INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGIA MOLECULAR

“La respuesta al estrés oxidante de *Candida glabrata* a través de la regulación génica de *CTA1*”

Tesis que presenta

Gabriel Guillermo Luna Arvizu

Para obtener el grado de

Maestro en Ciencias en Biología Molecular

Codirectores de la Tesis:

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San Luis Potosí, S.L.P., Septiembre de 2017

Constancia de aprobación de la tesis

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Créditos Institucionales

Esta tesis fue elaborada en el Laboratorio de Microbiología Molecular de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C., bajo la codirección de los doctores Alejandro De Las Peñas Nava y Guadalupe Gutiérrez Escobedo.

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Gabriel Guillermo Luna Arvizu

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Dedicatorias

A mis papás y hermanas por su apoyo y afecto conmigo durante mi vida.
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Resumen

La resistencia de *Candida glabrata* al estrés oxidante a través de la regulación de la expresión de *CTA1*

Candida glabrata es una levadura haploide patógena oportunista, que se encuentra como comensal en individuos sanos, pero puede causar infecciones severas en pacientes inmunocomprometidos. *C. glabrata* tiene mecanismos enzimáticos y no enzimáticos para contrarrestar al estrés oxidante. *C*. *glabrata* tiene solo una catalasa (Cta1) que le confiere resistencia a altas concentraciones de H_2O_2 *in vitro*. Esto sugiere que la regulación de la expresión de *CTA1* podría ser importante para entender la virulencia de esta levadura. Realizamos fusiones transcripcionales de la región intergénica entre *OYE2* y *CTA1* con GFP como gen reportero para mapear el promotor de *CTA1*, identificar los elementos en *cis* importantes para la regulación de este gen y determinar qué factores de transcripción son importantes para la inducción de *CTA1* en presencia de estrés oxidante. Generamos deleciones de 5' a 3' de la región intergénica entre *OYE2* y *CTA1* y analizamos la actividad del promotor de *CTA1* por citometría de flujo en diferentes fondos genéticos (cepa silvestre, *yap1Δ*, *skn7Δ*, *yap1 skn7Δ*, *msn2Δ*, *msn4Δ*, *msn2Δ msn4Δ*). El promotor basal se localiza entre -1 kb y -0.75 kb del ATG de *CTA1* y encontramos dos elementos regulatorios en *cis* localizados entre -4 kb y -3.3 kb, entre -1.34 kb y -1 kb. Además, determinamos que ambos factores de transcripción, Yap1 y Skn7, son necesarios para la inducción el promotor de *CTA1* en presencia de H_2O_2, mientras que Msn2 y Msn4 son dispensables. Adicionalmente, realizamos un ensayo de complementación heteróloga con pP_{ScCTA1}::Cg*CTA1*::3'UTR_{ScCTA1} en diferentes fondos genéticos de *S. cerevisiae*: cepa silvestre, *cta1*Δ, *ctt1*Δ, *cta1*Δ *ctt1*Δ. Tanto en fase estacionaria como en fase logarítmica de crecimiento, los diferentes fondos genéticos con el plásmido pP_{ScCTA1}::Cg*CTA1*::3'UTR_{ScCTA1} incrementan notablemente su resistencia a H_2O_2. Esto se debe a que la catalasa de *C*. *glabrata* presenta una mayor actividad enzimática.

Palabras clave: Estrés oxidante, regulación genética, catalasa

Abstract

Candida glabrata resistance to oxidative stress through _CTA1_ regulation

Candida glabrata is an opportunistic pathogenic haploid yeast that is found as a commensal in healthy individuals but can cause severe infections in immunocompromised patients. *C. glabrata* has enzymatic and non-enzymatic mechanisms to counteract oxidative stress. *C. glabrata* has only one catalase (Cta1) which confers resistance to high concentrations of H_2O_2 in vitro. This suggests that the regulation of *CTA1* expression might be important in understanding the virulence of this yeast. We did transcriptional fusions of the intergenic region between *OYE2* and *CTA1* and GFP as a reporter gene to map the *CTA1* promoter, to identify *cis*-acting regulatory elements and to determine which transcription factors are important for the induction of *CTA1* in the presence of oxidative stress. We generated 5' to 3' deletions of the intergenic region between *OYE2* and *CTA1* and analyzed the activity of the *CTA1* promoter by FACS (Fluorescence-Activated Cell Sorting) in different genetic backgrounds (wild type, *yap1*Δ, *skn7*Δ, *yap1*Δ *skn7*Δ, *msn2*Δ, *msn4*Δ, *msn2*Δ *msn4Δ*). The basal promoter is located between -1 kb and -0.75 kb from the ATG of *CTA1* and we found two *cis*-acting regulatory elements located between -4 kb and -3.3 kb, between -1.34 kb and -1 kb. In addition, we determined that both transcription factors, Yap1 and Skn7, are required for the induction of the *CTA1* promoter in the presence of H_2O_2, while Msn2 and Msn4 are dispensable. Additionally, we performed a heterologous complementation assay with p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UT$R_{Sc\mathit{CTA1}}$ on different genetic backgrounds of *S. cerevisiae*: wild type, *cta1*Δ, *ctt1*Δ, *cta1*Δ *ctt1*Δ). Both in stationary phase and in log phase of growth, the different genetic backgrounds with the plasmid p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UT$R_{Sc\mathit{CTA1}}$ remarkably increase their resistance to H_2O_2., due to *C. glabrata* catalase higher enzymatic activity.

Keywords. Oxidative stress, gene regulation, catalase

Running title: ***Candida glabrata*** **resistance to oxidative stress through *CTA1* regulation**

Keywords: Oxidative stress, gene regulation, catalase

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1. Abstract

Candida glabrata is an opportunistic pathogenic haploid yeast that is found as a commensal in healthy individuals but can cause severe infections in immunocompromised patients. *C. glabrata* has enzymatic and non-enzymatic mechanisms to counteract oxidative stress. *C. glabrata* has only one catalase (Cta1) which confers resistance to high concentrations of H_2O_2 in vitro. This suggests that the regulation of *CTA1* expression might be important in understanding the virulence of this yeast. We did transcriptional fusions of the intergenic region between *OYE2* and *CTA1* and GFP as a reporter gene to map the *CTA1* promoter, to identify *cis*-acting regulatory elements and to determine which transcription factors are important for the induction of *CTA1* in the presence of oxidative stress. We generated 5' to 3' deletions of the intergenic region between *OYE2* and *CTA1* and analyzed the activity of the *CTA1* promoter by FACS (Fluorescence-Activated Cell Sorting) in different genetic backgrounds (wild type, *yap1*Δ, *skn7*Δ, *yap1*Δ *skn7*Δ, *msn2*Δ, *msn4*Δ, *msn2*Δ *msn4Δ*). The basal promoter is located between -1 kb and -0.75 kb from the ATG of *CTA1* and we found two *cis*-acting regulatory elements located between -4 kb and -3.3 kb, between -1.34 kb and -1 kb. In addition, we determined that both transcription factors, Yap1 and Skn7, are required for the induction of the *CTA1* promoter in the presence of H_2O_2, while Msn2 and Msn4 are dispensable. Additionally, we performed a heterologous complementation assay with $pP_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'$UTR_{Sc\mathit{CTA1}}$ on different genetic backgrounds of *S. cerevisiae*: wild type, *cta1*Δ, *ctt1*Δ, *cta1*Δ *ctt1*Δ). Both in stationary phase and in log phase of growth, the different genetic backgrounds with the plasmid $pP_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'$UTR_{Sc\mathit{CTA1}}$ remarkably increase their resistance to H_2O_2., due to *C. glabrata* catalase higher enzymatic activity.

2. Introduction

The Fungi Kingdom is comprised of eukaryotic unicellular and multicellular organisms. Some have been identified as *Candida* which are unicellular fungi commonly found as commensals in the gastrointestinal and genitourinary tracts in humans (Gabaldón & Carrete, 2016).

2.1 *Candida glabrata*

C. glabrata is an opportunistic pathogenic yeast and its incidence has increased in the past two decades (Fidel et al., 1999) (Healey et al., 2016; Pfaller & Castanheira, 2016). *C. glabrata* has a 12.3 Mb genome organized in 13 chromosomes (A-M) encoding for 5293 open reading frames (ORFs) (as of June 2017), it has a G+C content of 38.8% and uses the standard genetic code. This yeast is haploid, monomorphic and has no described sexual cycle (Kaur, et al., 2005). *C*. *glabrata* belongs to the clade *Saccharomycetaceae*, thus it is closely related phylogenetically with *Saccharomyces cerevisiae* than with the other *Candida* species (Gabaldón & Carrete, 2016) (Figure S1).

2.2 Epidemiology

C. glabrata is the second most frequently isolated *Candida* yeast found in patients, causes up to 30% of all candidemias and has a higher attributable mortality (30-50%) than *C. albicans* (West et al., 2013; Linde et al., 2015). Results from sentinel and population-based surveillance from the United States and Europe show that the rank order for the five-major species of the *Candida* genus is *C. albicans* > *C. glabrata* > *C. parapsilosis* > *C. tropicalis* > *C. krusei*, which account for 95-97% of human fungal infections (Table S1) (Pfaller & Castanheira, 2016).

2.3 Virulence factors

The term virulence is the ability of an organism to cause a disease in a host. Pathogenic microbes have broad repertoire of virulence factors that are needed to succeed in a specific host-microbe interaction. For example, interaction with the host in specialized pathogens is part of their life cycle and opportunistic pathogens express their virulence under certain conditions (Gabaldón & Carrete, 2016). *C. glabrata*, an opportunistic pathogen yeast has been extensively studied and several of its virulence factors have been identified:

-*C. glabrata* is highly resistant to oxidative stress compared to other closely related yeasts, as it can neutralize the reactive oxygen species (ROS) by inducing antioxidant systems (catalase, superoxide dismutases, peroxidases, peroxiredoxins and glutathione) and the transcriptional regulation of the oxidative stress response (OSR) is controlled by the well-conserved transcription factors Yap1, Skn7, Msn2, Msn4 (Cuellar-Cruz et al., 2008; Cuéllar-Cruz et al., 2009; Roetzer et al., 2008; Roetzer et al., 2011). It has been reported that *C. glabrata* can survive and replicate inside of macrophages, which are the first line of defense of the immune system against pathogens. Phagocytosed yeasts prevent phagosome maturation, which allows them to replicate until the macrophage is lysed and cells are released (Kaur et al., 2007; Seider et al., 2011).

-Compared with other *Candida* species, *C. glabrata* has an innate resistance to azolic antifungals (Table S2). This resistance is due: a) to an increase in expression of ABC (ATP-binding cassette) transporters and other transporters of the MFS (Major Facilitator Superfamily), b) to *MSH2* disruption, which encodes a DNA repair protein that renders the cell with a mutator phenotype causing a higher frequency of

mutations enabling the cell to acquire multidrug resistance (MDR), and/or c) to an increased copy number of Erg11, the target protein of the azolic compound (Pfaller & Castanheira, 2016; Monk & Goffeau, 2008; Healey et al., 2016).

-*C. glabrata* can adhere to epithelial host cells through cell wall proteins (CWP, adhesins) and it has been proposed that this adherence is the first step towards a successful colonization. Adhesins are covalently bound to the cell wall through a GPI (glycosylphosphatidylinositol) domain and interact with the surface of epithelial cells through its N-terminal domain. *C*. *glabrata* has about 67 genes that encode for CWP: *PWPs*, *AWPs* and *EPAs* and only a few have been described in detail (de Groot et al., 2008). The *EPA* genes are present at subtelomeric regions and their expression is subject to chromatin based subtelomeric silencing mediated by the silencing machinery [Sir2-3-4, Rap1, Ku70/80, Rif1] and only *EPA1* is expressed *in vitro* in the BG14 strain background (Cormack, et al., 1999; De Las Peñas et al., 2003; Castaño et al., 2005). Analysis of the *EPA* genes family of adhesins has shown that *EPA1*, *EPA6* and *EPA7* mediate adherence to epithelial cells *in vitro* (Castaño et al., 2005; Zupancic et al., 2008). *EPA2* expression is induced *in vitro* in the presence of H_2O_2. *EPA2* expression is regulated by Yap1 and Skn7, and Msn4 (De Las Peñas et al., 2003; Kaur et al., 2005; Juárez-Cepeda et al., 2015). *EPA3* is induced during biofilm formation (Kraneveld et al., 2011).

-*C*. *glabrata* can form biofilms, which are a complex microbial community embedded in extracellular matrix that can form onto epithelial cells or bind to inert surfaces like catheters and other medical devices. Biofilms are resistant to antifungals and to the attack of the host immune system, therefore allowing the yeast to survive and persist inside the host (Fanning & Mitchell, 2012; Gabaldón & Carrete, 2016).

-*C. glabrata* can grow at elevated temperatures compared to other fungal species. *C*. *glabrata* can grow at standard human body temperature or higher (37-40°C).

2.4 Oxidative stress and reactive oxygen species (ROS)

ROS are a variety of highly reactive, partially reduced, oxygen species, which are generally unstable. They are produced as a result of O_2 excitation to form a singlet oxygen; or, from one, two or three electron transfer to O_2, to form respectively, superoxide radical (O_2–), hydrogen peroxide (H_2O_2) or hydroxyl radical (OH–) (Finkel & Holbrook, 2000; Balaban et al., 2005). ROS are produced in cell compartments such as mitochondria and peroxisomes and by enzymes such as the NADPH oxidases. However, the vast majority of cellular ROS (90%) is produced in mitochondria as a by-product of aerobic respiration (Balaban et al., 2005; Finkel & Holbrook, 2000). ROS can damage all biomolecules in the cell causing irreversible damage to DNA, proteins and lipids, resulting in cell death, however organisms have evolved enzymatic and non-enzymatic mechanisms to maintain the redox homeostasis inside the cell (Finkel & Holbrook, 2000; Briones et al., 2014; Dimri, et al., 2016).

