Combinatorial stress response of the fungal pathogen *Candida glabrata*

A thesis submitted for the degree of Doctor of Philosophy of

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II Declaration

I certify that this thesis and the research presented herein are the product of my own work, unless expressly stated. Any other information presented in this thesis, published or otherwise, is fully acknowledged and referenced in the bibliography.

Signed:

Melanie C. Puttnam, November 2012.

III Abstract

Candida glabrata is an opportunistic human fungal pathogen, with an increasing incidence of infection, as well as an innate resistance to antifungal drug therapies. It is more closely related to the model and non-pathogenic yeast, *Saccharomyces cerevisiae*, than other *Candida spp.* Previous studies have only focused on the response to independent stressors therefore little is known about the adaptive response to simultaneous stresses, even though this is likely to be more relevant in an ecological and pathophysiological setting e.g. upon macrophage engulfment. This study was conducted with the hypothesis that the response of *C. glabrata* to stressors applied simultaneously could not be explained by simply combining the response to single stresses. To investigate this hypothesis, the response of *C. glabrata* to hyperosmotic and oxidative stressors applied singly and in combination were examined by timecourse microarray analysis and functional genomics.

While genes involved in a HOG-like (High Osmolarity Glycerol) response were regulated by *C. glabrata* under hyperosmotic stress, many homologous genes are not observed to be regulated by *S. cerevisiae*. The phenotypes displayed by null mutants of the HOG pathway implicate this MAPK signalling pathway in not only hyperosmotic stress, but also cell wall integrity and metal ion resistance. Microarray analysis revealed a prolonged transcriptional regulation over time with increasing concentration of oxidative stress and other genes with a similar pattern of expression were identified and studied. Transcript profiling of a strain lacking the key oxidative stress regulator Yap1, along with bioinformatic analysis of its binding sites, identified possible targets of this transcription factor in *C. glabrata* under oxidative stress. This study has identified differentially regulated transcript profiles unique to simultaneous stress and not seen under single stress conditions, indicating that a specific transcriptional response is required for *C. glabrata* to respond and adapt to combinatorial stress; it is not simply the addition of two individual responses. Comparisons of the transcriptional analysis presented here with that of published macrophage engulfed *C. glabrata* cells revealed that combinatorial stress elicits a similar response as the host environment.

Combining functional genomics and transcript profiling under stress has allowed the identification and characterisation of genes involved in stress response as well as the construction of diagrams specific to the response of *C. glabrata* to stress.

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VIII Abbreviations

°C	degrees centigrade
µg	micro-gram
µl	micro-litre
ABC	ATP Binding Cassette
ANOVA	ANalysis Of Variance
ATCC	American Type Culture Collection
ATP	Adenosine Tri-Phosphate
$BaCl_2$	Barium Chloride
BLAST	Basic Local Alignment Search Tool
bp	base pair
bZIP	Basic Leucine Zipper Domain
$CaCl_2$	Calcium Chloride
$CdCl_2$	Cadmium Chloride
cDNA	complementary DNA
CHiP	CHromomatin immuno-Precipitate
$CoCl_2$	Cobalt Chloride
CsCl	Caesium Chloride
$CuCl_2$	Copper Chloride
Cy3	Cyanine dye; Cy3 fluoresces green (~550 nm)
Cy5	Cyanine dye; Cy5 fluoresces red (~650/670 nm)
DNA	DeoxyriboNucleic Acid
dSLAM	Diploid-based Synthetic Lethality Analysis on Microarrays
DTT	DiThioThreitol
EDTA	EthyleneDiamineTetraacetic Acid

ESR	Environmental Stress Response
EtBr	Ethidium Bromide
$FeCl_2$	Iron Chloride
g	gram
GFP	Green Fluorescent Protein
GO	Gene Ontology
GPR	GenePix Results
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione S-Transferase
H_2O_2	Hydrogen peroxide
kb	kilo base
KCL	Potassium Chloride
kDa	kilo Daltons (protein weight)
KEGG	Kyoto Encyclopedia of Genes and Genomes
KH	Klipp-Hohmann
kV	kilo volts
L	Litre
LB	Luria Broth
LiCl	Lithium Chloride
LOWESS	LOcally WEighted Scatterplot Smoothing
M	Molar
MAPK	Mitogen-Activated Protein Kinase; involved in MAPK signalling pathways, MAPKKKK phosphorylates MAPKKK which phosphorylates MAPKK which in turn phosphorylates MAPK
MAPKK	Mitogen-Activated Protein Kinase Kinase
MAPKKK	Mitogen-Activated Protein Kinase Kinase Kinase

MAPKKKK	Mitogen-Activated Protein Kinase Kinase Kinase Kinase
mg	milli-gram
$MgCl_2$	Magnesium Chloride
MIC	Minimum Inhibitory Concentration
min	Minutes
ml	milli-litre
mM	milli-molar
$MnCl_2$	Manganese Chloride
mRNA	messenger RNA
NaCl	Sodium chloride
$NADP^+$	Nicotinamide adenine dinucleotide phosphate; oxidised form
NADPH	Nicotinamide adenine dinucleotide phosphate; reduced form
NaOH	Sodium Hydroxide
NES	Nuclear Export Sequence
ng	Nano-gram
NLS	Nuclear Localisation Sequence
nm	Absorbance
ODγ	Optical Density; γ = absorbance (nm)
ORF	Opening Reading Frame
PCR	Polymerase Chain Reaction
PMN	PolyMorphonuclear Neutrophils
pg	pico-gram
RFP	Red Fluorescent Protein
RNA	RiboNucleic Acid
ROS	Reactive Oxygen Species
RPM	Revolutions per minute; rpm

RT	Reverse Transcriptase
SAPK	Stress Activated Protein Kinase
SC	Synthetic Complete
SDS	Sodium dodecyl sulphate
SGD	Saccharomyces Genome Database (http://www.yeastgenome.org/)
SPELL	Serial Pattern of Expression Levels Locator
TAP	Tandem Affinity Purification
TBE	Tris/Borate/EDTA
tBOOH	tert-butyl hydroperoxide
TE	Tris EDTA buffer
TF	Transcription Factor
TSA	Thiol-Specific Antioxidant
UTR	Un-Translated Region
UV	Ultra-Violet
V	Volts
w/v	Weight per volume
WT	Wild Type
YAP	Yeast Activator Protein
YFP	Yellow Fluorescent Protein
YGOB	Yeast Gene Order Browser (http://wolfe.gen.tcd.ie/ygob/)
YKO	Yeast KnockOut
YPD	Yeast Peptone Dextrose
YRE-A	Yeast Response Element – Adjacent
YRE-O	Yeast Response Element – Overlapping
$ZnCl_2$	Zinc Chloride

1 Introduction

1.1 General introduction

Candida glabrata is an opportunistic fungal pathogen of humans with an increasing incidence and worryingly high mortality rate, especially in immune-compromised patients. Stress response is especially important for pathogens such as *C. glabrata*, which must be able to adapt to the changing environments and defence mechanisms employed by the host in order to establish an infection. Regulators of stress response in fungal pathogens have been shown to be essential for virulence in infection models, linking stress adaptation to virulence. In a host setting, stresses would not occur singly, but simultaneously and in combination with each other. The response to stresses applied in combination has not been studied before, even though it is likely to be more biologically relevant for a pathogen. Therefore, this project was untaken to study and characterise the response of *C. glabrata* to combinatorial stress conditions by the understanding of the molecular biology of stress response.

To understand the response of *C. glabrata* to combinatorial stress, the response to stressors applied singly were first investigated. Hyperosmotic and oxidative stress were chosen to be examined and defined doses of the compounds used to elicit these stresses were established. Hyperosmotic stress has been extensively studied in other fungi, including the closely related model yeast *Saccharomyces cerevisiae* and is pertinent to a pathogen due to the varied sites in which *C. glabrata* can colonise. The response and adaptation of *C. glabrata* to hyperosmotic stress is presented in Chapter 4. The response to oxidative stress (Chapter 5) is also important for a pathogen like *C. glabrata* as this stress would be encountered when dealing with attack from the host immune system. Combinatorial stress response (simultaneous hyperosmotic and oxidative stress) was investigated to identify whether a unique transcriptional response would be observed compared to the response of single stresses or whether this response would resemble the addition of the two stressors applied singly (Chapter 6).

To characterise stress response in *C. glabrata*, transcriptional analyses and a functional genomics approach were used. Null mutants of genes identified as being involved in stress responses were created and phenotypically screened to elucidate their function in *C. glabrata*. Transcription profiling experiments were conducted over a time course under these defined stress conditions, singly and in combination. The transcriptional response of *C. glabrata* was

compared to published data of its close relative *S. cerevisiae* to stress and the response of *C. glabrata* engulfed by macrophages **[1, 2]**.

To understand the rationale behind this study, background information is presented in this chapter.

1.2 The fungal kingdom

In 1991, Hawksworth estimated that there were 1.5 million fungal species on Earth **[3]**. A more recent study concluded that while only 99,000 fungal species had been identified, the actual estimate was closer to 5.1 million, easily outnumbering plant species **[4]**. These numbers are increasing, with around 1000 new fungal species discovered every year. Fungi were originally assigned to the group, Thallophyta, which formed part of the Kingdom Plantae and as such the study of fungal species developed as a branch of botany. Although some fungi share similar characteristics to plants, sequence comparisons have shown that fungi actually diverged from a shared ancestor with animals approximately a billion years ago **[5]**. The major characteristic which separates fungi from plants and animals is that the fungal cell wall is mainly composed of glucans and chitin **[6]**. Glucans are also found in the cell wall of plants and chitin, in the exoskeleton of arthropods, however, fungi are the only organisms that can combine these together in their cell wall **[7]**.

The kingdom Fungi is one of the oldest and largest clades of living organisms and includes moulds, yeasts, mushrooms, smut and plant parasitic rusts. Fungi are remarkably diverse and can live as symbiotic, commensal and pathogenic organisms. Mycorrhiza is a symbiotic interaction between fungi and plant roots. There are more than 6000 fungi which are capable of forming mycorrahiza relationships, with the most common associated with arbuscular mycorrhizas from the *Glomus* spp. These fungi enhance the growth of the plant host, many crop plants included, by improving phosphate availability **[7]**. Fungi can be exploited by humans for very beneficial purposes. Not only do we use yeast to ferment wine, brew beer and make bread rise, but we also rely on fungi to produce many antibiotics, for example, Penicillin from *Penicillium chrysogenum* **[7]**.

Fungi have a wide range of morphologies, encompassing an array of taxonomic characteristics. Some species grow as single-celled yeasts that reproduce by budding or fission, such as the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively **[8]**. Other fungal morphologies include: dimorphic fungi which can switch between a yeast phase and a hyphal phase, such as *Candida albicans*; the aero-aquatic

Helicoon gigantisporum which produces tightly coiled conidia to trap air and allow the spore to be buoyant, facilitating growth in aquatic environments; and filamentous fungi, such as *Aspergillus* spp. which form long, branching hyphae structures and spore-producing bodies called conidiophores (reviewed in **[4, 7]**). These morphologies allow fungi to occupy a wide range of ecological niches, from soil and water dwelling to fruit, plant and animal hosts.

1.3 Pathogenic fungi

The relationship between humans and fungi can also be detrimental with the devastating impact of human mycoses and plant diseases. The rice blast fungus *Magnaporthe oryzae* causes lesions on the rice plant leaf and contributes to huge losses in crop yield (As reviewed in **[9]**). The three most prevalent pathogenic fungi of humans are *Candida* (72.8 million cases per year), *Cryptococcus* (65.5 million cases per year) and *Aspergillus* (12.4 million cases per year) species **[10]**. Colonisation by *Candida* spp causes candidosis which is the most common cause of HIV-related fungal infections **[7]**. *Cryptococcus* is associated with a severe form of meningitis and Aspergillosis, which is caused by inhalation of *Aspergillus* condida, causes a range of respiratory diseases as the spores become lodged in the lungs **[7]**. With these fungi having an average fatality rate of 23.3% **[10]**, biological knowledge and effective antifungal drug treatments are urgently needed.

1.4 *Candida* spp.

Candida resides in the phylum *Ascomycota*, the largest of the Fungi phyla. *Ascomycota* comprises almost 50% of all known fungal species and approximately 80% of the pathogenic and opportunistic species **[11]**. Many *Candida* spp. exist as commensal organisms of the normal human gut flora and are not associated with human morbidity. However, those that are pathogenic contribute to make *Candida* spp. the fourth most common bloodstream isolate in patients in the United States **[10]**. The *Candida* spp. identified from patients include *C. albicans* (62.3%), *C. glabrata* (12%), *Candida tropicalis* (7.5%), *Candida parapsilosis* (7.3%), *Candida krusei* (2.7%) and other *Candida* spp. (8.2%) **[10]**. *Candida* infections are associated with an increasing incidence and mortality rate, with disruption of the mucosal barrier, patients under immune suppression and the extremely young and old at high risk of infection. Non-*albicans Candida* spp. infections are also increasing, with the total incidence of *C. tropicalis* and *C. parapsilosis* increasing from 4.6% and 4.2% in 1997 to 7.5% and 7.3% in 2003, respectively **[10]**. Acquired resistance to antifungal drug therapies are increasing in *C. glabrata* clinical isolates, especially azole and echinocandin antifungal

compounds which are commonly used in the treatment of invasive Candidosis **[12]**. The need for drugs with new modes of action is essential as cross resistance to other azoles is also widespread **[10]**. The financial costs to healthcare institutions are high; it costs approximately $230 per patient per day to treat infections caused by *Candida* spp. and this cost is significantly increased as Candidiosis patients require extended hospital stays **[13]**.

1.5 Using a non-model organism – *Candida glabrata*

Candida glabrata is an opportunistic fungal pathogen of humans and resides in the subphylum *Saccharomycotina*. *C. glabrata,* after *C. albicans,* is the second most common cause of Candidosis in humans **[14]**. This opportunistic fungal pathogen commonly exists as a commensal organism as part of the normal human gut mucosal flora but can cause disease in a range of anatomical sites including the oral cavity, genitalia, gastrointestinal tract, urinary bladder and oesophagus which can lead to a systemic infection, candidemia **[14, 15]**.

The incidence of invasive Candidosis caused by *C. glabrata* has increased over the past 50 years, from 8% to 20% of systemic candidal infections and accounts for 20% to 24% of all *Candida* bloodstream infections in the US **[10, 14]**. This has been attributed to the increased use of antifungal prophylaxis, the higher number of immune suppressed patients; including an ageing population and increasing organ transplant operations, as well as new techniques to more accurately distinguish between *C. albicans* and other *Candida* spp. **[10, 16-18]**. The risk of infection in these individuals also increases with hospitalisation, due to the use of catheters and invasive surgery **[18]**.

Once the mucosal barriers are breached, the consequences of invasive Candidosis can be grave. *C. glabrata* has a high mortality rate of 29%, 30 days after admission to hospital and this has been found to be much higher when patients are left untreated **[10, 18]**. Other case studies have shown mortality rates for *C. glabrata* infections are as high as 75% **[19]**. *C. glabrata* has an innate and higher resistance than other *Candida* spp. to the commonly used antifungal drugs, the azoles. This is especially apparent with fluconazole, which has an average MIC_{50} of 16 µg/ml (67 isolates from 30 hospitals) compared to an average of only 0.25 µg/ml for *C. albicans* (178 isolates from 31 hospitals) **[14, 20]**. Resistance to antifungal drugs has been shown to increase with pre-treatment and cross resistance to other antifungal drugs also occurs in strains resistant to fluconazole **[17, 18]**. The innate drug resistance shown by *C. glabrata* to azoles has been attributed to the increased efflux of antifungal drugs out of the cell by pumps and the prevention of diffusion into the cell by changes to the cell

wall **[21, 22]**. Deletion of the genes responsible for regulating these pumps, notably *PDR1*, results in antifungal drug sensitivity **[23]**. Many clinical isolates exhibiting high resistance to fluconazole treatment have gain of function mutations in *PDR1* and these mutations confer an enhanced virulence **[24]**.

The genome of *C. glabrata* (ATCC 2001) was sequenced and made publicly available in 2004 by the Génolevures Project (http://www.genolevures.org/) **[25]**. The 5,213 proteins are encoded across 13 chromosomes totalling approximately 12.3 Mb **[26]**. A study which analysed the genome structure and stability of 40 clinical isolates of *C. glabrata*, including ATCC 2001, showed that although gene sequences were well conserved among different strains, their chromosome structures differed drastically, mainly through the translocation of chromosomal arms **[27]**. This genome plasticity correlated with antifungal drug resistance in these strains and hence is likely to confer an advantage in the host environment. There are also differences between ATCC 2001 and another frequently used *C. glabrata* strain, that of BG2, which will be discussed further in 1.6.1.

Evolutionarily, *C. glabrata* is more closely related to the model yeast, *S. cerevisiae* than *Candida albicans* and other *Candida* species, as shown in the phylogenetic tree (Figure 1.1) **[26, 28]**. The average sequence identity between orthologous proteins of *S. cerevisiae* and *C. glabrata* is approximately 65% **[26]**. *C. glabrata*, like *S. cerevisiae*, was part of a whole genome duplication event which occurred after diverging from their last shared ancestor with *C. albicans*. *C. glabrata* is also separated from the *Candida* clade as these yeast translate CTG as serine instead of leucine **[28]**. It is interesting that although its closest relatives are largely non-pathogenic, *C. glabrata* has diverged to become a human pathogen. Due to this similarity with the model organism *S. cerevisiae* and the limited number of studies using *C. glabrata*, the majority of information on gene function, gene ontology (GO) terms and pathways to date has been inferred from *S. cerevisiae*. As such, the gene ontology enrichment analysis presented in this study is inferred from homology with *S. cerevisiae* genes using sequence similarity and their syntenic context (using YGOB http://wolfe.gen.tcd.ie/ygob/ **[29]**). While this is informative, genes in *C. glabrata* which do not have a homologue in *S. cerevisiae* are automatically excluded from this analysis, as well as genes in *S. cerevisiae* which have no known function. There are also many studies which have shown key differences in protein function and regulation between closely related species, therefore the biology taken from these analyses must also be supported with other evidence, for example by utilising null mutant, protein-protein interactions and/or over expression studies **[30, 31]**.

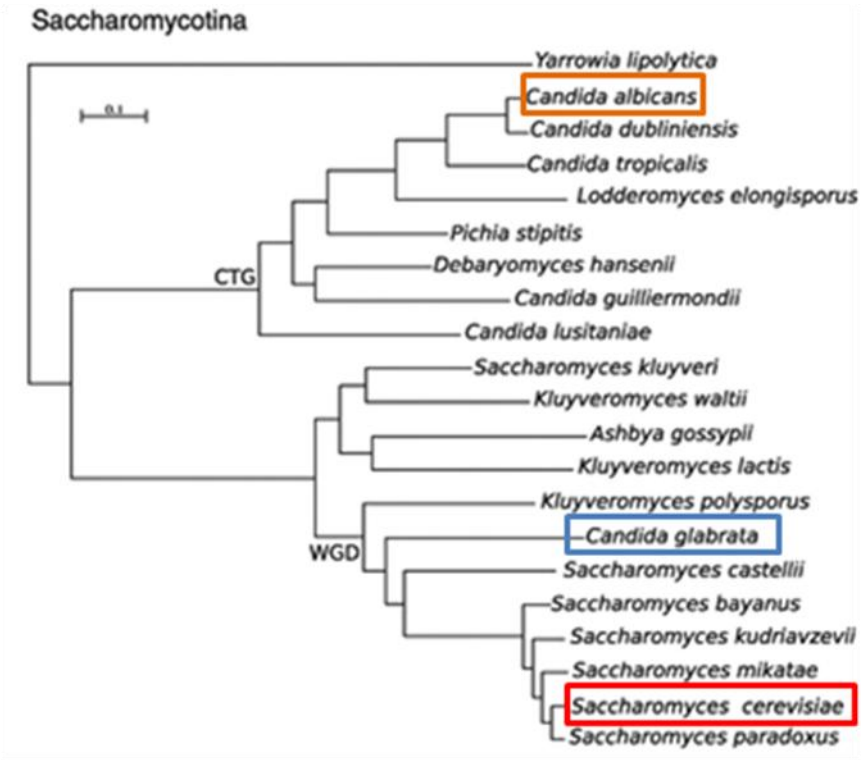


Figure 1.1: Phylogenetic tree of the subphylum *Saccharomycotina*. This phylogenetic tree shows that while named *Candida glabrata*, this organism is actually more closely related to *Saccharomyces cerevisiae*. It is not part of the *Candida* CTG clade and along with *S. cerevisiae* took part in the whole genome duplication event (WGD) (Taken from **[32]**). Scale bar indicates the number of amino acid changes per site. *C. albicans*, *C. glabrata* and *S. cerevisiae* are boxed in orange, blue and red, respectively.

Although *C. glabrata* and *S. cerevisiae* are closely related, there are many key differences; *C. glabrata* is an opportunistic fungal pathogen like *C. albicans*, whereas *S. cerevisiae* is non-pathogenic. *C. glabrata* can exhibit pseudohyphal growth but only on solid nitrogen starvation media, unlike *C. albicans* which is filamentous when grown at 37°C **[33]**. The similarities and differences between *C. glabrata*, *C. albicans* and *S. cerevisiae* are summarised in Table 1.1.

C. glabrata is a haploid organism thus making genetic analysis through the creation of null mutants easier than in diploid fungi as only one copy of a gene must be removed; however, this is not true for all haploid fungi as *Aspergillus fumigatus*, for example, has proven very difficult to genetically manipulate. Transformation protocols used for *S. cerevisiae* such as lithium acetate and electroporation methods have proved to work well in *C. glabrata* and permit the efficient generation of null mutants to test hypotheses generated from modelling and experimentation. As no sexual cycle has been observed in *C. glabrata*, these mutant strains cannot be backcrossed and hence there is a possibility of second site mutations. This is counteracted by creating 3-4 clones of each strain and screening these for similar phenotypic profiles as well as complementation. It is important to note that while a mating cycle is not

observed in *C. glabrata*, orthologues of many of the genes involved in mating in *S. cerevisiae* are present in *C. glabrata* and strains displaying the different mating types (a and α) have been identified **[34]**.

Table 1.1: Characteristics of *C. glabrata*, *S. cerevisiae* and *C. albicans*: a comparison.

	C. glabrata	*S. cerevisiae*	*C. albicans*	Reference
Taxonomy	Ascomycota (WGD clade)	Ascomycota (WGD clade)	Ascomycota (CTG clade)	**[28]**
Genome size	13 chromosomes, 5274 ORFs	16 chromosomes, 6607 ORFs	8 chromosomes, 6563 ORFs	**[26]**
Ploidy	Haploid	Haploid/diploid	Diploid/tetraploid	-
Codon usage	Standard	Standard	CTG encodes Serine rather than Leucine	**[28]**
Sexual cycle	Unknown	Known	Known (cryptic)	-
Mating genes	Present	Present	Present	**[35]**
Colony morphology	Small, white/creamy colonies	White, round shaped colonies	Smooth (+ wrinkled) colonies	-
Cell morphology	Monomorphic: budding yeast (pseudohyphae, only under nitrogen starvation)	Budding yeast (pseudohyphae)	Dimorphic: yeast, hyphae, pseudohyphae, chlamydospores	**[16]**
Biofilm formation	Present	Present	Present	**[36]**
Phenotypic switching	Present (colour change, visible on $CuSO_4$ media)	Absent	Present (yeast – hyphae; white – opaque)	**[37]**
Auxotrophy	Niacine, thiamine, pyridoxine	Absent	Absent	**[38]**
Sugar utilization	Glucose, trehalose	Glucose, maltose, galactose, sucrose, melibiose	Glucose, maltose, galactose, trehalose	**[39]**
Natural habitat	Microbial flora of humans	Plants, fruits (grapes)	Microbial flora of humans	**[40]**
Virulence	Opportunistic pathogen	Non - pathogenic	Opportunistic pathogen	-
Clinical significance	Up to 25 % of *Candida* isolates	-	50 % of *Candida* isolates	**[41]**
Site of infection	Faeces, urine, blood, vagina, oral, disseminated	-	Faeces, urine, blood, vagina, oral, disseminated	-
Azole resistance	Decreased susceptibility	Susceptible	Susceptible	**[10]**
Adhesins	Lectins (Epa family)	Sexual agglutinins, lectins (Flo family)	Lectins (Hwp1, Als family)	**[42]**

The characteristics of three yeast are compared and references are included (adapted from **[43]**).

The molecular tools available for *C. glabrata* are limited compared to that of *S. cerevisiae*. While the entire genome has been sequenced for the *C. glabrata* strain, ATCC 2001, and the little biological information known is freely available via the Génolevures database, the community is lacking a library of non-essential gene knockouts and therefore no large scale null mutant phenotypic screens have been conducted **[25, 44]**. Table 1.2 summarises the resources and tools available to the *C. glabrata* community compared to *S. cerevisiae*.

Table 1.2: The resources and tools available to study *C. glabrata* in comparison to *S. cerevisiae*.

	S. cerevisiae	***C. glabrata***
Genome sequenced	Entire genome of S228C was sequenced and is freely available from the *Saccharomyces* Genome Database (SGD) **[45]**	Entire genome of ATCC 2001 was sequenced in 2004 and is freely available from the Génolevures database **[25]**
Comprehensive information of molecular biology and genetics available	Extensive information available from SGD **[45]**	Limited information available from Génolevures
Large scale analysis data available e.g. Yeast-2-hybrid screens, CHIP analysis	Available from SGD **[45]** (gene deletions **[46]**, functional profiling **[47]**, protein localisation **[48]**)	-
Knockout library of non-essential genes	Created by a consortium of laboratories and commercially available from Open Biosystems **[46, 47]**	-
Library containing essential genes under the control of a tetracycline regulatable promoter	Created by the Ted Hughes laboratory and commercially available from Open Biosystems **[49]**	-
TAP-fusion library	Library constructed by Erin O'Shea and Jonathan Weissman at UCSF and is commercially available from Open Biosystems **[50]**	-
Green Fluorescent Protein (GFP) tagged library	Library constructed by Erin O'Shea and Jonathan Weissman at UCSF and is commercially available from Invitogen **[48]**	-
Yeast GST-tagged library	Constructed by the Andrews laboratory and commercially available from Open Biosystems **[51]**	-

Adapted from **[52]**.

The genome of the *S. cerevisiae* strain, S288C, was sequenced and is publicly available via the *Saccharomyces* Genome Database (SGD, www.yeastgenome.org). This is annotated with

as much biological and functional information as possible and is frequently updated. The sequencing of its genome led to a consortium of laboratories working together to create a library of deletion strains of every open reading frame (ORF) in *S. cerevisiae*. Over 20,000 null mutant strains of approximately 6000 unique genes have been created using a PCR based method where each gene was replaced with the *KanMX* cassette which selects for the antibiotic Geneticin. This, aptly named library, the Yeast Knockout Library (YKO) was constructed in both haploid mating types (a and α) and heterozygous and homozygous diploid backgrounds. These make up four collections which are commercially available from Open Biosystems **[46, 47]**. Each strain constructed has a unique bar code, facilitating their use in competitive growth assays by pooling large numbers of strains together for parallel experimentation. As this library is publicly available, many yeast researchers have made use of this invaluable tool and as these experiments are all conducted using the same strains, constructed in the same manner, comparisons between experiments can be drawn with more confidence than those made in different backgrounds.

The creation of the YKO library resulted in the discovery that approximately 1105 genes in *S. cerevisiae* were essential for growth at 30°C in rich media. A library (Tet-YKO) containing essential genes under the control of a tetracycline regulatable promoter was created by the Ted Hughes laboratory and is commercially available from Open Biosystems **[49]**. The native open reading frame of the essential gene was maintained and preceded by a Tet-promoter which, in the presence of doxycycline, results in the repression of the gene of interest. It has been shown that, in the low doses needed to repress gene expression, doxycycline has no effect on growth or gene expression in yeast. Using these strains, phenotypic analysis, microarray studies and synthetic genetic arrays have been carried out, revealing functionality for these essential genes in *S. cerevisiae* **[49]**.

A collection of ORFs tagged at the carboxyl terminal end using the coding sequence of *Aequorea victoria* green fluorescent protein (GFP) and a collection tagged at the carboxyl terminal end with a modified version of the tandem affinity purification (TAP) tag, were created under their native promoters by Erin O'Shea and Jonathon Weissman at UCSF **[48, 50]**. The production of these two collections has enabled the localisation of three-quarters of the *S. cerevisiae* proteome using the GFP library and absolute protein abundances to be determined by quantitative Western blot analysis using the TAP-tagged strains **[48, 50]**. The GFP- and TAP- tagged libraries are commercially available from Invitrogen and Open Biosystems, respectively.

The Brenda Andrews Laboratory at the University of Toronto has created a collection of approximately 5000 strains, covering over 80% of the *S. cerevisiae* genome, tagged with a Glutathione S-Transferase (GST) tag at the N terminal **[51]**. The library, constructed in plasmids with each ORF expressed under the control of the *GAL1/10* promoter, allows the over expression of a specific gene when grown in galactose. This library has been used to show that the over expression of most genes did not compromise growth rate and of the 15% which did reduce growth, many were cell cycle-regulating genes, signalling molecules and transcription factors **[51]**.

These gene knockout libraries made for *S. cerevisiae* have been analysed under different stresses to characterise their function and identify essentiality; however, the *C. glabrata* research community does not yet have these valuable molecular tools **[46, 47]**. In collaboration with the Kuchler and Cormack labs, the Haynes lab are developing a library of targeted gene deletions. This library of approximately 700 null mutants includes: 180 transcription factors as well as kinases, genes involved in MAPK signalling pathways, meiosis genes and ABC transporters.

While approximately 4500 genes in *C. glabrata* do have homology with *S. cerevisiae* genes, there are approximately 700 genes whose sequence, when compared against the entire genome of *S. cerevisiae* by reciprocal best hit BLAST search, showed no similarity to any gene. Some of these approximately 700 genes could be orthologues when considering their syntenic context. How can these genes have the same function in *S. cerevisiae* when their sequences are so diverged? There are also many *C. glabrata* specific genes, with no homology to *S. cerevisiae* and no possible homology through synteny. These *C. glabrata* specific genes may hold the key as to why *C. glabrata* is pathogenic, while *S. cerevisiae* is not, or how it is extremely stress resistant compared to other fungi. I will be touching on these genes and their possible roles in stress response in this study.

Functional genomics studies through the creation of null mutants and their phenotypic screening have proven to be essential in elucidating gene function, from using the libraries constructed in *S. cerevisiae* to screen on different phenotypic conditions, to dSLAM (Diploid-based Synthetic Lethality Analysis on Microarrays) using the barcodes incorporated into each deletion as markers for growth on DNA microarrays through competitive growth assays under stress conditions **[46, 47, 53, 54]**. Using targeted null mutants made in *C. glabrata* and those constructed in this study, functional analysis of genes involved in stress response in *C. glabrata* will be presented herein. Initially, the null mutants created in this study were

identified from the literature as being known to be involved in stress response in other fungi, namely *S. cerevisiae*. Those genes identified as being transcriptionally regulated under stress conditions in *C. glabrata* were also targeted for deletion and characterisation. This functional analysis was achieved by utilising a pinning robot (RoToR, Singer Instruments) to phenotypically screen these mutants on a wide range of conditions (Table 3.6, Materials and Methods). The prominent review of phenotypic data in *S. cerevisiae* by Hampsey, 1997 was used to guide the initial conditions and concentrations to use in these mutant screens **[55]**. Many of the concentrations previously used for *S. cerevisiae* needed to be increased for use with *C. glabrata*, as it is more stress resistant.

