depletion is included. (B) Genes induced (>4-fold) in menadione-associated oxidative stress response. (C) The overlap between the hydrogen peroxide and menadione stress patterns. Genes were selected with an induction >2-fold. Among 41 overlapping genes, Cgyap1Δ and Cgskn7Δ dependent genes (>2-fold) were determined. (D) Identification of genes upregulated upon glucose starvation and either menadione stress (Group a) or hydrogen peroxide stress (Group b) (>4-fold induction). Gene names correspond to C. glabrata systematic ORF designations and S. cerevisiae orthologues. (E) Number of Skn7 and Yap1 consensus sites present within –1200 bp upstream regions of C. glabrata genes of Groups 1, 2 and 3.
We compared susceptibility of *C. glabrata* and *S. cerevisiae* strains to menadione (Fig. 3D). In *S. cerevisiae*, expression of ScSOD1 is regulated by ScYap1 during oxidative stress [14]. Correspondingly, we observed increased sensitivity of *S. cerevisiae* Scyap1Δ mutants. In contrast, *C. glabrata* Cgyap1Δ mutants had similar sensitivity to menadione as the wild type. *C. glabrata* and *S. cerevisiae* sod1Δ mutants were both highly sensitive. We conclude that protection against superoxide by superoxide dismutase seems to be uncoupled from CgYap1 control in *C. glabrata*.

3.4. CgYap1 and CgSkn7 are interdependent for activation of expression of oxidative stress genes

Genes comprising Group 1 were dependent on both CgYap1 and CgSkn7 for full expression. We tested recruitment of CgYap1 and CgSkn7 to oxidative stress gene promoters in silico analyses of promoter regions of Group 1 genes revealed that Yap1/Skn7 binding site pairs located in close proximity (10–20 nucleotides from centre to centre of each binding site) were significantly enriched (Fischer exact test *P* < 0.01). Moreover, inter-motif distances in these pairs were not evenly distributed but presented peaks at 10, 14, and 19 nucleotide distances, which correspond to roughly, 50 base pairs. These pairs were not evenly distributed but presented peaks at 10–20 nucleotides from centre to centre of each binding site. Therefore, we chose CgYap1 and CgSkn7 as target gene promoters for ChIP.

After 24 h, engulfed cells were recovered on YPD plates. CgYap1Δ, CgSkn7Δ and CgYap1Δsod1Δ mutant cells (Fig. 5). C. *glabrata* cells were added to macrophages in a 1:1 ratio. After 24 h, engulfed *C. glabrata* cells were recovered on YPD plates. CgYap1Δ, CgSod1Δ and CgSkn7Δaap1Δ mutant cells displayed nearly diminished survival rate. However, the loss of both CgSod1Δ and CgYap1Δ had a major and significant effect on surviving phagocytes. The oxidative burst is part of the strategy of phagocytic cells to erase engulfed cells. Consequently, we analyzed the importance of CgYap1, CgSkn7 and CgSod1 during phagocytosis. We infected murine bone marrow derived macrophages (BMDM) with *C. glabrata* Cgyap1Δ, Cgyap1Δskn7Δ, Cgsod1Δ and Cgyap1Δsod1Δ mutant cells (Fig. 5). *C. glabrata* cells were added to macrophages in a 1:1 ratio. After 24 h, engulfed *C. glabrata* cells were recovered on YPD plates. CgYap1Δ, CgSod1Δ and CgSkn7Δaap1Δ mutant cells displayed nearly diminished survival rate. However, the loss of both CgSod1Δ and CgYap1Δ had a major and significant effect on surviving phagocytes.

### 4. Discussion

The preferred environments of *C. glabrata* and *S. cerevisiae* differ radically. *C. glabrata* lives preferably on mucosal surfaces competing with the microbial flora and may also encounter phagocytic cells of the innate immune system. These environments may have selected its high resistance against starvation, oxidative and chemical stress. We found that in *C. glabrata*, like in *S. cerevisiae*, Yap1 and Skn7 regulate core peroxide stress resistance genes. Different to *S. cerevisiae*, expression of *C. glabrata* superoxide dismutases was not regulated by CgYap1 but dependent on carbon source. Both CgYap1 and CgSod1 were required for optimal survival during macrophage phagocytosis.

### Table 3a

| Gene name | Systematic name | Function | wt†/Δys‡ |
|-----------|----------------|----------|----------|
| CgTRR1    | CAGLO002530g    | Thioredoxin reductase | 22.8 1.1 |
| CgTRR2    | CAGLO011668g    | Thioredoxin reductase | 30.2 0.0 |
| CgTRX2    | CAGLO000803g    | Thioredoxin | 16.6 1.7 |
| CgTS1     | CAGLO007271g    | Thioredoxin peroxidase | 11.4 0.0 |
| CgTS2     | CAGLO006259g    | Thioredoxin peroxidase | 22.3 1.6 |
| CgGPX2    | CAGLO01705g     | Glutathione peroxidase | 22.4 2.1 |
| CgCTA1    | CAGLO10868g     | Catalase | 17.2 1.3 |