2.5 Enzymatic and non-enzymatic response to ROS

Antioxidant enzymes play a fundamental role to maintain the redox homeostasis inside the cell. Superoxide dismutases (SOD) catalyze the conversion of superoxide to hydrogen peroxide, then the catalases and the glutathione peroxidases convert H_2O_2 to water (Briones-Martin-Del-Campo et al., 2014; Finkel & Holbrook, 2000). In addition to the enzymatic response to ROS there is also a non-enzymatic mechanism. Glutathione (GSH, a non-ribosomal tripeptide, glutamate-cysteine-glycine) maintains the reduced state of thiol groups, acts directly on ROS or as a

cofactor with glutathione peroxidase (GPx) or glutaredoxin (GRx) to detoxify the cell from ROS or xenobiotics (Gutiérrez-Escobedo et al., 2013; Holmgren et al., 2005).

2.6 Oxidative stress response

C. *glabrata*, as well as other pathogens, are able to survive within the host by recruiting these antioxidant enzymes and non-enzymatic molecules to neutralize ROS generated by macrophages and other phagocytic cells. This response is known as the oxidative stress response.

2.6.1 Regulation of the oxidative stress response (OSR)

OSR is mediated by proteins that detect external and intracellular stress and induce a response that affects cell physiology by modifying the transcriptional and translational profiles as well as proteolysis levels (Finkel & Holbrook, 2000; Chelikani, et al., 2004). Several well-conserved transcription factors (TF) that regulate the OSR in *C. glabrata* have been described: Yap1, Skn7, Msn2 and Msn4. Yap1 accumulates transiently in the nucleus during phagocytosis and activates, together with Skn7, the expression of *CTA1* (catalase 1), *TRR1/2* (thioredoxin reductase 1 and 2), *TSA1/2* (thioredoxin peroxidase 1 and 2), *TRX2* (thioredoxin 2), *GPX2* (glutathione peroxidase 2), *CCP1* (cytochrome c peroxidase 1) (Roetzer et al., 2010; Roetzer et al., 2011). However, in a murine model of systemic infection showed that Yap1 is dispensable (Chen et al., 2007). Skn7 is a highly conserved TF among fungal species and is activated in the presence of cell wall stress, oxidative stress and is required for oxidative stress adaptation (Singh et al., 2004; Cuéllar-Cruz et al., 2008; Ruprich-Robert et al., 2008). In addition, Skn7 regulates the expression of *TRX2*, *CTA1*, *TRR1*, *TSA1* and *GLR1* (glutathione oxidoreductase) and *TSA1*. In a murine model of disseminated candidiasis of *C. glabrata*, the

absence of Skn7 showed a reduced level of organ colonization (Saijo et al., 2010). Interestingly, both Yap1 and Skn7 are required for *EPA2* expression in the presence of oxidative stress (Juárez-Cepeda et al., 2015). Msn2 and Msn4 mediate, in part, the general and oxidative stress response. This TF share similar pathways with other fungal species like *S. cerevisiae* and *C. albicans* (Cuéllar-Cruz et al., 2008; Roetzer et al., 2008). DNA microarrays in *C. glabrata* have shown that Msn2 and Msn4 regulate genes involved in the osmotic, heat, carbon source and oxidative stress response (Roetzer et al., 2008, 2011).

2.7 Catalases

Catalases are metalloenzymes capable of degrading H_2O_2 to water and oxygen. These proteins are stable, highly resistant to denaturation and proteolysis (Bernroitner, et al., 2009; Chelikani et al., 2004). The overall reaction is:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

There are three protein families of these enzymes: heme-containing mono and bi-functional catalase, and manganese catalase. Mono-functional catalases are the most abundant of these enzymes, are found in every life domain and are divided into large-subunit (>75 kDa) and small-subunit (<60 kDa). The mono-functional catalases degrade only H_2O_2 and have different catalytic efficiencies. Bi-functional catalases can degrade H_2O_2 and other organic compounds, acting as electron donors, and they can differ in location, structure and properties. Manganese catalases have two Mn ions in their active site, are found only in bacteria and their activity is lower than other catalases (Chelikani et al., 2004; Zamocky et al., 2008, 2009).

Catalases localize in the mitochondria, peroxisomes and cytoplasm. Catalases can be found at high concentrations along with other antioxidant enzymes. For example, catalases are present in the peroxisome where fatty acids β-oxidation occurs generating toxic H_2O_2 (Roetzer et al., 2010; Smith & Aitchison, 2013). In addition, catalases which are present in the peroxisome require the peroxisomal targeting signal (PTS) to be imported into this organelle (Gould, et al., 1987, 1989).

Chemical properties vary among catalases. Catalases do not follow the Michaelis-Menten kinetics except at very low substrate concentrations and have k_{cat} (turnover number) values ranging from 54,000 reactions to 833,000 reactions per second (Chelikani et al., 2004). Catalases are catalytically active between 60-80°C at pH 7.0, show resistance to protein unfolding and resist proteolysis, probably due to a generally rigid structure (Switala & Loewen, 2002; Chelikani et al., 2004; Jia, et al., 2016).

Many pathogenic bacteria and fungi have shown reduced virulence in the absence of their catalases: in *C. albicans* in a murine model of disseminated candidiasis, in clinical isolates of *Exophiala*, in *Staphylococcus aureus* where it is even a marker for clinical diagnostics, in *Edwardsiella tarda* a fish pathogen, it is required for biofilm formation in *Pseudomonas aeruginosa* and in the plant pathogen *Xanthomonas citri* (Wysong et al., 1998; Över et al., 2000; Nakagawa et al., 2003; Srinivasa-Rao, et al., 2003; Sav et al., 2016; Chung et al., 2016; Tondo et al., 2016). However, other catalases have been shown to be dispensable for virulence as in *Aspergillus nidulans* and *Aspergillus fumigatus.* In *C*. *glabrata*, *CTA1* is induced in the phagocytic cells, however the *cta1*Δ mutant did not show a reduced colonization in a murine model of systemic infection. These results suggests the presence of other

antioxidant systems that compensate the absence of the catalases (Chang et al., 1998; Paris et al., 2003; Cuéllar-Cruz et al., 2008; Roetzer et al., 2010).

C. glabrata has one catalase, Cta1, a mono-functional 57 kDa heme-containing protein, classified as a small subunit catalase and is 85% similar to *S. cerevisiae* peroxisomal catalase (ScCta1). In *C*. *glabrata*, Cta1 mediates the high resistance to H_2O_2 (Cuéllar-Cruz et al., 2008). It is present in the cytosol at low H_2O_2 concentrations and accumulates in peroxisomes during respiration in spite of not having a canonical PTS. The expression of *CTA1* is induced in the presence of oxidative stress and carbon source deprivation and genetic analysis have shown that *CTA1* is regulated by Yap1 and Skn7 and partially by Msn2 and Msn4 (Cuéllar-Cruz et al., 2008; Roetzer et al., 2008, 2011). Interestingly, *S. cerevisiae*, a non-pathogenic yeast that is closely related phylogenetically to *C*. *glabrata*, has two catalases, ScCta1 (peroxisomal) and ScCtt1 (cytosolic), but *S. cerevisiae* is not as resistant to H_2O_2 as *C. glabrata*.

In this thesis, we aim to better understand how *CTA1*, which encodes for the catalase-1 in *C. glabrata*, is transcriptionally regulated in response to oxidative stress. We identified two *cis*-acting regulatory elements in the promoter region of *CTA1* located between -4 kb and -3.3 kb, and between -1.34 kb and -1 kb from the ATG. We determined that the basal promoter is located between -1 kb and 0.75 kb from the ATG. We found that both, Yap1 and Skn7, are required to induce the promoter in the presence of H_2O_2. In a heterologous complementation assay, we show that *CgCTA1* confers a notably higher resistance to H_2O_2 to *S. cerevisiae* due to a higher enzymatic activity.

Aim

To better understand the oxidative stress response through *CTA1* regulation in the opportunistic pathogen yeast *C. glabrata*.

Specific aims

- To identify *cis*-acting regulatory elements in the *CTA1* promoter region and the basal promoter
- To determine the role of the transcriptional factors Yap1, Skn7, Msn2 and Msn4 in the activation of the *CTA1* promoter
- To characterize the enzymatic activity of Cg*CTA1*

3. Materials and Methods.

Strains, plasmids and primers.

All strains, plasmids and primers used in this work are listed in Table S3, Table S4, and Table S5, respectively.

Media.

Yeasts were grown in standard yeast media with 2% agar added for plates. Synthetic complete (SC) contains 1.7 g/liter yeast nutrient base (YNB, without NH_2SO_4 and amino acids), 5 g/liter NH_2SO_4 and supplemented with 0.6% casamino acids (CAA) and 2% glucose. When needed, SC was supplemented with 25 mg of uracil/liter. Yeast extract-peptone-dextrose (YPD) medium contains 10 g/liter yeast extract, 20 g/liter peptone, and is supplemented with 2% glucose. Bacteria were grown in LB medium. LB medium contains 5 g/liter yeast extract, 10 g/liter tryptone, 5 g/liter NaCl and for plates, 2% agar were added. All strains were kept in freezer stocks at -80˚C in 15% glycerol.

Plasmid extraction.

Plasmid DNA minipreps were done using QIAGEN® QIAprep Spin Miniprep following manufacturer's instructions. All plasmid constructs were introduced into strain DH10 by electroporation and 100 µg/ml carbenicillin (Invitrogen TM) was added to select for the plasmids.

Yeast transformation.

Yeast transformations with supercoiled plasmids were performed as previously described using the LiOAc/single-stranded carrier DNA/PEG method (Castaño et al., 2003; Gietz & Schiestl, 2014).

FACS analysis of GFP expression.

C. glabrata strains were grown for 48 h (SP) at 30°C in SC medium. Cells were diluted into fresh media for logarithmic phase cells (LP) experiments and exposed to the non-lethal concentration of 5 mM of H_2O_2. GFP was used as the reporter gene to measure the activity of the *CTA1* promoter in terms of relative fluorescence units (RFU). 300 µL samples were taken and fluorescence was assessed by fluorescence activated cell sorter (FACS) using a FACSCalibur flow cytometer (BD Biosciences®). The fluorescence indicates the output value of the FL2 channel (green fluorescence detection). A total of 10,000 cells were analyzed for each sample and the geometric means were recorded. The negative control was the strain with a promoter-less GFP vector (CGM514). Experiments were repeated two or three times.

Mapping of the *CTA1* promoter.

We constructed a series of 5' to 3' deletions of the *CTA1* promoter. PCR fragments were cloned in pMC14 (*GFP*::3'UTR$_{CTA1}$) (Table S4, Figure 1). The resulting plasmids were transformed into BG14 (WT), *yap1*Δ, *skn7*Δ, *yap1*Δ *skn7*Δ, *msn2*Δ,

*msn4*Δ, *msn2*Δ *msn4*Δ strains and the activity of the *CTA1* promoter deletions was measured using GFP as reporter by FACS. Experiments were repeated at least three times.

H_2O_2 sensitivity assays.

All cultures were grown for 48 h in YPD at 30°C. H_2O_2 35 % (wt/wt) solution was obtained from Sigma-Aldrich®. For sensitivity assays of LP cells, saturated cultures were diluted in fresh media (YPD) in such a way that all strains went through seven doublings to reach an OD_{600nm} of 0.5 and for SP cells, 48 h saturated cultures were diluted into sterile water to an OD_{600nm} of 0.5. LP and SP cultures were then serially diluted in 96-well plates. Each dilution was spotted onto YPD plates and YPD with different concentrations of H_2O_2 (5, 10, 15 and 20 mM). The plates were incubated at 30°C for 48 h. Experiments were repeated at least three times.

Catalase activity assay.

Catalase activity assays were performed as described previously with slight modifications (Orta-Zavalza et al., 2013). Strains were grown for 48 h at 30°C in SC medium. Cells were diluted into spent medium and exposed to 1 or 5 mM H_2O_2 for 3 h at 30°C. Cells were collected by centrifugation, washed twice with distilled water and resuspended in lysis buffer [potassium phosphate (pH 7.0) supplemented with cOmplete™ Mini Protease Inhibitor Cocktail, Sigma-Aldrich®]. A volume of zirconia beads (BioSpec®) was added and cells were lysed with a FastPrep-24 homogenizer Instrument (MP Biomedicals®), applying a 60 s pulse at 6.0 m/s and placed on ice for 5 min (repeated six times). The lysate was centrifuged at 12,500 rpm for 20 min at 4°C to remove cell debris and the zirconia beads. The supernatant was used to measure the catalase activity and the protein content was determined with the

Bradford assay using bovine serum albumin (Sigma-Aldrich®) as standard. Catalase activity was determined spectrophotometrically by the breakdown of H_2O_2 by catalase at OD_{240nm}. The measurement was taken three times. The following volumes were used for the control solution: 1.99 ml of buffer PB, 1 ml 30 mM H_2O_2 and 0.01 ml of bovine liver catalase solution (to avoid bubbling). Measurements were set for 1 min and the activity was normalized to total protein content from the lysate and expressed as units per mg of protein. One unit is defined as the amount of catalase required for decomposition of 1 µmol H_2O_2 per minute.

4. Results

There are two regulatory elements and the basal promoter in the 4.6 kb intergenic region between *OYE2* and *CTA1*.