Even with the increasing incidence of *C. glabrata*, its innate resistance to antifungal drug treatment and an ever-increasing immune deficient population, far less is known about its virulence mechanisms than *C. albicans.* Due to this, it is important to study *C. glabrata* to understand its molecular determinants of virulence and how they co-operate to identify key molecules that can be exploited for therapeutic intervention.

1.6 Stress response is important for virulence

Stress occurs when there is an abrupt change in the environmental conditions necessary for the optimal growth and function of the organism. A rapid molecular response to these new environmental conditions is required, usually through transcriptional adaptation. For a pathogen, the host environment presents an array of different stresses which need to be sensed and adapted to in order to successfully establish an infection. Different stresses are encountered by a pathogen in the many and varied sites of infection of the human body. These stress conditions include temperature changes, iron deprivation, glucose starvation, low oxygen levels in tissues and varying pH across different host niches, as well as those this study will focus on; hyperosmotic and oxidative stress **[56, 57]**. The environmental signals regulating virulence determinates of bacterial pathogens have been well studied and have shown that stress conditions in the host activate genes essential for virulence (as reviewed in **[56, 57]**).

The mitogen-activated protein kinase (MAPK) Hog1, as well as the transcription factor Rim101, have both been shown to be essential for wild type virulence in murine models of candidosis caused by *C. albicans* and have critical functions in hyperosmotic and alkaline stress adaptation, respectively **[58-60]**. The transcription factor Skn7, which is involved in the oxidative stress response, has been shown to be pivotal in the virulence of many fungal

pathogens, *C. glabrata* included, as well as required for oxidative stress resistance **[61]**. These results suggest that virulence greatly depends upon a pathogen's ability to adapt to environmental stress conditions. The first stress to be investigated in this study is that of hyperosmotic stress. An adequate response to hyperosmotic stress has been identified in both fungal and bacterial pathogens as important for survival in the host **[57, 59]**.

1.6.1 Hyperosmotic stress

C. glabrata can infect a range of anatomical sites and this is just one example of its striking adaptability to the host environment. The ambient pH and hyperosmotic stress a pathogen encounters in a human host varies depending on the site of infection from near neutral in the blood to acidic in the vagina and an osmolarity equivalent to 0.3 M NaCl in the intestinal lumen and 0.15 M NaCl in the bloodstream **[57, 62]**. *C. glabrata* also encounters osmotic stress produced in the phagosome of its host. Rapid acidification of the phagosome occurs upon phagocytosis from pH 7.4 to below pH 5 **[63]**. This change in pH is caused by the movement of hydrogen and chloride ions across the membrane. KCl is one of the compounds found inside a phagosome, therefore the response of *C. glabrata* to osmotic stress as well as cationic stress is important to study in relation to host-pathogen interactions **[64]**.

Hyperosmotic stress has been studied extensively in *S. cerevisiae* by genome deletion analyses and functional profiling under different stress conditions **[46, 47, 65]**. A quantitative model of the hyperosmotic stress response pathway in *S. cerevisiae* was proposed by Klipp and Hohmann in 2005, along with a diagram illustrating the pathway components (Figure 1.2) **[66]**. Hyperosmotic stress is caused by an increase in the solute concentration around a cell caused by salts, sugars or other solutes. This causes the movement of water across the cell membrane and out of the cell to balance the water potential. To regain the turgor pressure lost, osmolytes (namely glycerol) must be synthesised to protect the cell from lysis (as reviewed in **[67]**).

To summarise the model in *S. cerevisiae* briefly: the MAPK Hog1 (**H**igh **O**smolarity **G**lycerol) is the main regulator of the hyperosmotic stress response. It is phosphorylated by the MAPKK Pbs2 when transducing the hyperosmotic stress stimulus from the Sho1 and Sln1 osmosensing branches in the cell membrane. Once phosphorylated, it is translocated to the nucleus where is regulates transcription factors such as Hot1 and Sko1 **[67]**. In the nucleus, phospho-Hog1 up regulates genes involved in the production of glycerol and reduces the efflux of glycerol by targeting the glycerol-aquaporin Fps1 for endocytosis, increasing turgor pressure inside the cell **[66, 67]**.

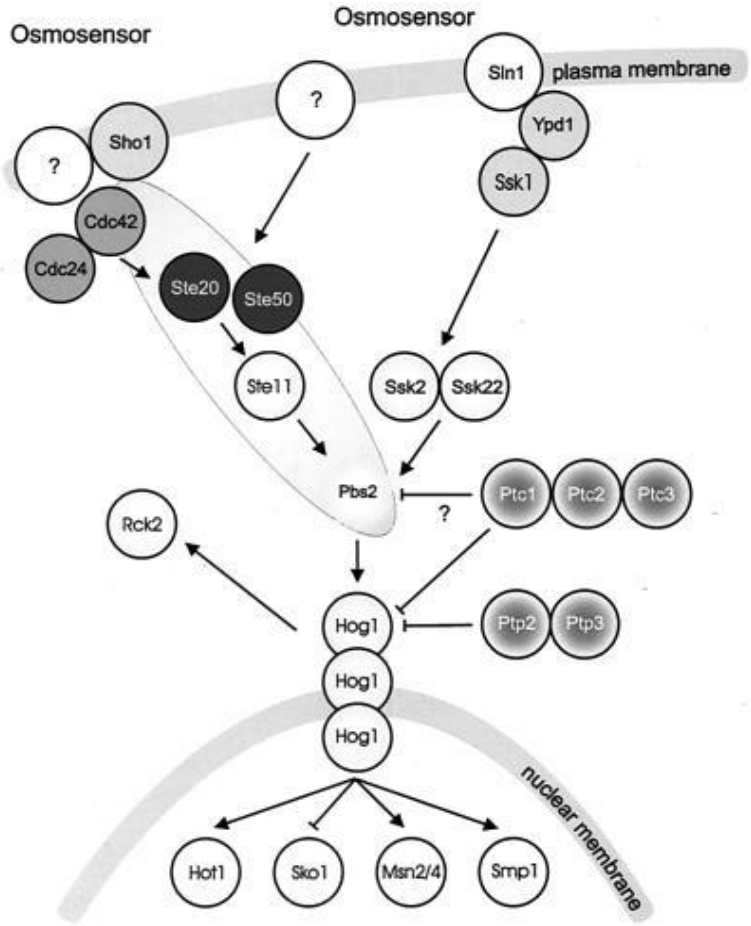


Figure 1.2: Pathway diagram of osmo-adaptation in *S. cerevisiae* described by Klipp and Hohmann. This diagram of hyperosmotic stress adaptation shows the two branches of the signalling pathway through the osmosensors, Sho1 and Sln1. This diagram also shows the protein tyrosine phosphatases, Ptp2/3 which act by de-phosphorylating Hog1 and the transcription factors which Hog1 regulates in the nucleus **[67]**.

The response to hyperosmotic stress has also been well studied in the fission yeast, *S. pombe*. While *S. pombe* and *S. cerevisiae* are distantly related, the Stress Activated Protein Kinases (SAPK) are among the most conserved stress signalling proteins in fungi **[68]**. The SAPK Sty1 is phosphorylated by the MAPKK Wis1 under stress conditions. While these yeast each contain a single SAPK and MAPKK, the number of MAPKKKs, which relay the stress signals to activate the pathway, differ; *S. cerevisiae* having three and *S. pombe* only two. Sty1 is also activated by a diverse range of stress conditions including oxidative stress, heat shock, cold stress, arsenite, UV light, nitrogen limitation and carbon source starvation (reviewed in **[69]**). The phosphorylation and activation of Hog1 in response to other stresses has not been studied in *C. glabrata*.

In the first instance, data mining of the Génolevures (http://www.genolevures.org/) and Yeast Gene Order Browser (http://wolfe.gen.tcd.ie/ygob/) was used to identify homologous components of the Klipp-Hohmann (KH) diagram in *C. glabrata*. From this, it was found that

the pathway components of *C. glabrata*'s response to hyperosmotic stress are very similar to that of *S. cerevisiae*, with many important proteins, such as Hog1 and Pbs2, found to be homologous to *C. glabrata*. There are however, key differences in the hyperosmotic stress pathway in *C. glabrata* compared to the KH diagram in *S. cerevisiae* from published data. The HOG pathway in *C. glabrata* consists of a MAPK signalling pathway with a MAPK, Hog1 and MAPKK, Pbs2. *C. glabrata* lacks one of the MAPKKKs present in *S. cerevisiae*, Ssk22 and studies have shown that ATCC 2001 has a point mutation in *SSK2* which encodes the MAPKKK Ssk2, resulting in an inactive, truncated protein to be made. As such ATCC 2001 lacks a functional Sln1 signalling pathway and all mutants made in this background should be considered to also be *SSK2* deletions **[70]**. Genome evolution has resulted in the reference strain of *C. glabrata* (ATCC 2001) containing only one MAPKKK functioning in the signalling pathway; that of Ste11, through the sensor Sho1 (Figure 1.3). This provides a simplification to the modelling as one of the signalling inputs of the hyperosmotic stress pathway has been removed. However, one must note that there is no difference in growth or viability under hyperosmotic stress between *C. glabrata* ATCC 2001 and BG2, a *C. glabrata* strain which does have a functioning Ssk2 (4.2.3).

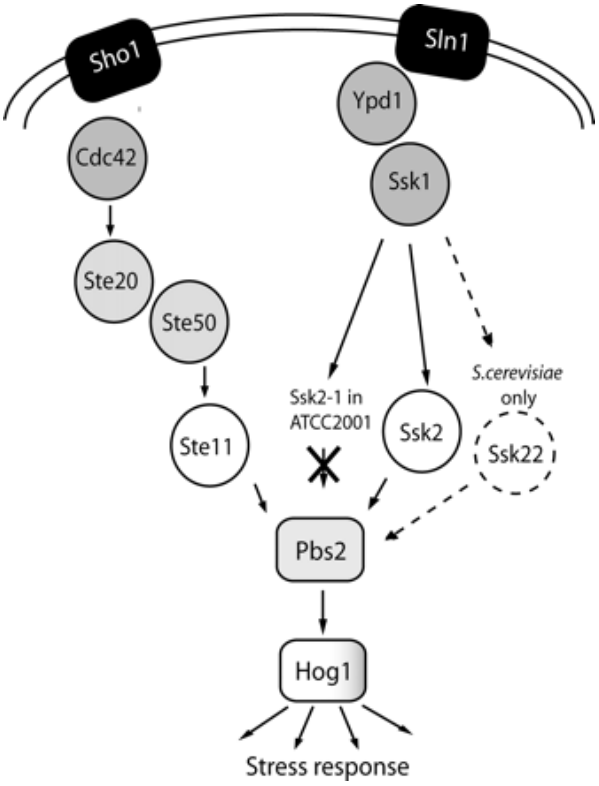


Figure 1.3: Hyperosmotic stress signalling pathway in *C. glabrata* (taken from [70]). This model of hyperosmotic stress response in *C. glabrata* shows the point mutation occurring in Ssk2 of the sequenced strain ATCC 2001 and the subsequent functionally redundant Sln1 branch of the hyperosmotic stress signalling pathway.

The MAPK Hog1 has been described as the main regulator of the hyperosmotic stress response in *S. cerevisiae* and is activated by phosphorylation. Previous studies by Gregori *et al*, 2007 and Kaloriti *et al*, 2012, show that Hog1 is phosphorylated under hyperosmotic stress in *C. glabrata* **[70, 71]**. This activation of Hog1 in *C. glabrata* under hyperosmotic stress is also HOG pathway dependent as removal of the signalling components Ste11 and Ste20 abolishes Hog1 phosphorylation **[72]**.

Glycerol production upon hyperosmotic stress has not been studied directly in *C. glabrata*. However, the genes responsible for its production in *S. cerevisiae* (*GPD1* and *GPP1*) have homologues in *C. glabrata* and hence its production is assumed to be conserved. Glycerol is a polyhydroxy alcohol (polyol) and as an osmolyte restores turgor pressure lost upon hyperosmotic stress conditions. Glycerol, as well as other osmolytes such as proline and trehalose, has also been shown to act as a protein stabiliser, forming weak protein/osmolyte interactions and in high concentrations forcing the folding of proteins and changing protein folding kinetics **[73, 74]**. Polyol compounds are produced by many fungi in response to stress; *S. cerevisiae* produces glycerol and trehalose while the marine dwelling fungus *Aureobasidium pullulans*, produces trehalose, glycerol and mannitol under hyperosmotic and heat stress conditions **[75-77]**. *S. cerevisiae* grows poorly on media with mannitol as the sole carbon source because of very low levels of mannitol dehydrogenase activity **[78]**. However, it has been shown that resistance to high osmolarity conditions can be restored in glycerol-deficient *S. cerevisiae* strains by the incorporation of a multicopy plasmid encoding the mannitol-1-phosphate dehydrogenase of *Escherichia coli*, indicating that not only can mannitol function as an osmolyte in *S. cerevisiae*, it can also substitute glycerol as the main osmolyte **[79]**. It has also been shown that mutants that produce low levels of mannitol in the pathogen *Cryptococcus neoformans* are sensitive to hyperosmotic and heat stress, as well as attenuated for virulence in a mouse model of infection **[80]**. A study using *C. glabrata* has shown that the addition of proline, an amino acid, to culture media increases hyperosmotic stress resistance **[81]**. Given this data, the production and utilisation of alternative osmolytes by *C. glabrata* under hyperosmotic stress is a possibility and will be investigated in this study.

Previous transcription profiling experiments conducted under hyperosmotic stress conditions in *S. cerevisiae* show that a large number of genes are involved in the response to hyperosmotic stress. O'Rourke and Herskowitz have shown that 2277 genes in *S. cerevisiae* are regulated after exposure to hyperosmotic stress, with most genes (1789) being repressed

under this stress condition **[82]**. They also identified 579 genes, representing approximately 10% of the yeast genome, that are dependent on Hog1. With this data in mind, we proposed to examine if a similar trend is observed in *C. glabrata* and this will be further discussed in Chapter 4.

1.6.2 Oxidative stress

When *C. glabrata* is engulfed by macrophages it encounters oxidative stress due to the production of reactive oxygen species (ROS) such as superoxides, hydrogen peroxide and hypochlorous acid by cells of the immune system. These damage DNA and cause oxidation of proteins and lipids, as well as inducing apoptosis in *C. albicans* **[83-85]**. Studies have shown that polymorphonuclear neutrophils (PMN) kill the pathogen *C. neoformans* by oxidative mechanisms **[86]**. The transcriptional response of the pathogenic fungi *C. albicans* to phagocytosis by macrophages has been well studied and shown to induce genes involved in starvation, DNA damage and the oxidative stress response, as well as the repression of translation activities **[87]**. This is in stark contrast to the response of the model yeast *S. cerevisiae* to phagocytosis in which the same study showed few genes are regulated in response to the host environment. A microarray dataset for macrophage engulfed *C. glabrata* cells is available and will be utilised in this study to compare the transcriptional response to stresses applied *in vitro* to that of the host environment **[2]**.

It has been shown that the concentration of H_2O_2 inside a macrophage is only 0.4 mM and *C. glabrata* can easily grow in concentrations exceeding 25 times that (this study) **[88]**. Studies have shown that *C. glabrata* is particularly resistant to hydrogen peroxide, compared to *S. cerevisiae* and even clinical isolates of *C. albicans* **[89]**. It has also been shown that this resistance is increased in stationary phase cultures of *C. glabrata* compared to cells growing in log phase **[89]**.

Hydrogen peroxide, the compound we use for oxidative stress, is detoxified in three main pathways; by catalase, the glutathione system and the thioredoxin system. Superoxides are also converted to hydrogen peroxide by superoxide dismutases, the first step in detoxifying the cell of oxidative stressors **[90]**. Hydroxyl radicals and superoxide anions are also produced by Fenton and Fenton-like reactions involving a range of metal ions including iron and copper **[91]**. Cadmium causes lipid peroxidation leading to plasma and mitochondrial membrane damage and the oxidation of glutathione and protein sulfhydryls reducing the ability of the cell to detoxify oxidative stressors **[91, 92]**. This damage and oxidation of

proteins and lipids causes an overall depletion of ATP and NADP(H) levels in the cell, as the processes of repairing and reducing these compounds is energy expensive.

Many of the genes involved in the response to oxidative stress in *S. cerevisiae* have homologues in *C. glabrata*. An abundance of biological information on oxidative stress can also be inferred from that known in *C. albicans*. As *C. albicans* is also a pathogenic yeast, its response to oxidative stress has been extensively studied in an effort to understand its interactions with the host. Data mining of studies of the oxidative stress response of *S. cerevisiae* and *C. albicans* helped to construct the model of the oxidative stress signalling pathway in *C. glabrata* shown below (Figure 1.4).

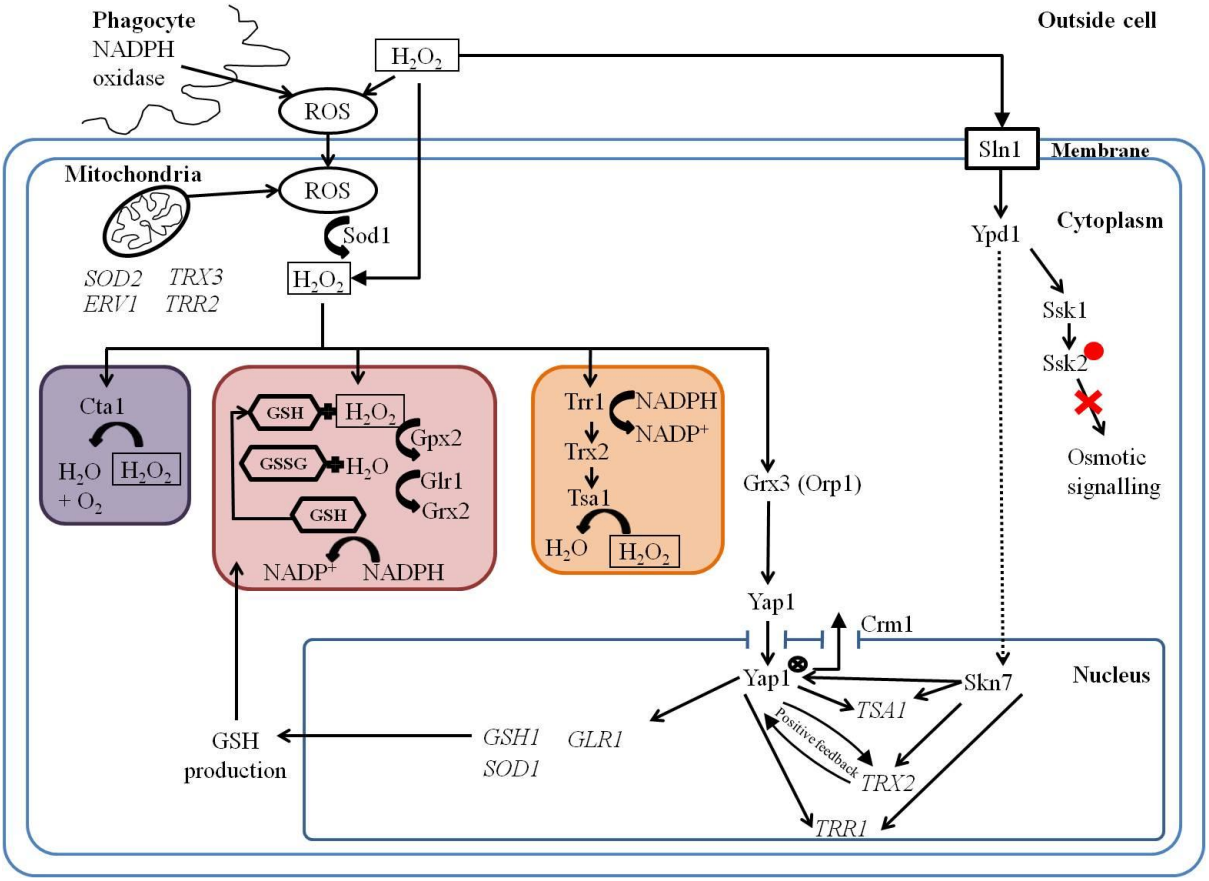


Figure 1.4: Model of the oxidative stress signalling pathway in *C. glabrata*. This model was made through mining the literature of *S. cerevisiae*, *C. albicans* and where possible, *C. glabrata*. It shows the production of ROS by the phagocyte and mitochondria, as well as the conversion of superoxides into H_2O_2 by Sod1. The three main H_2O_2 detoxification pathways are boxed; catalase (purple), glutathione (red) and thioredoxin (orange). The two main transcription factors which regulate the oxidative stress response and some of their known targets are shown. It also shows the proposed overlap between the hyperosmotic and oxidative stress pathways through Sln1-Ypd1 to Skn7 (dashed line). The point mutation in Ssk2 in *C. glabrata* is denoted by a red circle and the red cross shows the lack of signal transduction to the hyperosmotic stress pathway. The conformational change to Yap1, which allows it to accumulate inside the nucleus, is denoted by a circle with a cross through it. **[93-96]**

Catalases break down H_2O_2 to water and oxygen (boxed in purple Figure 1.4). *S. cerevisiae*, has two catalases; *CTT1* encodes for the cytoplasmic catalase of which Cat1 in *C. albicans* is

the orthologue and *CTA1*, which encodes for a peroxisomal catalase and is the orthologue of the only catalase in *C. glabrata* **[25, 45, 97]**. Cat1 has been linked to virulence in *C. albicans* and as such, provides a link between oxidative stress and virulence **[98]**. While it has been shown that Cta1 is essential for hydrogen peroxide resistance in *C. glabrata*, *cta1* mutants in *C. glabrata* however, do not have an effect on virulence in murine models of infection **[89]**. Studies in *C. glabrata* have shown that *CTA1* is transcriptionally induced by 0.4 mM H_2O_2 treatment and its protein is localised to the cytoplasm and peroxisomes upon phagocytosis by macrophages, as well as 0.4 mM H_2O_2 treatment **[88]**.

The glutathione oxidoreductase and thioredoxin systems detoxify H_2O_2 in complex and energy expensive reactions as they require a large amount of NADPH (boxed in red and orange, respectively in Figure 1.4). Glutathione (GSH) acts as an electron donor by converting H_2O_2 to water resulting in an oxidised form of GSH, glutathione disulfide (GSSG) **[94]**. This reaction is catalysed by the glutathione peroxidase enzymes Gpx1 and Gpx2. GSSG is reduced back to GSH, by the glutathione reductase, Glr1, using NADPH as an electron donor **[94]**. The glutaredoxins Grx1 and Grx2 may also be involved in the maintenance of a high GSH to GSSG (reduced to oxidised) ratio. *GSH1* and *GSH2*, in *S. cerevisiae*, have been shown to be involved in GSH biosynthesis and although GSH deficient mutants are sensitive to H_2O_2, they are still able to induce an adaptive stress response to H_2O_2, suggesting that GSH does not play a role in sensing oxidative stress (as reviewed in **[93]**).

The thioredoxin pathway in *S. cerevisiae* consists of a redox relay involving Trr1 and Trx1, leading to the oxidation of the thioredoxin peroxidase, Tsa1, which converts H_2O_2 to H_2O. Trr1 is a thioredoxin reductase, which reduces the thioredoxin, Trx1, using NADPH. This reduced Trx1 oxidises Tsa1, which is then able to convert H_2O_2 to H_2O. Detoxification of H_2O_2 leads to the reduction of Tsa1 back to its reduced form **[99]**. *S. cerevisiae* has been shown to possess five peroxiredoxins; three cytoplasmic: Tsa1, Tsa2 and Ahp1, one mitochondrial: Prx1 and one nuclear: Dot5 **[100, 101]**. All of these peroxiredoxins have homologues in *C. glabrata* by sequence similarity and syntenic context **[45]**. In *S. cerevisiae*, Tsa1 is expressed at relatively high basal levels even in unstressed cells. Although the transcript abundance of Tsa2, an isoenzyme of Tsa1, is induced by more than 120 fold upon H_2O_2 treatment and Tsa1 is only induced by approximately 3 fold, the actual molecules per pg of RNA is nearly three times that of Tsa2 **[102]**. *tsa1* mutants in *S. cerevisiae* show sensitivity to H_2O_2, tBOOH and diamide suggesting it acts as a critical antioxidant to a range

of oxidative stressors, which is not surprising with its high abundance and relatively strong antioxidant activity **[101, 103]**. Tsa1 has also been linked to genome stability in *S. cerevisiae* and this has been thought to be due the damaging effect ROS can have on DNA **[103, 104]**. Interestingly, other proteins involved in the oxidative stress response have also been implicated in mutation suppression and genome stability in *S. cerevisiae* including Sod1 and partially, Skn7 and Yap1 **[104]**. *tsa2* mutants are not sensitive to oxidative stressors, instead showing a slow-growth phenotype in *S. cerevisiae* from the accumulation of cells in G1 of the cell cycle **[101, 103]**. *ahp1* mutants in *S. cerevisiae* however, only show sensitivity to tBOOH, an alkyl hydroperoxide, which is explained by the alkyl hydroperoxide specific kinetic properties of Ahp1, for which it is named **[100, 101]**. Trx1 and Tsa1 have been shown to be involved in the regulation of the oxidative stress response in *C. albicans*, with both proteins involved in the H_2O_2-induced regulation of the SAPK Hog1 **[96]**. Components of the thioredoxin pathway in *C. glabrata*, including one of the two possible *TSA1* homologues, have been shown to be regulated upon H_2O_2 stimulation **[61]**.

Yap1 and Skn7 are the main transcription factors which regulate genes involved in the detoxification of H_2O_2. It has been shown that Skn7 regulates some of the major oxidative stress response genes such as those involved in the thioredoxin system, as well as *CTA1* in *C. glabrata*; however it is unclear how Skn7 is activated in oxidative stress conditions **[61, 89]**. It has been proposed that activation of Skn7 is through the Sln1 – Ypd1 signalling pathway in *S. cerevisiae*, however Sln1 does not possess an oxidative signalling domain such as that found in Gpx3 which activates Yap1 **[105]**. While Yap1 translocates from the cytoplasm to the nucleus under oxidative stress, it has been found that Skn7 is constitutively localised to the nucleus in *C. glabrata* **[106]**. *SKN7* has been shown to be required for resistance to oxidative stress in *C. glabrata* and *skn7* mutants show attenuated virulence in a *C. glabrata* murine model of infection **[61, 89]**. It has been shown that *C. glabrata* cells pre-treated with a low dose of hydrogen peroxide are more resistant to subsequent treatments with higher doses and that this adaptive response is dependent on Yap1 and Skn7 **[89]**. Yap1 and Skn7 have independent roles in regulating oxidative stress adaptation in *C. glabrata*, but also co-operate to regulate many genes and this is similar to *S. cerevisiae* **[89, 106]**.

In *S. cerevisiae*, Gpx3 (Orp1) acts as a key sensor of oxidative stress inside the cell; as in the presence of H_2O_2 it is reduced **[105]**. This reduced form of Gpx3 then oxidises Yap1, hiding the nuclear export sequence and allowing Yap1 to accumulate inside the nucleus instead of being continually shuffled between the cytoplasm and nucleus by the nuclear export pump

Crm1 **[95]**. This accumulation of Yap1 leads to the transcriptional regulation of genes including *GSH1* in *S. cerevisiae* **[107]**. Studies have shown that in *C. glabrata*, Yap1 is nuclear localised after only 10 minutes H_2O_2 treatment and also accumulates inside the nucleus of *C. glabrata* cells when phagocytised by macrophages **[88]**. It has been shown in *C. glabrata* that loss of Yap1 renders cells highly sensitive to oxidative stress and unable to adapt to subsequent oxidative stress treatment **[89]**. Studies in a murine model of infection using *C. glabrata* cells lacking *YAP1* showed no difference in fungal burden **[108]**. Sod1, the cytoplasmic superoxide dismutase, is also required for oxidative stress resistance in *C. glabrata* however, only a double *sod1, yap1* mutant is efficiently killed by primary mouse macrophages with no statistically significant effect observed in the single deletions **[106]**.

As a transcription factor (TF), Yap1 contains a DNA binding domain which binds to specific DNA sequences upstream of the gene it regulates. TFs control gene expression by promoting or blocking the recruitment of RNA polymerases, which transcribe DNA to mRNA in the nucleus. Six DNA binding motifs recognised by Yap1 in *S. cerevisiae* have been characterised so far and are referred to as Yap Response Elements (YREs) **[109, 110]**. These motifs are seven or eight base pairs in length and palindromic or pseudo palindromic around a central (G/C) base pair **[111]**. Kuo *et al*, 2010, described these YREs as "half sites" with the central (G/C) base pair positioned either adjacent (TTACGTAA), referred to as YRE-A, or overlapping (TTA(C/G)TAA), referred to as YRE-O **[30]**. It has been shown in *S. cerevisiae* that Yap1 preferentially binds the perfect YRE-O sequence: TTA(C/G)TAA **[109]**. YAP proteins are basic-leucine zipper (bZIP) transcription factors, with DNA binding domains consisting of basic and leucine-rich regions. It has been shown that residue 12 in the basic region of the YAP family of proteins in *S. cerevisiae* (eight in total) is important for binding specificity between the YRE-A and YRE-O binding motifs. By mutating the DNA binding domain of Yap1 and Yap4 (Cin5) in *S. cerevisiae,* it was found that the presence of an arginine at residue 12 confers binding to YRE-O motifs, while a lysine confers binding preference to YRE-A motifs **[30]**.

The homologue of *YAP1* in *C. glabrata* is *CAGL0H04631g* **[25]**. Sequence comparisons between *S. cerevisiae* and *C. glabrata* show that while overall their sequence similarity is low (37% identical), the functional domains are conserved between the species (Figure 1.5). This includes the nuclear localisation sequence (NLS) and nuclear export sequence (NES) which are used to target the import and export of Yap1 to and from the nucleus, as well as the pairs of cystine residues which form disulphide bonds upon oxidative stress treatment, either

directly by H_2O_2 or through the binding of Gpx3. The disulphide bonds formed block the NES, stopping the export of Yap1, leading to its accumulation inside the nucleus **[95, 112]**.