### Table 3b

| Gene name | Systematic name | Function | H2O2 | O2⁻ |
|-----------|----------------|----------|------|------|
| CgYAH1    | CAGLO000660g    | Ferredoxin | 2.4 1.3 | 2.4 1.3 |
| CgGPX2    | CAGLO01705g     | Glutathione peroxidase | 22.4 2.1 | 22.3 2.1 |
| CgADH6    | CAGLO14037g     | NADPH dehydrogenase | 2.1 0.0 | 8.8 2.2 |
| CgPYC1    | CAGLO14037g     | Pyruvate carboxylase | 2.7 1.2 | 2.2 1.3 |
| CgSMO2    | CAGLO04061g     | Core Sin protein | 2.8 0.0 | 2.2 1.3 |
| CgTS2     | CAGLO006259g    | Thioredoxin peroxidase | 22.3 1.6 | 2.4 1.4 |
| CgGRX7    | CAGLO003545g    | Monothiol glutaredoxin | 3.0 1.3 | 2.0 0.0 |
| YOR111W   | CAGLO002882g    | Unknown function | 2.2 1.1 | 2.9 0.0 |
| CgTRR2    | CAGLO01705g     | Thioredoxin reductase | 30.2 0.0 | 3.4 0.0 |
| YJR11C    | CAGLO04873g     | Unknown function | 2.4 1.4 | 2.3 0.0 |
| YOR111W   | CAGLO002882g    | Unknown function | 2.2 1.1 | 2.9 0.0 |

### Footnotes:

† Fold induction.
‡ Fold induction.
4.1. The C. glabrata core peroxide stress response is related to S. cerevisiae

In S. cerevisiae, expression of more than 70 genes is increased within minutes upon exposure to hydrogen peroxide [44]. ScYap1 controls about thirty genes of the S. cerevisiae oxidative stress regulon [14,41]. Fifteen of these proteins required both Skn7 and Yap1 for induction. This is in agreement with an earlier report on CgSkn7 regulated genes [39]. Two distinct Yap1 regulons were defined in S. cerevisiae, covering oxidative stress response, the second involved in the metabolic pathways regenerating the main cellular reducing power, GSH and NADPH [14]. In C. glabrata the core response to oxidative stress included thioredoxin peroxidases (CgTsa1, CgTsa2), thioredoxin reductases (CgTrr1, CgTrr2), the thioredoxin cofactor CgTrx2, the glutathione peroxidase CgGpx2, and the catalase CgCta1. Cgyap1Δ mutant cells displayed higher susceptibility to hydrogen peroxide. In contrast to S. cerevisiae, CgYap1 had only a small effect on the susceptibility to superoxide anions [45]. This is in line with the observation that the DNA recognition pattern recognized CgYap1 in C. glabrata is changed due to a point mutation from ScYap1 [29]. Similar to our results, CgSkn7 has been shown recently to be important for peroxide stress protection and for the induction of CgTRX2, CgTRR1, CgTSA1 and CgCTA1 [39].

4.2. CgYap1 and CgSkn7 cooperate for promoter binding

In S. cerevisiae, genetic and in vitro evidence suggested that a direct interaction between Yap1 and Skn7 is necessary for induction of a number of oxidative stress response genes [15,27]. Our ChIP data for two genes showed that cooperation occurs at the level of promoter recognition. This kind of interdependence was previously observed in vitro. Electrophoretic mobility shift assays demon-
strated the presence of a Skn7–Yap1 complex with the promoter DNA of TSA1 [14]. Here, we showed the first time in vivo, that Yap1 and Skn7 cooperatively bind to the upstream region of core oxidative stress genes.

4.3. Dual control by oxidative stress and carbon source

Twentyseven genes of the oxidative stress regulon are upregulated during glucose starvation [42]. *C. albicans*, *S. cerevisiae* and...
C. glabrata cells grown to stationary phase exhibit increased resistance against menadione and hydrogen peroxide [28,40,46]. Thus, adaptation to these environmental changes causes a simultaneous upregulation of a set of genes, beneficial for both oxidative stress and glucose starvation. The transcription factor mediating this activation in C. glabrata remains to be uncovered. CgMsn2/4 are most probably not involved since carbon source control of CgCta1 is also observed in the double mutant (Roetzer and Schüller, unpublished observation). Interestingly, nutrient limitation can also increase resistance to oxidative stress and to pH stress in bacteria such as Staphylococcus aureus and Salmonella typhimurium. An Afsod1ΔAfsod2Δ afsod3Δ triple mutant and Afskn7 and AfsYap1 mutant strains showed no defect in pathogenicity in murine infection models despite being sensitive against menadione and peroxide [47]. This might point to a comparable additive protection mechanism in A. fumigatus.

4.4. The distinct oxidative stress regulons have a synergistic impact on virulence

In a primary mouse BMDM macrophage model Cgyap1Δ and Cgyap1 Δskn7Δ mutant cells were similar resistant as the wild type. Accordingly, Cgyap1 Δskn7Δ cells can easily overcome 0.4 mM H2O2 stress, the concentration that C. glabrata cells most probably experience inside the mammalian host [28,48]. CgSod1Δ mutants had no severe decrease of survival upon phagocytosis. However, the combined loss of superoxide protection with loss of hydrogen peroxide response (Cgyap1 Δsod1Δ) made cells much more sensitive to BMDM internalization. We suggest that the production of superoxides inside the phagolysosome is intercepted by Cgsod1. Only if both Cgsod1 and Cgyap1 are absent, C. glabrata cells are more sensitive to the oxidative burst. It will be interesting to test whether this relates to the mechanism how C. glabrata cells can suppress ROS production upon internalization by macrophages [49]. In summary, in C. glabrata regulation of oxidative stress protective factors supports survival of phagocytosis conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.12.006.

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