The catalase of *Candida glabrata* is central in the resistance to oxidative stress generated by H_2O_2. To understand how *CTA1* expression is regulated, we constructed a transcriptional fusion in a replicative plasmid of the intergenic region between *OYE2* and *CTA1* and GFP. In addition, we performed 5' to 3' deletions of this intergenic region and analyzed the activity of the *CTA1* promoter in the presence of H_2O_2 by FACS (Figure 1A). Recently, a 219 bp ORF (*CMC4*) was annotated and 7 of these plasmids contain this gene (Figure 1A). All plasmids were transformed into the BG14 parent strain (Table 1). The strains were grown in SC medium to stationary phase. The cells were diluted in fresh medium to an $O.D._{600nm}$ of 0.5 and samples were taken every 2 hours in the absence and presence of H_2O_2 (See Materials and Methods and the legend to Figure 1). In the absence of H_2O_2, we observed that the plasmids -4.56 kb, -4 kb, -3 kb, and -2 kb have a high constitutive

activity and their expression is maintained constant during the 6 h of the experiment (Figure 1B). The -4 kb and -2 kb plasmids exhibit induction upon dilution in fresh medium and the -3 kb has a lower expression, but is induced approximately two-fold and the expression is maintained high (Figure 1B). The expression of the -1 kb plasmid is low and constitutive and the -0.75 kb, -0.5 kb, 0.2 kb and empty vector have no activity (Figure 1B). In the presence of 5 mM H_2O_2, we identified three groups of plasmids (Figure 1C). Plasmids -4.56 kb and -4 kb (Figure 3C, Group I) are induced around two-fold. Plasmids -3.3 kb, -3 kb, -2.6 kb, -2.0 kb, -1.6 kb and -1.34 kb have a low basal expression, but respond to H_2O_2 (Figure 3C, Group II). The -1.0 kb, -0.75 kb and -0.2 kb group did not respond to H_2O_2 (Figure 3C, Group III). These data indicate that in the *CTA1* promoter there are two *cis*-positive regulatory elements located between -4 kb and -3.3 kb and between -1.34 kb and -1 kb, and the basal promoter is located between -1 kb and 0.75 kb (Figure 1D).

Yap1 and Skn7 are required to induce the expression of *CTA1* in the presence of H_2O_2.

Previously, genetic analyses have shown that the transcription factors of *C. glabrata* Yap1 and Skn7 and Msn2 and Msn4 are involved in the response to oxidative stress (Cuéllar-Cruz et al., 2008). To determine whether the regulation of *CTA1* expression in *C. glabrata* is mediated by these transcription factors, we analyzed the *CTA1* promoter activity in strains lacking these transcription factors in the absence and presence of H_2O_2 by FACS. The plasmid collection described in Figure 1A was transformed into the mutant strains *yap1*Δ, *skn7*Δ, *yap1*Δ *skn7*Δ, *msn2*Δ, *msn4*Δ, and *msn2*Δ *msn4*Δ (Table 1). The strains were grown as described in the legend to Figure 2 in the absence and presence of H_2O_2 (See Materials and Methods and

Legends to Figures 2 and 3). In the absence of H_2O_2, there is a constitutive expression of the *CTA1* promoter expression in all the analyzed plasmids in *yap1*Δ, *skn7*Δ and *yap1*Δ *skn7*Δ. Even the slight induction that is seen in Figure 1B in the first hour after the dilution in fresh medium is eliminated. In the presence of 5 mM H_2O_2 there is no induction of *CTA1* expression in *yap1*Δ, *skn7*Δ and *yap1*Δ *skn7*Δ strains (Figure 2A-F). In addition, the *skn7*Δ strain has a lower basal expression than the *yap1*Δ strain in both conditions (Compare Figures 2A, 2B and 2C, and Figures 2B, 2D and 2F). These results indicate that both Yap1 and Skn7 are required for induction of the *CTA1* promoter. Furthermore, Skn7 is required for the basal expression of *CTA1*. In contrast, the role of Msn2 and Msn4 in the expression of *CTA1* is different. The constitutive expression of *CTA1* in the absence of H_2O_2 is maintained in the strains *msn2*Δ and *msn4*Δ, however, there is a slight reduction in the basal expression in *msn2*Δ *msn4*Δ (Compare Figures 3A, 3C and 3E with Figure 1C). In contrast, the absence of both, Msn2 and Msn4, does not affect the induction of the *CTA1* promoter in the presence of H_2O_2 (Compare Figures 3B, 3D and 3F with Figure 1C). The induction by H_2O_2 in the mutants *msn4*Δ and *msn2*Δ *msn4*Δ is higher, however, the induction is higher because the basal levels are lower; the same induction level is reached in the three mutants. These data indicate that both Msn2 and Msn4 are required for the basal expression of *CTA1* and are dispensable for the induction of *CTA1* by H_2O_2.

Heterologous expression of Cg*CTA1* in *S. cerevisiae* increases H_2O_2 resistance.

Cta1 confers resistance to *C. glabrata* at high concentrations of H_2O_2 in both stationary phase and in log phase. In contrast, *S. cerevisiae*, a non-pathogenic yeast

but closely related phylogenetically to *C. glabrata*, has two catalases ScCta1 (peroxisomal) and ScCtt1 (cytoplasmic) but does not exhibit a high H_2O_2 resistance as *C. glabrata*. To determine whether the high H_2O_2 resistance of *C. glabrata* is mainly due to *CTA1*, we evaluated the resistance of *S. cerevisiae* expressing Cg*CTA1*. We constructed a replicative plasmid containing the Cg*CTA1* ORF under the Sc*CTA1* promoter and the 3'UTR of Sc*CTA1* ($P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UTR$_{Sc\mathit{CTA1}}$). We transformed the plasmid into the catalase mutants of *S. cerevisiae*: WT, *cta1*Δ, *ctt1*Δ and *cta1*Δ *ctt1*Δ (Table 1), and evaluated the resistance to H_2O_2 in stationary and in log phase cells. Strains were grown in SC to stationary phase and the cells were diluted in conditioned medium to an O.D.$_{600nm}$ of 1. Cells were exposed to different concentrations of H_2O_2 for 3 h. The cells were washed and serial dilutions were made in YPD medium. (See Materials and Methods and Legends to Figures 4 and 5). For logarithmic phase, the strains were grown in SC to stationary phase and diluted in fresh medium so that after 7 duplications the cultures reached an O.D.$_{600nm}$ of 1. Cells were exposed to different concentrations of H_2O_2 for 3 h. The cells were washed and serial dilutions were made in YPD medium. (See Materials and Methods and Legends to Figures 4 and 5). The strains Sc WT pVector and Sc *ctt1*Δ pVector grow to 6mM H_2O_2 (Figure 4A and C). In contrast, the strains Sc *cta1*Δ and Sc *cta1*Δ *ctt1*Δ grow to 2 mM H_2O_2 (Figure 4B and D). However, these *S. cerevisiae* strains carrying the plasmid p$P_{Sc\mathit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\mathit{CTA1}}$ become highly resistant to H_2O_2. The strains Sc WT p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UTR$_{Sc\mathit{CTA1}}$, Sc *cta1*Δ p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UTR$_{Sc\mathit{CTA1}}$, Sc *ctt1*Δ p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UTR$_{Sc\mathit{CTA1}}$ and the Sc *cta1*Δ *ctt1*Δ p$P_{Sc\mathit{CTA1}}$::Cg*CTA1*::3'UTR$_{Sc\mathit{CTA1}}$ grow to 300mM H_2O_2 (Figure 4A-D). Consistent with this increase in resistance of stationary phase cells carrying

pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$, log phase cells also increase their resistance from 0.1 mM to 1 mM H_2O_2 (Figure 5A-D) . In addition, the presence of pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$ does not affect the growth of *S. cerevisiae* strains. Doubling times (Figure 6) are very similar between all *S. cerevisiae* strains (Table 2). These data indicate that expression from the Sc*CTA1* promoter of the *Cg CTA1* in *S. cerevisiae* confers the higher increase in resistance to H_2O_2.

*Cg*Cta1 has a higher activity than *S. cerevisiae* catalases and is responsible for the increase in H_2O_2 resistance.

To determine whether the increase in resistance of *S. cerevisiae* to H_2O_2 expressing Cg*CTA1* is due to an increase in enzymatic activity, we decided to measure the enzymatic activity of the catalase in *S. cerevisiae* strains WT pVector, *cta1*Δ pVector, *ctt1*Δ pVector, *cta1*Δ *ctt1*Δ pVector, *cta1*Δ *ctt1*Δ pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$, clinical isolate YJM128, clinical isolate YJM336 and the *C. glabrata* strains, BG14 and *cta1*Δ. The strains were grown as described to the legend of Figure 7 (See Materials and Methods). In the strains of *S. cerevisiae* WT pVector, *cta1*Δ pVector, *ctt1*Δ pVector and *cta1*Δ *ctt1*Δ pVector, the catalase activity is so low that it cannot be measured even in the presence of 1mM of H_2O_2 (Figure 7). In contrast, in the *S. cerevisiae ctt1*Δ *cta1*Δ pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$ strain in the absence or presence of H_2O_2, there is a remarkable increase in the catalase activity (Figure 7). Basal enzyme activity in *C. glabrata* is the same as in the *S. cerevisiae* pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$, however, catalase activity is induced in the presence of 5mM H_2O_2 (Figure 7, CgWT). Consistent with the idea that resistance to oxidative stress is important for virulence, clinical isolates from *S. cerevisiae*, YJM128 and YJM336, have a higher catalase activity in the presence of 5mM H_2O_2

(Figure 7). These data indicate that the higher resistance to H_2O_2 in the *S. cerevisiae* strain carrying Cg*CTA1* is due to the higher enzymatic activity of *C. glabrata* catalase. In addition, the clinical isolates of *S. cerevisiae* have a higher enzymatic activity that could be important for survival and persistence within the phagocytic cells of the host.

5. Discussion

Fungal pathogens are eliminated inside phagocytic cells by the induction of reactive oxygen species (ROS), damaging all biomolecules; however, these organisms have evolved mechanisms to counteract the oxidative stress generated inside the cells of the host. These fungal pathogens reprogram their transcriptional profile through conserved transcription factors (Yap1, Skn7, Msn2 and Msn4) and in combination with the synthesis of antioxidant enzymes (Ctas, SODs, GPxs, etc.) and antioxidant molecules (GSH), can survive inside phagocytic cells.

Catalases are ubiquitous enzymes acting as scavengers of H_2O_2 and is central in maintaining the redox balance inside the cell. In some microbial pathogens, it has been shown that catalases play a crucial role in virulence (Wysong et al., 1998; Över et al., 2000; Nakagawa et al., 2003; Paris et al., 2003). Elimination of ROS is of utmost importance for fungal pathogens and this has been extensively studied. *C. glabrata* is no exception, as it has a robust oxidative stress response (OSR) that contributes to its virulence. This opportunistic fungal pathogen is among the most frequently isolated species in fungal infections. Thus, major insights in the mechanisms and factors that affect colonization and survival inside the host are needed.

In this work, we focused on the single catalase of *C. glabrata* (Cta1) that mediates the high resistance to oxidative stress. Our results showed that the basal promoter of *CTA1* is located within -1 kb from the ATG and its expression is regulated by the presence of *cis*-acting regulatory elements in this promoter region. Moreover, both Yap1 and Skn7 are required for the induction of the *CTA1* in the presence of H_2O_2, whereas Msn2 and Msn4 are dispensable for the activation of the *CTA1* in the presence of H_2O_2; however, both are required for the constitutive expression of *CTA1* in the absence of H_2O_2. In addition, the heterologous expression of Cg*CTA1* in *Saccharomyces cerevisiae* renders the cells more resistant to H_2O_2, due to a higher catalase enzymatic activity (Figure 8).

CgCTA1 is regulated by transcriptional factors Yap1 and Skn7 upon exposure to oxidative stress.

Previously, it has been demonstrated by genetic analysis that the transcriptional response to oxidative stress is mediated in part by the well-conserved transcription factors Yap1, Skn7, Msn2 and Msn4 however the quadruple mutant is not sensitive as *cta1*Δ (Cuéllar-Cruz et al., 2008). This suggests that there are additional transcriptional factors controlling the expression of genes encoding antioxidant proteins. In addition, microarray analysis data showed that *CTA1* is dependent on Yap1 and Skn7 (Roetzer et al., 2011). One report showed that Yap1 is located in the cytoplasm in unstressed conditions in *C. glabrata* but accumulated in the nucleus upon exposure to oxidative stress (Roetzer et al., 2010). Homologous transcriptional factors to Yap1 had been shown to directly regulate expression of catalases in other yeasts. In *Schizosaccharomyces pombe*, Pap1 is involved in the induction of catalase following oxidative stress and its enzyme activity is much higher

in Pap1-positive than Pap1-negative cells (Kim et al., 2008). In a ChIP-chip profiling in *C. albicans* , it was found that Cap1 binding was enriched at the *CAT1* locus and other genes known to be involved in the OSR (Znaidi et al., 2009). Moreover, other reports show that *skn7*Δ has decreased tolerance to H_2O_2 in stationary and log phase cells (Cuéllar-Cruz et al., 2008; Saijo et al., 2010). In addition, in *C*. *glabrata*, a qRT-PCR assay showed that *CTA1* is not induced in the presence of H_2O_2 in a *skn7*Δ background consistent with our data showing that both Skn7 and Yap1 are required for induction of *CTA1* in the presence of H_2O_2 (Figure 2 and 3). Interestingly, the *skn7*Δ strain showed attenuated virulence in a murine model of disseminated candidiasis, indicating that in addition to Cta1, there are additional genes controlled by Skn7 (and presumably by Yap1) important for virulence since the *cta1*Δ strain showed no effect in virulence (Cuéllar-Cruz et al., 2008; Saijo et al., 2010). In *C. neoformans skn7*Δ are more susceptible to ROS in vitro and are less virulent than the WT strain suggesting a role in virulence (Wormley et al., 2005). This genetic interaction between Skn7 and Yap1 suggests a physical interaction between these transcription factors. In fact, in *S. cerevisiae* it has been shown the physical association of the receiver domain of ScSkn7 and the cysteine-rich domain of ScYap1 (Mulford & Fassler, 2011). However, preliminary work in *C*. *glabrata* has shown that Yap1 and Skn7 do not interact physically even in the presence of H_2O_2. ScMsn2 and ScMsn4 are paralogous zinc finger transcription factors regulating the general stress response, whose functions are largely redundant (Boy-Marcotte et al., 2006). In addition, resistance to oxidative stress in *C. glabrata* stationary phase cells is dependent on the roles of Yap1, Skn7 and Msn4 and the adaptive response is dependent on Yap1, Skn7 and partially on Msn2 and Msn4 (Cuéllar-Cruz et al.,

2008). However, our analysis suggests that Msn2 and Mns4 are not required for the induction of *CTA1* in the presence of H_2O_2 (Figure 2 and 3). Interestingly, our data showed that Msn2 and Msn4 are required for basal expression of *CTA1*.