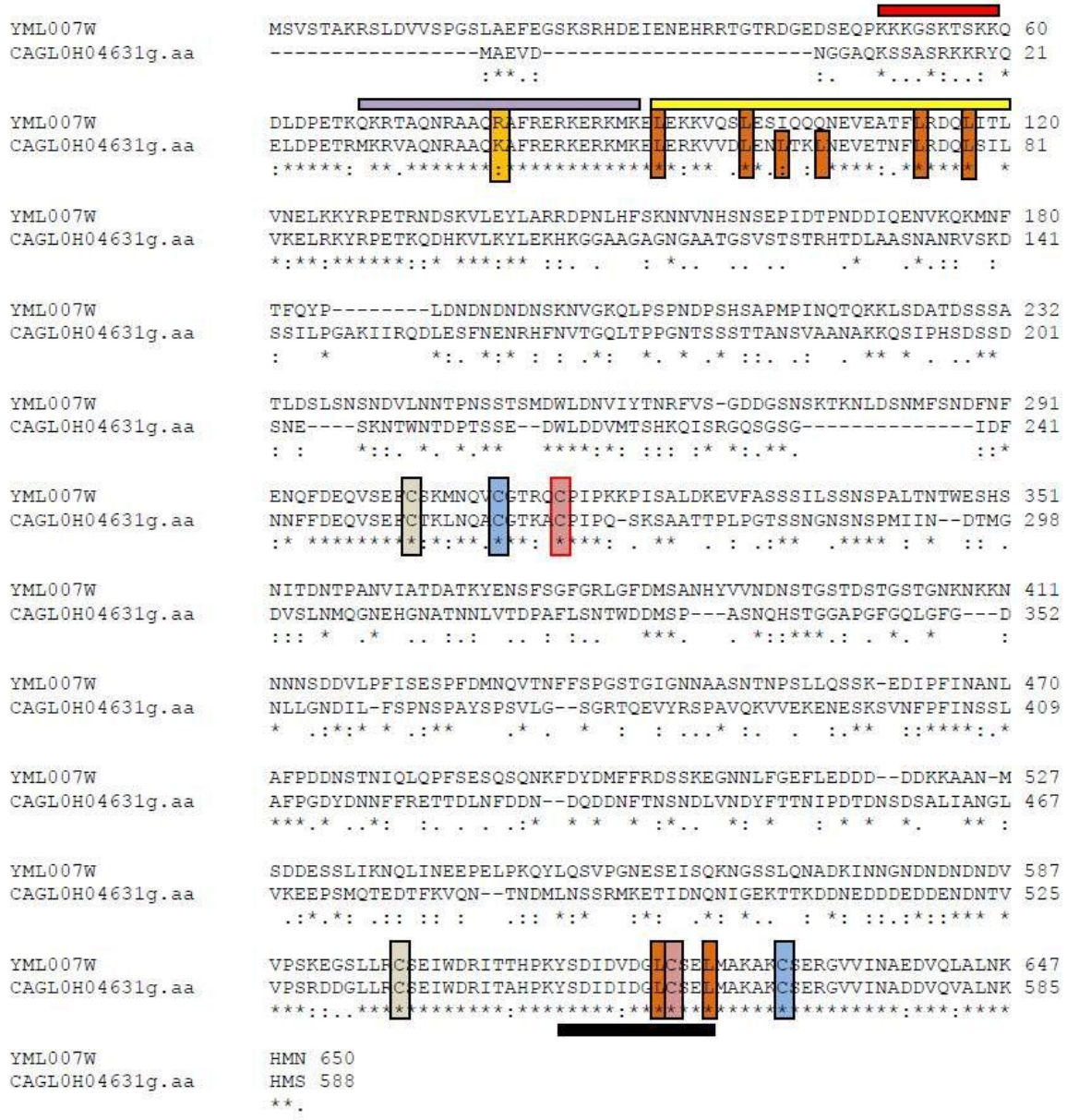


Figure 1.5: Protein sequence comparison between *S. cerevisiae* Yap1 and its homologue in *C. glabrata*. Protein sequences were obtained from Génolevures and SGD **[25, 45]**. Alignment of sequences was conducted using ClustalW **[113]**. Coloured labels denote: Basic region (purple), leucine zipper region (yellow), change from arginine to lysine (light orange), leucine residues (dark orange), disulphide bond formed between Cys 303 – Cys 598 (grey), disulphide bond formed between Cys 310 – Cys 629 (blue), disulphide bond formed between Cys 315 – Cys 620 (red), the nuclear localisation sequence (NLS) (red) and nuclear export sequence (NES) (black). Cys315 is boxed in red to highlight that it is not conserved in other fungal species. Symbols denote: * (asterisk) - identical amino acid, **:** (colon) – conserved substitution (same group), **.** (period) – semi-conserved substitution (group with similar properties).

The basic regions of *S. cerevisiae* and *C. glabrata* Yap1 have diverged; the *S. cerevisiae* Yap1 has an arginine at residue 12 while Yap1 in *C. glabrata* has a lysine (highlighted in light orange in Figure 1.5) **[30]**. This mutation in *C. glabrata* has been shown not to be strain

specific; sequencing of ATCC 2001 and an independent isolate revealed both to contain this diverged sequence **[30]**. Comparisons of Yap1 orthologues across 23 fungal species revealed that all possessed an arginine at residue 12, except for *C. glabrata* and *Neurospora crassa*, possessing a lysine and glutamine, respectively **[30]**. However, comparisons of residue 12 of other YAP proteins in *C. glabrata* and *S. cerevisiae* show the presence of either an arginine or lysine to be conserved between orthologues **[30]**. While Kuo *et al*, 2010, proposed that Yap1 in *C. glabrata* binds YRE-A motifs exclusively, Goudot *et al*, 2011, showed this protein to bind both YRE-A and YRE-O motifs **[30, 111]**. *C. glabrata YAP1* has also been shown to functionally complement the loss of *YAP1* in *S. cerevisiae* **[108]**. As Yap1 targets are largely conserved between *S. cerevisiae* and *C. glabrata* but the motifs they bind to are not, it has been proposed that these targets have co-evolved with compensatory mutations maintaining gene expression of the same set of targets **[30]**.

Using mutant microarray analyses and ChIP-chip experiments, 98 genes were shown to be targets of Yap1 in *C. glabrata* under benomyl treatment **[111]**. The transcriptional response of *C. glabrata* differs depending on the oxidative stressor used; Roetzer *et al*, 2010, showed little overlap between the transcriptional response of *C. glabrata* to H_2O_2 compared to that of menadione treatment **[106]**. Therefore, to characterise the specific targets of Yap1 in *C. glabrata* upon H_2O_2 treatment, mutant microarray analyses under H_2O_2 treatment were conducted.

Hog1, the MAPK involved in hyperosmotic stress resistance has also been implicated in the oxidative stress response of *C. albicans* **[114]**. CaHog1 is phosphorylated under hyperosmotic and oxidative stress conditions and cells lacking *HOG1* form filaments under non hyphae inducing conditions **[115, 116]**. As mentioned previously, the phosphorylation of Hog1 in *C. glabrata* has not been studied under different stresses, however its potential role in oxidative stress will be discussed in Chapter 5.

1.6.3 Crosstalk between signalling pathways

Crosstalk between different stress response pathways should be considered when investigating any kind of signalling pathway. Crosstalk is when one or more components of a signalling pathway affect a different pathway, with this usually occurring in signalling cascades. Signalling pathways are not linear and these pathways overlap and interconnect to form complex dynamic networks with many signalling components shared between different pathways. As previously mentioned, Hog1 as well as other MAPKs can be dually activated

under multiple stress conditions in many fungi. Components of the hyperosmotic stress pathway in *S. cerevisiae* are also involved in the cell wall integrity and mating/pheromone pathways **[67]**.

Cells must be able to process multiple signalling inputs in parallel and combine this information in order to trigger the appropriate response. Signalling pathways interact with each other and as Figure 1.6 shows, a large amount of cross talk is possible in the MAPK pathways in *S. cerevisiae.* Many proteins shown in the MAPK pathway model below are components of more than one pathway and act in different ways, suppressing or activating, depending on the pathway. As *C. glabrata* and *S. cerevisiae* are evolutionarily very close and have many homologous genes, *C. glabrata*'s pathway architecture may also be similar, as may the level of crosstalk between pathways.

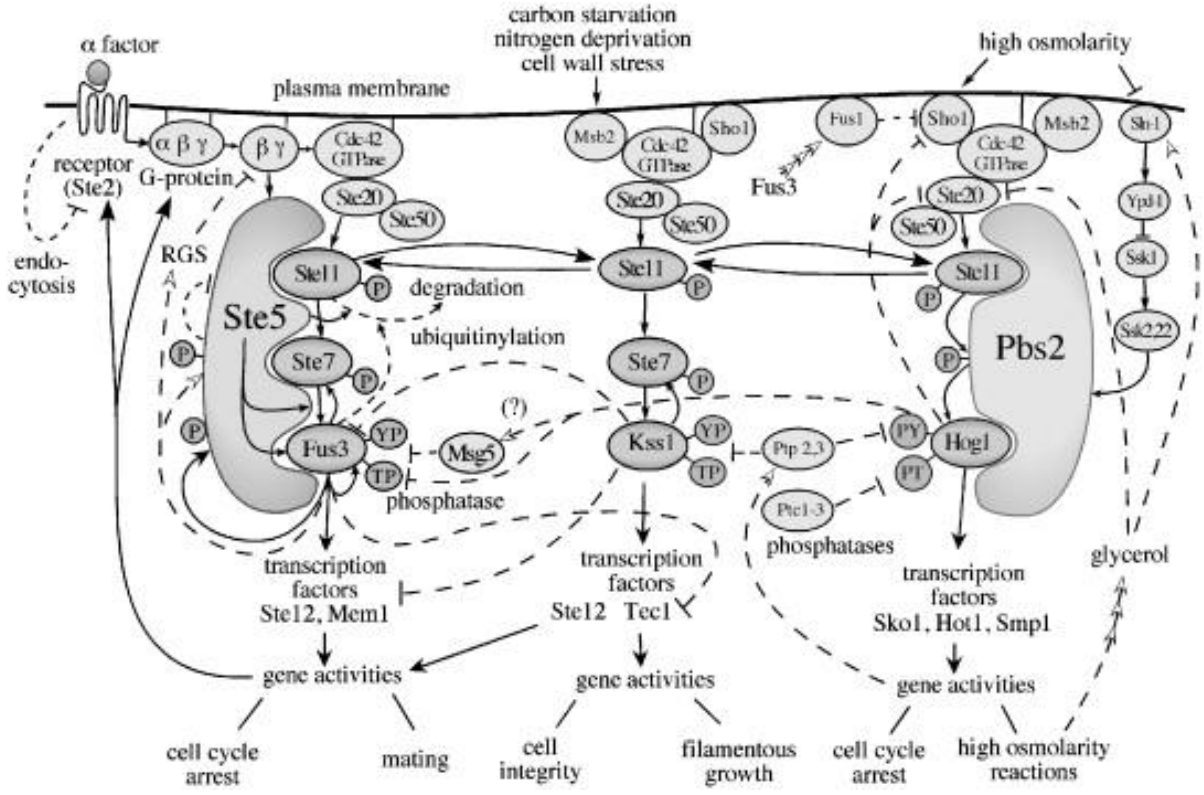


Figure 1.6: Model of MAPK regulation in *S. cerevisiae*. Mating/pheromone response (left), invasive filamentous growth (middle) and high osmolarity glycerol response (right). Diagram taken from **[117].**

The cross talk described above may explain the set of genes consistently regulated under varying stress conditions in *S. cerevisiae*. Gasch *et al*, 2000, found approximately 900 genes were regulated by a diverse range of environmental stress conditions including: hyper- and hypo-osmotic stress, a range of oxidative stress causing compounds, heat shock, temperature shift and nutrient starvation conditions **[1]**. These core stress response genes, referred to as

the environmental stress response (ESR), have also been reported in *C. glabrata* and will be examined in this study **[118]**.

1.6.4 Why study combinatorial stress?

As discussed in the previous sections on hyperosmotic and oxidative stress, these stresses are both found in cells of the host immune system. Environmental stresses often occur simultaneously in the host, and it is more informative to study *C. glabrata* under combinatorial stress if predictions of genetic contribution to virulence are to be made from *in vitro* studies **[119]**. To date there are only a small number of *in vivo* whole genome screens for *C. glabrata* survival in hosts and these experiments are expensive and technically difficult **[2, 120]**. To increase this knowledge base an alternative is to identify host-like environments *in vitro* that provide combinatorial stress conditions to study the pathogen. To aid the reading of this thesis, "combinatorial stress" refers to the simultaneous addition of hyperosmotic and oxidative stressors in this study.

Experiments on solid media have shown that while there is a moderate effect on viability under single stress conditions, when those stressors are applied simultaneously, this has a severe effect on growth when using high concentrations (an example is shown in Figure 1.7). For the array data presented in this study, lower concentrations of hyperosmotic and oxidative stress were used so that the *Candida* cells were stressed, but still able to grow, although this growth was inhibited (Figure 6.1). This striking phenotype shows the importance of studying combinatorial stress response in *C. glabrata*.

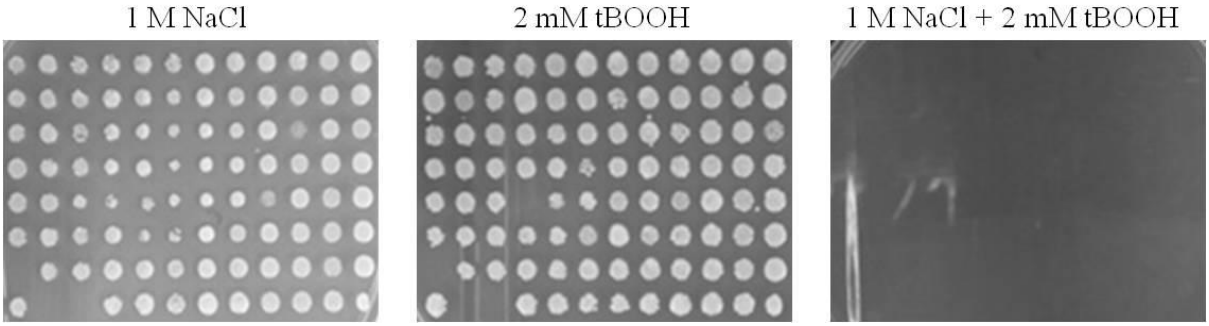


Figure 1.7: The effect of hyperosmotic and oxidative stress applied singly and in combination to null mutants in *C. glabrata*. Labels denote: 1 M NaCl; hyperosmotic stress, 2 mM tBOOH; oxidative stress and 1 M NaCl + 2 mM tBOOH; combinatorial stress (both stressors applied simultaneously). Single targeted gene deletions, as well as parental controls, were replica plated on to YPD media containing the indicated stress and incubated at 30°C for three days (Lauren Ames, personal communication).

While there have been some studies which have looked at the effect of combination drug therapies on cancer as well as the treatment of *Candida* infections, very few studies have been published looking at the response of any organism to combinations of simultaneous

environmental stress **[121, 122]**. A few studies have looked at the effect of serial additions of stress (treatment with one stress, then another at a later time) in respect to cross protection and adaptation, primarily by looking at the effect on viability, but not the simultaneous addition of hyperosmotic and oxidative stress. Gasch *et al*, 2000 conducted transcriptional analysis by pre-treating *S. cerevisiae* cells with sorbitol and then transferring to media at a higher temperature, while Jaimeson, 2007, showed that pre-treatment with a low dose of one oxidative stressor, increased resistance to subsequent challenges by different oxidative stressors **[1, 123]**.

Transcription profiling has usually been restricted to the study of the response to a single stress and while the transcript profile of *S. cerevisiae*, for example, has been extensively studied under many diverse environmental stresses, these have not been combined. Organisms are exposed simultaneously to multiple stresses in the host environment, as reviewed by Brown *et al*, 2009, such as the oxidative and nitrosative stress which occurs after macrophage engulfment **[85, 119].** Leading from these initial datasets, we have studied the response to stress under single and combinatorial stress conditions. Most transcription profiling studies are also restricted to one time point under a single stress condition and as such only give a snap shot of the transcriptional response at a single moment in time, an example being Schuller's experiments **[124]**. Our experiments were conducted over one and two hour time courses and this allows the adaptation of the organism to stress over time to be analysed, as well as the immediate response and at specific time points. Combining this analysis with time course growth assays under stress allows comparisons between gene regulation and growth to be made.

Transcript profiling has been used extensively to study *S. cerevisiae*. From DNA microarrays and next generation sequencing, the expression and regulation of RNA transcripts of different strains, single and double mutants and different stress conditions have been experimentally determined and used to build our knowledge of the yeast transcriptome. SPELL (**S**erial **P**attern of **E**xpression **L**evels **L**ocator) is an online repository of *S. cerevisiae* published microarray datasets (spell.yeastgenome.org). Transcriptional analysis generates large amounts of data on mRNA levels of an organism on a global scale. This allows the collection of data on 1000s of genes at a time, rather just the few genes that are able to be analysed through other methods, such as qRT-PCR. These targets of PCR based methods must also be chosen before conducting the experiments, potentially skewing results and leaving little room for genes with novel functions. This is especially important with organisms such as *C.*

glabrata that have little functional gene annotation. While these studies are informative, all molecular tools have their drawbacks. While it is thought that levels of mRNA and their corresponding protein abundances correlate, it has been shown in yeast that this is not always the case **[125, 126]**. The stability and decay of the RNA being determined should also be considered, as different mRNAs have been shown to have widely varying half lives in yeast, from 3 to 90 minutes **[127]**.

2 Aims

C. glabrata is the second most prevalent cause of Candidosis in humans and yet little is known about its molecular biology. Although it is more closely related to the non-pathogenic yeast *S. cerevisiae*, the specific characteristics that allow *C. glabrata* to be a successful fungal pathogen remain largely elusive. *C. glabrata* is resistant to environmental stressors and this has been shown by the range of infection sites and ecological niches it can colonise. Studies have shown that stress response proteins are important for virulence and therefore the study of this opportunistic pathogen to environmental stress conditions could aid in the development of a pathogenicity model. Environmental stressors would not occur singly in a host setting, but simultaneously and in combination. Therefore the study of *C. glabrata* under not only single hyperosmotic and oxidative stress but also combinatorial stress conditions is pertinent to the host niche.

The aim of this study was to define and characterise the response of *C. glabrata* to combinatorial stress conditions by transcript profiling and functional genomics analyses. This included:

- Transcript profiling under single and combinatorial stress conditions over a time course.
- Construction of null mutants of stress response genes identified from the literature and transcriptional analysis.
- Phenotypic screening and characterisation of these null mutants.
- Construction of models to describe the response of *C. glabrata* to environmental stresses.

3 Materials and Methods

All molecular biology products and reagents were obtained from Sigma, unless otherwise stated. Standard methods for molecular biology were used as per Sambrook, 1989 **[128]**.

3.1 Culturing strains

3.1.1 Yeast strains

Strains were grown in YPD medium (2 % yeast extract, 1 % Bacteriological peptone, 2 % glucose) at 30°C, unless otherwise stated.

3.1.2 Bacterial strains

LB (Luria Broth) was used to grow bacterial strains in this study at 37°C.

3.2 Yeast, bacterial strains and plasmids

3.2.1 Bacterial strains

The bacterial strains used in this study are listed in Table 3.1.

Table 3.1: Bacterial strains used in this study.

Strain	Genotype	Description	Source
Top10	*mcrA*, Δ(*mrr-hsd*RMS-*mcrBC*), Phi80*lacZ(del)M15*, Δ*lacX74*, *deoR*, *recA1*, *araD139*, Δ(*ara-leu*)7697, *galU*, *galK*, *rpsL(SmR)*, *endA1*, *nupG*	Used for general cloning with a high transformation efficiency	Lab stock

3.2.2 Plasmids

The plasmids used in this study are listed in Table 3.2.

Table 3.2: Plasmids used in this study.

Name	Marker	Description	Source
pCR2.1-SAT	ampR, NAT	Used to amplify the NAT cassette	B. Cormack[1]
pBM51	ampR, Sc*HIS*	Contains *HIS* gene used to create pMP10. Cg*CEN/ARS*.	Lab strain
pRD16	ampR, Sc*URA3*	*FLP*-expression cassette (Cg*EPA1* promoter, *FLP1* and Cg*HIS3* 3' UTR), Cg*CEN/ARS*.	K. Kuchler[2]
pMP10	ampR, Sc*HIS*	Used to create double mutants by recycling the NAT cassette	This study

1. Johns Hopkins, Maryland, USA. 2. Medical University of Vienna, Austria.

3.2.3 Yeast strains

The yeast strains used in this study are listed in Table 3.3.

Table 3.3: Yeast strains used in this study.

Strain	Genotype	Description	Source
ATCC 2001	-	Clinical isolate, type strain, sequenced strain	ATCC[1]
CG2001 *hisΔ*	*his3Δ*	Derivative of ATCC 2001, parental strain of mutants constructed in this study	K. Kuchler[2]
CG2001 *HTLΔ*	*his3Δ, trp1Δ, leu2Δ*	Derivative of ATCC 2001, parental strain of mutants constructed in Kuchler mutant library	K. Kuchler[2]
BG2	-	Clinical isolate, no mutation in *SSK2*	Lab strain
aft2Δ	CG2001 *his3Δ, aft2Δ*::*NAT*	*aft2Δ* mutant	Lab strain
asg1Δ	CG2001 *hisz3Δ, trp1Δ, leu2Δ, asg1Δ*::*NAT*	*asg1Δ* mutant	K. Kuchler[2]
cta1Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, cta1Δ*::*NAT*	*cta1Δ* mutant	K. Kuchler[2]
ecm1Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, ecm1Δ*::*NAT*	*ecm1Δ* mutant	K. Kuchler[2]
ecm13Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, ecm13Δ*::*NAT*	*ecm13Δ* mutant	K. Kuchler[2]
ecm33Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, ecm33Δ*::*NAT*	*ecm33Δ* mutant	K. Kuchler[2]
gpd1Δ	CG2001 *his3Δ, gpd1Δ*::*NAT*	*gpd1Δ* mutant	This study
gpd2Δ	CG2001 *his3Δ, gpd2Δ*::*NAT*	*gpd2Δ* mutant	This study
gpdΔ2, NATΔ	CG2001 *his3Δ, gpd2Δ*::FRT	*gpd2Δ* mutant with NAT cassette removed for recycling of disruption cassette	This study
gpd2Δ, gpd1Δ	CG2001 *his3Δ, gpd2Δ*::FRT, *gpd1Δ*::*NAT*	*gpd1Δ, gpd2Δ* mutant	This study
gsf2Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, gsf2Δ*::*NAT*	*gsf2Δ* mutant	K. Kuchler[2]
gsm1Δ	CG2001 *his3Δ, gsm1Δ*::*NAT*	*gsm1Δ* mutant	Lab strain
hal1Δ	CG2001 *his3Δ, hal1Δ*::*NAT*	*hal1Δ* mutant	This study
hog1Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, hog1Δ*::*NAT*	*hog1Δ* mutant	Lab strain
hor7Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, gsm1Δ*::*NAT*	*hor7Δ* mutant	K. Kuchler[2]
hsp12Δ	CG2001 *his3Δ, hsp12Δ*::*NAT*	*hsp12Δ* mutant	This study
msb2Δ	CG2001 *his3Δ, msb2Δ*::*NAT*	*msb2Δ* mutant	This study
NiSODΔ	CG2001 *his3Δ, NiSODΔ*::*NAT*	*NiSODΔ* mutant	This study
opy2Δ	CG2001 *his3Δ, opy2Δ*::*NAT*	*opy2Δ* mutant	This study
pbs2Δ	CG2001 *his3Δ, pbs2Δ*::*NAT*	*pbs2Δ* mutant	This study
pck1Δ	CG2001 *his3Δ, trp1Δ, leu2Δ,*	*pck1Δ* mutant	K. Kuchler[2]

	pck1Δ::*NAT*		
rim9Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, rim91Δ*::*NAT*	*rim9Δ* mutant	K. Kuchler[2]
scm4Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, scm4Δ*::*NAT*	*scm4Δ* mutant	K. Kuchler[2]
sho1Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, sho1Δ*::*NAT*	*sho1Δ* mutant	K. Kuchler[2]
skn7Δ	CG2001 *his3Δ, skn7Δ*::*NAT*	*skn7Δ* mutant	Lab strain
sln1Δ	CG2001 *his3Δ, sln1Δ*::*NAT*	*sln1Δ* mutant	This study
sod1Δ	CG2001 *his3Δ, sod1Δ*::*NAT*	*sod1Δ* mutant	This study
ste11Δ	CG2001 *his3Δ, trp1*Δ, *ste11*Δ *::HIS3*	*ste11Δ* mutant	Lab strain
ste20Δ	CG2001 *his3Δ, trp1*Δ, *ste20*Δ *::HIS3*	*ste20Δ* mutant	Lab strain
ste20βΔ	CG2001 *his3Δ, ste20βΔ*::*NAT*	*ste20βΔ* mutant	This study
ste50Δ	CG2001 *his3Δ, trp1Δ, leu2Δ, ste50Δ*::*NAT*	*Δste50* mutant	K. Kuchler[2]
TFΔ	CG2001 *his3Δ, TFΔ*::*NAT*	*TFΔ* mutant	This study
tsa1Δ	CG2001 *his3Δ, tsa1Δ*::*NAT*	*tsa1Δ* mutant	This study
tsa1Δ, NATΔ	CG2001 *his3Δ, tsa1Δ*::FRT	*tsa1Δ* mutant with NAT cassette removed for recycling of disruption	This study
tsa1Δ, tsa2Δ	CG2001 *his3Δ, tsa1Δ*::FRT, *tsa2Δ*::*NAT*	*tsa1Δ, tsa2Δ* mutant	This study
tsa2Δ	CG2001 *his3Δ, tsa2Δ*::*NAT*	*tsa2Δ* mutant	This study
yap1Δ	CG2001 *his3Δ, trp1Δ, yap1Δ*:: *HIS3*	*yap1Δ* mutant	J. Quinn[3]
ypd1Δ	CG2001 *his3Δ, ypd1Δ*::*NAT*	*ypd1Δ* mutant	This study
BG2 *opy2Δ*	BG2, *opy2Δ*::*NAT*	*opy2Δ* mutant	This study
BG2 *sho1Δ*	BG2, *sho1Δ*::*NAT*	*sho1Δ* mutant	This study
BG2 *ypd1Δ*	BG2, *ypd1Δ*::*NAT*	*ypd1Δ* mutant	This study
BY4741*	MAT*a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*	Derivative of S288C, parental strain of YKO library	ATCC[1]
*tsa1Δ**	MAT*a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, tsa1Δ*::*KanMX*	*tsa1Δ* mutant	YKO library
*tsa2Δ**	MAT*a, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, tsa2Δ*:: *KanMX*	*tsa2Δ* mutant	YKO library
DBY7268*	MATa, *ura3-52 GAL2*	Strain used in Gasch *et al*, 2000 **[1]**	A. Gasch[4]

1. American Type Culture Collection. 2. Medical University of Vienna, Austria. 3. Newcastle University, UK. 4. Dr. Audrey Gasch, University of California, Berkeley. An asterisk denotes *S. cerevisiae* strains. **NB.** It is important to note that while some of the *C. glabrata* strains used in this study have different auxotrophies, these have no bearing on virulence **[129]**. The single (*Δhis3*; *Δtrp1*; and *Δleu2)* as well as triple auxotrophic (*Δhis3, Δtrp1, Δleu2*) strains had little effect, compared to wild type (ATCC 2001), on the ability of *C. glabrata* to colonise and persist in a mouse model of infection.

3.3 Molecular techniques

3.3.1 Extraction of DNA from yeast cells

Genomic extractions of DNA from yeast cells were performed using EpiCentre MasterPure Yeast DNA Purification Kit, as per the manufacturer's instructions, or modified as described below.

A single yeast colony was taken from an agar plate and re-suspended thoroughly by either vortex mixing or pipetting in a microcentrifuge tube containing 300 µl of Yeast Cell Lysis Solution. The suspended cells were incubated at 65°C for 15 minutes then chilled on ice for 5 minutes. Then, 150 µl of MPC Protein Precipitation Reagent was added and the solution mixed by vortexing. The unwanted cellular debris was pelleted by centrifugation for 10 minutes at ≥10,000 rpm and the supernatant transferred to a clean microcentrifuge tube containing 500 µl of isopropanol. This solution was mixed by inverting the microcentrifuge tube 6-8 times; harsh mixing can cause shearing of the DNA. The DNA was pelleted by centrifugation for 10 minutes at ≥10,000 rpm and the supernatant removed by pipetting and discarded. The DNA pellet was washed with 500 µl of 70 % ethanol and centrifuged again, if necessary. The ethanol was removed by careful pipetting, discarded and the tube left open until any remaining ethanol had evaporated. The DNA pellet was re-suspended in 35 µl of TE Buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8) and stored at either +4°C or -20°C.

3.3.2 Southern blot analysis

Restriction enzyme digested DNA was run in a DNA gel (1 % agarose (w/v)) without ethidium bromide (EtBr) in TBE buffer at 100V for 2-3 hours, until the samples had reached the end of the gel. The gel was submerged in TBE buffer containing EtBr (1:100) for 30 minutes and imaged using the G Box imager from Syngene. The gel was de-purinated in 250 mM HCl for 15 minutes, at room temperature with shaking and then washed for 20 minutes with distilled water. The gel was then de-natured using denaturation solution (1.5 M NaCl, 0.5 M NaOH) by washing twice for 15 minutes with shaking then rinsing for 10 minutes with distilled water. The gel was washed with neutralisation solution (0.6 M Tris-HCL, 1.5 M NaCl, pH 7.2) for 15 minutes twice with agitation and then rinsed for 10 minutes with distilled water. The gel was equilibrated in 20x SSC (0.44 M Tris, 0.44 M boric acid, 0.01 M EDTA) for 10 minutes, before being set up for transfer on to a positively charged nylon membrane (Roche) overnight as described by Southern, 1975 **[130]**. After transfer, the blot was dismantled, the membrane placed (DNA side up) onto Whatman paper and UV cross

linked using the Stratagene UV Stratalinker 1800. The DIG Easy Hyb solution (Roche) was pre-warmed to the hybridisation temperature (T_{hyb}) for the probe being used which is worked out using the equations below.

$$T_m = 49.82 + 0.41(\%\ G + C) - \frac{600}{length}$$

$$T_{hyb} = T_m - (20 \text{ to } 25°C)$$

The membrane was pre-hybridised at the T_{hyb} in DIG Easy Hyb solution for 1 - 3 hours with shaking. Probes for southern hybridisation were amplified using DIG Probe Synthesis Kit (Roche) following the manufacturer's instructions. The primers used to amplify the NAT cassette probe from pCR2.1-SAT (B. Cormack, John Hopkins, Maryland, USA) can be found in Table 3.5 (NAT-5'-R and NAT-3'-F). Approximately 50 ng of probe was added to 50 µl of PCR grade water, boiled for 5 minutes to de-nature the probe and immediately chilled on ice. This mix was then added to 20 ml of pre-warmed DIG Easy Hyb solution at the T_{hyb}. The membrane was incubated overnight (16 hours) with agitation at the T_{hyb}. The membrane was washed twice in low stringency buffer (2x SSC, 0.1 % SDS) at room temperature with agitation for 5 minutes and then washed twice for 15 minutes in high stringency wash buffer (0.5x SSC, 0.1 % SDS) pre-heated to 65°C with agitation. The membrane was then washed for 2 minutes in washing buffer (0.3 % Tween in Maleic Acid buffer, pH 7.5) at room temperature with shaking. Blocking solution (1 % blocking in Maleic acid buffer, pH7.5, using Blocking Reagent, Roche) was added to the membrane for a minimum of 30 minutes (maximum of 3 hours) at room temperature with shaking. The membrane was incubated with 3 µl of Anti-Digoxigenin-AP Fab antibody (Roche) in 30 ml of blocking solution for 30 minutes with agitation. The membrane was washed twice with washing buffer for 15 minutes at room temperature with shaking and then allowed to equilibrate in detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5) for 3 minutes at room temperature with shaking. The chemiluminescent substrate, CDP-Star (Roche), was added and incubated as per the manufacturer's instructions at room temperature for 5 minutes in the dark and the blot developed using a G Box from Syngene.

3.3.3 *C. glabrata* transformation by electroporation

The parental *C. glabrata Δhis3* strain was cultured from a freezer stock in 5 ml YPD overnight at 30°C with shaking at 180 rpm. This overnight culture was diluted to an OD_{600} of 0.2 in 50 ml of fresh YPD and grown at 30°C for 3-4 hours with shaking at 180 rpm to a final OD_{600} of 1.0. The cells were harvested by centrifugation at 3000 rpm for 5 minutes and then

washed with 25 ml of sterile H_2O. The cells were resuspended gently in 8 ml of sterile H_2O, 1 ml 10x TE (100 mM Tris-HCl, pH 8.0, 10 mM EDTA) and 1 ml 1 M Li-acetate and incubated at 30°C, 180 rpm for 30 minutes. To the cell mixture, 125 µl of 2 M DTT was added and incubated at 30°C, 180 rpm for 1 hour. Then, 40 ml of autoclaved water was added to the cell suspension, centrifuged at 3000 rpm for 5 minutes and re-suspended in 25 ml cold sterile water by gently shaking. Once centrifuged at 3000 rpm for 5 minutes, the cells were re-suspended in 5 ml cold 1 M sorbitol by gently shaking. The cells were then centrifuged at 3000 rpm for 5 minutes and re-suspended in 500 µl of 1 M sorbitol. These are the competent *C. glabrata* cells used for transformations. Using pre-chilled, sterile electroporation cuvettes (2 mm gap), 55 µl of competent cells and 6 µl of the deletion construct DNA were added and left on ice for 5-10 minutes. Electroporation was performed using a BioRad gene pulser (200 Ω, 25 µF, 1.5 kV) and 950 µl YPD was immediately added to the cuvette and transferred to a 1.5ml microcentrifuge tube. Cells were incubated at 30°C, 180 rpm for 4 hours. After 4 hours, the transformed cells were centrifuged at 13,000 rpm for 30 seconds, re-suspended in 200 µl of sterile water and spread on to YPD agar supplemented with 200 µg/ml of CloneNat (Nourseothricin, WernerBioAgents). The plates were then incubated at 30°C for 2-3 days.