Heterologous expression of *CgCTA1* confers to *S. cerevisiae* an increase in resistance to H_2O_2.

Unlike mammalian cells that express only one type of catalase in the peroxisomes, *S. cerevisiae* has two catalase isoenzymes, peroxisomal ScCta1 and cytosolic ScCtt1 (Cohen et al., 1988). These isoenzymes are part of the mono-functional class and differentially regulated (Cohen et al., 1985; Marchler et al., 1993). In spite of having two genes encoding catalases, *S. cerevisiae*, a non-pathogenic yeast, has lower oxidative stress resistance than other fungal pathogenic yeast like *C. albicans* or *C. glabrata*. Interestingly, a *S. cerevisiae* clinical isolate has increased resistance to H_2O_2, albeit not at the same level as *C. glabrata* (Cuéllar-Cruz et al., 2008). *ScCTA1* and *ScCTT1* are not essential under logarithmic growth conditions and, although, the catalase genes are only moderately induced by H_2O_2, both are important for resistance to H_2O_2 in stationary phase (Izawa et al., 1996; Jamieson, 1998). ScCta1 is thought to play an important role in the survival of mother cells under conditions of increased oxygen (Hiltunen et al., 2003). In addition, lack of peroxisomal catalase affects the developmental program and aging of *Caenorhabditis elegans* (Petriv & Rachubinski, 2004). *ScCTT1* plays a central role under osmotic, heat and oxidative stress and water loss tolerance (Schüller et al., 1994; França et al., 2005). During cell division ScCtt1 has an important role in daughter cells to eliminate ROS (Erjavec & Nyström, 2007). Other reports suggests that loss of ScCtt1 has a small effect on viability (Longo et al., 1996).

In this work, we have shown that both Sc*CTA1* and Sc*CTT1* play a minor role in resistance to H_2O_2 in stationary and log phase, since the double mutant is slightly more sensitive than WT or the single mutants (Figure 4 and 5). Heterologous expression of Cg*CTA1* in *S. cerevisiae* in both log and stationary phase, the increase in resistance to H_2O_2 is remarkable. (Figure 4 and 5). Our results in log phase experiments are in accordance with previous studies that establish that catalases are dispensable in log phase and, therefore, the resistance to H_2O_2 seen in *S. cerevisiae* is at very low levels of H_2O_2 and due to other antioxidant mechanisms (Izawa et al., 1996)

Catalase activity is responsible for the increased resistance to H_2O_2 in *S. cerevisiae* and CI of *S. cerevisiae* exhibit enzyme activity even in the absence of H_2O_2.

Many types of stresses, such as oxidative stress and caloric restriction, and entering in stationary phase induce the expression of catalase in *S. cerevisiae* (Izawa et al., 1996). Measurements in various *S. cerevisiae* strains (WT, *cta1*Δ, *ctt1*Δ, *cta1*Δ*ctt1*Δ) of stationary phase cells exhibit catalase activity without H_2O_2 treatment, except in the double mutant; log phase cells increase the enzyme activity when exposed to H_2O_2 (not detected in the double mutant strain), but it is detected at much lower levels than stationary phase (Izawa et al., 1996). Interestingly, another report used the same background strain as this previously mentioned work, in addition to the BY4741 strain, and, when exposed to different H_2O_2 concentrations, the catalase activity in general, was slightly decreased in this genetic background (Martins & English, 2014). In *C. glabrata* catalase activity is regulated by oxidative stress and nutrient deprivation, and the increased H_2O_2 resistance is linked to the increase of

catalase activity in the absence of sirtuin, Cg*HST1*, involved in transcriptional repression of several genes (Roetzer et al., 2011; Orta-Zavalza et al., 2013). In two different studies conducted in *C. albicans*, it is demonstrated that heterozygous mutants lacking one or two alleles of WT catalase have reduced catalase activity and the homozygous null mutant had no detectable activity (Wysong et al., 1998; Nakagawa et al., 2003). In this work, we could not detect any catalase activity in any of the *S. cerevisiae* strains carrying the pVector. Surprisingly, in the case of the WT strain (BY47471), this contrasts with some of the previously mentioned studies where catalase activity is detected. The use of SC growth medium which differs in nutrient composition from complex growth medium could cause lower protein synthesis levels, therefore limiting detection during the readouts. Another cause can be that most of the studies were performed in log phase, in contrast to our measurements done in stationary phase, because of the evident increased resistance to H_2O_2 in budding yeast. One additional reason for this undetectable catalase activity in the WT strain can be due to the H_2O_2 concentration used in this work for *S. cerevisiae* cultures, since one study used lower concentrations and had higher catalase activity (Martins & English, 2014).

In the heterologous expression of Cg*CTA1* in *S. cerevisiae*, a higher catalase enzyme activity is present. The stability of the mRNA of *CgCTA1* could be higher or the *Cg*Cta1 enzyme is uniquely stable that can withstand stationary phase conditions, a period of rapid protein turnover and elevated protease levels (Chelikani et al., 2004). Future work should provide better insights of how this catalase of *C. glabrata* achieves higher levels of enzyme activity that causes an increase in

resistance to H_2O_2 in *S. cerevisiae* and *C. glabrata* (Figure 4 and 5; Cuéllar-Cruz et al., 2008)

Interestingly, Clinical Isolates (CI) of the non-pathogenic *S. cerevisiae* showed higher catalase activity upon exposure to oxidative stress compared to the reference strain, although not at the same levels of activity as in *C. glabrata* (Figure 7). CI of *S. cerevisiae* are more resistant to H_2O_2 in stationary and log phase compared to the reference strain (Cuéllar-Cruz et al., 2008). These results are in accordance with our findings, but more importantly, further confirm the idea that pathogens need a robust OSR in order to survive under hostile conditions encountered inside the mammalian host.

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7. Tables, figures legends and figures.

7.1 Tables

Table 1. Doubling times of *S. cerevisiae* WT, *cta1*Δ, *ctt1*Δ and *cta1*Δ *ctt1*Δ strains carrying the empty vector (pRS416) and with the pP$_{Sc}$*CTA1*-Cg*CTA1* (pCV27). *

***S. cerevisiae* strain**	**Doubling time (h)**
WT pVector	1.617
*cta1*Δ pVector	1.618
*ctt1*Δ pVector	1.701
*cta1*Δ *ctt1*Δ pVector	1.634
WT pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$	1.618
*cta1*Δ pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$	1.574
*ctt1*Δ pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$	1.606
*cta1*Δ *ctt1*Δ pP$_{ScCTA1}$::Cg*CTA1*::3'UTR$_{ScCTA1}$	1.624

***One biological replicate shown**

7.2 Figure legends

Figure 1. Activity of the *CTA1* promoter.

A) Map of the plasmids of the intergenic region between *OYE2* and *CTA1*. The *CMC4* gene is a recently reported small ORF. B and C) Analysis of the activity of the *CTA1* promoter by FACS in the absence and presence of H_2O_2. Strains were grown for 48 h in SC medium to stationary phase. Cells were diluted in fresh medium to an $O.D._{600nm}$ of 0.5 and samples were taken every 2 hours (0, 1, 2, 4 and 6 h) in the absence (B) and presence (C) of 5 mM H_2O_2. Samples were analyzed by FACS (See Materials and Methods). D) Schematic of the *cis*-regulatory elements of the *CTA1* promoter. The result of two biological replicates is shown.

Figure 2. Regulation of *CTA1* by Yap1 and Skn7.

Analysis of the activity of the *CTA1* promoter by FACS in the absence of the transcription factors Yap1 and Skn7, in the absence and presence of H_2O_2. Strains were grown for 48 h in SC medium to stationary phase. The cells were diluted in fresh medium to an $O.D._{600nm}$ of 0.5 and samples were taken every hour (0, 1, 2, and 3 h) in absence (A) and presence of 5 mM H_2O_2 (B) in the *yap1*Δ mutant; in the absence (C) and presence of 5mM H_2O_2 (D) in the mutant *skn7*Δ; and in the absence (E) and presence of 5mM of H_2O_2 (F) in the mutant *yap1*Δ *skn7*Δ. Samples were analyzed by FACS (See Materials and Methods). The results of three biological replicates are shown, except for the mutant strain *yap1*Δ, with two biological replicates.

Figure 3. Regulation of *CTA1* by Msn2 and Msn4.

Analysis of the activity of the *CTA1* promoter by FACS in the absence of the transcription factors Msn2 and Msn4, in the absence and presence of H_2O_2. Strains

were grown for 48 h in SC medium to stationary phase. The cells were diluted in fresh medium to an $O.D._{600nm}$ of 0.5 and samples were taken every hour (0, 1, 2, and 3 h) in absence (A) and presence of 5 mM H_2O_2 (B) in the *msn2*Δ mutant; in the absence (C) and presence of 5mM H_2O_2 (D) in the mutant *msn4*Δ; and in the absence (E) and presence of 5mM H_2O_2 (F) in the mutant *msn2*Δ *msn4*Δ. Samples were analyzed by FACS (See Materials and Methods). The result of three biological replicates is shown.

Figure 4. Resistance of *S. cerevisiae* to H_2O_2 in stationary phase in the presence of Cg*CTA1*.

Heterologous complementation of the *cta1*Δ, *ctt1*Δ and *cta1*Δ *ctt1*Δ strains of *S. cerevisiae* with the empty vector (pRS416) and the plasmid pP_{ScCTA1} :: Cg*CTA1* :: $3'UTR_{ScCTA1}$ (pCV27). Strains were grown in SC medium for 48 h to stationary phase and the cells were diluted in conditioned medium to an $O.D._{600nm}$ of 1. Cells were exposed to different concentrations of H_2O_2 for 3 h. The cells were washed and serial dilutions were spotted on YPD agar plates. (See Materials and Methods). A) *S. cerevisiae* WT pVector (empty vector) and WT pP_{ScCTA1} :: Cg*CTA1* :: $3'UTR_{ScCTA1}$. B) *cta1*Δ pVector and *cta1*Δ pP_{ScCTA1} :: Cg*CTA1* :: $3'UTR_{ScCTA1}$. C) *ctt1*Δ pVector and *ctt1*Δ pP_{ScCTA1} :: Cg*CTA1* :: $3'UTR_{ScCTA1}$. D) *cta1*Δ *ctt1*Δ pVector and *cta1*Δ *ctt1*Δ pP_{ScCTA1} :: Cg*CTA1* :: $3'UTR_{ScCTA1}$. Plates were incubated at 30°C for 48 h (See Materials and Methods).

Figure 5. Resistance of *S. cerevisiae* to H_2O_2 in log phase in the presence of Cg*CTA1*.

Heterologous complementation of the *cta1*Δ, *ctt1*Δ and *cta1*Δ *ctt1*Δ strains of *S. cerevisiae* with the empty vector (pRS416) and the plasmid pP_{ScCTA1} :: Cg*CTA1* ::

3'UTR$_{Sc\textit{CTA1}}$ (pCV27). The strains were grown in SC medium for 48 h to stationary phase and diluted in fresh medium so that after 7 duplications an O.D.$_{600nm}$ of 1 was reached. Cells were exposed to different concentrations of H_2O_2 for 3 h. The cells were washed and serial dilutions were spotted on YPD agar plates. A) *S. cerevisiae* WT pVector (empty vector), and WT pP$_{Sc\textit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\textit{CTA1}}$. B) *cta1*Δ pVector and *cta1*Δ pP$_{Sc\textit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\textit{CTA1}}$. C) *ctt1*Δ pVector and *ctt1*Δ pP$_{Sc\textit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\textit{CTA1}}$. D) *cta1*Δ *ctt1*Δ pVector and *cta1*Δ *ctt1*Δ pP$_{Sc\textit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\textit{CTA1}}$. Petri plates were incubated at 30°C for 48 h (See Materials and Methods).

Figure 6. Growth curve of *S. cerevisiae* WT strains, *cta1*Δ, *ctt1*Δ, *cta1*Δ *ctt1*Δ with empty vector (pRS416) and pP$_{Sc\textit{CTA1}}$ :: Cg*CTA1* :: 3'UTR$_{Sc\textit{CTA1}}$ (pCV27).

The strains were grown in SC medium for 48 h to stationary phase and diluted in fresh medium to O.D.$_{600nm}$ of 0.3. Incubation and optical density measurements were performed on the Bioscreen equipment. Measurements were recorded every 15 min with shaking for 48 h at 30°C. The result of a biological replicate is shown.

Figure 7. Enzymatic activity of different strains of *S. cerevisiae* and *C. glabrata* in stationary phase.

The strains were grown in SC medium for 48 hrs to stationary phase and diluted in conditioned medium at O.D.$_{600nm}$ of 5 for *S. cerevisiae* strains and O.D.$_{600nm}$ of 2.5 for *C. glabrata* cultures. Cells were treated in the absence and presence of H_2O_2 for 1 h and extraction of soluble total protein was performed. The amount of protein was quantified by Bradford assay. The enzymatic activity was measured by spectrophotometry to O.D.$_{240nm}$. The strains *S. cerevisiae* pVector (pRS416) and *C. glabrata cta1*Δ were treated for 1 h with 1 mM H_2O_2. *S. cerevisiae* strains *cta1*Δ *ctt1*Δ

pP_{Sc}*CTA1* :: Cg*CTA1* :: $3'UTR_{Sc}$*CTA1*, clinical isolates YJM128 and YJM336 and *C. glabrata* WT (BG14) were treated for 1 h with 5 mM H_2O_2. Sc: *S. cerevisiae*; Cg: *C. glabrata*; CI: clinical isolate; WT: wild type. The results of three biological replicates are shown (See Material and Methods).

Figure 8. Model of *CTA1* regulation upon oxidative stress exposure.

In this model, the transcriptional regulation of *CTA1* is mediated by the presence of two *cis*-acting regulatory elements and its basal promoter located between -1 kb and -0.75 kb. Both Yap1 and Skn7 transcription factors are required for the induction of the *CTA1* promoter in the presence of H_2O_2; Skn7 is also required for the basal expression of *CTA1* in the absence of H_2O_2. Msn2 and Msn4 are dispensable for the activation of the CTA1 promoter in the presence of H_2O_2; however, both are required for the basal expression of CTA1 in the absence of H_2O_2. Msn2 and Msn4 may be acting as homo or heterodimers to modify basal expression levels of *CTA1* and Msn4 may have a negative effect regarding activation of *CTA1* promoter. On the other hand, heterologous expression of Cg*CTA1* confers an increased resistance to H_2O_2 to *S. cerevisiae* in both stationary and log phase because the catalase of *C. glabrata* has higher enzymatic activity thus conferring the higher resistance to H_2O_2.

7.3 Figures

Figure 1

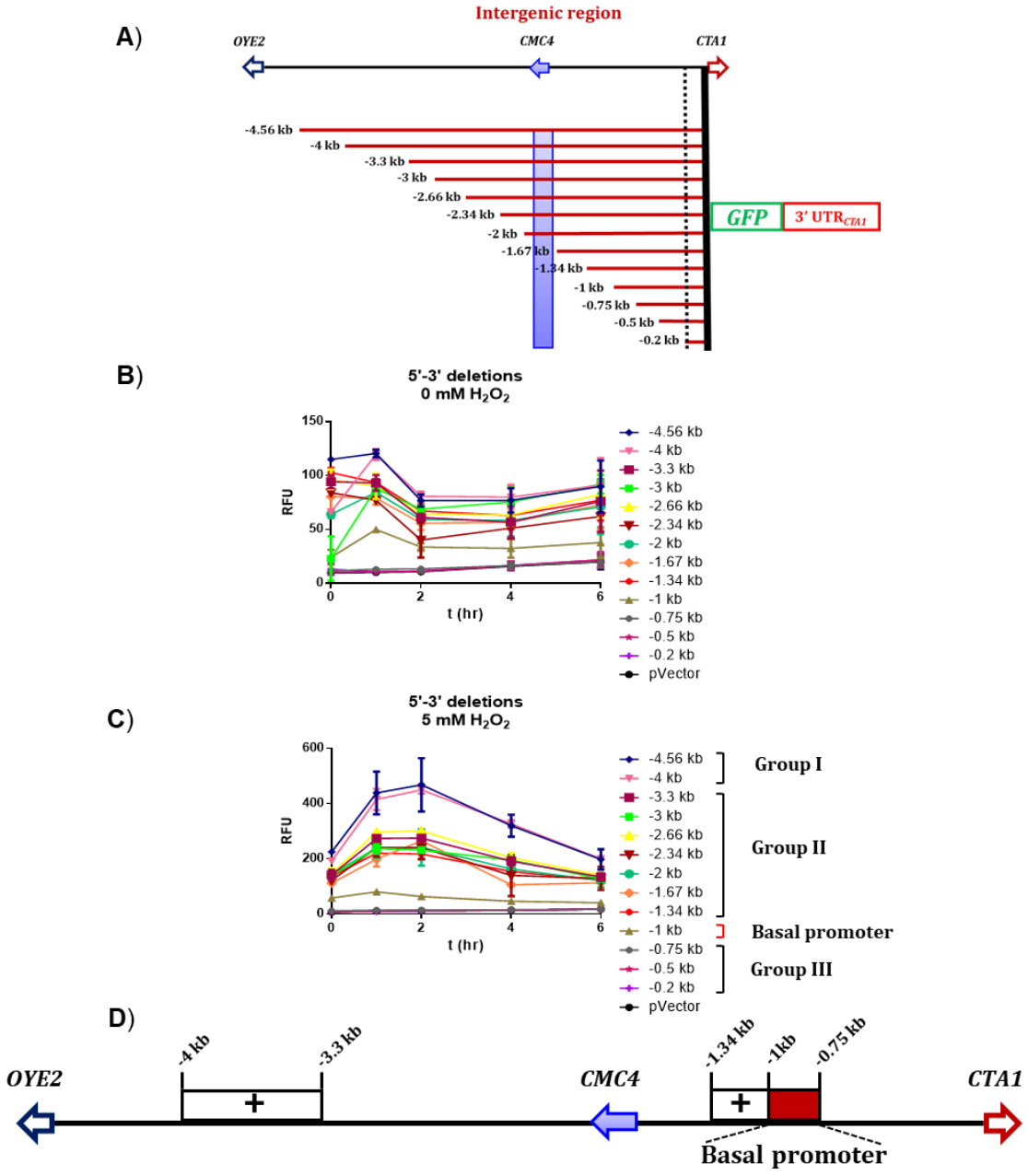


Figure 2

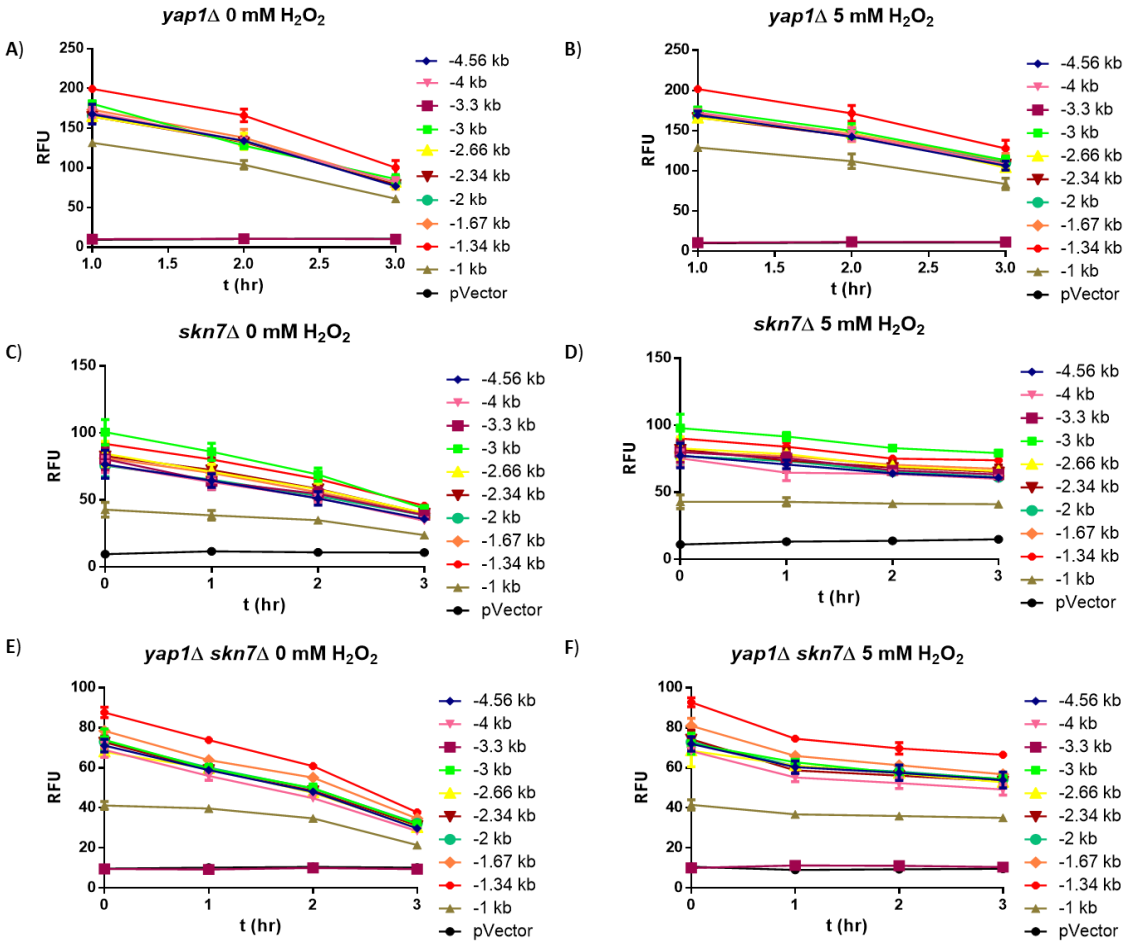

A)
yap1Δ 0 mM H2O2
B)
yap1Δ 5 mM H2O2
C)
skn7Δ 0 mM H2O2
D)
skn7Δ 5 mM H2O2
E)
yap1Δ skn7Δ 0 mM H2O2
F)
yap1Δ skn7Δ 5 mM H2O2
RFU
t (hr)
-4.56 kb
-4 kb
-3.3 kb
-3 kb
-2.66 kb
-2.34 kb
-2 kb
-1.67 kb
-1.34 kb
-1 kb
pVector

Figure 3

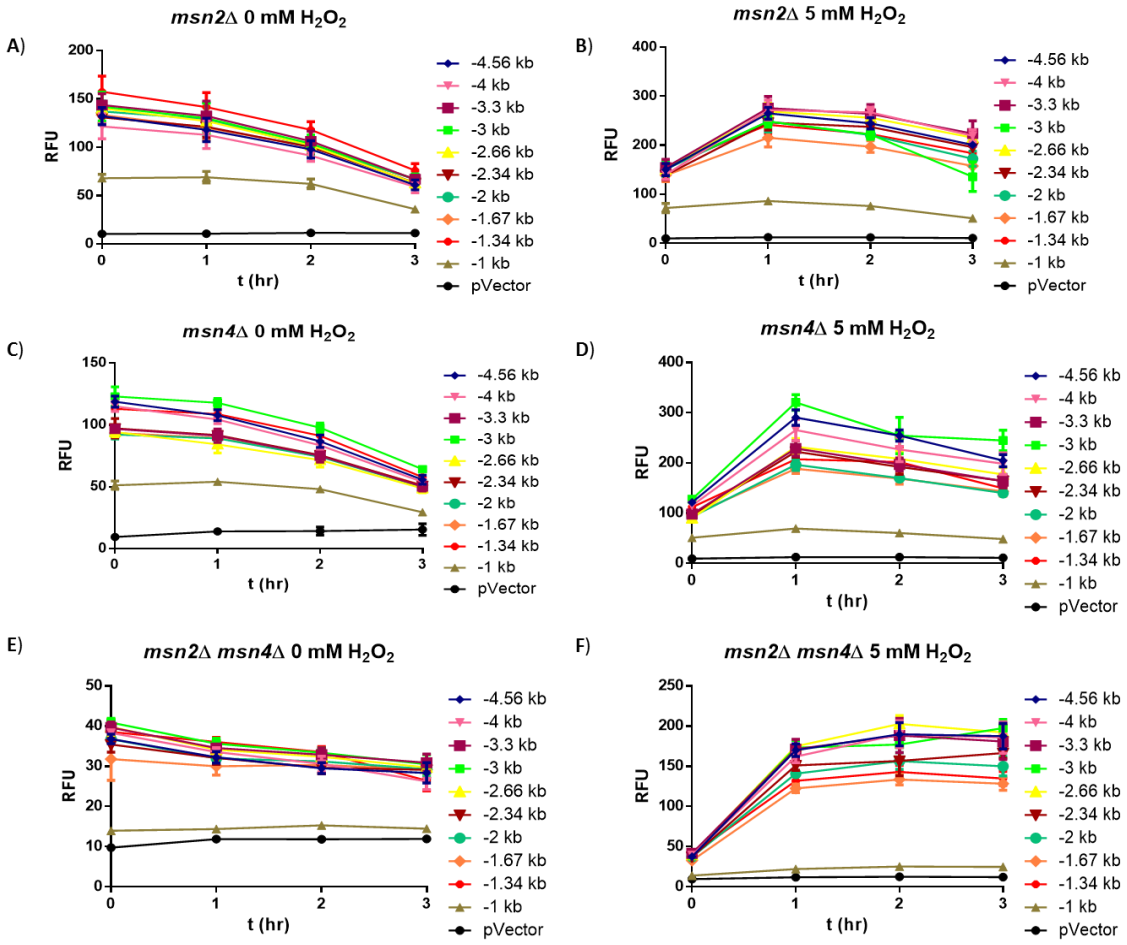

A)
msn2Δ 0 mM H2O2
B)
msn2Δ 5 mM H2O2
C)
msn4Δ 0 mM H2O2
D)
msn4Δ 5 mM H2O2
E)
msn2Δ msn4Δ 0 mM H2O2
F)
msn2Δ msn4Δ 5 mM H2O2
RFU
t (hr)
-4.56 kb
-4 kb
-3.3 kb
-3 kb
-2.66 kb
-2.34 kb
-2 kb
-1.67 kb
-1.34 kb
-1 kb
pVector