3.3.4 Preparation of competent *E. coli* cells and *E. coli* transformation

Top10 *E. coli* cells were made competent for transformation using the method described here. Top10 cells were cultured from a freezer stock in 5 ml LB overnight at 37°C with shaking at 200 rpm. These cells were subcultured in 100 ml LB in a 1 L flask and grown for 1 hour. These cells were chilled on ice for 5 minutes, centrifuged at 3000 rpm for 5 minutes at 4°C, the supernatent removed and the cells resuspended gently in 40 ml ice cold Transformation Buffer 1 (30 mM KAc, 100 mM KCL, 10 mM $CaCl_2$, 50 mM $MnCl_2$, 15 % glycerol, pH 5.8). After being chilled on ice for 5 minutes, the cells were centrifuged at 3000 rpm for 5 minutes at 4°C, the supernatent removed and the cells resuspended gently in 4 ml ice cold Transformation Buffer 2 (10 mM MOPs, 75 mM $CaCl_2$, 10 mM KCL, 15 % glycerol, pH 6.5). The cells were chilled on ice for 15 minutes and then aliquoted into sterile tubes and stored at -80°C.

Plasmids were transformed into competent *E. coli* cells by heat shock as described here. The competent *E. coli* cells were removed from -80°C storage and thawed on ice. For each transformation reaction, 50 ng of plasmid DNA was added to 50 µl of competent cells in a microcentrifuge tube and incubated on ice for 15 minutes. The mixture was heat shocked for 45 seconds in a 42 °C water bath and immediately chilled on ice for 5 minutes. The cells

were incubated in 1 ml of LB media for an hour at 37°C. The cells were briefly centrifuged and most of the media removed, re-suspended gently in the remaining media and spread on to LB plates containing the antibiotic Ampicillin. Plates were incubated overnight at 37°C.

3.3.5 Oligonucleotides

The oligonucleotides used in this study are listed in Table 10.1, p.213, Appendix I and were synthesised by Sigma.

3.3.6 Polymerase Chain Reaction (PCR)

Polymerase chain reactions were conducted as per the manufacturer's instructions for each polymerase used (Table 3.4).

Table 3.4: Polymerase enzymes used in this study.

Polymerase enzyme	Manufacturer	Use in study
GoTaq	Promega	Amplifying UTRs for null mutant creation; checking of putative null mutants
Ex-Taq	TaKaRa	Creation of the disruption cassette
Bio-X-Act Short	Bioline	Amplifying the NAT cassette from plasmid DNA

3.3.7 DNA gel electrophoresis

Agarose gels were made using 1 % (w/v) agarose in TBE buffer (45 mM Tris-borate, 1 mM EDTA) dissolved by heating on half power in a microwave for 4 minutes. This solution was left to cool to approximately 50°C and either EtBr or SyberSafe (Invitrogen) was added (1:1000). The gel mixture was poured into a gel cast using the appropriate sized combs and allowed to set for 20-30 minutes. The combs were then removed, TBE buffer was added to the tank to cover the gel and the DNA samples loaded into the wells. The gel was run at 80V for 45 minutes until the dye front reached the bottom of the gel. Images were obtained using a G Box from Syngene. DNA samples were loaded using a 5x DNA loading buffer (5 % (w/v) sucrose, 0.3 % (w/v) bromophenol blue). Either a 1 kb or 100 bp DNA ladder from New England BioLabs was used a marker.

3.3.8 Purification of PCR products

PCR products were purified using the Gel Purification Kit from Sigma, following the manufacturer's instructions.

3.3.9 Purification of plasmid DNA from *E. coli* cells

Plasmid DNA was extracted and purified from *E. coli* cells using Plasmid MiniPrep Kit from Sigma, as per the manufacturer's instructions.

3.3.10 Restriction enzyme digests

Restriction enzymes were purchased from either Promega or Fermentas and reactions were set up as per the manufacturer's instructions. If appropriate, digests were checked for the correct size by gel electrophoresis. DNA digests were purified using the PCR Purification Kit from Sigma, as per the manufacturer's instructions.

3.3.11 Ligation

Ligation reactions were carried out as per the manufacturer's instructions using T4 DNA ligase from Fermentas. The concentration of insert (ng) to add to 50 ng of vector DNA was determined using the equation below. The ratio of insert to vector used was either 3:1, 10:1, 1:1, 1:3 or 1:10.

$$\frac{\text{50 ng of vector DNA x size of insert DNA (kb)}}{\text{Size of the vector DNA (kb)}} \quad \text{x} \quad \text{Ratio of } \frac{\text{insert}}{\text{vector}}$$

3.3.12 Quantification of RNA and DNA

RNA and DNA extracted from yeast cells were quantified using a NanoDrop ND-1000 Spectrophotometer from Labtech. Samples were only used if the 260/280 ratio was 1.8 for DNA and 2.0 for RNA and the 260/230 ratio was >2.0.

3.4 Construction of null mutants in *C. glabrata*

Null mutants were created based on methods from Noble, 2005 and as described below **[131]**. Figure 3.1 depicts this process and the primers used in this study can be found in Table 10.1, p.213, Appendix I.

3.4.1 Construction of null mutants

C. glabrata orthologues of *S. cerevisiae* were identified by BLAST search and by checking their chromosomal context **[25, 29]**. 500 bp products of the 5' and 3' UTR of each gene were generated by PCR, using primers -500-F/-1-NAT-R and +500-R/+1-NAT-F. The NAT cassette, which confers resistance to Nourseothricin, was amplified from the plasmid pCR2.1-SAT (B. Cormack, Johns Hopkins, Maryland, USA) using U2-BC-NAT/D2-BC-NAT primers (Table 3.5). Disruption cassettes comprising of the NAT cassette flanked by the 500 bp 5' and 3' UTR were generated by PCR using -500-F/+500-R primers. These disruption cassettes were introduced into CG2001 *Δhis3* by electroporation and selected for by plating onto YPD medium supplemented with 200 mg/µl Nourseothricin (WernerBioAgents).

Correct incorporation of the NAT cassette was verified by PCR using primers -600-F/NAT-5'-R and +600-R/NAT-3'-F and the elimination of the target gene using primers, int F/int R.

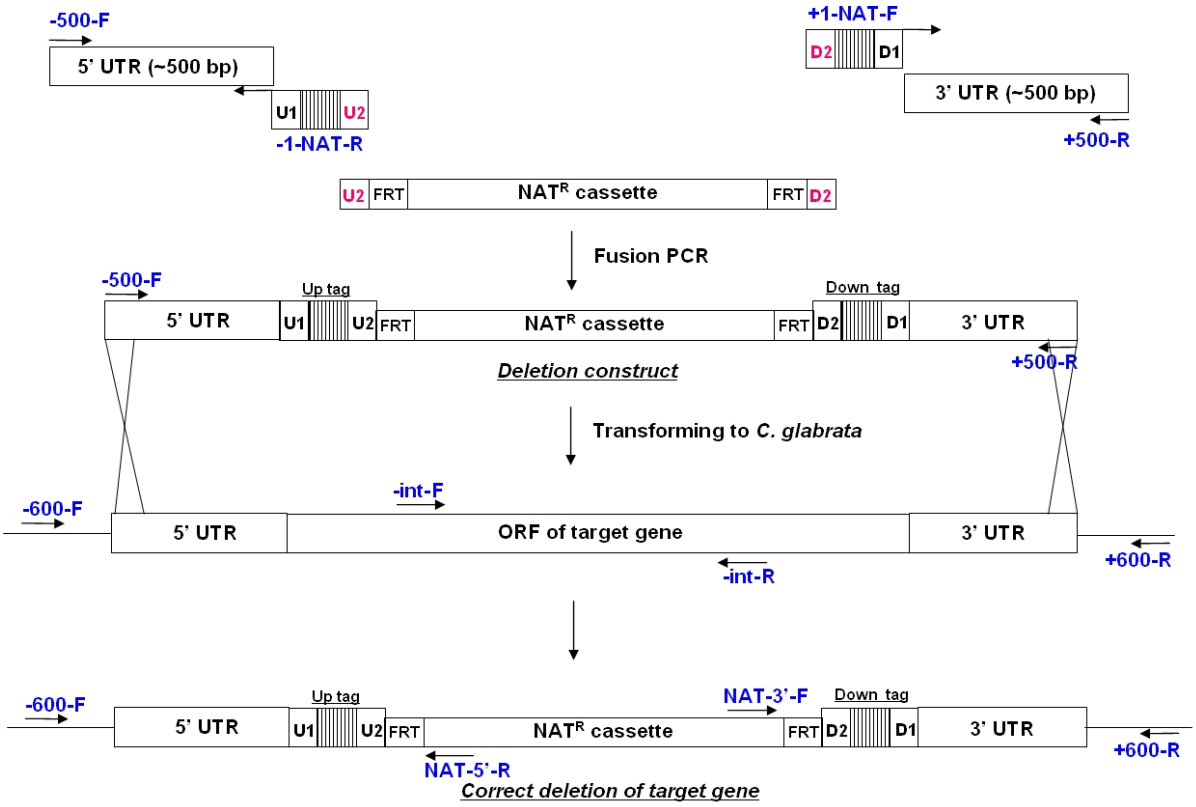


Figure 3.1: Diagram illustrating the process of creating null mutants in *C. glabrata* using the NAT disruption cassette. From (Biao Ma, personal communication).

Table 3.5: Primers used in this study: to amplify the NAT disruption cassette and check for the correct genomic incorporation of the NAT cassette.

Primer Name	Sequence (5' to 3')
NAT-5'-R	catccaaagtagtagac
NAT-3'-F	atgtctatgccatgtcc
U2-BC-NAT	cgtacgctgcaggtcgacgcCTTCCGCTGCTAGGCGC
D2-BC-NAT	ctacgagaccgacaccgtcgGGCCGCTGACGAAGTTC

Southern blot analysis was conducted to check the correct incorporation of only one NAT cassette. DNA extracted from two independent isolates of each null mutant strain were restriction enzyme digested using the enzymes shown in Figure 10.2, p.219, Appendix I. Southern blot analysis was conducted using a probe for the NAT cassette which is present in all null mutants. Correct null mutant isolates displayed a single band of DNA the correct size for the restriction enzyme used. If more than one band was observed this would indicate a random insertion of the disruption cassette into the genome. No isolates displayed more than

one NAT cassette of the 21 strains (with 2 isolates each) or parental controls tested. It is of note that strains made using pMP10 (this study) to remove the NAT cassette did not display a band when probed for NAT. An example of the experimental design of these Southern blot analyses (for *opy2*) is shown in Figure 3.2.

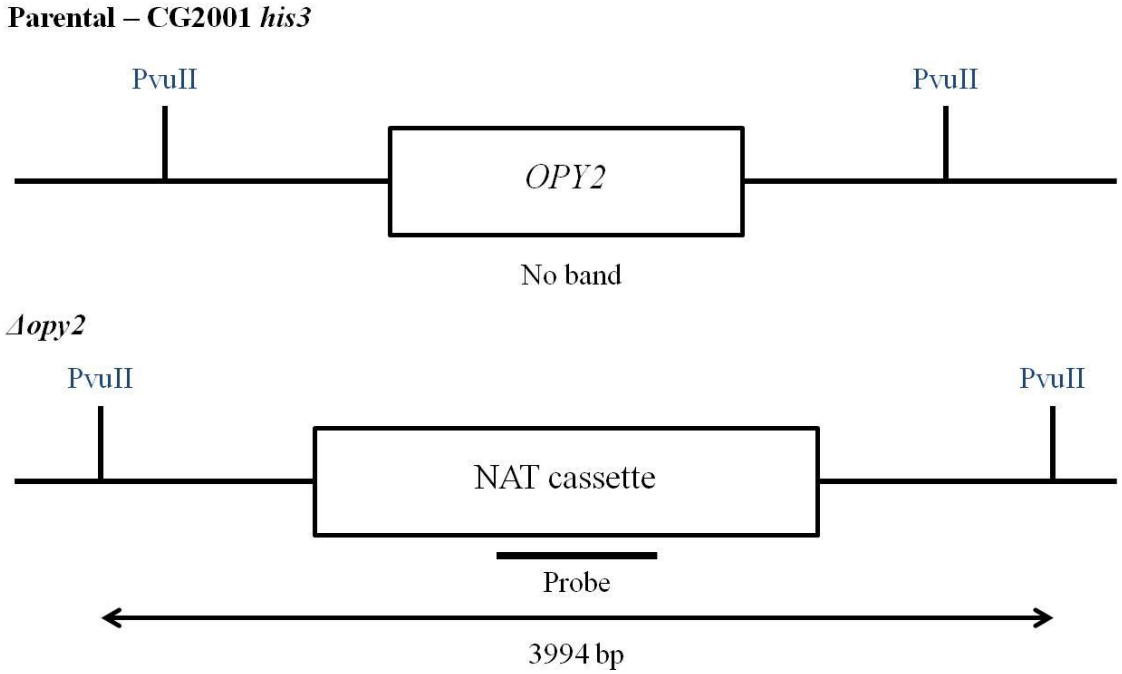


Figure 3.2: An example of the design of Southern blot experiments to confirm correct null mutants. Using a probe specific for the disruption cassette (NAT), restriction enzyme digested DNA was analysed by Southern blot. No band was expected or observed in the parental strain. A single band was observed in the correct null mutants; in the case of *opy2*, a band of approximately 4 kb was displayed. For this example, the restriction enzyme PvuII was used. The Sothern blot image associated with this diagram can be found in Figure 10.2, p.219, Appendix I.

Barcodes (Up or Down tags) and the sequences to amplify these unique tags for screening, were incorporated into each mutant. Barcodes can be amplified using U1-BC-NAT/U2-BC-NAT and D1-BC-NAT/D2-BC-NAT. The Up/Down tags are taken from the *S. cerevisiae* gene deletion project, for which microarrays are available to screen the mutants using these tags **[47]**. If there were two *C. glabrata* ORFs which both share homology with a single *S. cerevisiae* gene, tags corresponding to a *S. cerevisiae* gene that does not have a *C. glabrata* homologue were used for one of the copies.

3.5 Plasmid construction

pMP10 (Figure 3.3) was made by removing the *URA* gene from pRD16 (K. Kuchler, Medical University of Vienna, Austria) and replacing it with the *HIS* gene from pBM51 (lab strain). pRD16 (vector) and pBM51 (insert) were double restriction enzyme digested with SbfI and NgoMIV. Vector and insert were ligated and transformed into competent Top10 *E. coli* cells.

Correct plasmid constructs were checked by restriction enzyme digest and sent off for sequencing using the primers in Table 10.2, p. 218, Appendix I. pMP10 allows the recycling of the NAT disruption cassette as it contains the *FLP* gene (under a *C. glabrata* EPA promoter) which recognises the FRT (Flippase Recognition Target) sites either side of the NAT cassette and removes it, allowing sensitivity to Nourseothricin and restoring the ability to grow on media lacking histidine. The plasmid can then be removed by streaking to single colonies on YPD plates containing 50 mM 3-AT which competitively inhibits the *HIS* gene, encouraging the removal of pMP10. Correct strains will be unable to grow on media lacking histidine or media containing Nourseothricin and can now be used for the creation of double mutants by re-using the NAT cassette. This plasmid allowed the creation of the double mutants *gpd1*, *gpd2* and *tsa1*, *tsa2*.

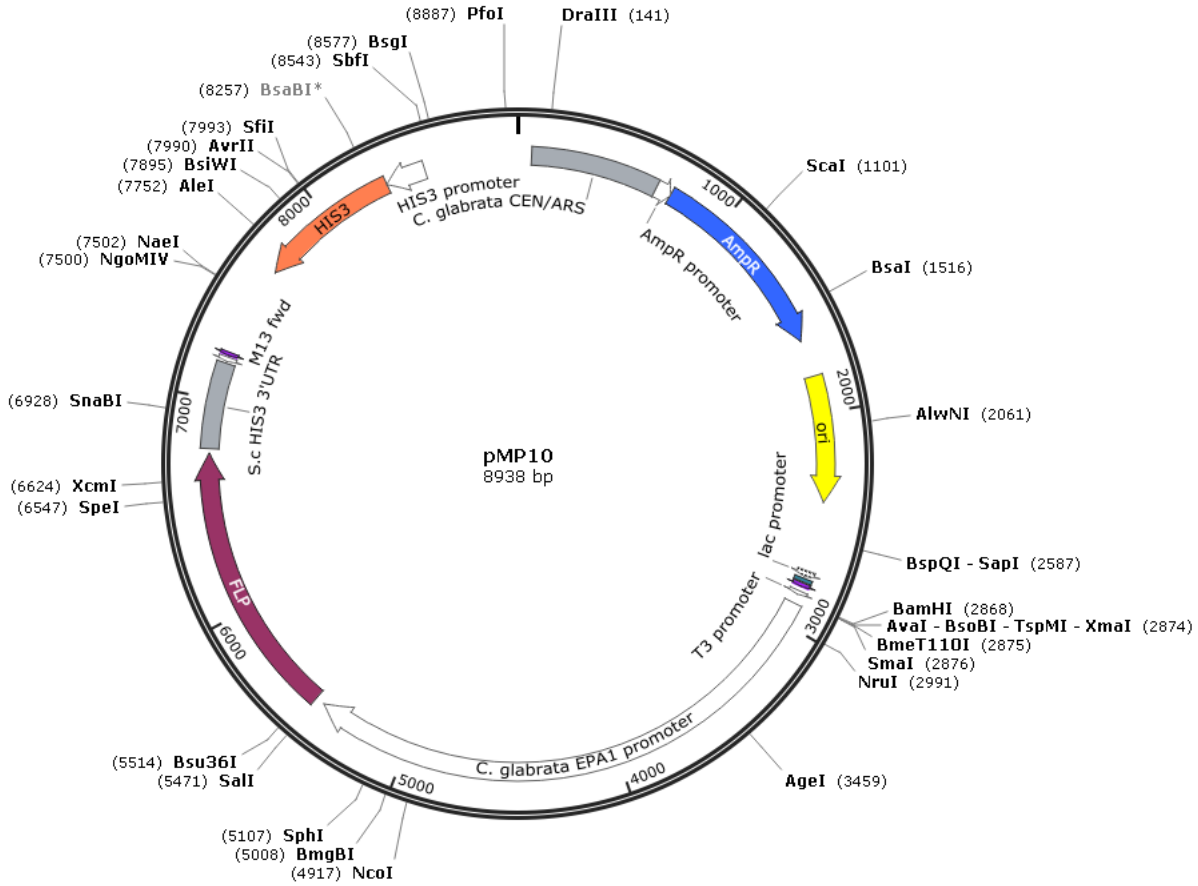


Figure 3.3: Plasmid map of pMP10. This plasmid was used to remove the NAT disruption cassette using the FLP (flippase) gene, allowing recycling of this selectable marker. Commonly used restriction enzymes are shown. Features of this plasmid denote: AmpR - β-lactamase gene (ampicillin resistance), ori - Origin of replication, FLP – Flippase gene (flanked by the *C. glabrata* EPA1 promoter and *S. cerevisiae* HIS3 3'UTR), HIS3 – HIS3 selectable marker, CEN/ARS – *C. glabrata* centromere and autonomously replicating sequence.

3.6 Protein expression

3.6.1 Protein extraction from yeast

Protein extractions were performed as described by Kushnirov, 2000 and here briefly **[132]**. Yeast cells were harvested by centrifugation from liquid cultures after the appropriate treatment. The cells were re-suspended in 100 µl of PCR grade water, 100 µl 0.2 M NaOH was added and then incubated for 5 minutes at room temperature. The cells were pelleted, the supernatant discarded and re-suspended in 50 µl SDS sample buffer (0.06 M Tris-HCl, pH 6.8, 5 % glycerol, 2 % SDS, 4 % β mercaptoethanol, 0.0025 % bromophenol blue). The samples were boiled for 3 minutes and centrifuged briefly to separate the cell debris from protein, before loading.

3.6.2 Western blot analysis

The same volume (20ul) from each sample was loaded in to a SDS-PAGE gel. Pre-cast 4-12 % NuPAGE Bis-Tris Gels were purchased from Invitrogen. These gels were set up in an XCell SureLock Mini Cell electrophoresis tank from Invitrogen, as per the manufacturer's instructions. The samples were loaded and the gel run at 150V in running buffer (25 mM Tris, 192 mM glycine, 0.1 % SDS) until the protein samples have reached the bottom of the gel. The protein marker, ColorPlus PreStained Protein Marker (BioLabs) with bands ranging between 7-175 kDa, was used. Correct loading of similar amounts of protein in the gel was checked by UV exposure using a G Box imager from Syngene (Figure 10.1, p.218, Appendix I).

The PVDF membrane (Invitrogen) was activated by wetting in 100 % methanol for 30 seconds, rinsing in water and equilibrating in transfer buffer (25 mM Tris, 192 mM glycine, 10 % methanol) before being assembled into the XCell II Blot Module (Invitrogen) as per the manufacturer's instructions. The inner chamber was filled with transfer buffer to just cover the blot, while the outer chamber was filled with cold water and ran for 2 hours at 30V.

The blot module was disassembled and the membrane rinsed in PBS. The membrane was blocked in PBS-Tween (PBS, 1 % Tween20) with 2.5 % dried milk powder for 30 minutes at room temperature with shaking. The primary antibody Prdx3 (Dr. Janet Quinn) was mixed in 10 ml PBS-T with 2.5% dried milk powder (1:5000) overnight at 4°C with shaking. The next day, the membrane was washed five times, each for 5 minutes with PBS-T. The secondary antibody, anti-rabbit-IgG was added (1:5000) to the membrane in 10 ml PBS-T with 2.5%

dried milk powder for 1 hour at room temperature with shaking. The membrane was then washed three times for 5 minutes each in PBS-T and incubated for 1 minute at room temperature in the dark with ECL (Amersham). The membrane was developed using a G Box imager from Syngene.

3.7 Yeast growth assays

3.7.1 Liquid growth assays

Yeast cells were cultured in liquid YPD media incubated overnight at 30°C. YPD media with the indicated stress (1.1 x) was dispensed into a 96 well plate (90 µl) and 10 µl of cells were added to a final OD_{600} of 0.1. Growth assays were performed using a plate reader (VersaMax Microplate Reader, Molecular Devices) with shaking and measurements taken at 595 nm every 30 minutes over a 24 or 48 hour timecourse. Results were visualised in Excel.

3.7.2 Solid growth assays

Solid plate assays were performed using YPD agar (un-buffered), with the indicated stress and incubated at 30°C, unless stated, for 1 or 2 days, then photographed. Strains were cultured overnight in YPD at 30°C in 96-well plates. Cells were diluted in autoclaved water and pinned on to solid agar plates using a RoToR pinning robot from Singer Instruments. Each strain was pinned 4 times in a square formation with 2 technical replicates and 2 or 3 biological replicates for each condition.

The conditions screened in this study are shown in Table 3.6, along with the functional implications of each phenotype. The osmolarities shown in Table 4.2 were calculated using the equation below:

$$\text{mOsmol/L} = \gamma \times n \times C$$

where:

γ is the osmotic coefficient, its degree of disassociation, between 0 and 1, where 1 is 100% disassociation **[133]**.

n is the number of ions the compound disassociates into. E.g. NaCl has *n* of 2.

C is the concentration in mM.

Table 3.6: Conditions screened in this study.

Phenotype	Condition	Functional implication	Reference
Slow growth	YPD 30°C	General growth defect	**[55]**
Heat sensitivity	YPD 42°C	General growth defect; altered plasma membrane; involved in protein folding	**[134]**
Sensitivity to heat shock	YPD 55°C for 1 hour then 30°C for 2 days	Defects in RAS-adenlyate cyclase signal transduction pathway	**[55]**
Cold sensitivity	YPD 16°C	General protein defect	
Respiratory activity	Tetrazolium overlay	Defects in mitochondrial function	**[135]**
Osmotic sensitivity	1M sorbitol	Cell wall or cytoskeletal defects	**[55]**
	YPD + 3 % glycerol		
Cation sensitivity	0.5 M NaCl, 1 M NaCl and 1.5 M NaCl	Plasma membrane defects	This study
	200 mM KCL		
	80 mM LiCl		
Divalent cation and heavy metal sensitivity	100 µM $FeCl_2$	Altered expression of plasma membrane ATPases; defects in many other biological processes depending on cation or heavy metal used	**[55]**, This study
	250 mM CsCl		
	2 mM $CoCl_2$		
	50 mM $BaCl_2$		
	8 mM $ZnCl_2$		
	0.5 M $CaCl_2$		
	10 mM $CuCl_2$		
	2 mM $CdCl_2$		
	5 mM $MnCl_2$		
	0.6 M $MgCl_2$		
Oxidative stress	2 mM H_2O_2 and 5 mM H_2O_2	Defects in the de-toxifcation of reactive oxygen species	This study
	5 mM tBOOH		
	100 µM menadione		
Combinatorial	0.5 M NaCl + 2 mM H_2O_2	Defects in the response to oxidative and hyperosmotic/cation stress	This study
Heat and oxidative stress	42°C + 2 mM H_2O_2	Defects in heat sensitivity and oxidative stress	This study
Ethanol sensitivity	6 % ethanol	General protein defect	**[55]**
Caffeine	10 mM and 20 mM caffeine	Defective MAP kinase signalling pathways; other defects	
Cyclohexamide	1 µg/ml cyclohexamide	Cell cycle protein synthesis	
Caspofungin	100 ng/ml and 25	Cell wall; β-glucan synthase inhibitor	

	ng/ml caspofungin		
SDS	0.05% SDS and 0.1% SDS	Cell wall defects	**[55]**
Nystatin	5 µg/ml Nystatin	Defective sterol biosynthesis	**[55]**
Calcofluor white	1 mg/ml calcofluor white	Cell wall; chitin and B-glucan synthase defect. Mutants with lowered cell wall chitin levels are more resistant.	**[136]**
Congo red	250 µg/ml congo red	Cell wall; chitin and B-glucan synthase defect. Mutants with lowered cell wall chitin levels are more resistant.	**[136]**
Benomyl	0.5 µg/ml benomyl	Defective microtubule function	**[55]**
Arsenic	1 mM arsenic oxide	Sensitivity reveals defects in folding of actin, tubulin, and other proteins. Resistance reveals impaired ribosome function.	**[137]**
Vanadate	2.5 mM sodium vanadate	Resistance shows defective protein glycosylation and secretory defects; sensitivity reveals vacuolar defects	**[55, 138]**
Hygromycin	50 µg/ul hygromycin	Antibiotic resistance	
UV light	10 joules and 15 joules UV	Defective repair of UV-induced DNA damage	**[55]**
No ammonium sulphate	SC with no ammonium sulphate	Low nitrogen	This study
Maltose fermentation	YPM + bromocresol purple	Defective in carbon catabolite repression	**[55]**
YP + carbon source; anaerobic conditions for increased stringency	2% ethanol	Utilisation of alternative carbon source	This study
	2% lactose		
	2 % starch		
	2% xylose		
	2% sodium acetate		
	2% sorbitol		
	2% maltose		
	2% galactose	Defective in transcriptional activation	**[55]**
	2% raffinose	Defective in carbon catabolite repression; mutants generally defective in transcriptional regulation	**[55]**
	2% glycerol	Failure to produce respiratory competent mitochondria	**[55]**
	2% sucrose	Defective in carbon catabolite repression; mutants generally defective in transcriptional regulation	**[55]**

3.8 Transcription profiling

All microarray experiments conducted in this study are shown in Table 3.7. All samples, treated and untreated, for each time point were compared on each array against an untreated time zero reference sample. Reference samples were made in a large batch to provide enough material to be pooled across all stress conditions and replicates.

Table 3.7: Microarray experiments conducted in this study.

Stress condition	Treatment	Time points (minutes)	*C. glabrata* strain	Number of Replicates
Untreated	YPD	All of the below	ATCC 2001[1]	1 – 3
Hyperosmotic	0.1 M, 0.5 M and 2 M NaCl	15, 30, 60, 90, 120, 150 180, 240	ATCC 2001[1]	1
	0.5 M NaCl	5, 15, 30, 45, 60, 120	ATCC 2001[1]	3
Oxidative	1 mM, 10 mM, 100 mM H_2O_2	15, 60	ATCC 2001[1]	3
Combinatorial	0.5 M NaCl + 1 mM H_2O_2	15, 60	ATCC 2001[1]	3
Untreated	YPD	15	ATCC 2001 *Δhis3, Δtrp1,Δyap1*::*HIS3*[2]	2
Oxidative	1 mM H_2O_2	15	ATCC 2001 *Δhis3, Δtrp1,Δyap1*::*HIS3*[2]	3

1. American Type Culture Collection. 2. Newcastle University, UK.

3.8.1 Cell harvesting

Cells were cultured in liquid media using YPD buffered with Tris HCL to pH 7.4, either with or without the indicated stress at 30°C. Growth kinetics were obtained by measuring absorbance at OD_{600} over the timecourse. The pH of the media did not change over the time course (data not shown).

From an overnight culture, *C. glabrata* ATCC 2001 was inoculated to an OD_{600} of 0.2 and the culture was then incubated at 30°C, 180 rpm for 3.5 - 4 hours. 62.5 ml of OD_{600} = 0.8 cells were added to each of the flasks containing 187.5 ml of pre-warmed stress media containing the appropriate stress and incubated at 30°C, 180 rpm. At each time-point the following steps were conducted: 50 ml aliquots were taken from each stress media and the cells pelleted, washed with cold, sterile PBS, transferred to 2 ml screw cap tubes and snap frozen in liquid nitrogen then stored at -80°C.

3.8.2 Extraction of RNA from yeast cells

RNA was extracted using RNeasy Mini Kit with an on column DNase1 treatment (both Qiagen), as per the manufacturer's instructions from either fresh yeast cells, or those previously snap frozen in liquid nitrogen and stored at -80°C.

3.8.3 cDNA synthesis and labelling

Using the extracted RNA, cDNA was synthesized and fluorescently labelled using ChipShot Direct Labelling and Clean-Up System (Promega). The protocol was followed as per the manufacturer's instructions using 10 µg of total RNA. Treated samples were labelled with Cy5 dye, which fluoresces red and reference samples were labelled with Cy3 dye, which fluoresces green (both GE Healthcare). RNA for the reference samples were pooled together before use in the labelling reactions. The labelled samples were stored in an opaque box to protect from light at -80°C. cDNA samples were quantified using a NanoDrop1000 spectrophotometer to measure the concentration of cDNA and dye incorporation. If the concentration or dye incorporation was found to be too low, the samples were not used. The same amount (825 ng/µl) of each treated and reference sample was mixed in a new 1.5 ml microcentrifuge tube, vacuum concentrated and re-suspended in the same volume of PCR grade water (41.8 µl). Hybridization buffer was added to the samples, vortexed and centrifuged briefly. Samples were added to the microarray slides as per the manufacturer's instructions (Agilent).