Figure 4

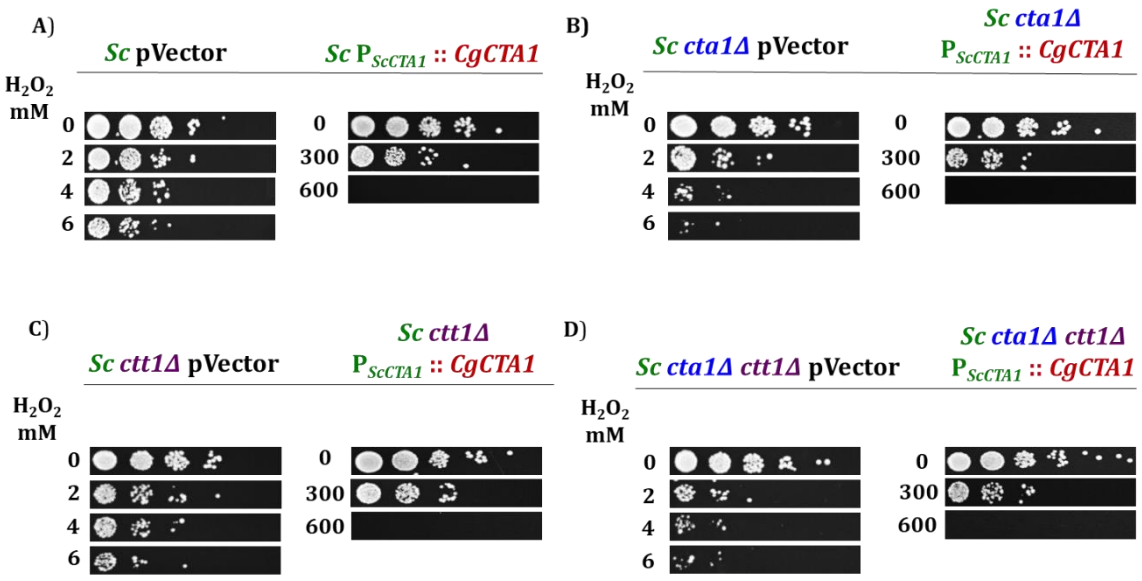

A)
Sc pVector
Sc P ScCTA1 :: CgCTA1
H2O2 mM
0
2
4
6
0
300
600
B)
Sc cta1Δ pVector
Sc cta1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
2
4
6
0
300
600
C)
Sc ctt1Δ pVector
Sc ctt1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
2
4
6
0
300
600
D)
Sc cta1Δ ctt1Δ pVector
Sc cta1Δ ctt1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
2
4
6
0
300
600

Figure 5

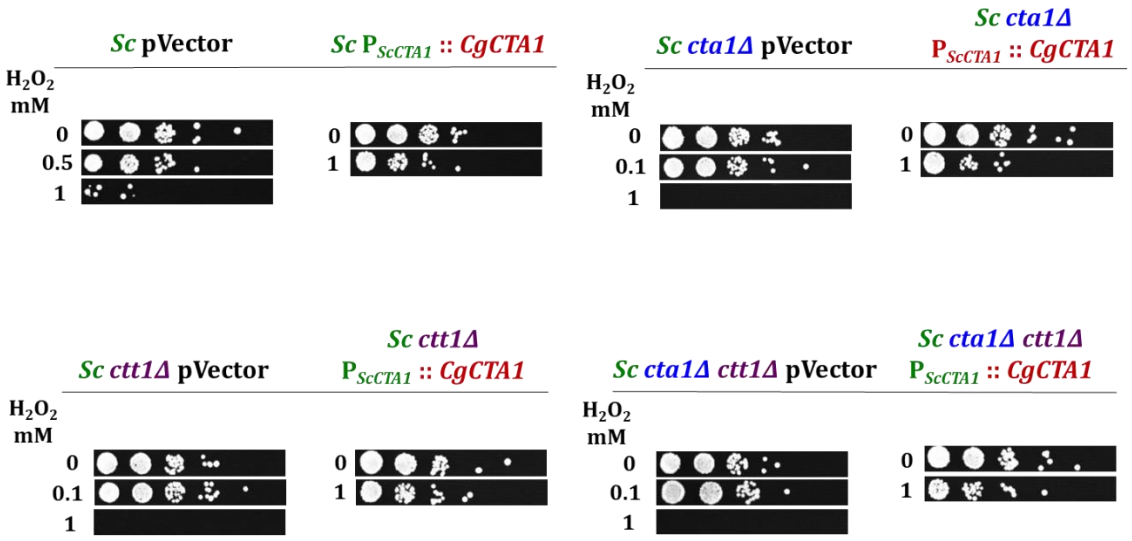

Sc pVector
Sc P ScCTA1 :: CgCTA1
H2O2 mM
0
0.5
1
0
1
Sc cta1Δ pVector
Sc cta1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
0.1
1
0
1
Sc ctt1Δ pVector
Sc ctt1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
0.1
1
0
1
Sc cta1Δ ctt1Δ pVector
Sc cta1Δ ctt1Δ
P ScCTA1 :: CgCTA1
H2O2 mM
0
0.1
1
0
1

Figure 6

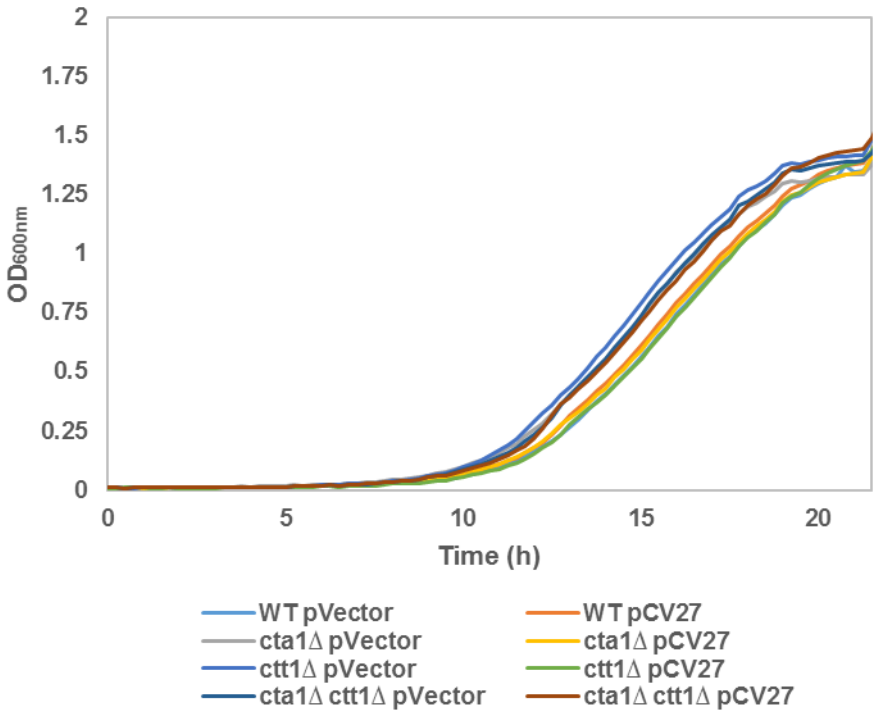

2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
OD600nm
0
5
10
15
20
Time (h)
WT pVector
WT pCV27
cta1Δ pVector
cta1Δ pCV27
ctt1Δ pVector
ctt1Δ pCV27
cta1Δ ctt1Δ pVector
cta1Δ ctt1Δ pCV27

Figure 7

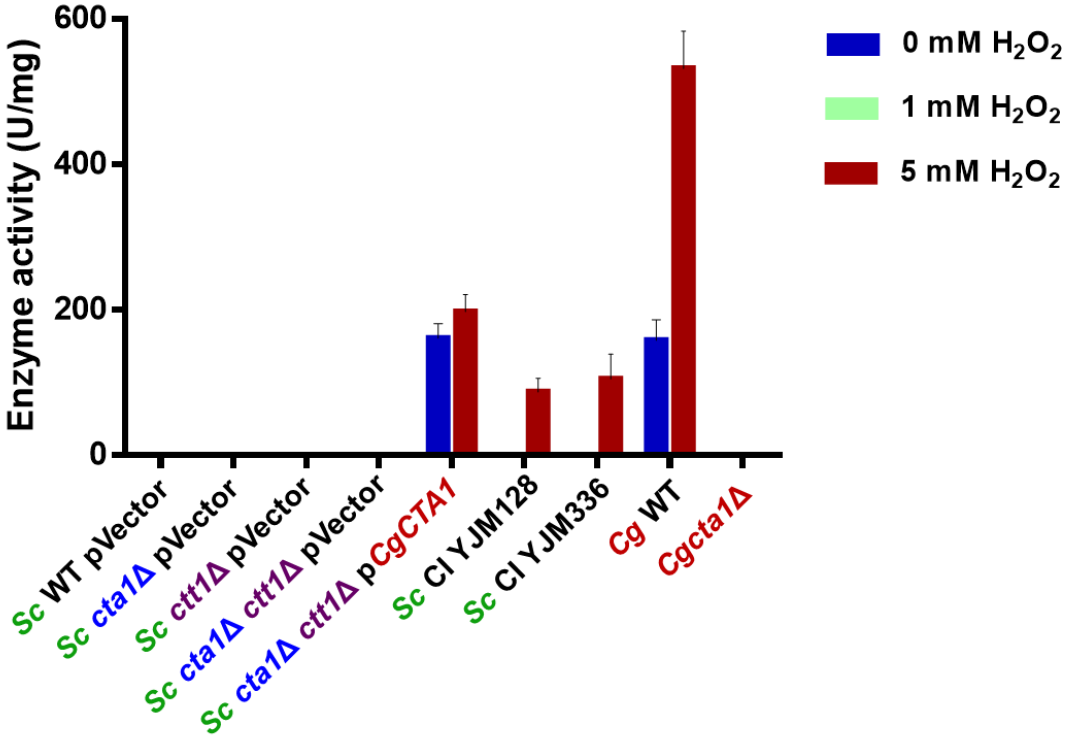
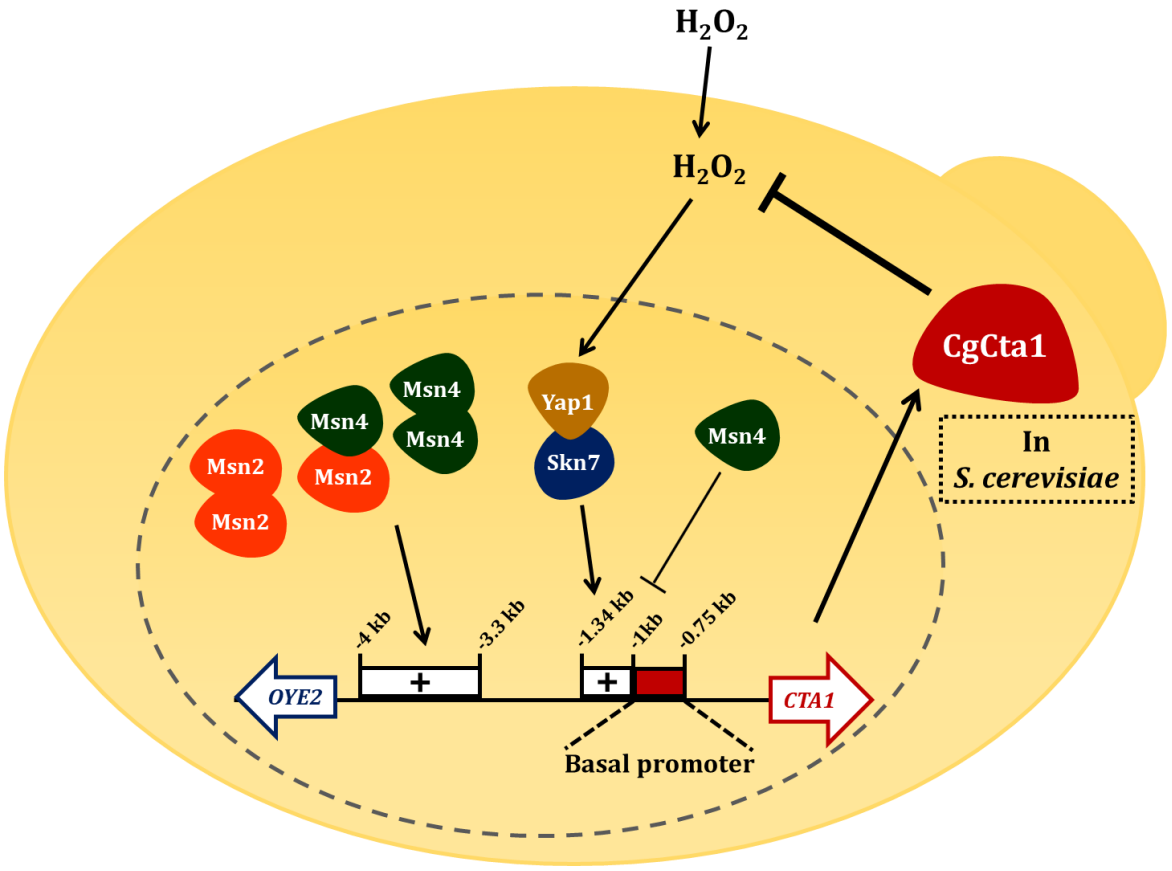

600
400
200
0
Enzyme activity (U/mg)
0 mM H2O2
1 mM H2O2
5 mM H2O2
Sc WT pVector
Sc cta1Δ pVector
Sc ctt1Δ pVector
Sc cta1Δ ctt1Δ pVector
Sc cta1Δ ctt1Δ pCgCTA1
Sc CI YJM128
Sc CI YJM336
Cg WT
Cgcta1Δ

Figure 8


H_2O_2
H_2O_2
CgCta1
In
S. cerevisiae
Msn4
Msn4
Msn4
Msn2
Msn2
Msn2
Yap1
Skn7
Msn4
-4 kb
-3.3 kb
-1.34 kb
-1kb
-0.75 kb
OYE2
CTA1
Basal promoter

8. Supplementary information

Table S1. Species distribution of *Candida* isolates from patients with candidemia: results from sentinel and population-based surveillance.

	% of total by surveillance program (# of isolates)		
Species	**SENTRY (860)**	**PATH (3,648)**	**CDC (2,209)**
C. albicans	47	42	41
C. glabrata	18	27	27
C. parapsilosis	18	16	18
C. tropicalis	11	9	9
C. krusei	1	3	2
Other	5	3	3

SENTRY: Antimicrobial Surveillance Program; PATH: Prospective Antifungal Therapy; CDC: Centers for Disease Control and Prevention **(Pfaller & Castanheira, 2016).**

Table S2. *Candida* species antifungal susceptibility profile.

	Susceptibility category as determined by CLSI Interpretive Criteria				
Species	**AMB**	**FLC**	**ITR**	**VOR**	**Echinocandins**
C. albicans	S	S	S	S	S
C. tropicalis	S	S	S	S	S
C. parapsilosis	S	S	S	S	S/?*
C. glabrata	S/NS	SDD/R	SDD/R	S/NS	S/R
C. krusei	S/NS	R	SDD/R	S	S

CLSI: Clinical and Laboratory Standards Institute; AMB: amphotericin B; FLC: fluconazole; ITR: itraconazole; VOR: voriconazole; S: susceptible; NS: non-susceptible; SDD: susceptible dose dependent; R: resistant.

*MIC for echinocandins and *C. parapsilosis* are elevated compared to other species. Fluconazole is the preferred agents for this species **(Pfaller & Castanheira, 2016).**

Table S3. *Escherichia coli*, *Candida glabrata* and *Saccharomyces cerevisiae* strains used in this study.

***E. coli* strain**	**Relevant genotype or description**	**Reference**
DH10B	*F⁻ mcrAΔ* (mrr-hsdRMS-mcrBC) *φ*80d*lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139Δ (ara,leu)7697 galU galKλ⁻ rpsL nupG*	(Calvin & Hanawalt, 1988)

***C. glabrata* strain**	**Parent**	**Relevant genotype or description**	**Reference**
BG2		Clinical isolate; WT	(Cormack, et al., 1999)
BG14 (Lab collection CGM1)	BG2	*ura3*Δ::Tn*903* G418^R Ura$^-$	(Cormack et al., 1999)
CGM295	BG14	*ura3*Δ::Tn*903* G418^R *cta1*Δ::*hph* Hyg^R Ura$^-$	(Cuéllar-Cruz et al., 2008)
CGM297	BG14	*ura3*Δ::Tn*903* G418^R *yap1*Δ::*hph* Hyg^R Ura$^-$	(Cuéllar-Cruz et al., 2008)
CGM306	BG14	*ura3*Δ::Tn*903* G418^R *skn7*Δ::*hph* Hyg^R Ura$^-$	(Cuéllar-Cruz et al., 2008)
CGM310	CGM297	*ura3*Δ::Tn*903* G418^R *yap1*Δ *skn7*Δ::*hph* Hyg^R Ura$^-$	(Cuéllar-Cruz et al., 2008)
BG1739	BG14	*ura3*Δ::Tn*903* G418^R *msn2*Δ Hyg^S Ura$^-$	(Mundy & Cormack, 2009)
BG1740	BG14	*ura3*Δ::Tn*903* G418^R *msn4*Δ Hyg^S Ura$^-$	(Mundy & Cormack, 2009)
BG1742	BG14	*ura3*Δ*::*Tn*903* G418^R *msn2*Δ *msn4*Δ::*hph* Hyg^R	(Mundy & Cormack, 2009)
CGM2935	BG14	*ura3*Δ::Tn*903* G418^R pCV35 Ura$^+$	This work
CGM2937	BG14	*ura3*Δ::Tn*903* G418^R pCV36 Ura$^+$	This work
CGM2939	BG14	*ura3*Δ::Tn*903* G418^R pCV37 Ura$^+$	This work
CGM2941	BG14	*ura3*Δ::Tn*903* G418^R pCV39 Ura$^+$	This work
CGM2943	BG14	*ura3*Δ::Tn*903* G418^R pCV41 Ura$^+$	This work

CGM2945	BG14	*ura3*Δ::Tn*903* G418^R pCV52 Ura$^+$	This work
CGM2967	BG14	*ura3*Δ::Tn*903* G418^R pCV25 Ura$^+$	This work
CGM2969	BG14	*ura3*Δ::Tn*903* G418^R pCV43 Ura$^+$	This work
CGM2971	BG14	*ura3*Δ::Tn*903* G418^R pCV54 Ura$^+$	This work
CGM2973	BG14	*ura3*Δ::Tn*903* G418^R pCV56 Ura$^+$	This work
CGM2975	BG14	*ura3*Δ::Tn*903* G418^R pCV58 Ura$^+$	This work
CGM2977	BG14	*ura3*Δ::Tn*903* G418^R pCV60 Ura$^+$	This work
CGM2979	BG14	*ura3*Δ::Tn*903* G418^R pCV64 Ura$^+$	This work
CGM2981	BG14	*ura3*Δ::Tn*903* G418^R pCV66 Ura$^+$	This work
CGM2983	BG14	*ura3*Δ::Tn*903* G418^R pCV68 Ura$^+$	This work
CGM2990	BG14	*ura3*Δ::Tn*903* G418^R pCV62 Ura$^+$	This work
CGM2992	BG14	*ura3*Δ::Tn*903* G418^R pCV70 Ura$^+$	This work
CGM2994	BG14	*ura3*Δ::Tn*903* G418^R pCV72 Ura$^+$	This work
CGM2996	BG14	*ura3*Δ::Tn*903* G418^R pCV74 Ura$^+$	This work
CGM2998	BG14	*ura3*Δ::Tn*903* G418^R pCV76 Ura$^+$	This work
CGM3000	BG14	*ura3*Δ::Tn*903* G418^R pCV78 Ura$^+$	This work
CGM3002	BG14	*ura3*Δ::Tn*903* G418^R pCV80 Ura$^+$	This work
CGM3004	BG14	*ura3*Δ::Tn*903* G418^R pCV82 Ura$^+$	This work
CGM3006	BG14	*ura3*Δ::Tn*903* G418^R pCV84 Ura$^+$	This work
CGM3268	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV25 Ura$^+$	This work
CGM3270	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV35 Ura$^+$	This work
CGM3272	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV36 Ura$^+$	This work
CGM3274	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV37 Ura$^+$	This work
CGM3276	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV41 Ura$^+$	This work
CGM3278	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV64 Ura$^+$	This work
CGM3280	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV66 Ura$^+$	This work
CGM3282	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV68 Ura$^+$	This work
CGM3284	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV70 Ura$^+$	This work
CGM3286	CGM297	*ura3*Δ::Tn*903 yap1*Δ G418^R pCV72 Ura$^+$	This work

CGM3288	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV25 Ura$^+$	This work
CGM3290	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV35 Ura$^+$	This work
CGM3292	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV36 Ura$^+$	This work
CGM3294	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV37 Ura$^+$	This work
CGM3296	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV41 Ura$^+$	This work
CGM3298	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV64 Ura$^+$	This work
CGM3300	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV66 Ura$^+$	This work
CGM3302	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV68 Ura$^+$	This work
CGM3304	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV70 Ura$^+$	This work
CGM3306	CGM306	*ura3*Δ::Tn*903 skn7*Δ G418^R pCV72 Ura$^+$	This work
CGM3313	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV25 Ura$^+$	This work
CGM3315	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV35 Ura$^+$	This work
CGM3317	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV36 Ura$^+$	This work
CGM3319	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV37 Ura$^+$	This work
CGM3321	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV41 Ura$^+$	This work
CGM3323	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV64 Ura$^+$	This work
CGM3325	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV66 Ura$^+$	This work
CGM3327	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV68 Ura$^+$	This work
CGM3329	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV70 Ura$^+$	This work
CGM3331	CGM833	*ura3*Δ::Tn*903 msn2*Δ G418^R pCV72 Ura$^+$	This work

CGM3333	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV25 Ura$^+$	This work
CGM3335	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV35 Ura$^+$	This work
CGM3337	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV36 Ura$^+$	This work
CGM3339	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV37 Ura$^+$	This work
CGM3341	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV41 Ura$^+$	This work
CGM3343	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV64 Ura$^+$	This work
CGM3345	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV66 Ura$^+$	This work
CGM3347	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV68 Ura$^+$	This work
CGM3349	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV70 Ura$^+$	This work
CGM3351	CGM835	*ura3*Δ::Tn*903 msn4*Δ G418^R pCV72 Ura$^+$	This work
CGM3353	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV25 Ura$^+$	This work
CGM3355	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV35 Ura$^+$	This work
CGM3357	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV36 Ura$^+$	This work
CGM3359	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV37 Ura$^+$	This work
CGM3361	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV41 Ura$^+$	This work
CGM3363	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV64 Ura$^+$	This work
CGM3365	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV66 Ura$^+$	This work
CGM3367	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV68 Ura$^+$	This work
CGM3369	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV70 Ura$^+$	This work
CGM3371	CGM310	*ura3*Δ::Tn*903 yap1*Δ *skn7*Δ G418^R pCV72 Ura$^+$	This work

CGM3373	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV25 Ura$^+$	This work
CGM3375	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV35 Ura$^+$	This work
CGM3377	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV36 Ura$^+$	This work
CGM3379	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV37 Ura$^+$	This work
CGM3381	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV41 Ura$^+$	This work
CGM3383	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV64 Ura$^+$	This work
CGM3385	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV66 Ura$^+$	This work
CGM3387	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV68 Ura$^+$	This work
CGM3389	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV70 Ura$^+$	This work
CGM3391	CGM837	*ura3*Δ::Tn*903 msn2*Δ *msn4*Δ G418^R pCV72 Ura$^+$	This work

***S. cerevisiae* strain**	**Parent**	**Relevant genotype or description**	**Reference**
BY4741		*MAT*a *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	(Brachmann et al., 1998)
BY4742		*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*	(Brachmann et al., 1998)
L74	BY4741	*MAT*a *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	(Winzeler et al., 1999)
L75	BY4742	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *YDR256C*::*KanMX4*	(Winzeler et al., 1999)
L76	BY4742	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 ctt1*Δ *YGR088W*::*KanMX4*	(Winzeler et al., 1999)
L77	L75	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *ctt1*Δ	(Winzeler et al., 1999)
L82	L74	*MAT*a *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0* pRS416 Ura$^+$	Lab collection
L83	L75	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *YDR256C*::*KanMX4* pRS416 Ura$^+$	Lab collection

L84	L76	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 ctt1*Δ *YGR088W*::*KanMX4* pRS416 Ura$^+$	Lab collection
L85	L77	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *ctt1*Δ pRS416 Ura$^+$	Lab collection
L90		Clinical isolate (YJM128)	(Clemons, et al., 1994)
L93		Clinical isolate (YJM336)	(Clemons et al., 1994)
L307	L74	*MAT*a *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0* pCV27 Ura$^+$	This work
L309	L75	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *YDR256C*::*KanMX4* pCV27 Ura$^+$	This work
L311	L76	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 ctt1*Δ *YGR088W*::*KanMX4* pCV27 Ura$^+$	This work
L313	L77	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 cta1*Δ *ctt1*Δ pCV27 Ura$^+$	This work

Table S4. Plasmids used in this study.

Plasmid	Relevant genotype or description	Reference
pGRB2.0	Replicative cloning vector. *URA3 CgCEN/ARS* Amp^R	(Domergue et al., 2005)
pRS416	Replicative cloning vector. *URA3 ScCEN/ARS* Amp^R	(Sikorski & Hieter, 1989)
pMC14	A 0.717 kb *Bam*HI/*Eco*RI fragment carrying the GFP and a 0.29 kb *Eco*RI/*Xho*I fragment carrying the $3'UTR_{CTA1}$ were cloned in pGRB2.0. *URA3 CgCEN ARS* Ap^R. Promotorless GFP vector	Lab collection
pMC71	A 2.145 kb fragment carrying Cg*CTA1* under the control of Sc*CTA1* promoter and the $3'UTR_{ScCTA1}$ (P_{ScCTA1}::GFP::$3'UTR_{ScCTA1}$) was cloned into pRS416. *URA3 CgCEN/ARS* Amp^R	Lab collection
pCV27	A 1.533 kb *Xba*I/*Eco*RI PCR fragment (primers 248/1018) carrying the Cg*CTA1* was cloned into pMC71. *URA3 CgCEN/ARS* Amp^R Cg*CTA1* under the control of Sc*CTA1* promoter and the $3'UTR_{ScCTA1}$ (P_{ScCTA1}::GFP::$3'UTR_{ScCTA1}$)	This work
pCV25	A 3 kb *Sac*I/*Bam*HI PCR fragment (primers 1026/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}3kb}$::GFP::$3'UTR_{CTA1}$)	This work
pCV35	A 1 kb *Sac*I/*Bam*HI PCR fragment (primers 1024/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1kb}$::GFP::$3'UTR_{CTA1}$)	This work
pCV36	A 4 kb *Sac*I/*Bam*HI PCR fragment (primers 1027/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}4kb}$::GFP::$3'UTR_{CTA1}$)	This work
pCV37	A 4.56 kb *Sac*I/*Bam*HI PCR fragment (primers 1028/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}4.56kb}$::GFP::$3'UTR_{CTA1}$)	This work
pCV39	A 0.5 kb *Sac*I/*Bam*HI PCR fragment (primers 1023/1022) carrying the promoter region of *CTA1* was	This work

	cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.5kb}$::GFP::3′UTR$_{CTA1}$)	
pCV41	A 2 kb *Sac*I/*Bam*HI PCR fragment (primers 1025/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}2kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV43	A 0.2 kb *Xba*I/*Bam*HI PCR fragment (primers 1450/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.2kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV52	A 3.6 kb *Sac*I/*Spe*I PCR fragment (primers 1028/1457) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}3.8kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV54	A 2.1 kb *Sac*I/*Xba*I PCR fragment (primers 1026/1022) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}2.3kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV56	A 0.5 kb *Sac*I/*Xba*I PCR fragment (primers 1028/1455) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.7kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV58	A 1.5 kb *Sac*I/*Xba*I PCR fragment (primers 1028/1454) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1.7kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV60	A 4.08 kb *Sac*I/*Nhe*I PCR fragment (primers 1028/1451) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}4.28kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV62	A 3.53 kb *Sac*I/*Nhe*I PCR fragment (primers 1028/1481) carrying the promoter region of *CTA1* was cloned into pCV43. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}3.73kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV64	A 3.3 kb *Sac*I/*Bam*HI PCR fragment (primers 1594/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP	This work

	under the control of *CTA1* promoter ($P_{CTA1\text{-}3.3kb}$::GFP::3′UTR$_{CTA1}$)	
pCV66	A 2.65 kb *Sac*I/*Bam*HI PCR fragment (primers 1595/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}2.65kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV68	A 2.34 kb *Sac*I/*Bam*HI PCR fragment (primers 1596/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}2.34kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV70	A 1.67 kb *Sac*I/*Bam*HI PCR fragment (primers 1597/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1.67kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV72	A 1.34 kb *Sac*I/*Bam*HI PCR fragment (primers 1598/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1.34kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV74	A 0.75 kb *Sac*I/*Bam*HI PCR fragment (primers 1599/1022) carrying the promoter region of *CTA1* was cloned into pMC14. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.75kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV76	A 1.31 kb *Sac*I/*Nhe*I PCR fragment (primers 1025/1600) carrying the promoter region of *CTA1* was cloned into pCV56. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1.51kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV78	A 0.8 kb *Sac*I/*Nhe*I PCR fragment (primers 1025/1601) carrying the promoter region of *CTA1* was cloned into pCV56. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}1kb}$::GFP::3′UTR$_{CTA1}$)	This work
pCV80	A 0.6 kb *Sac*I/*Nhe*I PCR fragment (primers 1025/1602) carrying the promoter region of *CTA1* was cloned into pCV56. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.8kb}$::GFP::3′UTR$_{CTA1}$)	This work

pCV82	A 0.4 kb *Sac*I/*Nhe*I PCR fragment (primers 1603/1025) carrying the promoter region of *CTA1* was cloned into pCV56. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.6kb}$::GFP::3′UTR_{CTA1})	This work
pCV84	A 0.2 kb *Sac*I/*Nhe*I PCR fragment (primers 1025/1604) carrying the promoter region of *CTA1* was cloned into pCV56. *URA3 CgCEN/ARS* Amp^R GFP under the control of *CTA1* promoter ($P_{CTA1\text{-}0.4kb}$::GFP::3′UTR_{CTA1})	This work

Table S5. Primers used in this study.