3.8.4 Hybridisation, washing and scanning

Hybridisation and washing of the samples on our custom microarray slides was performed as per the manufacturer's instructions. The custom microarray slides used are available from Agilent Technologies. These microarray slides were produced using published sequence data from Génolevures **[25]**. Each microarray slide comprised 4 arrays, with each array containing 4 60-mer oligonucleotide probes for each of the 5210 genes.

Each microarray slide was scanned using a GenePix4000B semi-confocal scanner and features extracted using GenePix Pro 4.0, both available from MDS Analytical Technologies. Pixel size was set at 10 µm and lines to average at 2. The photomultiplier tube (PMT) voltage for each channel (532 nm, Cy3 and 635 nm, Cy5) was adjusted to give an overall intensity ratio across each slide of 1. Arrays were quality controlled for properties including: signal-to-background ratio, feature variation, background variation, flagged features, features with saturated pixels and where the PMT threshold had been exceeded. Any array which failed a

number of these criteria was removed from the data set. Feature extraction was performed semi-automatically by GenePix Pro 4.0. Data sets were exported as GenePix Results (GPR) files.

3.8.5 Microarray analysis

GPR files were loaded into GeneSpring GX11 (Aglient Technologies), a statistical program for the visualization and analysis of expression data. Data was normalised in GeneSpring by LOWESS (**LO**cally **WE**ighted **S**catter plot **S**moothing) originally proposed by Cleveland, 1979 **[139]**. The *C. glabrata* genome was annotated with *S. cerevisiae* gene names, functions and GO terms using annotation from Génolevures (http://www.genolevures.org/) and SGD (http://www.yeastgenome.org/) **[45, 140]**. GO term analysis was performed in GeneSpring with a cut off p value of 0.05. ANOVA statistical analysis was conducted to find significantly regulated transcripts with a p value <0.05 and fold change compared to untreated of >2. Venn Diagrams were constructed in GeneSpring. Hierarchical clustering was performed using Cluster3 (http://bonsai.hgc.jp/~mdehoon/software/cluster/) and visualised in TreeView (http://jtreeview.sourceforge.net/) **[141, 142]**. All regulated transcripts are denoted using orange (up) and blue (down) in figures for those who are colour blind.

All normalised microarray data can be found on the CD attached with this thesis.

4 Hyperosmotic stress adaptation in *C. glabrata*

4.1 Chapter overview

Hyperosmotic stress refers to an increase in osmotic pressure encountered by a cell. This stress response has been extensively studied and mathematically modelled in the model organism, *S. cerevisiae* and as such presented a starting point in the study of hyperosmotic stress response in *C. glabrata*. Hyperosmotic stress is encountered by a pathogen in the phagosome of cells of the immune system, hence it is an important stress response to study and characterise in the fungal pathogen of humans, *C. glabrata*. Inside the phagosome, hyperosmotic stress has been shown to occur by an increase in the concentration of KCl and a dramatic change in pH **[63, 64]**. This goes to partly explain why we use NaCl instead of sorbitol to impart hyperosmotic stress. While sorbitol elicits an osmotic stress, with no ionic component, it can also be used as an alternative carbon source by many organisms, *C. glabrata* included. Therefore, the metabolism of the organism may be affected when responding to hyperosmotic stress using sorbitol; it has been shown that sorbitol regulates a distinct set of genes from that regulated by NaCl at the same osmolarity in *S. cerevisiae* **[143]**. High concentrations are needed to inhibit growth of *C. glabrata* cells as they are so stress resistant but when adding high concentrations of sorbitol to culture media, the viscosity increases and effective experimentation with it becomes impossible (this study, data not shown). NaCl has been used in many studies of hyperosmotic stress response of *S. cerevisiae* **[1, 66, 143, 144]**. In order to compare the response of *C. glabrata* to that of other fungi, the use of NaCl allows comparisons to be made while retaining a cationic environment, similar to that observed in the phagosome, as well as a hyperosmotic stress.

Genes involved in the hyperosmotic stress pathway, referred to as HOG pathway components (Table 4.1) were identified from previously published data described in the introduction. Null mutants of these genes were created using the NAT disruption cassette conferring resistance to Nourseothricin and potential mutants were confirmed by PCR and Southern blot (Chapter 3). The hyperosmotic stress resistance of HOG pathway component mutants were first tested to increasing concentrations of hyperosmotic stress, on solid and in liquid media. To uncover functionality, these null mutants were subsequently screened on over 60 conditions including: temperature; hyperosmotic stress; oxidative stress; alternate carbon sources; cell wall stressors; metal stress and drugs. The phenotypes displayed by these mutants, as well as their

functional implications will be discussed in this chapter. Genes found to be regulated transcriptionally in response to hyperosmotic stress (presented in this study) were also targeted for gene deletion and phenotypically screened using the same methods.

Table 4.1: HOG pathway components considered in this study

***S. cerevisiae* Standard Name**	***C. glabrata* Systematic Name**	**Null mutant available?**
MSB2	*CAGL0F08833g*	This study
OPY2	*CAGL0D01276g*	This study
SHO1	*CAGL0G03597g*	Mutant library, this study
SLN1	*CAGL0H06567g*	This study
YPD1	*CAGL0K04961g*	This study
STE20	*CAGL0K02673g*	Mutant library
	CAGL0M10153g	This study
STE11	*CAGL0B02739g*	Lab strain
STE50	*CAGL0B00858g*	Mutant library
PBS2	*CAGL0L05632g*	This study
HOG1	*CAGL0M11748g*	Lab strain
SSK1	*CAGL0D02882g*	Not available
CDC42	*CAGL0F05269g*	Essential in *S. cerevisiae*

C. glabrata homologues were identified using Génolevures **[25]**.

Three concentrations of sodium chloride were established that had varying effects on cell growth of wild type *C. glabrata* cells: 0.1, 0.5 and 2 M NaCl (taken from **[71]**). Figure 4.1 shows the cell density over time under these defined doses of hyperosmotic stress. Little effect or even slightly better growth than untreated cells is observed in *C. glabrata* cells treated with 0.1 M NaCl. While 2 M NaCl treated cells do not show an increase in cell density over the time course, treatment with 0.5 M NaCl results in growth inhibition but cell density does increase over time.

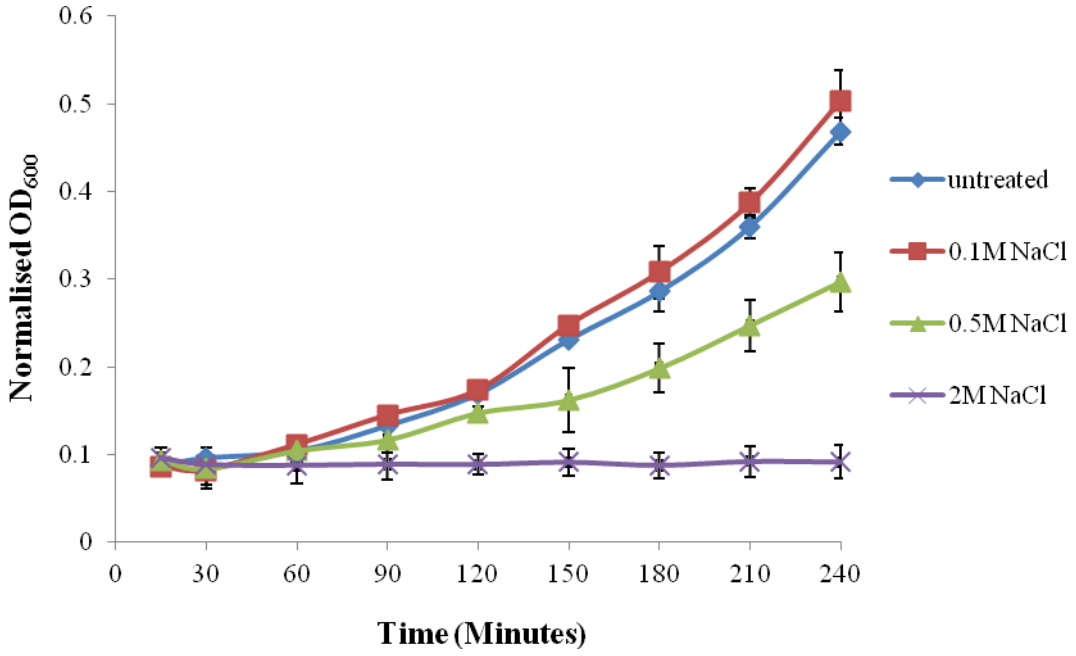


Figure 4.1: Hyperosmotic stress inhibits growth of *C. glabrata*. Wild type (CG2001) *C. glabrata* cells were grown in either untreated YPD or YPD containing the indicated concentration of NaCl and OD_{600} measurements were taken at each point up to 4 hours. OD_{600} measurements were normalised by subtracting the background absorbance of blank media. Error bars for the standard deviation of three biological replicates are shown.

Initially, in collaboration with Dr. Andrew McDonagh, the first set of microarray experiments were conducted under these three concentrations of hyperosmotic stress with 8 time points over a four hour time course and one replicate. These transcript profiling experiments under hyperosmotic stress were conducted using *C. glabrata* custom made DNA microarrays from Agilent Technologies (as described in Chapter 3). The generated microarray data provided initial insights into the hyperosmotic stress response of *C. glabrata* to a range of different osmolarities. This was the first look at the transcriptional regulation of *C. glabrata* to our defined doses of hyperosmotic stress and proved to be useful in guiding our subsequent microarray analyses. As this dataset only has one replicate, little statistical analysis was possible and as such, the results here should be viewed with caution and were used only as an indication. The number of genes regulated by more than 2 fold compared to untreated

samples at 15 minutes were calculated and the overlap between different concentrations of hyperosmotic stress are shown in Figure 4.2.

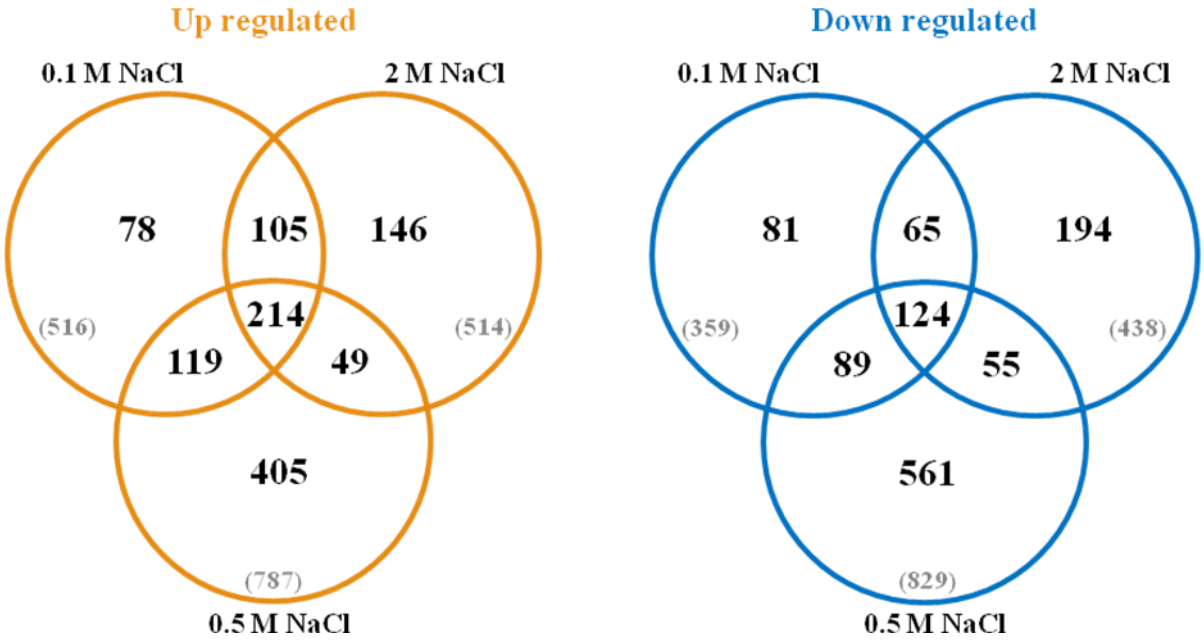


Figure 4.2: Venn diagram showing the number of genes regulated, compared to untreated, under different concentrations of hyperosmotic stress in *C. glabrata* after 15 minutes treatment. Genes regulated by more than 2 fold compared to untreated are shown: up regulated genes on the left; down regulated genes on the right. The total number of genes regulated under each stress condition is shown in brackets.

The Venn diagrams in Figure 4.2 show that while there are a number of genes regulated by all of the hyperosmotic stress conditions studied in *C. glabrata* (214 up regulated and 124 down regulated), there are also many genes regulated uniquely by each concentration of NaCl. Treatment with 0.5 M NaCl shows the highest number of total genes regulated under any stress condition and also has the highest number of uniquely regulated genes. GO term analysis performed on these genes showed that although many genes are shared between all hyperosmotic stress conditions, the up regulated genes are not associated with stress response, instead being involved in the regulation of endocytosis, as well as vacuolar and lysosomal transport, proteolytic protein processing and the assembly of protein complexes. Genes up regulated uniquely by 0.5 M NaCl treatment are associated with the response to stress, including osmotic and oxidative stress genes.

It was found that homologues of *S. cerevisiae* genes involved in hyperosmotic stress were regulated under our medium stress condition (0.5 M NaCl) and therefore this concentration of hyperosmotic stress was taken forward for further analysis. Microarray analysis using 0.5 M NaCl with 6 time points taken over a two hour timecourse and three biological replicates was conducted and all other transcriptional analysis presented in this study uses this more

expansive dataset, unless expressly stated. The time points were chosen as these were used in the prominent Gasch *et al*, 2000, paper on the transcriptional regulation of *S. cerevisiae* to a diverse range of environmental stresses **[1]**. By conducting our microarray experiments in this way, it has allowed the transcriptional response to hyperosmotic stress to be compared between these two closely related yeast. Unless otherwise stated, all microarray analysis was conducted using GeneSpring software (Agilent.com).

The transcript profiling and functional genomics analysis presented in this study have allowed the creation of an updated model of the HOG pathway in *C. glabrata* and the identification of new components involved in the hyperosmotic stress response.

4.2 Results

4.2.1 Removal of Hog1 or other signalling components of the HOG pathway results in hyperosmotic stress sensitivity

Homologous genes known to be involved in the HOG pathway in *S. cerevisiae* were identified in *C. glabrata* using sequence comparisons and their syntenic context **[25, 29]**. Null mutants of these HOG pathway components were constructed and tested for sensitivity to varying concentrations of hyperosmotic stress using NaCl. Detailed methods can be found in Chapter 3. *sln1* and *ypd1* null mutants made in the ATCC 2001 background show no sensitivity to hyperosmotic stress (Figure 11.1, p.220, Appendix II). Ste20, one of the MAPKKKKs in the HOG pathway has a paralogue in *C. glabrata* by sequence comparison and as such these genes, *CAGL0K02673g* and *CAGL0M10153g* are named *STE20α* and *STE20β*. Phenotypic screening of null mutants shows that s*te20β* is not sensitive to hyperosmotic stress nor shows a phenotype on any other condition screened, while *ste20α* shows sensitivity to a wide range of compounds. All HOG pathway components tested, except *msb2*, show varying degrees of sensitivity to increasing concentrations of hyperosmotic stress (Figure 4.3).

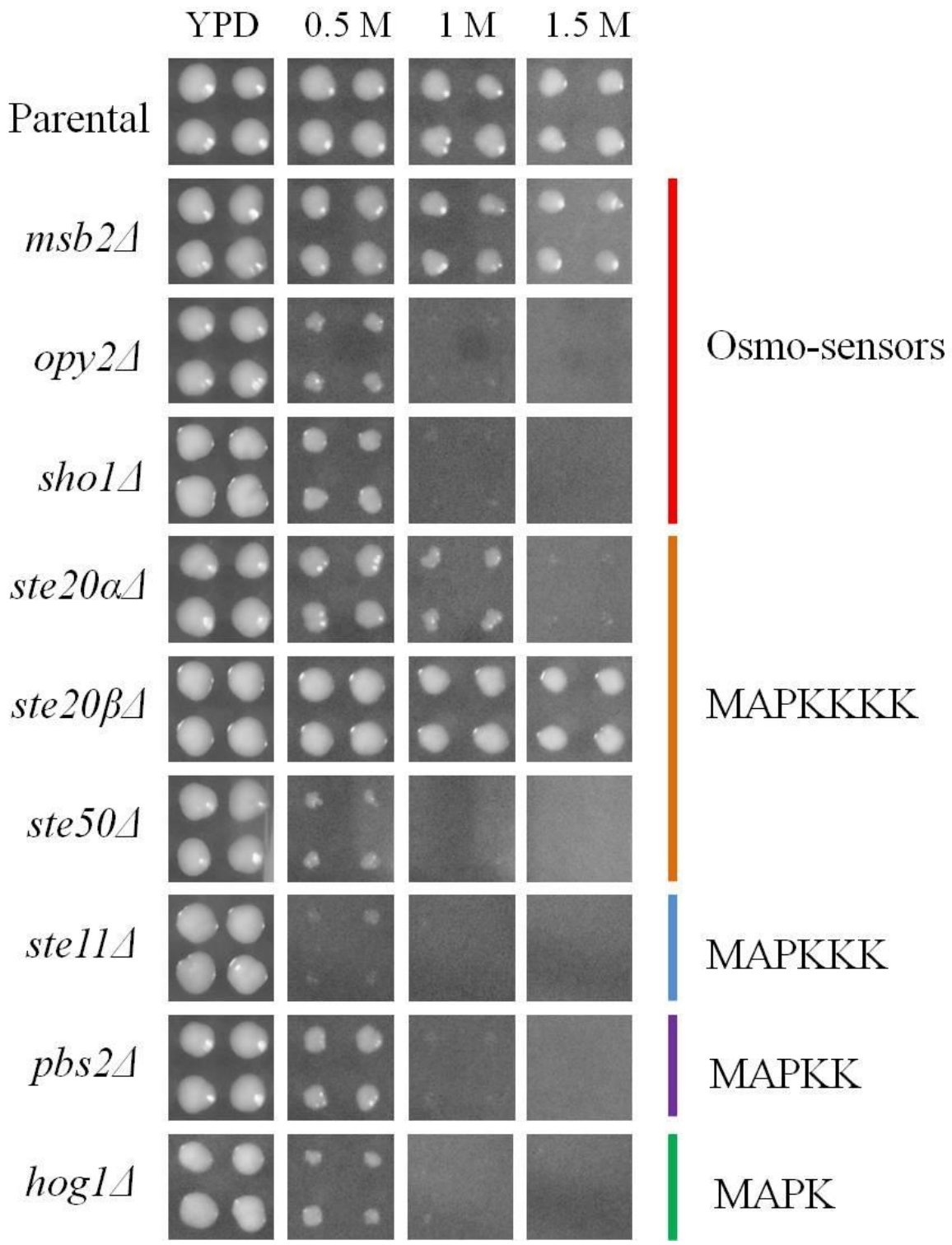


Figure 4.3: Sensitivity of HOG pathway component null mutants under increasing concentration of sodium chloride. Each mutant strain was spotted four times in a square on to media containing the indicated concentration of stress. Pictures were taken after 2 or 3 days.

4.2.2 Phenotypic screening of HOG pathway mutants reveal other functions

HOG pathway null mutants were screened on over 50 phenotypic conditions (see Table 3.6 in Chapter 3). Table 4.2 summarises the growth phenotypes of null mutants of the HOG signalling pathway. Osmolarities were calculated and each phenotype is arranged by increasing osmolarity, left to right.

HOG pathway mutants are viable and display a similar growth phenotype to the parental strain in rich media at 30°C. *ste20α* and *ste50* were sensitive to both 16°C and 42°C, while *opy2* is also sensitive to 42°C. *sho1*, a second osmosensor of the HOG pathway as well as *hog1* show no sensitivity to thermo variance. However, both *hog1* and *sho1* are sensitive to an elevated temperature of 42°C in combination with oxidative stress. While *ste11* is sensitive to 16°C and 42°C with oxidative stress, it was not observed to be sensitive to 42°C alone. It is important to note that no HOG pathway mutants are sensitive to oxidative stress elicited on its own (Figure 11.2, p.221, Appendix II).

The HOG pathway mutants display increasing inhibition of growth to increasing concentrations of hyperosmotic stress elicited by cationic stress (NaCl and KCL). Mutants that are sensitive to 0.5 M NaCl (all except *msb2* and *ste20α*) are also sensitive to 1 M sorbitol which elicits hyperosmotic stress, at a similar osmolarity, without also being a cationic stress.

The HOG pathway components were also observed to display a phenotype of sensitivity to divalent cations and heavy metals. Interestingly, while *msb2* mutants are not sensitive to hyperosmotic stress up to even 1.5 M NaCl, *msb2* cells did show sensitivity to divalent cations and heavy metals which were used at much lower osmolarities. *msb2* was also sensitive to the cell wall stressors, SDS and calcofluor white. Other HOG pathway components were also sensitive to divalent cations and heavy metals where varying degrees of sensitivity were observed on media containing $CoCl_2$, $MnCl_2$, CsCl, $CaCl_2$ and $MgCl_2$. It is of interest to note that *hog1* cells were not sensitive to $CdCl_2$, $ZnCl_2$ and $BaCl_2$ containing media, while other HOG pathway components were.

All HOG pathway components are also sensitive to the cell wall stressors SDS and calcofluor white, with the exception of *pbs2* cells to calcofluor white. There are nine conditions in which *hog1* mutants are more sensitive than *pbs2* mutants; 42°C in combination with oxidative stress, sorbitol, $CoCl_2$, $MnCl_2$, $CdCl_2$, caffeine, SDS, calcofluor white and cyclohexamide.

Table 4.2: Summary of phenotypes of HOG pathway mutants

Phenotype	Control	Temperature		Oxidative + temperature	Oxidative stress	Cationic stress				Osmotic stress	Divalent cations and heavy metals								Cell wall defects			Carbon source	MAPK pathway	Cell cycle protein synthesis	Protein glycosylation	Sterol biosynthesis	Protein folding defects	
Osmolarity (mOsm/L)	-	-	-	-	-	368	930	1860	2790	980	-	-	-	-	159	455	1290	1602	-	-	-	386	-	-	-	-	-	-
Condition	30° C	16° C	42° C	42° C + 2 mM H_2O_2	2 mM H_2O_2	200 mM KCL	0.5 M NaCl	1 M NaCl	1.5 M NaCl	1 M sorbitol	1 mM $CoCl_2$	2 mM $CdCl_2$	8 mM $ZnCl_2$	30 mM $MnCl_2$	50 mM $BaCl_2$	250 mM CsCl	0.5 M $CaCl_2$	0.6 M $MgCl_2$	0.05% SDS	1% SDS	1 mg/ml Calcofluor white	2% NaAc	20 mM caffeine	1 µg/ml cyclohexamide	2.5 mM vanadate	5 µg/ml Nystatin	1 mM Arsenic oxide	0.5 mM meta-arsenite
Strain																												
Parental	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
msb2	N	N	N	N	N	N	N	N	N	N	S	N	S	S	N	S	S	S*	S	L	L	N	N	N	S	N	N	N
opy2	N	N	S	N	N	S	S	L	L	S	L	S	L	L	S	L	L	S	S	L	L	S	N	S	L	N	L	L
sho1	N	N	N	S	N	S	S	L	L	S	L	S	S	S	S	S	L	S	L	L	L	N	N	S	L	N	S	L
ste20α	N	S	S	N	N	N	N	S	L	N	L	N	S	L	N	S	L	S*	L	L	L	S	N	N	L	S	L	L
ste50	N	S	S	N	N	S	S	L	L	S	L	S	S	L	S	L	L	L	L	L	L	S	N	L	L	S	L	L
ste11	N	S	N	S	N	S	S	L	L	S	L	L	S	L	S	L	L	L	L	L	L	S	N	L	L	N	L	L
pbs2	N	N	N	N	N	N	S	L	L	S	S	N	N	S	N	S	L	S	S	L	N	N	N	N	S	N	L	L
hog1	N	N	N	S	N	N	S	L	L	L	L	S	N	L	N	S	L	S	L	L	S	N	S	S	S	N	L	L

Labels denote: N: normal growth (grey); S*: slight growth defect (light orange); S: sensitive (orange); L: lethal (dark pink). Growth defect of 1 or 2 strain isolates and 2 or 3 biological replicates. Osmolarity was calculated as described in the Methods and the values shown are additional to the YPD media. A hyphen (-) denotes no/negligible additional osmolarity.

The deletions showing the most inhibitory phenotypes were *ste50* and *ste11* and these were observed to be more sensitive than *hog1* to many conditions: 16°C, 42°C, KCl, $CdCl_2$, $ZnCl_2$, $BaCl_2$, CsCl, $MgCl_2$, calcofluor white, NaAc, cyclohexamide and Nystatin.

opy2, *sho1*, *ste50*, *ste11* and *hog1* all showed growth inhibition to cyclohexamide which is an inhibitor of protein biosynthesis. HOG pathway mutants were also sensitive to arsenic compounds and sodium vanadate. *ste20α* and *ste50* are the only null mutants of the HOG pathway to show sensitivity to Nystatin.

4.2.3 ATCC 2001 vs. BG2 – null mutants have different phenotypes

ATCC 2001 is the sequenced strain and as previously described has a point mutation in *SSK2* which encodes the MAPKKK Ssk2, causing the Sln1 branch in which it resides to be non-functioning in this strain. BG2 is a widely used lab strain, originally isolated as a clinical isolate, which has been shown not to carry this mutation in Ssk2 and therefore have both signalling branches of the HOG pathway functioning **[70]**. Figure 4.4 shows that the type strain, ATCC 2001, displays no observed difference in hyperosmotic stress tolerance compared to BG2, even though one of the signalling branches is non functional.

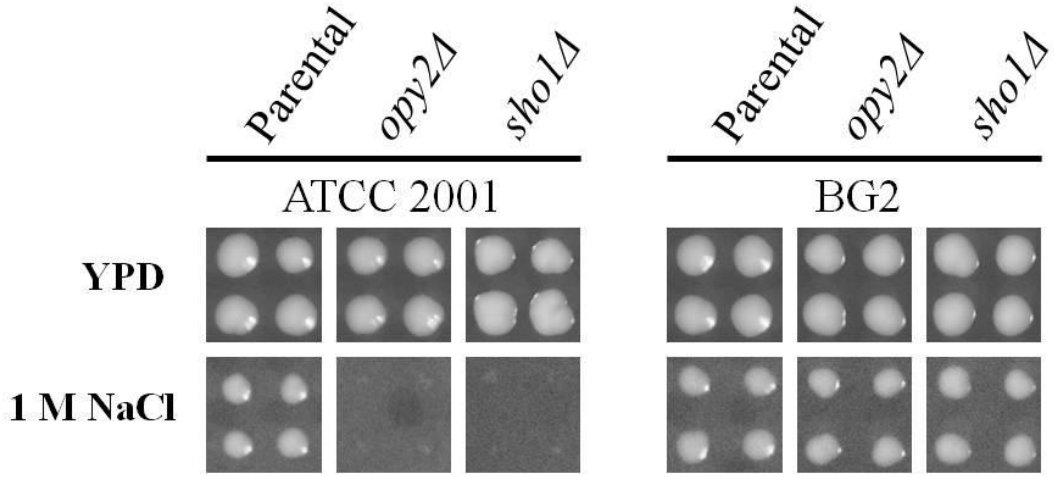


Figure 4.4: Hyperosmotic stress sensitivities of *opy2* and *sho1* in different *C. glabrata* strains. Each mutant strain was spotted four times in a square on to media containing the indicated concentration of stress. Pictures were taken after 2 or 3 days.

HOG pathway mutants made in the *C. glabrata* strain BG2 do not display the same phenotypes as the same null mutants created in an ATCC 2001 background. Figure 4.4 shows that *sho1* and *opy2* are sensitive to hyperosmotic stress in an ATCC 2001 background but not a BG2 background. Null mutants of *ypd1* and *sln1* were created in ATCC 2001 and *ypd1* in a BG2 background. Construction of a *sln1* strain in a BG2 background proved unsuccessful. It is important to note that that *sln1* and *ypd1* strains created in *S. cerevisiae* are inviable. *sln1*

and *ypd1* strains made in *C. glabrata* were not sensitive to hyperosmotic stress (Figure 11.1, p.220, Appendix II). Phenotypic screening of these mutants on over 50 conditions also showed no other sensitivities, except that of *sln1* in an ATCC 2001 background which shows a slight inhibition of growth on 1 % SDS (Figure 11.1, p.220, Appendix II).

ATCC 2001 and BG2 parental strains show different phenotypes on a number of different conditions. These include BG2 being resistant to 42°C, LiCl, SDS, $CoCl_2$, arsenic oxide and metarsenite, as well as sensitivity to CsCl and $MnCl_2$, as shown in Figure 4.5. These phenotypes are also apparent in all mutants made in the BG2 background.

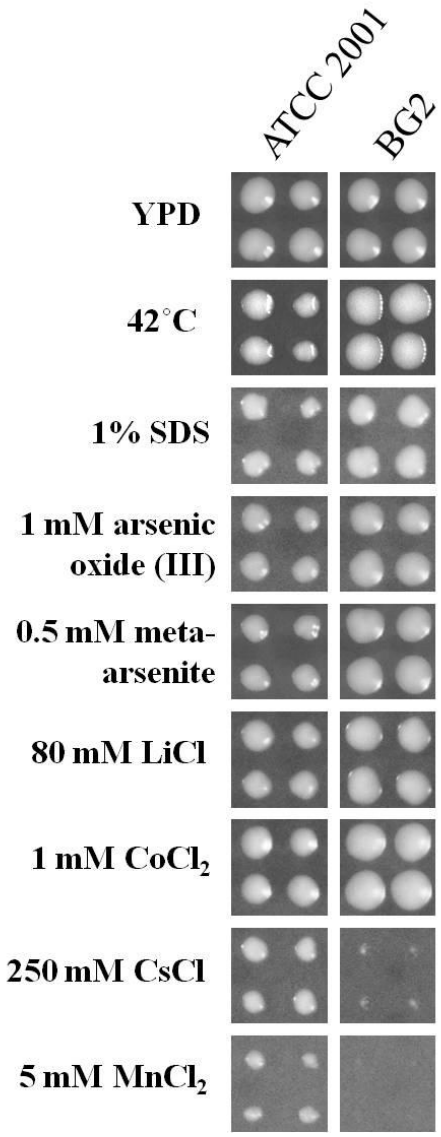


Figure 4.5: Differences in phenotypes of the *C. glabrata* strains, ATCC 2001 and BG2. Each strain was spotted four times in a square on to media containing the indicated concentration of stress. Pictures were taken after 2 or 3 days.

4.2.4 The immediate transcriptional response – 0.5 M NaCl, 15 minutes

Using microarray data with three replicates from cells treated with 0.5 M NaCl for 15 minutes, statistical analysis using 2-way ANOVA was applied. This found that approximately 21 % (1101 genes) of the total *C. glabrata* genome was significantly regulated under hyperosmotic stress conditions (>2 fold compared to untreated cells at 15 minutes, p-value <0.05) of which 486 were up regulated and 615 down regulated.

GO term enrichment analysis using FunSpec (http://funspec.med.utoronto.ca/) (p-value <0.01), using gene ontology inferred from homology with *S. cerevisiae*, was conducted on the significantly up (Table 4.3) and down (Table 4.4) regulated genes **[145]**. GO terms associated with these up regulated genes include those involved in the response to stress, as well as heat, osmotic and oxidative stress. Genes involved in trehalose and proline synthesis are also up regulated, along with the pentose-phosphate pathway.