Primer #	Sequence (5'-3')	Description or restriction site
248	CTAG**tctaga**AAAATGTCCGCTAATCCAACTAACAC	*Xba* I
1018	AAA**gaattc**TTAGATCTTAGCAG	*EcoR* I
1022	TAGC**ggatcc**GTTTTTTTTCAATTG	*BamH* I
1023	CTT**gagctc**CGGCAATAGAC	*Sac* I
1024	GGG**gagctc**GTCGTTCAAC	*Sac* I
1025	CGGA**gagctc**TATCCCATATG	*Sac* I
1026	CTTT**gagctc**ATTAAAGATTGACC	*Sac* I
1027	CACA**gagctc**TTAACATACCGGG	*Sac* I
1028	GAAT**gagctc**TTGTTATGTTGTAG	*Sac* I
1451	TCCAAA**tctaga**GCAGTGTCTATTGCCGAAG	*Xba* I
1452	GAATGGGAT**tctaga**GGG	*Xba* I
1453	CGCAAT**tctaga**TGTGCACCTAGGAGATGC	*Xba* I
1454	GTTTAG**tctaga**GTTTTGAAAGCAGGTGAGG	*Xba* I
1455	ATGTAA**tctaga**GGTGAAAATGCATGGC	*Xba* I
1481	CGCA**gctagc**CCGTAGAACTCACAACAC	*Nhe* I
1594	GCCT**gagctc**GTTTACATTCAC	*Sac* I
1595	GAAC**gagctc**TAAGCTCAGAC	*Sac* I
1596	CAGA**gagctc**GAATATCGGACG	*Sac* I
1597	TGTT**gagctc**TTTATGTGCGC	*Sac* I
1598	CAGT**gagctc**TGTCCAACTTCG	*Sac* I
1599	CTTA**gagctc**GCCCTTTATCCTAC	*Sac* I
1600	GAGC**gctagc**TAGTAAAGGG	*Nhe* I
1601	GATA**gctagc**GTGAGTTGTAC	*Nhe* I
1602	CTGA**gctagc**CAGGAGTATAACTGC	*Nhe* I
1603	CCAA**gctagc**TTGTCCACATATCATCTG	*Nhe* I
1604	CGAT**gctagc**TACAAGTGTTGTAAG	*Nhe* I

Table S6. Conserved amino acids between *C. glabrata* (Cg*CTA1*) and *S. cerevisiae* (Sc*CTA1*) catalases.

Conserved domain	Conserved amino acids	Non-conserved amino acids	% of conserved amino acids
Heme binding group	H65, S104, N138, F143, F151, R346, Y350	None	100 (7/7)
NADPH binding site	P141, H186, I190, S193, R195, H227, V294, W295, P296, Q440, L444	H205N, K229L, Q297H, NXD	73 (11/15)
Tetramer interface	N63, G111, S112, G131, N132, D147, P148, S149, K150, F151, H153, H156, P162, Q163, T164, N165, L166, M171, D174, A246, G247, D251, Q254, Q255, A281, K282, S287, F289, L315, F318, Q323, T331, A339, P341, Q344, L347, F348, D352, A353, Y356, R357, L358, N361, H363, Q364, I365, P366, P370, F375, F376, P378, D382, P384, M385, N386, Y397, A399, G468, R476	N58R, R167K, E240D, T243V, F258Y, F317Y, Q320S, T330N, S349A, P360A, T396N, L398Y, N400S, D401N, E467A, C470D, Q472E	79 (59/75)

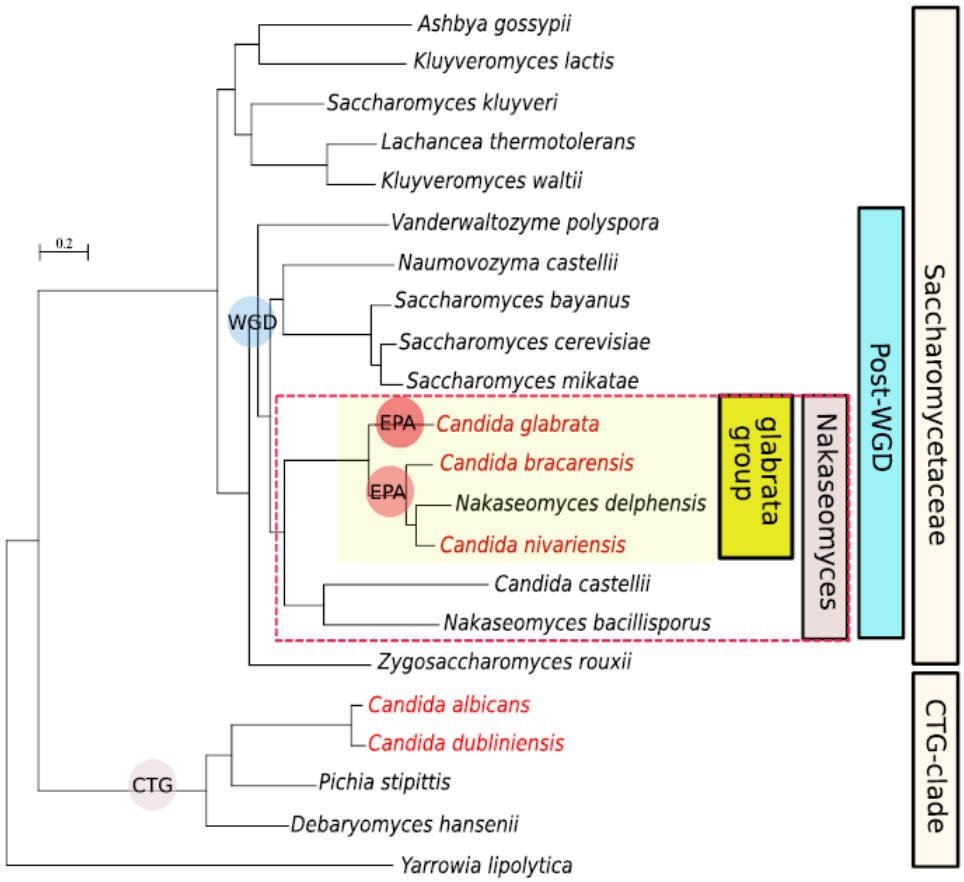


Figure S1. Phylogenetic relations between *C. glabrata*, Nakaseomyces clade species and Saccharomycotina subphylum species.

Pathogenic species (red), relevant taxonomic groups and significant evolutionary events (right side of the tree and colored circles, respectively). CTG: transition of the genetic code; WGD: ancestral whole-genome duplication/hybridization; *EPA*: occurrence of two independent expansions of *EPA* genes **(Gabaldón & Carrete, 2016).**

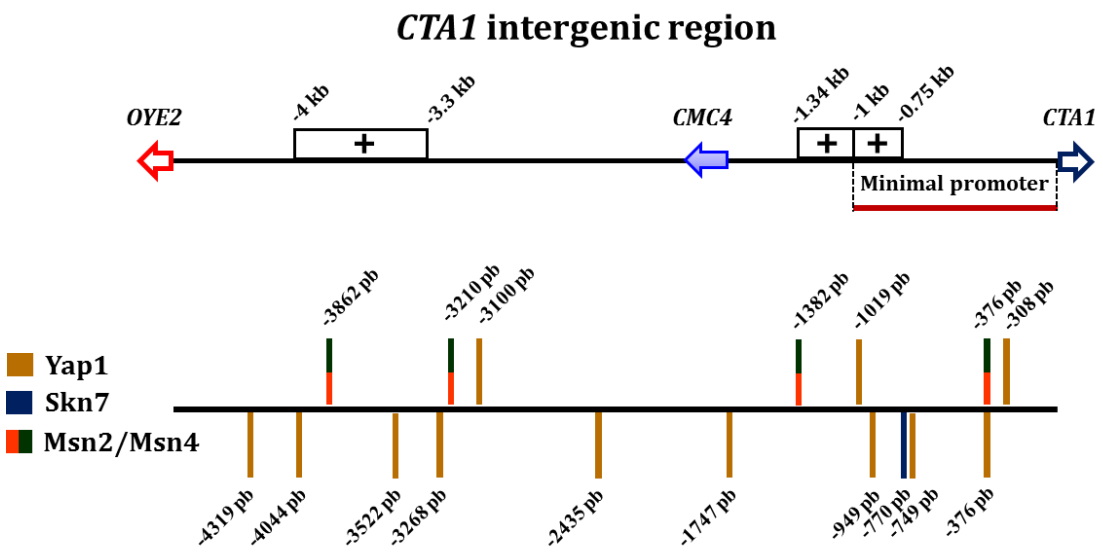


Figure S2. Putative binding sites for Yap1, Skn7, Msn2 and Msn4 in the *OYE2* / *CTA1* intergenic region.

Schematic representation of the *OYE2* / *CTA1* intergenic region where several putative binding sites for Yap1, Skn7, Msn2 and Msn4 were identified using YEASTRACT (Yeast Search for Transcriptional Regulators and Consensus Tracking, (www.yeastract.com/). Using this software, we found twelve putative Yap1 binding sites, one putative Skn7 binding site, and four putative binding for Msn2/Msn4. The orientation of transcription is represented by the direction of the arrows, the nucleotide position for predicted binding sites are shown in base pairs (pb) and depicts either the upper or the lower strand and these *cis*-acting elements for protein binding are color coded and shown on the left side.

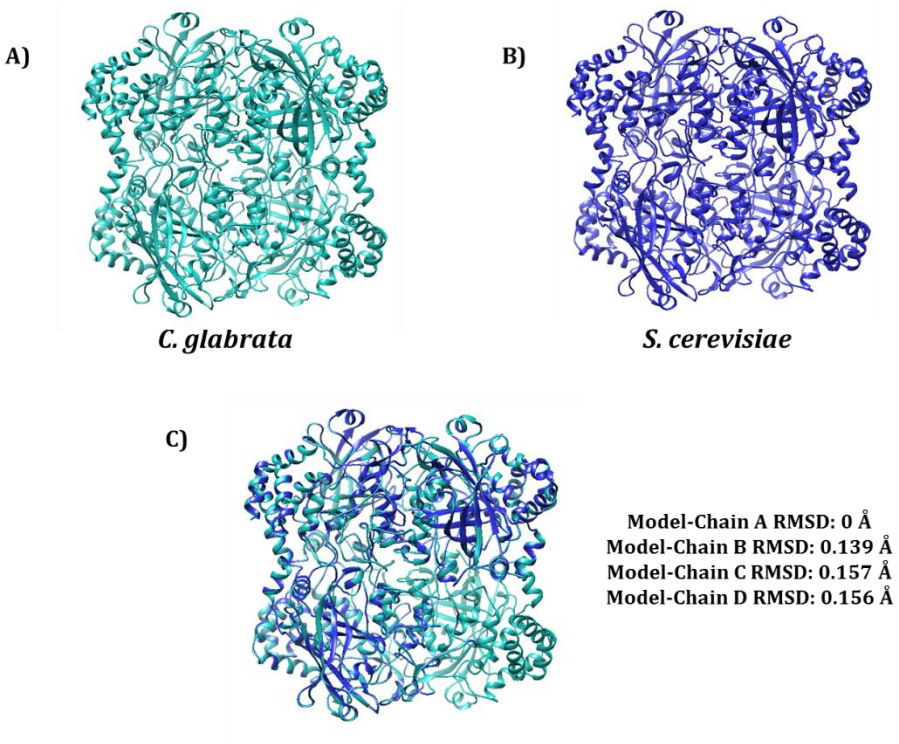


Figure S3. Protein structure prediction of the *Cg*Cta1 by homology modelling.

A) Predicted structure of *Cg*Cta1. B) Structure of *Sc*Cta1 retrieved from PDB database (accession code: 1A4E). C) Superimposition of *Cg*Cta1 predicted structure with *Sc*Cta1 (model) deposited structure; RMSD values for chain A, B, C and D respective to the model are shown on the right side.

Homology modelling was performed using PHYRE2 software (Protein Homology/analogY Recognition Engine, http://www.sbg.bio.ic.ac.uk/phyre2/) and structure superimposition was done using UCSF Chimera software (https://www.cgl.ucsf.edu/chimera/). Note that RMSD values closer to 0 Å represent a better match between the predicted structure and the model.