GO terms associated with these down regulated genes include ribosome biogenesis and the biosynthesis of cellular components, as well as GO terms for genes involved in RNA polymerase I complex and rRNA processing. Null mutants of many genes down regulated under hyperosmotic stress conditions were also found to have slow growth phenotypes in *S. cerevisiae*.

Some transcripts were found to be highly induced in response to hyperosmotic stress by *C. glabrata*, indicating their importance in this stress response. The top 20 genes up regulated under hyperosmotic stress treatment in *C. glabrata* as well as their fold change compared to untreated cells are shown in Table 4.5, along with their homologue and its description in *S. cerevisiae*.

Table 4.3: GO terms associated with up regulated genes under hyperosmotic stress (0.5 M NaCl, 15 minutes).

GO Term	p-Value	Genes	Number of Genes Regulated	Number of Genes in GO Term
Trehalose biosynthetic process	2.26 e^{-5}	*TPS1 TPS2 UGP1 PGM2 TPS3*	5	7
Oxidative stress response	4.06 e^{-5}	*FRT2 UGA2 PST2 CTA1 GRX2 BLM10 HSP12 TRX2 MCR1 FMP46 GAD1 GRE2 GLR1*	13	55
Osmosensing and response	5.60 e^{-5}	*PTC3 STE50 PTC1 DOA4 PTP3 HSP12 SLT2 SIS2 CIN5 GCY1*	10	35
Response to stress	0.000131	*FRT2 SSA3 TPS1 PTC1 NTH1 TPS2 HSP42 HSP78 HSP31 HSP12 GRE3 XBP1 MSN4 MNN4 UTH1 SSA2 HSP104 UBI4 DDR48 TPS3 TIR4 TIR2 ATH1*	23	152
Heat shock response	0.001366	*HSP12 PIL1 GRE3 GRE2 GAC1 LSP1*	6	20
Proline biosynthetic process	0.002552	*PRO1 PUT2 PRO2*	3	5
Inactivation of MAPK activity involved in osmosensory signaling pathway	0.002552	*PTC3 PTC1 PTP3*	3	5
Pentose-phosphate pathway	0.003007	*GND1 TAL1 PGM2 ZWF1 SOL1 TKL1*	6	23

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae* using FunSpec **[145]**.

Table 4.4: GO terms associated with down regulated genes under hyperosmotic stress (0.5 M NaCl, 15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	1.00 e^{-14}	*UTP20 MAK5 ENP1 REI1 DBP10 TSR1 NOP14 SAS10 FAP7 NHP2 NOP6 FAL1 MAK21 RRP17 UTP6 SNU13 NOP16 NUG1*	132	170
DNA-directed RNA polymerase I complex	1.00 e^{-14}	*RPB5 RPA14 RPC10 RPA34 RPA12 RPC19 RPA49 RPB10 RPB8 RPA43 RPA190 RPA135 RPC40 RPO26*	14	14
rRNA processing	1.00 e^{-14}	*UTP20 MAK5 ENP1 RRP7 SPB1 KRR1 RRP43 PWP2 RSA4 NOP1 DBP10 TSR1 RRP42 NOP14 SAS10 FAP7*	129	169
Cellular biosynthetic process	3.22 e^{-5}	*PRS2 PRS3 PRS1 PRS5 MRI1*	5	6
Slow-growth phenotype	0.000132	*FUN12 FEN1 BUD23 FAL1 BIM1 DBP3 ARC1 PRS3 SSZ1 DYS1 BIG1 MPP10 RAD27 YAR1 FHL1 TIF3*	39	237

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 4.5: Top twenty genes up regulated by hyperosmotic stress treatment in *C. glabrata*.

C. glabrata **systematic name**	***S. cerevisiae*** **systematic name**	***S. cerevisiae*** **standard name**	**Description in *S. cerevisiae***	**Fold change**
CAGL0J11550g	*YNL195C*	-	Putative protein of unknown function, detected in highly purified mitochondria	88.4
CAGL0J04202g	*YFL014W*	*HSP12*	Plasma membrane protein involved in maintaining membrane organization in many stress conditions; regulated by HOG and Ras-Pka pathways	51.9
CAGL0M11000g	*YNR034W-A*	-	Putative protein of unknown function; expression is regulated by Msn2p/Msn4p	46.3
CAGL0G05269g	*YDR070C*	*FMP16*	Putative protein of unknown function; involved in responding to stress; detected in highly purified mitochondria	44.0
CAGL0G01738g	*YGR086C*	*PIL1*	Associated with endocytosis; null mutants show activation of Pkc1p/Ypk1p stress resistance pathways; detected in phosphorylated state in mitochondria	34.7
CAGL0H02101g	*YHR087W*	*RTC3*	Protein of unknown function involved in RNA metabolism	31.7
CAGL0L04378g	*YOR161C*	*PNS1*	Protein of unknown function	27.0
CAGL0G05544g	-	-	-	26.2
CAGL0G03289g	*YBL075C*	*SSA3*	ATPase involved in protein folding and the response to stress; member of the heat shock protein 70 (HSP70) family	25.6
CAGL0J04004g	*YOR228C*	-	Protein of unknown function, localized to the mitochondrial outer membrane	22.4
CAGL0F07953g	*YGR236C*	*SPG1*	Protein required for survival at high temperature during stationary phase; detected in highly purified mitochondria	20.5
CAGL0D05082g	*YLL039C*	*UBI4*	Ubiquitin, becomes conjugated to proteins, marking them for selective degradation via the ubiquitin-26S proteasome system; essential for the cellular stress response	19.6
CAGL0K10164g	*YDR077W*	*SED1*	Major stress-induced structural GPI-cell wall glycoprotein in stationary-phase cells, associates with translating ribosomes, possible role in mitochondrial genome maintenance	19.1
CAGL0K03459g	*YMR107W*	*SPG4*	Protein required for survival at high temperature during stationary phase; not required for growth on	18.0

			nonfermentable carbon sources	
CAGL0K05247g	*YBL101C*	*ECM21*	Protein involved in regulating the endocytosis of plasma membrane proteins	16.3
CAGL0H02387g	*YMR261C*	*TPS3*	Regulatory subunit of trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate trehalose; expression is induced by stress conditions and repressed by the Ras-cAMP pathway	16.1
CAGL0E01881g	*YLR120C*	*YPS1*	Aspartic protease, member of the yapsin family of proteases involved in cell wall growth and maintenance; attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor	15.6
CAGL0H02563g	-	-	-	15.5
CAGL0C05027g	*YAR035W*	*YAT1*	Outer mitochondrial carnitine acetyltransferase, minor ethanol-inducible enzyme involved in transport of activated acyl groups from the cytoplasm into the mitochondrial matrix	15.1
CAGL0F07777g	*YMR170C*	*ALD2*	Cytoplasmic aldehyde dehydrogenase, involved in ethanol oxidation and beta-alanine biosynthesis; uses NAD+ as the preferred coenzyme; expression is stress induced and glucose repressed	14.0

For each *C. glabrata* gene their *S. cerevisiae* homologue and its description from SGD are given **[45]**. The fold change was calculated by comparing cells treated with 0.5 M NaCl to untreated cells at 15 minutes. Only genes which were statistically significant are included (2-way ANOVA, p-value <0.05).

Two of the genes most highly regulated upon hyperosmotic stress treatment are *C. glabrata* specific genes with no homology to *S. cerevisiae*, including *CAGL0H02563g.* There are also six genes, which while they have homology to *S. cerevisiae*, encode putative proteins with unknown functions including the highest up regulated transcript *CAGL0J11550g* and *CAGL0M11000g*. Table 4.5 also contains *UBI4*, which encodes an ubiquitin, as well as glycosylphosphatidylinositol (GPI) -linked cell wall proteins encoded by *SED1* and *YPS1*. There are also three other *YPS* genes regulated under hyperosmotic stress in *C. glabrata* and the implications of these adhesion genes will be discussed.

4.2.5 Oxidative stress genes are regulated under hyperosmotic stress conditions

GO term enrichment analysis showed that genes involved in the oxidative stress response were up regulated under hyperosmotic stress conditions (4.2.4). While the response of *C. glabrata* to oxidative stress will be covered in Chapter 5, these genes, shown in Table 4.6,

encode key players of the oxidative stress response, such as Cta1, the only catalase *C. glabrata* possesses, as well as Trx2, and Grx2 and Glr1 which are involved in the thioredoxin and glutaredoxin pathways, respectively.

Table 4.6: Oxidative stress genes regulated under hyperosmotic stress conditions.

C. glabrata **systematic name**	***S. cerevisiae*** **systematic name**	***S. cerevisiae*** **standard name**	**Description in *S. cerevisiae***	**Fold change**
CAGL0K10868g	*YDR256C*	*CTA1*	Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix.	6.9
CAGL0K00803g	*YGR209C*	*TRX2*	Cytoplasmic thioredoxin isoenzyme of the thioredoxin system which protects cells against oxidative and reductive stress, acts as a cofactor for Tsa1p, required for ER-Golgi transport and vacuole inheritance	5.6
CAGL0J07612g	*YNL241C*	*ZWF1*	Glucose-6-phosphate dehydrogenase (G6PD), catalyzes the first step of the pentose phosphate pathway; involved in adapting to oxidatve stress.	4.6
CAGL0L05434g	*YKR042W*	*UTH1*	Mitochondrial outer membrane and cell wall localized SUN family member required for mitochondrial autophagy; involved in the oxidative stress response, life span during starvation, mitochondrial biogenesis, and cell death	3.3
CAGL0K05813g	*YDR513W*	*GRX2*	Cytoplasmic glutaredoxin, thioltransferase, glutathione-dependent disulfide oxidoreductase involved in maintaining redox state of target proteins, also exhibits glutathione peroxidase activity, expression induced in response to stress	2.4
CAGL0D05434g	*YPR065W*	*ROX1*	Heme-dependent repressor of hypoxic genes; contains an HMG domain that is responsible for DNA bending activity	2.4
CAGL0H05665g	*YPL091W*	*GLR1*	Cytosolic and mitochondrial glutathione oxidoreductase, converts oxidized glutathione to reduced glutathione; mitochondrial but not cytosolic form has a role in resistance to hyperoxia	2.1

For each *C. glabrata* gene regulated, their *S. cerevisiae* homologue and its description from SGD **[45]** are given. The fold change was calculated by comparing cells treated with 0.5 M NaCl to untreated cells at 15 minutes. Only genes which were statistically significant are included (2-way ANOVA, p value <0.05).

As these genes were regulated under hyperosmotic stress treatment it could be suggested that this regulation is mediated through the HOG pathway. An oxidative stress phenotype in these null mutants would be expected if the HOG pathway were to play a major or indispensible role in oxidative stress resistance in *C. glabrata*, as it does in *C. albicans* **[114]**. To test this, null mutants of the signalling components of the HOG pathway were tested for sensitivity to oxidative stresses, using hydrogen peroxide and tert-butyl hydroperoxide (tBOOH) (Figure 11.2, p.221, Appendix II). This revealed that, as previously discussed in 4.2.1, null mutants of the HOG pathway are sensitive to hyperosmotic stress, however, they are not sensitive to oxidative stressors.

4.2.6 Comparing the hyperosmotic stress response of *C. glabrata* and its close relative, *S. cerevisiae*

S. cerevisiae microarray data published by Gasch *et al*, 2000, was used to investigate whether the transcriptional response to hyperosmotic stress is well conserved between these two fungi **[1]**. The *S. cerevisiae* microarray study was conducted over a time course under hyperosmotic stress using 1 M sorbitol. Data was used where signal intensities had been normalised and the fold change compared to untreated cells had been calculated. The number of genes up and down regulated by each organism after 15 minutes treatment with hyperosmotic stress are compared in the Venn diagrams shown below (Figure 4.6).

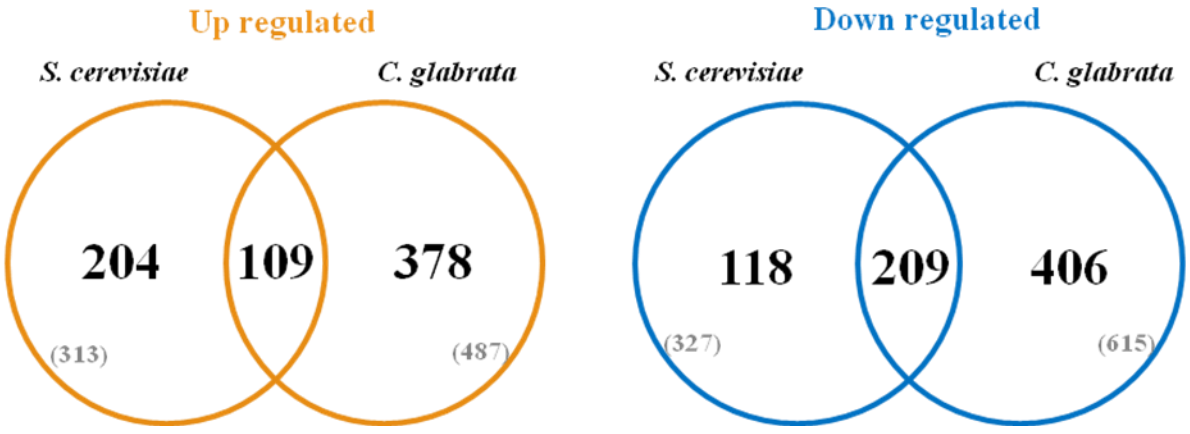


Figure 4.6: Venn diagrams comparing genes regulated under hyperosmotic stress in *S. cerevisiae* and *C. glabrata*. The number of genes regulated by more than 2 fold under hyperosmotic stress compared to untreated cells for each organism are shown. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

Of those regulated by more than 2 fold by hyperosmotic stress treatment in *S. cerevisiae*, 398 genes were up regulated while 392 genes were down regulated. Of the genes regulated by hyperosmotic stress in *S. cerevisiae*, 313 genes up regulated and 327 down regulated genes

were identified as having homologues in *C. glabrata*. Those genes in *S. cerevisiae* without homologues in *C. glabrata* were excluded.

The response of *C. glabrata* to hyperosmotic stress is not well conserved with that of *S. cerevisiae* as only 22.4% of the *C. glabrata* genes up regulated under hyperosmotic stress were also up regulated by *S. cerevisiae*. 34% of genes were observed to be down regulated in *S. cerevisiae* and *C. glabrata* upon hyperosmotic stress treatment. These commonly regulated genes can be found in Table 11.1 and Table 11.2, p.222 and p.229, Appendix II. Genes up regulated by both yeasts in response to hyperosmotic stress treatment include: the stress-induced reductases, *GRE2* and *GRE3*; trehalose synthases, *TPS1* and *TPS2*; a transcription factor of the yAP-1 family, *CIN5*; one of the glycerol-1-phosphatases, *RHR2* (*GPP1*); and many genes which encode heat shock proteins (descriptions inferred from homology with *S. cerevisiae* and taken from SGD (http://www.yeastgenome.org/).

GO term enrichment analysis was conducted for the sets of genes in Figure 4.6 and these genes and selected GO terms can be found in Table 11.3 - Table 11.12, p.243 and p.319, Appendix II. Genes uniquely regulated under hyperosmotic stress in *S. cerevisiae* include *GPD1*, the glycerol-3-phosphate dehydrogenase, a key enzyme in the production of glycerol. In *C. glabrata*, *CAGL0K01683g*, the homologue of *GPD1*, is not induced by hyperosmotic stress treatment however, its paralogue, *CAGL0C05137g*, homologue of *GPD2*, is induced by hyperosmotic stress. This suggested that these genes may have switched roles in *C. glabrata*. Sequence comparison, as well as their syntenic context, revealed that the protein sequences of these genes are very similar (76% identical, 89% similar) and that they are annotated correctly (Figure 11.3 and Figure 11.4, p.319 and p.320, Appendix II). As *gpd1* and *gpd2* strains in *S. cerevisiae* have different phenotypes, *gpd1* and *gpd2*, as well as double *gpd1gpd2* mutants were constructed in *C. glabrata* to test this hypothesis.

Phenotypic screening of *gpd1*, *gpd2* and *gpd1gpd2* to hyperosmotic stress show that these genes have switched roles in *C. glabrata*, compared to their *S. cerevisiae* homologues (Figure 4.7). *gpd1* mutants show no sensitivity to hyperosmotic stress in *C. glabrata*, whereas *gpd2* null mutants show severe growth defects on hyperosmotic stress media. It is important to note that the opposite phenotypes are shown by *GPD* mutants in *S. cerevisiae* **[77]**. This sensitivity is also extended to hyperosmotic stress elicited by compounds other than NaCl, including CsCl, sorbitol, $CaCl_2$ and $MgCl_2$, but not other metal ions such as CdCl, which were used at much lower concentrations. 0.5 M NaCl and 1 M sorbitol have a similar effect on growth (see Figure 4.7). However, the sensitivity of *gpd2* and *gpd1gpd2* cells to CsCl and $CaCl_2$ are much

more severe than the osmolarity they produce should cause. The osmotic pressure elicited by 250 mM CsCl (455 mOsm/L) although lower than 0.5 M NaCl (980 mOsm/L) causes a more severe growth defect and a similar effect is seen in 0.5 M $CaCl_2$ treatment compared to 0.6 M $MgCl_2$.

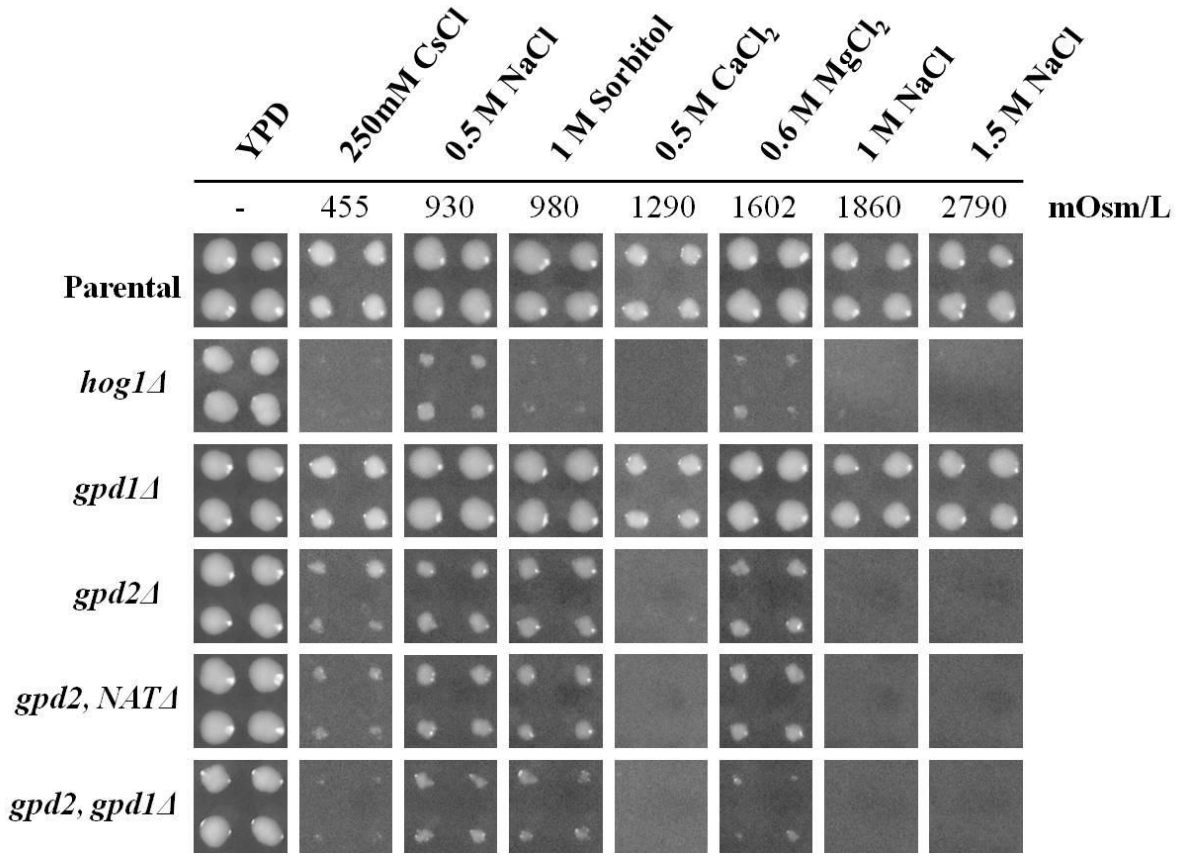


Figure 4.7: Phenotypes of null mutants of the glycerol-3-phosphate dehydrogenases and the MAPK *HOG1* in *C. glabrata*. Phenotypic conditions are arranged by increasing osmolarity, from left to right. Each strain was spotted four times in a square on to media containing the indicated concentration of stress. Pictures were taken after 2 or 3 days. *gpd2, NATΔ* indicates a *GPD2* null mutant with the NAT disruption cassette removed for subsequent construction of a double *gpd2, gpd1* mutant. Osmolarity is in addition to media and was calculated as shown in the Methods.

While the growth of these null mutants on solid media are very similar (Figure 4.7), the growth of *gpd2* and *gpd1gpd2* mutants compared to *hog1* cells in liquid media supplemented with 1 M NaCl are strikingly different (Figure 4.8). *gpd2* and *gpd1gpd2* mutants are more sensitive to hyperosmotic stress than *hog1* mutants, showing very slight or no increase in optical density over a 24 hour time period of hyperosmotic stress treatment while *hog1* cells do grow under hyperosmotic stress, albeit at a slower rate and reach a lower final biomass than wild type cells. It is difficult to infer if the hyperosmotic stress sensitivity observed is increased by removal of both glycerol-3-phosphate dehydrogenases in *C. glabrata* as there no significant difference in liquid cultures (Figure 4.8).

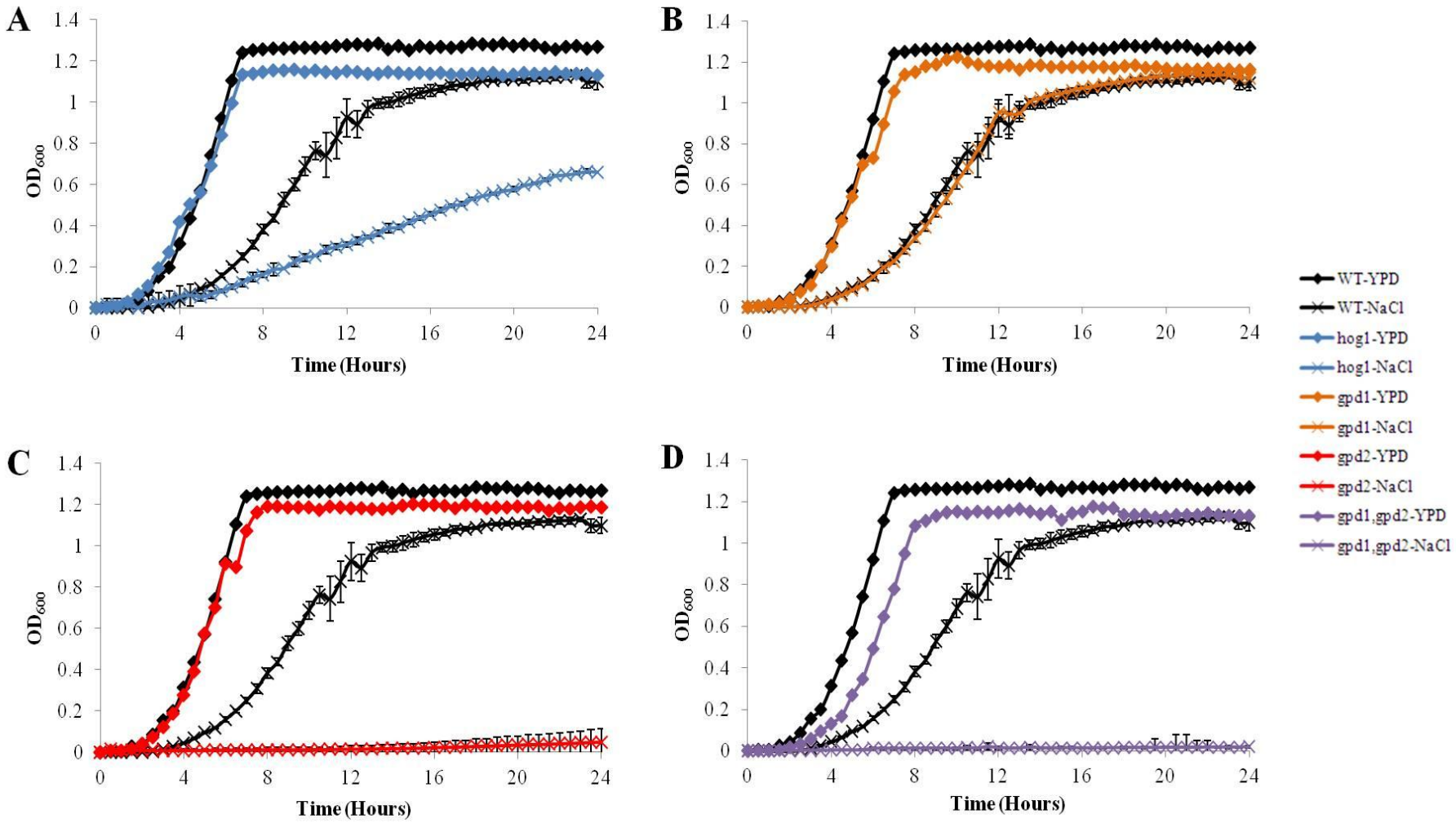


Figure 4.8: Growth of *hog1* and glycerol-3-phosphate dehydrogenase null mutants under hyperosmotic stress compared to wild type. Strains were diluted from overnight cultures, re-suspended in the appropriate media and grown at 30°C for 24 hours, with OD_{600} measurements taken every 30 minutes. Strains are coloured: wild type (black); *hog1* (blue); *gpd1* (orange); *gpd2* (red); *gpd1gpd2* (purple). Shapes denote treatment: ♦ represents YPD (untreated control) and x represents 1 M NaCl treatment. For ease of comparison, each mutant is shown compared to the wild type on each graph: A, wild type and *hog1*, B, wild type and *gpd1*, C, wild type and *gpd2*, D, wild type and *gpd1gpd2*. Error bars of the standard deviation of three technical replicates and two biological replicates are shown.

As shown in Figure 4.6, many genes regulated under hyperosmotic stress are only regulated in *C. glabrata* and not seen to be regulated by the model yeast, *S. cerevisiae*. These genes could therefore play a role in the increased stress resistance observed in *C. glabrata* compared to *S. cerevisiae.* Genes up regulated uniquely in *C. glabrata* by hyperosmotic stress treatment include GO terms for cell wall biogenesis and NADPH regeneration including the up regulation of genes involved in the pentose-phosphate shunt and NADP metabolic processes. Genes involved in proline metabolism and transport are also uniquely up regulated by *C. glabrata*. These overrepresented GO terms may explain the increased resistance of *C. glabrata* to hyperosmotic stress compared to *S. cerevisiae.* Genes down regulated uniquely by both *C. glabrata* and *S. cerevisiae,* as well as those commonly down regulated by both organisms are involved in ribosome biogenesis and RNA processes.

Many of the genes (71/378) uniquely regulated by *C. glabrata* in response to hyperosmotic stress are *C. glabrata* specific genes (which have no homology to genes in *S. cerevisiae*) or those with an unknown function in *S. cerevisiae.* This means inferred biological knowledge from the model yeast *S. cerevisiae* is ineffectual when investigating these particular genes in *C. glabrata*. These genes may however, hold the key as to how *C. glabrata* has evolved to become a human pathogen and these will be presented in 4.2.8.

4.2.7 The long term adaptation of *C. glabrata* to hyperosmotic stress

Transcript profiling experiments of *C. glabrata* treated with hyperosmotic stress were conducted over a two hour time course (three biological replicates). Analysis revealed that 93 genes are statistically and significantly regulated under hyperosmotic stress at the earliest time point (5 minutes) and 76 and 47 transcripts regulated at the later time points of 60 and 120 minutes, respectively (Figure 4.9). This is in contrast to the 1102 genes statistically and significant regulated after 15 minutes hyperosmotic stress treatment.

As discussed earlier, the GO terms associated with genes regulated at 15 minutes shows that while *C. glabrata* is up regulating genes involved in the response to stress, it also down regulates genes involved in ribosome biogenesis and cellular biogenesis. This regulation is seen at the 15 minute time point however, by 60 minutes few genes are regulated. The low level of gene regulation observed at 60 and 120 minutes implies that there is no significant difference between untreated and stressed cells by these late time points.

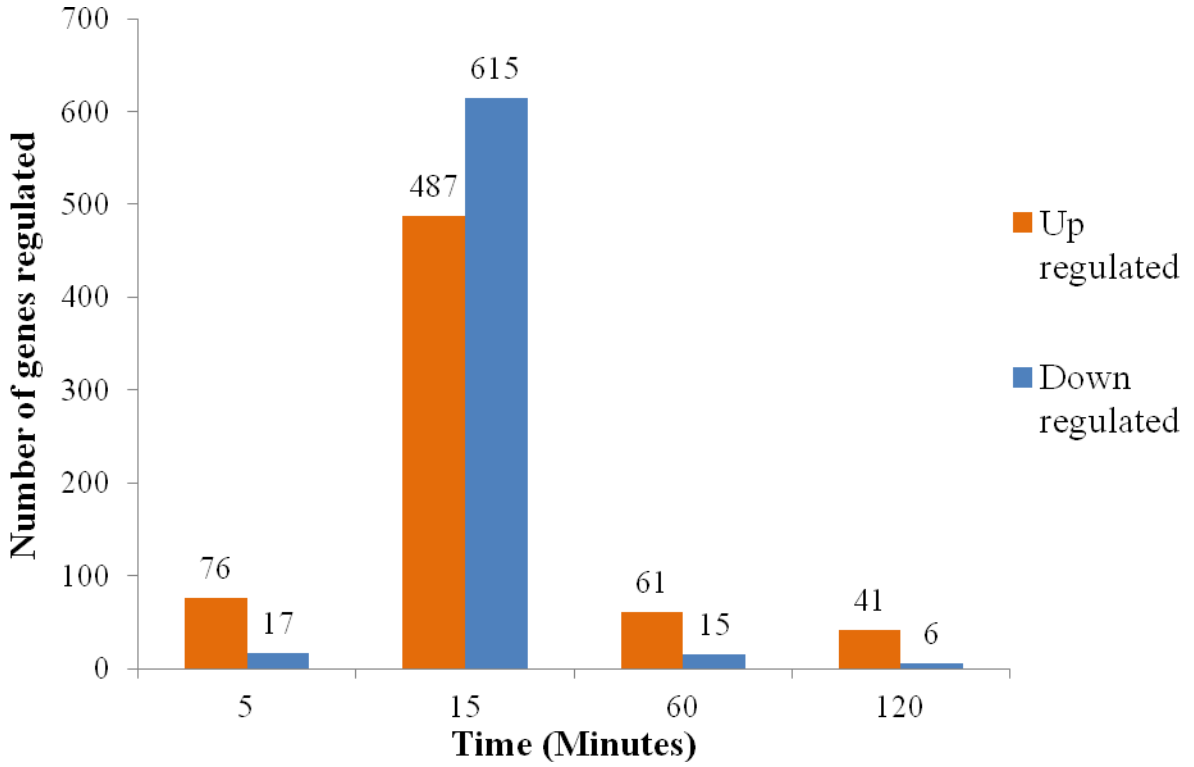


Figure 4.9: Number of gene statistically and significantly regulated under hyperosmotic stress over time. The graph shows the number of genes statistically (2-way ANOVA, p-value <0.05) and significantly regulated by more than 2 fold compared to untreated at that time point. The genes are split into up (orange) and down (blue) regulated genes, as shown.

The genes that are regulated at these later time points may be involved in the long term adaptation of *C. glabrata* to hyperosmotic stress. While there are too few genes to find statistically significant GO terms, by comparing the genes regulated at 60 and 120 minutes some biological information can be gleaned regarding those which may be needed for long term adaptation to hyperosmotic stress (Figure 4.10).

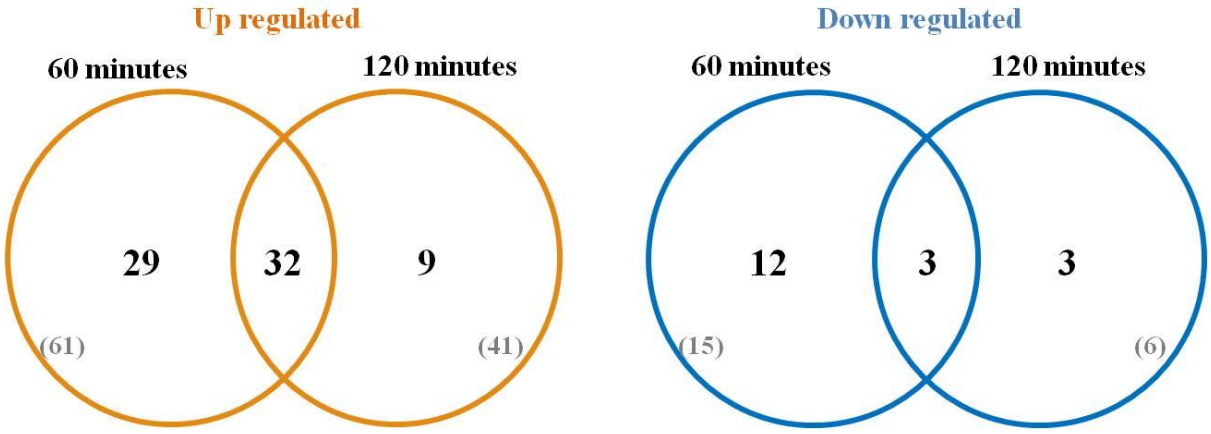


Figure 4.10: Venn diagram comparing genes significantly regulated at 60 and 120 minutes under hyperosmotic stress. The number of genes regulated by more than 2 fold under hyperosmotic stress compared to untreated cells at that time point. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

Stress response genes such as *CIN5*, *CTA1*, *BTN2* and *MSN4* are up regulated at 60 minutes but not after 120 minutes of treatment with hyperosmotic stress. *HSP12* and *ALD2*, as well as many non-othologous genes, are up regulated at both 60 and 120 minutes. In *C. glabrata*, the homologue of Hsp12 is *CAGL0J04202g*. *HSP12* is highly regulated over the timecourse under hyperosmotic stress conditions. A *hsp12* null mutant was created to examine its function in *C. glabrata*. *hsp12* mutants made in *C. glabrata* showed no phenotypes to any of the conditions screened in this study and this is in complete contrast to the many phenotypes reported in *hsp12* mutants in *S. cerevisiae*. Sequence comparisons between *C. glabrata* and *S. cerevisiae* show that these proteins are very similar (Figure 11.5, p.320, Appendix II). *FPS1*, which encodes the aqua-glycerol porin, is also down regulated at both 60 and 120 minutes.

4.2.8 Assigning functions to un-annotated and *C. glabrata* specific genes

The custom made Agilent microarrays used in this study have probes for 5210 ORFs. A number of these are *C. glabrata* specific genes; those not found to have homology or synteny with that of its close relative *S. cerevisiae*. There are a number of genes in *C. glabrata*, which have dubious homology, with very weak sequence similarity or those not found at the syntenic loci of their homologous gene in *S. cerevisiae*. Some genes which have homology with *S. cerevisiae* encode proteins of unknown function and so add no biological information as to their regulation under hyperosmotic stress. Any gene which falls into any of these categories has been excluded from most of the functional analysis so far presented in this study, especially when utilising GO term enrichment analysis as these genes do not have GO terms associated with them.

This study has already shown that out of the top twenty highest up regulated genes upon 15 minutes of hyperosmotic stress treatment, seven genes fall into the group described above. This included *CAGL0M11000g*, homologous to *YNR034W-A*, which although its biological function is still unknown, has been shown to be regulated by the stress activated transcription factors Msn2/4 in *S. cerevisiae* **[45]**. *CAGL0H02563g* was also highly regulated under hyperosmotic stress. It is not a true non-homologous gene as a search of the Génolevures database (http://www.genolevures.org/) shows it has some sequence homology with a characterised gene, *YDR524C-B*. However, the syntenic context of *CAGL0H02563g* aligns with that of *YMR251W-A* (*HOR7)* in *S. cerevisiae* and its protein sequence better matches that of *HOR7* than *YDR524C-B* (Figure 11.6 - Figure 11.8, p.320 – 321, Appendix II). A null mutant of this gene was phenotypically screened revealing it is not required for resistance to hyperosmotic stress (Figure 11.9, p.321, Appendix II) or any other condition screened.

There are 119 and 48 non-homologous or functionally unknown genes which are significantly up and down regulated, respectively, under hyperosmotic stress treatment in *C. glabrata* after 15 minutes treatment (Table 11.13 and Table 11.14, p.321 and p.325, Appendix II). To try and assign biological function to some of these genes, structural predictions were made (Table 4.7). This revealed these genes may be putative transcription factors, protein kinases and involved in protein transport. Unfortunately, none of these were available in the *C. glabrata* null mutant library. As such, phenotypic screening of these stress regulated, non-homologous and functionally unknown genes was not possible.

Table 4.7: Predicted domains of non-homologous genes regulated by hyperosmotic stress.

C. glabrata **Systematic Name**	**Predicted Domains**[a]
CAGL0I08151g	Pirin, iron binding, transcription factor in humans with unknown function
CAGL0K04719g	DNA binding
CAGL0G07645g	DNA polymerase-like
CAGL0M12474g	RNA binding
CAGL0D03938g	Protein binding, ER to Golgi vesicle-mediated transport
CAGL0K04631g	Zinc-finger, C2H2-type, DNA binding
CAGL0I07887g	Rub1-like, ubiquitin-related family, stabilising post-translational modification
CAGL0H03311g	Protein kinase
CAGL0G06182g	Pleckstrin homology, involved in signal transduction and cytoskeletal function
CAGL0M07007g	PI31-like, regulator of proteasome formation
CAGL0L00473g	Zinc-finger, C3HC4 type, ubiquitination pathway
CAGL0M02299g	Serine/threonine protein kinase
CAGL0M12881g	Dihydroorotate dehydrogenase, pyrimidine biosynthesis
CAGL0L08008g	ATPase proteolipid, plasma membrane H^+ ATPase
CAGL0J05786g	Zinc finger, B-box-type, transcription factor and Fe,Mn superoxide dismutase
CAGL0I09196g	Ubiquitin-protein ligases

[a.] Domain predictions conducted by Mark Wass, Imperial College London (Personal Communication). Those genes which produced no or low confidence predictions were removed. Up regulated genes are shown in the top part of the table, while down regulated genes are shown in the bottom half.

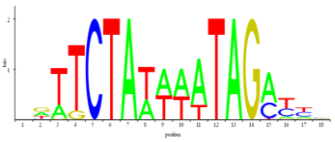
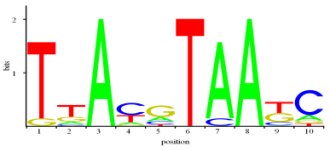
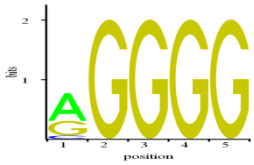
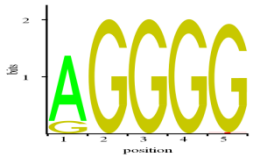
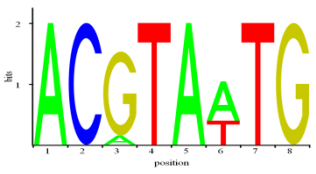
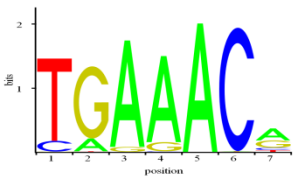
When comparing the genes up regulated at the later time points of the timecourse (Figure 4.10), it was found that 16 out of the 41 genes up regulated by the 120 minute time point are non-homologous or functionally unknown genes. These included *CAGL0L09251g* which was annotated as a *C. glabrata* specific ORF. BLAST search of this gene's sequence against the entire *S. cerevisiae* genome found no possible homologue. However, when comparing it's syntenic context, using YGOB (http://wolfe.gen.tcd.ie/ygob/), with that of *S. cerevisiae*, it was found that *CAG0L09251g* is homologous to *HAL1* **[29]**.

The length of the Hal1 proteins are similar in both *C. glabrata* and *S. cerevisiae* (282 and 294 amino acids, respectively) and by comparing the protein sequences, no conserved region, which could indicate a shared domain, was found. Hal1 in *C. glabrata* shares only 16% identical amino acids to that of Hal1 in *S. cerevisiae* (sequences from Génolevures (http://www.genolevures.org/) and sequence comparison using ClustalW **[25, 113]**). A null mutant of *CAGL0L09251g* was constructed and phenotypically screened (see Chapter 3 for complete list). This showed that, as in *S. cerevisiae*, *hal1* null mutants are viable and have no apparent phenotype.

By using the open-source software JASPAR, the upstream region of *HAL1* in *C. glabrata* was parsed for all known yeast binding motifs **[146]**. This found that the untranslated region upstream of *HAL1* in *C. glabrata* contains binding motifs for a range of transcription factors, including those known to be involved in regulating the hyperosmotic stress response as well as specifically *HAL1* in *S. cerevisiae*. The binding motifs predicted in the upstream region of *HAL1* in *C. glabrata* are shown in Table 4.8, along with their transcriptional regulation under hyperosmotic stress conditions. Predicted binding motifs in the upstream region of *HAL1* include: Rlm1, Cin5, Msn2, Msn4 and Ste12.

By combining transcriptional analysis with bioinformatic tools, the putative functions of non-homologous and functionally unknown genes have been predicted. The functionality predicted for these genes, as well as their observed regulation upon hyperosmotic stress could help to explain their roles in stress response.

Table 4.8: Predicted binding motifs in the upstream untranslated region of *HAL1* in *C. glabrata*.

C. glabrata **systematic name**	***S. cerevisiae*** **standard name**	**Description in *S. cerevisiae*[a]**	**Binding motif in *S. cerevisiae*[b]**	**Fold change**
CAGL0M06325g	Rlm1	MADS-box transcription factor, component of the MAP kinase pathway involved in the maintenance of cell integrity; phosphorylated and activated by the MAP-kinase Slt2p		7.5
CAGL0H08173g	Cin5	yAP-1 family; mediates pleiotropic drug resistance and salt tolerance; nuclearly localized under oxidative stress		4.6
CAGL0F05995g	Msn2	Transcriptional activator related to Msn4p; activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress response elements of responsive genes, inducing gene expression		0.06
CAGL0M13189g	Msn4	Transcriptional activator related to Msn2p; as above		4.8
CAGL0J06182g	Sko1	Forms a complex with Tup1p and Cyc8p to both activate and repress transcription; cytosolic and nuclear protein involved in osmotic and oxidative stress responses		2.1
CAGL0H02145g	Ste12	Activated by phosphorylation by kinases Fus3 and Kss1, involved in mating or pseudohyphal/invasive growth pathways		0.05

[a.] Description from SGD **[45]**, [b.] Binding motifs in *S. cerevisiae* from JASPAR **[146]**. Fold change calculated from 0.5 M NaCl treated cells compared to untreated cells at 15 minutes.

4.3 Discussion

This chapter has explored the adaptation of *C. glabrata* to hyperosmotic stress through transcript profiling and functional genomics analyses. Phenotypic screening of null mutants of the HOG pathway revealed that they function in more than just the hyperosmotic stress response. The implications of these additional functions will be discussed. Phenotypic screening of different strains of *C. glabrata* displayed the importance of choosing the appropriate background strain when investigating any organism. The transcriptional response of *C. glabrata* over time to hyperosmotic stress conditions was analysed and these results showed the regulation of genes involved in glycerol, trehalose and proline biosynthesis, as well as oxidative stress. Little differential gene expression was observed by 60 minutes treatment with hyperosmotic stress suggesting that *C. glabrata* had adapted to the environment and now resembled a growing culture of cells. The transcriptional response of *C. glabrata* to hyperosmotic stress was also compared to published data in *S. cerevisiae* and this identified *GPD2* as transcriptionally regulated in *C. glabrata*, in contrast to *S. cerevisiae* where *GPD1* is induced. Null mutants of *gpd1*, *gpd2* and *gpd1gpd2* were constructed and phenotypically screened, linking this observation with stress resistance and revealing functionality. *C. glabrata* specific and un-annotated genes involved in hyperosmotic stress were investigated and their possible roles will also be discussed. By combining the transcriptional and phenotypic analyses, a model to illustrate the hyperosmotic stress response of *C. glabrata* was created.

4.3.1 The effect of hyperosmotic stress on the growth and viability of *C. glabrata*

Null mutants of the HOG pathway in *C. glabrata* were screened on hyperosmotic stress conditions; those which displayed a phenotype indicated their involvement in the response to this stress. Null mutants of the functioning branch (Sho1) of the HOG pathway in *C. glabrata* show sensitivity to hyperosmotic stress, while the null mutants *sln1* and *ypd1* (part of the Sln1 branch) show no sensitivity to hyperosmotic stress. This is to be expected, as this branch is non-functioning in the ATCC 2001 strain **[70]**.

A homologue of Ste20, known to be involved in the MAPK signalling pathways of *S. cerevisiae*, has been identified and characterised in *C. glabrata* as being involved in hyperosmotic stress response and cell wall integrity, as well as virulence **[147]**. However, data mining of the Génolevures database (http://www.genolevures.org/) suggested the

possibility of a paralogue: *CAGL0M10153g* **[25]**. Null mutants of *CAGL0K02673g* and *CAGL0M10153g*, so named *ste20α* and *ste20β*, respectively, were phenotypically screened on a wide range of conditions. This showed that s*te20β* had no phenotype on hyperosmotic stress or any of the other conditions screened, revealing that as previously published, *STE20α* is the functioning gene in *C. glabrata*. This screening also found new phenotypes, not described in the literature for Ste20 in *C. glabrata*, such as those for arsenic and metal stressors.

Phenotypic screening of null mutants to hyperosmotic stress conditions has confirmed the function of components of the HOG pathway in *C. glabrata* from their inferred functions in *S. cerevisiae*. In *S. cerevisiae* it has been reported that Opy2 acts as an osmo-sensor in the plasma membrane **[148]**. Bioinformatic analysis using Génolevures (http://www.genolevures.org/) and YGOB (http://wolfe.gen.tcd.ie/ygob/) revealed a homologue of Opy2 in *C. glabrata*: *CAGL0D01276g* **[25, 29]**. A null mutant of this gene displays sensitivity to hyperosmotic stress, suggesting it is functionally similar to its homologue in *S. cerevisiae* and is involved in the response to hyperosmotic stress in *C. glabrata* (Figure 4.3).

4.3.2 Phenotypic screening of HOG pathway mutants reveals functionality

Functional genomics studies by phenotypically screening null mutants have been crucial in linking genes with their function in *S. cerevisiae*. The phenotypic screening of HOG pathway mutants conducted in this study has helped to elucidate their function in *C. glabrata* (see Sections 4.2.1 and 4.2.2).

As all HOG pathway mutants are viable and grow similarly to the parental strain at 30°C, this shows they have no general or slow growth defect. *ste20α* and *ste50* mutants are sensitive to both 16°C and 42°C, indicating that their protein products have important functions in the response to heat stress, while *opy2* is also sensitive to 42°C suggesting that it too has an a important role in heat stress in *C. glabrata*. Heat stress is known to result in the misfolding and aggregation of proteins, as well as affecting the plasma membrane in *S. cerevisiae* **[134]**. *opy2*, *ste20α* and *ste50* mutants are not sensitive to 42°C with oxidative stress which shows that oxidative stress somehow rescues their heat sensitivities. This rescue could be due to the heat shock proteins which are seen to be up regulated in *C. glabrata* by oxidative stress treatment (see Chapter 5). *sho1*, another and supposedly the main osmosensor of the HOG pathway as well as *hog1*, display no sensitivity to lowered or elevated temperature indicating their proteins are not required for resistance to heat stress. However, *hog1* and *sho1* are sensitive to an elevated temperature of 42°C with oxidative stress. While *ste11* is sensitive to

16°C and 42°C with oxidative stress, it is not sensitive to 42°C alone. Interestingly, no HOG pathway mutants are sensitive to oxidative stress elicited on its own. 42°C with oxidative stress may elicit an oxidative burst as hydrogen peroxide is less stable at higher temperature and would disassociate at an increased rate, than when used at 30°C. This increased temperature with oxidative stress may somewhat replicate the environment inside a macrophage, linking this phenotype with pathogenicity in a host setting.

As shown in 4.2.2, mutants that are sensitive to 0.5 M NaCl (all except *msb2* and *ste20β*) are also sensitive to 1 M sorbitol which elicits hyperosmotic stress without also being a cationic stress. The concentrations 0.5 M NaCl and 1 M sorbitol used have similar osmolarities (as shown in Table 4.2) and this shows that the hyperosmotic stress phenotype seen in these mutants is not just an effect of cationic stress.

As *msb2* mutants are not sensitive to hyperosmotic stress but are sensitive to divalent cations, heavy metals and cell wall stressors, this shows that Msb2 is involved in metal resistance and cell wall integrity, but is not essential for hyperosmotic stress. *CAGL0F03003g* was first identified in this study as a possible paralogue of *MSB2*, however, its syntenic context, using YGOB (http://wolfe.gen.tcd.ie/ygob/), revealed its annotation as *HKR1* **[29]**. A null mutant of this gene is available in the *C. glabrata* null mutant library and although it is not required for hyperosmotic stress resistance (data not shown), its other phenotypes are unknown. As such, phenotypic screening of *hkr1* would be informative and help to elucidate its function, specifically in regard to its reported functional redundancy with Msb2 in the HOG pathway of *S. cerevisiae* but differential regulation of the filamentous growth pathway compared to Msb2 **[149, 150]**. It is also important to note that the phenotypes displayed in this study by *msb2* mutants in *C. glabrata* have not been shown in the corresponding *S. cerevisiae* mutants, as per SGD (http://www.yeastgenome.org/). Other HOG pathway components are also sensitive to divalent cations and heavy metals and as this sensitivity does not increase with increasing osmolarity, it suggests that HOG pathway components are involved in heavy metal resistance separately from the hyperosmotic stress response.

HOG pathway components are also sensitive to cell wall stressors. Mutants sensitive to calcofluor white are associated with chitin and β-glucan synthase defects and sensitivity is linked to increased chitin levels in the cell wall, while the opposite is also true; mutants resistant to calcofluor white have been linked to decreased chitin levels **[151]**. This could be confirmed by staining cells with calcofluor white which binds to chitin in the cell wall and comparing these to wild type cells.

hog1 cells are sensitive to caffeine, which is not unexpected as sensitivity to caffeine reveals defects in MAPK signalling pathways. However, it is surprising that no other components of the HOG pathway showed sensitivity to caffeine as this is a MAPK signalling pathway. As no other MAPKK has been shown to phosphorylate Hog1 other than Pbs2 in *S. cerevisiae* and as the functionality of Hog1 has been intrinsically linked with its phosphorylation, a *pbs2* strain should have the same phenotypes as a *hog1* strain **[152]**. Studies have shown that Hog1 is not phosphorylated in a *pbs2* null mutant under hyperosmotic stress in *C. glabrata* therefore in *C. glabrata* it is also thought to be the only MAPKK to phosphorylate Hog1 **[70]**. If this is the case in *C. glabrata*, *pbs2* mutants should have the same phenotypes as *hog1* mutants. There are however, nine conditions in which *hog1* mutants are more sensitive than *pbs2* mutants (Table 4.2) and this would suggest that Hog1, in *C. glabrata*, may have functions in its un-phosphorylated form. Creation of non-phosphorylatable forms of Hog1 in *C. glabrata* would help to explore this theory.

ste50 and *ste11* have the most phenotypes, and also the most severe growth defects, being more sensitive than *hog1* under many conditions. This could be due to the fact that as previously mentioned, Ste50 (MAPKKK) and Ste11 (MAPKKKK), are shared with other MAPK pathways, namely the cell wall integrity pathway, as well as the filamentous growth pathway in *S. cerevisiae*. It also supports the argument that Hog1 is not the most important component in these stress responses.

opy2, *sho1*, *ste50*, *ste11* and *hog1* are all sensitive to cyclohexamide suggesting their involvement in cell cycle protein synthesis **[55]**. This suggests that the HOG pathway is involved in cell cycle regulation, which links hyperosmotic stress response to cell cycle progression in *C. glabrata*. Studies in *S. cerevisiae* have implicated the HOG pathway in the cell cycle delay and arrest into the G1 phase in response to hyperosmotic stress **[153]**. HOG pathway mutants also display severe sensitivity to arsenic compounds, revealing their role in actin, tubulin and protein folding **[137]**.

HOG pathway mutants are sensitive to sodium vanadate suggesting vacuolar defects **[138]**. This could be explored by microscopy using vacuole specific dyes (for example FITC) looking for defects in the size and number of vacuoles in these mutants compared to wild type. *ste20α* and *ste50* are also sensitive to Nystatin, which reveals defective sterol biosynthesis. Nystatin is used as an antifungal drug and binds to ergosterol in the plasma membrane causing the formation of pores and leading to loss of membrane integrity **[55]**.

Functional genomics analysis using a wide ranging phenotypic screen has revealed previously unknown functions of the HOG pathway, implicating components of the HOG pathway in cell wall integrity, metal ion toxicity and the cell cycle, as well that which it is named for, hyperosmotic stress.

4.3.3 ATCC 2001 and BG2: more than just a single point mutation

ATCC 2001 is the sequenced *C. glabrata* strain which, as discussed in 4.1, has a point mutation in *SSK2*, rendering the Sln1 signalling branch of the HOG pathway non-functional. BG2, another lab strain, does not possess this mutation and so has both branches functioning **[70]**. In *S. cerevisiae*, *sln1* and *ypd1* null mutants prove to be inviable. This has been shown to be because Sln1 acts as a repressor; in *sln1* cells, the signalling branch is constitutively active and leads to an over phosphorylation of Hog1 resulting in cell death **[154]**. Studies have shown that in *S. cerevisiae*, removal of downstream pathway components such as Pbs2 or Hog1, as well as over expression of *PTP2,* encoding a Protein Tyrosine Phosphatase, which de-phosphorylates Hog1, allows the creation of a viable *sln1* mutant **[155, 156]**. As already discussed, null mutants of *sln1* and *ypd1* constructed in an ATCC 2001 strain are not sensitive to hyperosmotic stress and were able to be made as the downstream target, Ssk2, is disrupted in this strain. Unlike in *S. cerevisiae* however, a *ypd1* mutant was able to be created in a strain (BG2) which does not possess any disruptions in the downstream targets of the Sln1 signalling pathway. A *ypd1* null mutant displays no phenotype on any condition, including hyperosmotic stress and while this is to be expected as the Sho1 signalling branch is still functioning in this strain, it does not help to elucidate its function in *C. glabrata*, which must be different from that of its homologue in *S. cerevisiae*. Attempts to construct a *sln1* null mutant in the BG2 strain proved to be unsuccessful, even after many attempts. This suggests that Sln1 in *C. glabrata* has a similar function as its homologue in *S. cerevisiae* and must act through the signalling pathway down to Hog1, but not through Ypd1. As an *ssk1* null mutant has not been created in *C. glabrata*, it is not possible to know whether Sln1 circumvents just Ypd1 or the complete signalling pathway down to Ssk2. Another possibility is that Sln1 acts by signalling through another, as yet unknown, protein down to Hog1. This unknown protein however, if one should exist, must at least signal through Ssk2 down to Hog1, otherwise the Sho1 branch would not be the only functioning pathway and therefore the null mutants made in it, would not be sensitive to hyperosmotic stress. These observations suggest that the Sln1 branch of the signalling pathway may have an independent role from that of the Sho1 branch in *C. glabrata*.

This study has shown that, as previously reported, *sho1* mutants do not show sensitivity to hyperosmotic stress when constructed in a BG2 background, unlike when created in an ATCC 2001 background **[70]**. Other phenotypes displayed by *sho1* mutants in an ATCC 2001 background are also not present in a BG2 background. This study has shown that Opy2 is not only an osmosensor as in *S. cerevisiae*, and involved in hyperosmotic, heavy metal, divalent cation and cell wall stress in *C. glabrata*, but that these phenotypes are also not present when constructed in a BG2 background **[148]**. These results show that, as previously reported, removal of one branch of the HOG pathway is not sufficient to render *C. glabrata* cells sensitive to hyperosmotic stress, as well as the other phenotypes displayed, if the other signalling branch is present and functional **[70]**.

While conducting these phenotypic screens, it became apparent that there are more differences between the ATCC 2001 and BG2 strains than simply a single point mutation. BG2, as well as all mutants made in a BG2 background, display increased resistance to a number of conditions, as well as sensitivity to a few others compared to the ATCC 2001 strain. BG2, although now a lab strain, is used by many in the *Candida* community as a clinical isolate and as with most *C. glabrata* strains, was originally isolated from an infected human host. The resistance displayed by BG2 to an elevated temperature of 42°C may have helped this strain withstand the temperature in the host environment. It may also possess more or have mechanisms in place to increase the regulation and expression of proteins involved in the response to temperature, such as heat shock proteins. BG2 is also more resistant to arsenic oxide and sodium metarsenite which suggests that it has impaired ribosome function compared to ATCC 2001 **[137]**. A null mutant in *S. cerevisiae* of *tim18* and the over expression of *ARR3* (*ACR3*) have been shown to be resistant to arsenic, as well as other apoptotic stimuli **[157, 158]**. Either of these mutations could be present in BG2 and if so, could explain this phenotype. This resistance may have helped BG2 resist killing in the host via apoptosis signals. Sequencing of the BG2 *C. glabrata* strain and comparison to the sequenced ATCC 2001 strain would help to identify these strain differences.

The phenotypes observed on cationic and heavy metal stresses are a little confounding; BG2 is resistant to LiCl and $CoCl_2$ but sensitive to CsCl and $MnCl_2$. Manganese and cobalt are both transition metals, have a charge of 2 and have similar crystal and ionic radii sizes **[159]**. Lithium and caesium are alkali metals, have a single charge but differ in atomic size. Caesium is further down Group 1 in the periodic table and as such has a much larger crystal and ionic radii compared to lithium **[159]**. As will be further discussed in 4.3.5, the response

to caesium is regulated by the HOG pathway in *S. cerevisiae* **[160]**. As BG2 has both signalling branches of the HOG pathway, this could be affecting its sensitivity to CsCl. While in low concentrations, free Mn^{2+} and Mg^{2+} ions act as co-factors of pyruvate carboxylase, an excess of free Mn^{2+} or Mg^{2+} ions has been shown to inhibit this activity **[161]**. The inhibition of pyruvate carboxylase would inhibit the process of gluconeogenesis from pyruvate, limiting the generation of glucose. The sensitivity of BG2 to $MnCl_2$ may be due to this limitation of glucose production or be due to differences in transporters in the plasma membrane between BG2 and ATCC 2001, causing increased and therefore toxic concentrations of Mn^{2+} ions. High concentrations of Mn^{2+} have also been shown to increase the error rate of DNA polymerases in *E. coli* leading to increased mutagenesis, which may also be occurring in the BG2 strain **[162]**.

BG2 is more resistant to the cell wall stressor SDS than ATCC 2001 suggesting that their cell wall compositions differ. This may be a consequence of BG2 possessing both signalling branches of the HOG pathway. This study has shown that components of the HOG pathway are required for cell wall integrity as well as that Sln1, part of the non-functioning signalling branch in ATCC 2001, may have a role in cell wall stress separate from the HOG pathway. This is due to *sln1* mutants created in an ATCC 2001 strain displaying some sensitivity to 1% SDS.

The differences in stress resistance observed in these two strain isolates of *C. glabrata* indicate the importance of choosing the most appropriate background strain when investigating a species. In *S. cerevisiae*, the main lineages used such as W303, 1278B and S288C also display different phenotypes and sequencing has revealed these each contain different mutations, which must be considered before experimentation **[163]**. Although ATCC 2001 shows different phenotypes to BG2, as well as the published *SSK2* mutation, it is the sequenced *C. glabrata* strain and therefore the most appropriate to use for this study; the microarrays were made using this sequence data and the null mutant library was constructed in this background.

4.3.4 The immediate transcriptional response of *C. glabrata* to hyperosmotic stress

Analysis of the immediate transcriptional response revealed that approximately 21% of the total genome of *C. glabrata* is statistically and significantly regulated in response to 15 minutes treatment with hyperosmotic stress (0.5 M NaCl). GO term enrichment analysis (p-

value <0.01) was conducted on those regulated genes after 15 minutes treatment and this found the up regulation of stress responses such as abiotic, heat and oxidative stress and the down regulation of ribosome biogenesis.

While the response to abiotic stress is to be expected as non-ideal growth conditions have been applied, the response to oxidative and heat stress are unexpected. Key players of the oxidative stress response are regulated under hyperosmotic stress conditions in *C. glabrata*. As these genes are regulated under hyperosmotic stress treatment it could be suggested that this regulation is mediated through the HOG pathway. Null mutants of the HOG pathway were tested for sensitivity to oxidative stressors and as seen in the many other stresses that they are involved, HOG pathway null mutants would be expected to have some phenotype to oxidative stressors if they do indeed play a major role. This screening revealed that null mutants of the HOG pathway are not sensitive to oxidative stress, implying that the HOG pathway in *C. glabrata* does not play a significant role in regulating the oxidative stress response. This is completely distinct from the HOG pathway in *C. albicans*, in which Hog1 plays an essential role in oxidative stress response **[114]**. It is also important to note that in *S. cerevisiae* and *C. albicans*, Hog1 is phosphorylated under oxidative stress conditions, whereas this is unknown in *C. glabrata* **[114, 164]**. The regulation of oxidative stress genes under hyperosmotic stress conditions in *C. glabrata* could be part of an environmental stress response (ESR); the regulation of a set of genes regardless of the stress condition imposed. ESR has been reported in *S. cerevisiae* and *C. glabrata*, and it is still under some debate in *C. albicans* **[1, 118, 165, 166]**. While further transcriptional analysis of *C. glabrata* to many varying stresses would be needed to make a confident conclusion, this will be further discussed in Chapter 6.

Genes regulated after 15 minutes treatment with hyperosmotic stress are also involved in the response to heat shock. It may be pertinent to note that the regulation of genes involved with the response to heat cannot be an artefact of the process of cell harvesting and the movement of cells from the bench environment at room temperature to the 30°C incubator. All treated samples are compared to the untreated control, which has been processed in exactly the same way at the same time, removing any effects the addition of cells to new media and temperature changes may have during the experimental procedure. Many of the genes up regulated which are involved in the response to heat and stress GO terms encode heat shock proteins, such as Hsp42, Hsp78, Hsp31, Hsp104 and Hsp12. While they are involved in the response to changes in temperature with roles in facilitating the proper folding of proteins

which may have been affected by an increase in temperature, they also act as chaperones, correctly folding newly synthesised proteins and have roles in protein degradation and are induced under varying stress conditions in *S. cerevisiae* **[167]**. This is an example of one of the few similarities between the transcriptional response of *C. glabrata* to hyperosmotic stress and that seen in *S. cerevisiae*. Hsp12 is a small heat shock protein known to be involved in the response of *S. cerevisiae* to many stresses **[168]**. This study has shown that *HSP12* is not only regulated immediately upon the addition of hyperosmotic stress in *C. glabrata,* it is also one of the highest regulated genes along with *UBI4* which encodes ubiquitin, known to function with HSPs and play an important role in stress response **[169]**. This high induction of *HSP12* is seen throughout the timecourse over the 2 hours analysed and it is also one of the few genes still regulated after 120 minutes treatment. It is a cytosolic and plasma membrane associated protein involved in regulating membrane function to stress conditions and is known to be induced by heat shock, oxidative stress, hyperosmotic stress, stationary phase, glucose depletion and alcohol, as well as being regulated by the HOG and Ras-Pka pathways in *S. cerevisiae* **[168]**. This continued regulation over time may be due to the continued production of its transcript, but is more likely to be due to its RNA stability. Although many have much shorter half lives, yeast mRNAs have been shown to have half lives of up to 90 minutes **[127]**. As Hsp12 is known to be regulated by the HOG pathway in *S. cerevisiae* and Hog1 is dephosphorylated and therefore inactivated by 45 minutes in *C. glabrata*, it is more likely that the high transcript abundance after 120 minutes is due to an increased RNA half life **[71]**. While *hsp12* mutants in *S. cerevisiae* are sensitive to oxidative and cell wall stress, as well as metal toxicity and ethanol, *hsp12* mutants constructed in *C. glabrata* show no phenotypes to any of these conditions or any other tested in this study **[170, 171]**. As the sequences of these two proteins are very similar, this would suggest that their functions should also be similar and therefore their phenotypes. There are however other heat shock proteins regulated under hyperosmotic stress in *C. glabrata*, such as those encoded by *HSP42*, *HSP78*, *HSP31* and *HSP104* and these could be compensating for the lack of Hsp12 in null mutants. Null mutants of these heat shock proteins and construction of double mutants would be needed to ascertain this. Cross complementation by reconstituting the *S. cerevisiae hsp12* mutant with the C. *glabrata HSP12* gene and testing for rescue of its phenotypes could be untaken. This would test whether the *C. glabrata HSP12* gene is functionally similar to its *S. cerevisiae* homologue if it can restore growth.

The up regulation of genes involved in trehalose and proline metabolism suggest that these could be being synthesised as osmolytes, alternative to the traditional glycerol, to regain turgor pressure inside the cell upon hyperosmotic stress. While genes involved in trehalose metabolism are seen in hyperosmotic stress treated *S. cerevisiae* microarray experiments, genes involved in proline synthesis and transport are uniquely regulated by *C. glabrata*. Proline is known to accumulate in plants in response to a range of stresses and act as an osmolyte as well as heat protectant **[172]**. There are also some indications that the induction of genes involved in proline synthesis in response to hyperosmotic stress are *HOG1* dependent in *C. glabrata* **[72]**. Studies by researchers in China have shown that the addition of proline increases the resistance of *C. glabrata* to hyperosmotic stress **[81]**. Taken together, these results suggest that glycerol may not be the only or most important compound produced by *C. glabrata* to adapt to hyperosmotic stress, like it has been reported to be in *S. cerevisiae*. So why produce glycerol if it may not to be used as an osmolyte? The regulation of genes involved with the pentose phosphate shunt and the regeneration of NADPH may be key to this observation. As the final biomass of stress treated cells are always lower than that of untreated cells, this shows that responding and adapting to stress environments is very energetic, which would lead to the production of ROS though respiration **[173]**. Studies have shown that metabolites, such as glycerol and mannitol, both shown to be produced by yeast upon hyperosmotic stress treatment, and proline, which is important in plants, can be used as antioxidants, scavenging ROS and their intermediates produced through respiration and by the host immune cells **[76, 80, 86, 172]**. As the response to any stress, hyperosmotic stress included, is very energy expensive, glycerol may be being used as an antioxidant rather than an osmolyte. The glycerol produced may also be playing a role in helping the regeneration of NADPH by removing the ROS produced. The up regulation of genes involved in NADPH regeneration, the pentose-phosphate shunt and NADP metabolic process suggest that although the response to hyperosmotic stress by *C. glabrata* is energy expensive, it is able to sufficiently regenerate the energy used and grow under the stress conditions. Glycerol accumulation assays conducted in hyperosmotic stress mutants would be informative. This data could also be compared to the ratio of NADP:NADPH in *C. glabrata* wild type and HOG pathway mutants under stress conditions.

Genes involved in the fungal cell wall are also up regulated; including the kinase encoded by *SLT2*, which is involved in the cell wall integrity pathway and prominent genes *SED1* and *CWP1*, often used as indicators of cell wall stress in *S. cerevisiae* **[174, 175]**. *RLM1*, a known

target of Slt2 and known to be involved in cell wall integrity in *C. glabrata*, is also regulated under hyperosmotic stress in this study **[176]**. This suggests a remodelling of the cell wall in response to hyperosmotic stress and this may help *C. glabrata* resist the osmotic pressure encountered under hyperosmotic stress conditions. This gene regulation links to the cell wall stress phenotypes which are seen when components of the HOG pathway are removed. This regulation of genes involved with the cell wall is specific to *C. glabrata* and not seen to be regulated by *S. cerevisiae* in response to hyperosmotic stress using data from Gasch *et al*, 2000 **[1]**.

There is also up regulation of four *YPS* genes, one of which is in the top twenty highest regulated transcripts after 15 minutes treatment with hyperosmotic stress. *YPS* genes are closely related to the *YPS* (Yapsin) genes of *S. cerevisiae*. *C. glabrata* encodes 11 predicted GPI-linked aspartyl proteases, three of which have homology with corresponding *YPS* genes in *S. cerevisiae*, while the other 8 are found in a cluster on Chromosome E **[2, 25]**. Studies have shown that the *C. glabrata YPS* genes are required for cell wall integrity, adherence to mammalian cells, survival in macrophages and virulence **[2]**. They are also transcriptionally regulated in *C. glabrata* cells engulfed by macrophages, drawing a direct link between the hyperosmotic stress response of *C. glabrata* with that of its response to the host immune cells **[2]**.

GO terms associated with genes down regulated immediately upon hyperosmotic stress include ribosome biogenesis and the biosynthesis of cellular components, suggesting a down regulation of growth under hyperosmotic stress conditions. GO terms for genes involved in gene expression and various RNA processes are also overrepresented. These suggest that unwanted transcripts are being prevented from being transcribed to allow new, stress response related proteins to be expressed. Many of these genes are also commonly down regulated under hyperosmotic stress conditions in *S. cerevisiae* implying that the down regulation of growth upon exposure to stress is not unique to *C. glabrata* and occurs with the down regulation of similar genes.

Analysis of the transcriptional response of *C. glabrata* to hyperosmotic stress has shown that while genes with inferred hyperosmotic stress functionality from *S. cerevisiae* are regulated, so too are genes involved in oxidative stress. Phenotypic screening of HOG pathway null mutants showed they were not required for oxidative stress resistance and therefore unlikely to be major regulators of the oxidative stress response. Genes involved (from homology with *S. cerevisiae*) in proline biosynthesis and transport were up regulated in response to

hyperosmotic stress, and comparisons with published *S. cerevisiae* data showed that this induction was *C. glabrata* specific. Taken together with published data from Xu, 2010, this suggests the production and import of proline as an alternative osmolyte in *C. glabrata* **[81]**. As genes involved in cell wall remodelling were up regulated, along with the sensitivity displayed by HOG pathway mutants to cell wall stressors, this implied that the adaptation of *C. glabrata* to hyperosmotic stress requires not only the HOG pathway but it also co-operates with other signalling pathways, namely the cell wall integrity pathway. Hyperosmotic stress treatment of *C. glabrata* regulates genes which are also found in macrophage engulfed *C. glabrata* cells, linking *in vitro* hyperosmotic stress response to *ex vivo* conditions.

4.3.5 Hyperosmotic stress response is not well conserved between the closely related yeasts, *C. glabrata* and *S. cerevisiae*

In *S. cerevisiae*, glycerol is the main osmolyte produced upon hyperosmotic stress treatment **[67]**. In comparing the response of *C. glabrata* to that of its close relative *S. cerevisiae*, it was found that *GPD1* which encodes a glycerol-3-phosphate dehydrogenase, a key enzyme in the production of glycerol, is uniquely regulated under hyperosmotic stress in *S. cerevisiae* and not regulated in *C. glabrata*. However, it was found that *GPD2*, encoding another glycerol-3-phosphate dehydrogenase only regulated under anoxic conditions in *S. cerevisiae*, is regulated in response to hyperosmotic stress in *C. glabrata*. In *S. cerevisiae*, null mutants of these genes display differing phenotypes; *gpd1* cells were sensitive to hyperosmotic stress and *gpd2* cells grew at a decreased rate under anaerobic conditions **[77]**. In the first instance, bioinformatic analysis was undertaken to confirm that these genes were annotated correctly and this annotation was confirmed. The transcriptional regulation of *GPD2* and not *GPD1* under hyperosmotic stress in *C. glabrata* suggested that these genes may have switched roles. Single, as well as double, null mutants were constructed in *C. glabrata* to examine this and these too showed differing phenotypes. In complete contrast to that of *S. cerevisiae*, *gpd1* mutants in *C. glabrata* are not sensitive to hyperosmotic stress but *gpd2* mutants are. Null mutants of *gpd1* display no phenotypes to any of the conditions tested in this study, while *gpd2* mutants have a number of phenotypes. 0.5 M NaCl and 1 M sorbitol have a similar effect on the growth of *gpd2* mutants and at the concentrations used in this study, exert a similar osmolarity. This would suggest that the sensitivity of *gpd2* mutants are due to an increased hyperosmotic stress environment, rather than an ionic or metal ion toxicity effect.

The sensitivity of *gpd2* and *gpd1gpd2* cells to CsCl and $CaCl_2$ are much more severe than the osmolarity they produce should cause. This would suggest that for these compounds, caesium

being a heavy metal and calcium being involved in cell signalling, Gpd2 in *C. glabrata* has a distinct role from just hyperosmotic stress response. CsCl sensing and signalling has been linked to the HOG and cell wall integrity pathways in *S. cerevisiae*. CsCl treatment results in Hog1 phosphorylation and the regulation of targets of the HOG and cell wall integrity pathways in *S. cerevisiae* **[144]**. It has been shown that the response to CsCl is dependent on Hog1 and the transcription factor Yaf9, although there is also independent and Hog1/Yaf9 interdependent regulation as well **[160]**. As discussed previously, null mutants of HOG pathway components are also sensitive to CsCl, which might be expected if the response to this alkali metal cation is Hog1 dependent in *C. glabrata* as it has been shown to be in *S. cerevisiae*. Studies have shown that upon CsCl treatment, Gpp1 and Gpp2, which encode isoforms of glycerol 3-phosphatase, are regulated in *S. cerevisiae* **[160, 177]**. Gpp1 and Gpp2 function in the biosynthesis of glycerol and are the final step in its synthesis after the glycerol 3 phosphate dehydrogenases, Gpd1 and Gpd2 **[177]**. This may help to explain the sensitivity of *gpd2* mutants to CsCl, if glycerol production is needed to effectively adapt to CsCl treatment.

The sensitivity of *gpd2* mutants to $CaCl_2$ would suggest that Gpd2 is involved in calcium signalling. It has been shown in *S. cerevisiae* that *gpd1* mutants show a severe growth defect to $CaCl_2$, however this was part of a large scale screening study and as such no indications of why this phenotype was found was presented **[178]**. Calcium ions are one of the most versatile signalling molecules, found in a range of eukaryotic cells from *S. cerevisiae* to the mammalian heart and their homeostasis and signalling pathways are crucial for the normal growth of *S. cerevisiae*. In *S. cerevisiae*, high levels of external Ca^{2+} ions are transported into the cell by some known and unknown transporters and then sequestered into the vacuole by the vacuolar Ca^{2+} transporters, Pmc1 and Vcx1 to regulate Ca^{2+} signals and to prevent calcium toxicity **[179]**. Mutants of these transporters cause $CaCl_2$ sensitively in *S. cerevisiae* and would suggest that Gpd2 in *C. glabrata* plays some function, directly or in a regulatory role, in the tolerance and transport of Ca^{2+} ions **[180]**. While Gpd2 may play some role in calcium signalling and Ca^{2+} ion tolerance, a much more thorough investigation would be needed to elucidate this. This could involve investigating whether the $CaCl_2$ phenotype displayed by *gpd2* mutants is rescued in the presence of FK506, which has been shown in *S. cerevisiae* to allow the growth of *pmc1* mutants on media containing $CaCl_2$ **[180]**. Interestingly, *gpd2* and the double *gpd1gpd2* cells are more sensitive to hyperosmotic stress

than *hog1* mutants, suggesting, again, that Hog1 is not the most important component of the HOG pathway in *C. glabrata*.

Comparisons of the transcriptional responses of *C. glabrata* and *S. cerevisiae* lead to the identification of a switch in the roles of the glycerol-3-phosphatate dehydrogenases. Not only was *GPD2* required for hyperosmotic stress resistance in *C. glabrata* (in contrast to *S. cerevisiae* where it is *GPD1*) but phenotypic screening revealed it is also required for resistance to $CaCl_2$ and CsCl, implicating it in calcium signalling and HOG pathway-mediated caesium resistance.

4.3.6 The long term transcriptional response of *C. glabrata* to hyperosmotic stress

Analysis of the long term transcriptional regulation of *C. glabrata* to hyperosmotic stress revealed that, as previously seen in other fungi under stress conditions, most of the transcriptional regulation occurs between 15 and 45 minutes after addition of stress **[143]**. The little regulation seen at the very early time point of only 5 minutes is not surprising as it can take some minutes for transcriptional responses to be activated and those signals interpreted into gene expression.

An important mechanism to survive non-ideal environmental conditions is the ability to stop growth while under stress. This can be seen in the delay in growth of *C. glabrata* when exposed to stress. Analysis of the GO terms associated with genes regulated at the 15 minute time point under hyperosmotic stress revealed that while *C. glabrata* is up regulating genes involved in response to stress, it also down regulates genes involved in growth, such as ribosome biogenesis and cellular biogenesis. As this pattern of gene regulation is not seen at the 60 and 120 minute time points it indicates that there is no difference between untreated and stressed cells by these late time points. This suggests that *C. glabrata* cells treated with hyperosmotic stress have recovered by 60 minutes and that their transcriptional activity is now similar to that of a growing population of cells.

By investigating the genes which are regulated at the later time points of the response to hyperosmotic stress it was hoped that information on the long term adaptation and memory of *C. glabrata* could be discovered. By comparing the genes statistically and significantly regulated at 60 minutes with those at 120 minutes, it was found that many stress response genes that are up regulated at 60 minutes are not regulated by 120 minutes treatment with hyperosmotic stress. This gene regulation may be the remnants of the stress response of *C.*

glabrata and as such, not needed by 120 minutes as the cells have started growing again. *FPS1*, which encodes the aqua-glycerol porin, is also commonly down regulated at both 60 and 120 minutes and this down regulation of may be important in maintaining turgor. An *fps1* mutant has been created in our lab and will the screened in the future; the phenotypes it displays will help characterise its function in *C. glabrata*. This gene regulation could be an indication of the long term adaptation of *C. glabrata* to hyperosmotic stress and the fact that 12 of the 32 genes commonly regulated genes are non-orthologues and so their functions are as of yet unknown, could go towards explaining why *C. glabrata* is more resistant to hyperosmotic stress than *S. cerevisiae*.

4.3.7 Attempts to functionally annotate *C. glabrata* genes

The various analysis methods applied in this study have shown that *C. glabrata* specific genes do have some important functions in the response to hyperosmotic stress. Through the transcriptional analysis performed in this study, these *C. glabrata* specific genes can be annotated as being regulated under hyperosmotic stress. This study has shown that many non-homologous or functionally unknown genes are regulated under hyperosmotic stress and these may help explain the increased stress resistance of *C. glabrata* and how it has become a human pathogen.

CAGL0H02563g was identified as having unknown functionality however, through this study it was found to have sequence similarity and be encoded at the syntenic loci of *HOR7* in *S. cerevisiae*. The HOR (HyperOsmolarity Responsive) genes were originally identified as a group of 7 genes which were regulated under hyperosmotic stress conditions. *HOR1, 3, 4, 5* and *6* were found to encode the glycerol-3-phosphate dehydrogenase (Gpd1), the glucokinase (Glk1), the hexose transporter (Hxt1), a heat shock protein (Hsp12) and the cation-ATPase transporter (Ena1), respectively **[181]**. While *HOR2* was later found to encode an isoform of the glycerol 3-phosphatase, Gpp2, the function of Hor7 however remains unknown, except that it's over expression rescues Ca^{2+} sensitivity mutants and increases the resistance of *S. cerevisiae* to high NaCl treatment **[177, 182]**. It has also been shown to be Hog1 dependently regulated in *S. cerevisiae* under hyperosmotic stress **[160]**. As *CAGL0H02563g* is regulated under hyperosmotic stress conditions, its null is not required for resistance to hyperosmotic stress and its protein sequence and syntenic context is homologous to that of *HOR7* in *S. cerevisiae*, this suggests it may be the functional homologue of *HOR7* in *C. glabrata*. To confirm this, an over expression strain of *HOR7* in *C. glabrata* could be constructed and tested for increased resistance to NaCl and the ability to rescue Ca^{2+} sensitive mutants.

Although sequence searches against all genes in *S. cerevisiae* revealed no genes similar to that of *CAG0L09251g* in *C. glabrata*, its syntenic context revealed its homologue to be that of *HAL1*. In *S. cerevisiae*, Hal1 is involved in halo tolerance; decreasing intracellular sodium ions by increasing efflux through the sodium pump Ena1 and increasing intracellular potassium ions by decreasing efflux. Studies have shown that its expression is induced by NaCl, KCl and sorbitol through Gcn4 and overexpression of *HAL1* improves growth under NaCl stress in *S. cerevisiae* **[183]**. Null mutants of *hal1* in *S. cerevisiae* are however, not sensitive to hyperosmotic stress or show any other phenotype **[183]**. Null mutants of *hal1* in *C. glabrata* also display no sensitivity to hyperosmotic stress or any other phenotypic screen condition. A search of the upstream region of *HAL1* in *C. glabrata* for potential DNA binding motifs found it contained predicted sites for Rlm1, Cin5, Msn2/4 and Sko1. These transcription factors are all transcriptionally regulated under hyperosmotic stress conditions in *C. glabrata* and are known to be involved in the response to hyperosmotic stress in *S. cerevisiae*, making them all potential regulators of *HAL1*. As in *S. cerevisiae*, a Ste12 binding motif was also found in the upstream region of *HAL1* in *C. glabrata* **[45]**. While *STE12* is not transcriptionally regulated under hyperosmotic stress in *C. glabrata*, its protein product is known to be activated by phosphorylation, rather than an increase in abundance, so may still be regulating *HAL1* in *C. glabrata*. Testing the phosphorylation state of Ste12 under hyperosmotic stress conditions by western blot, as well as its protein binding partners using a Yeast-2-Hybrid system would help to elucidate its function in *C. glabrata* upon hyperosmotic stress. Dr. Hsueh-lui Ho (a member of the Haynes lab) has created a collection of approximately 3000 *C. glabrata* ORFs that have been cloned into Gateway entry vectors for use with the Gateway System (Invitrogen). This allows the ORF of interest to be shuttled into any destination vector facilitating its use in localisation studies (GFP, RFP, YFP), Yeast-2-Hybrid assays (bait and prey) and over-expression studies (constitutive and inducible promoters). This system could be used with *HAL1* and *STE12* to explore their protein binding targets, cellular localisation and the affect of their over-expression. As the over-expression of *HAL1* in *S. cerevisiae* results in increase hyperosmotic stress resistance, over-expression studies of *HAL1* in *C. glabrata* would reveal if it has a similar function.

Many other non-homologous and functionally unknown genes are regulated by *C. glabrata* in response to hyperosmotic stress. Structural predictions of these genes revealed interesting putative functions including protein kinases and transcription factors. If these predictions hold true, these protein kinases may be novel components of the MAPK pathways in *C.*

glabrata. As null mutants of these genes were unavailable in the current *C. glabrata* null mutant library, phenotypic screening could not be conducted and therefore the creation of null mutants of these genes and the subsequent phenotypic screening would be the next step in elucidating their function in *C. glabrata*.

Characterisation attempts of functionally unknown and *C. glabrata* specific genes allowed the identification of novel genes involved in hyperosmotic stress response. These included putative protein kinases and transcription factors, as well as the identification of homologues in *C. glabrata* for *HOR7* and *HAL1*, both known to be involved in hyperosmotic stress response in *S. cerevisiae*.

4.3.8 A model of the hyperosmotic stress response specific to *C. glabrata*

The model below was produced by combining the functional genomics and transcriptional analysis of *C. glabrata* presented in this chapter (Figure 4.11). It illustrates the functional implications of the phenotypes discussed, the potential overlap between stress response pathways and transcriptional re-wiring of the HOG pathway in *C. glabrata* compared to its close relative, *S. cerevisiae*. This diagram also gives a framework for the further investigation of the response of *C. glabrata* to hyperosmotic stress.

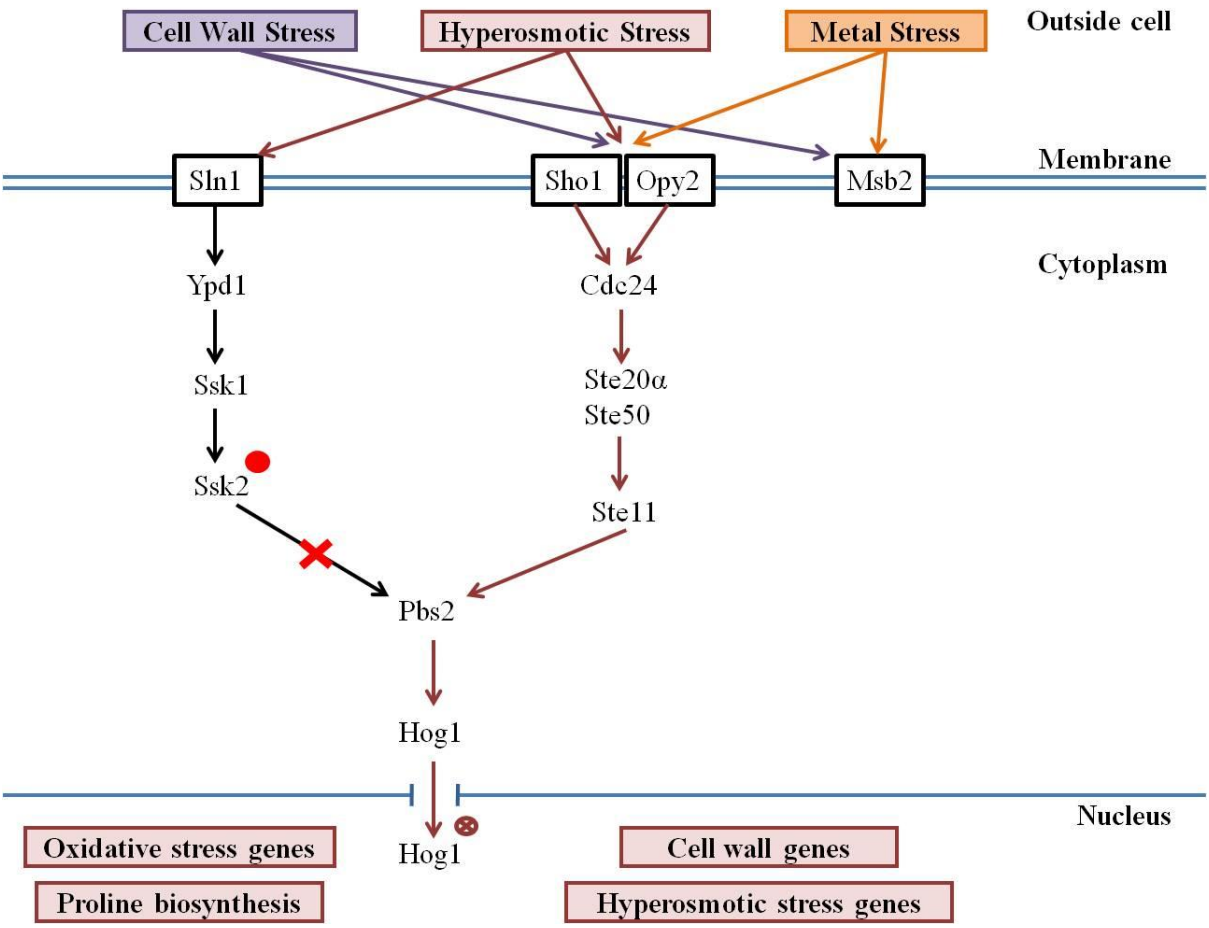


Figure 4.11: Model of the HOG pathway in *C. glabrata* ATCC 2001. The stresses conditions which the osmotic sensors in the membrane were observed to be sensitive to during phenotypic screening are indicated with correspondingly coloured arrows. The transcriptional response of *C. glabrata* to hyperosmotic stress is shown using red coloured arrows through the HOG pathway and resulting in transcriptional regulation of the boxed groups of genes. The cross enclosed by a circle represents the phosphorylation of Hog1 under hyperosmotic stress. The circle and red cross represent the point mutation in Ssk2 and subsequent loss of signalling though the Sln1 pathway.

5 Oxidative stress adaptation in *C. glabrata*

5.1 Chapter overview

Oxidative stress occurs when the concentration of reactive oxygen species present is higher than which the organism can readily detoxify or repair the damage resulting from this oxidative insult. As discussed in the introduction, *C. glabrata* is a pathogen which is engulfed by macrophages. During this process it encounters oxidative stress from the production of reactive oxygen species (ROS) by cells of the immune system, which damage DNA and biomolecules. Proteins involved in oxidative stress resistance have been implicated in the maintenance of virulence in other pathogenic fungi. Therefore, it is important to study the oxidative stress response in the human pathogen, *C. glabrata*.

Studies of the transcriptional response of *C. glabrata* to oxidative stress have shown that this response is dependent on the oxidative stressor used, for example, menadione compared to hydrogen peroxide **[88]**. Many other studies have used hydrogen peroxide to simulate oxidative stress conditions including those conducted in *S. cerevisiae* which will be used to compare to the oxidative stress response of *C. glabrata* **[1]**. Using the same compound to elicit oxidative stress as many other published microarray data sets allows more accurate comparisons to be made.

Three concentrations of hydrogen peroxide were established that had varying effects on the growth of wild type *C. glabrata* cells: 1, 10 and 100 mM H_2O_2 (taken from **[71]**). Figure 5.1 shows the cell density over time under these defined doses of oxidative stress. 1 mM H_2O_2 treatment results in a small growth inhibition compared to untreated cells, while 10 mM H_2O_2 treated cells display a more prominent growth defect. 100 mM H_2O_2 treated cells do not show an increase in cell density over time.

From previously published data, key proteins involved in the oxidative stress response of *C. glabrata* were identified and targeted for gene deletion, if they were not available from the mutant library. *YAP1* and *SKN7* encode the two main transcription factors involved in oxidative stress regulation and null mutants of these genes were phenotypically screened, along with the null mutants *cta1* and *sod1*, which encode catalase and a superoxide dismutase, respectively. The phenotypes observed and their functional implications will be presented.

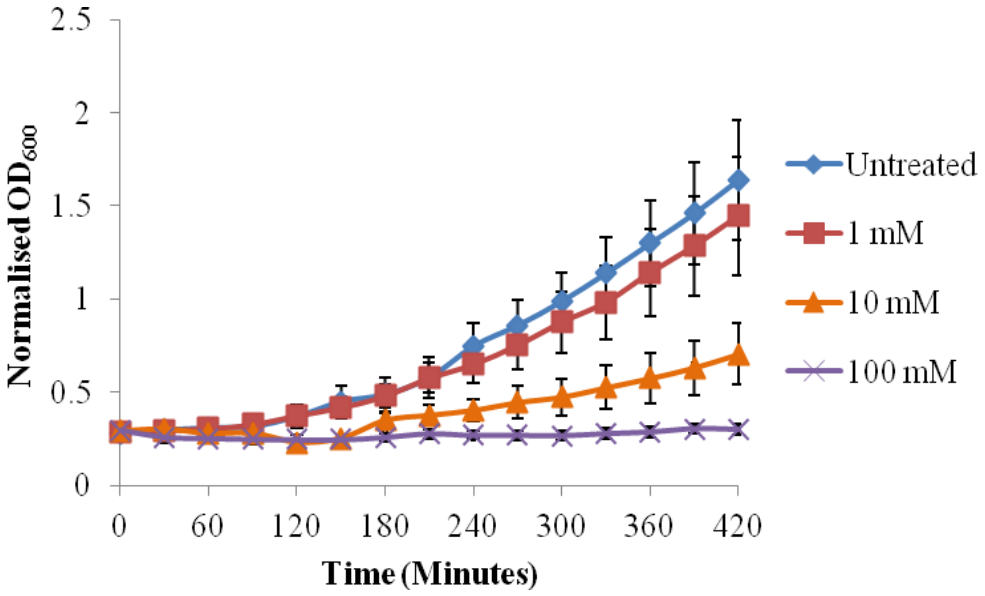


Figure 5.1: Growth of wild type *C. glabrata* to defined doses of oxidative stress. Wild type *C. glabrata* were grown in either untreated YPD or YPD containing the indicated concentration of H_2O_2 and OD_{600} measurements were taken every 30 minutes over a 420 minute timecourse. OD_{600} measurements were normalised by subtracting the background absorbance of blank media. Error bars for the standard deviation of three biological replicates are shown.

DNA microarray experiments were conducted with *C. glabrata* using the three defined concentrations of H_2O_2 (as described in Chapter 3) over an hour timecourse with time points at 15 and 60 minutes. Analysis of this microarray data showed that little gene regulation occurred in the 100 mM H_2O_2 treated cells and the expression of many transcripts did not change over time. This is in contrast to *C. glabrata* cells treated with 1 and 10 mM H_2O_2 which displayed many significantly regulated genes. This lack of gene expression over time correlates with the complete inhibition of growth of 100 mM H_2O_2 treated *C. glabrata* cells. As a result, the 100 mM H_2O_2 treated microarray data set was not used in this study.

The microarray experiments conducted in this study under oxidative stress conditions have been compared to the response of *C. glabrata*'s close relative, *S. cerevisiae*, to elucidate whether this stress response is conserved. As oxidative stress is encountered by *C. glabrata* when engulfed by macrophages, genes significantly regulated by the addition of oxidative stress have also been compared to published data on the response of *C. glabrata* when ingested by macrophages.

As discussed in the introduction, Yap1 is one of the main transcription factors governing the oxidative stress response in fungi. In *C. glabrata*, the DNA binding domain of Yap1 is different from the *S. cerevisiae* Yap1; a lysine has been substituted for an arginine. This change has been reported to affect the transcriptional targets of Yap1 in *C. glabrata* **[30]**. While Yap1 dependent targets of *C. glabrata* have been identified in response to benomyl

treatment using a range of techniques, these targets have not been characterised so thoroughly in response to hydrogen peroxide induced oxidative stress **[110]**. As such, microarray experiments were conducted in *C. glabrata*, using a *yap1* mutant under oxidative stress conditions induced by hydrogen peroxide. Genes affected by the loss of Yap1 under oxidative stress were identified and compared to those known to be targets in *S. cerevisiae* and those identified under benomyl stress in *C. glabrata* **[1, 110]**.

The transcript profiling and functional genomics analyses presented in this study have helped to characterise the oxidative stress response of this human pathogen as well as allowing the identification of new components involved in the oxidative stress response of *C. glabrata*.

5.2 Results

5.2.1 *YAP1*, *SKN7*, *SOD1* and *CTA1* are required for oxidative stress resistance

Null mutants of key genes involved in the oxidative stress response of *C. glabrata* were either constructed in this study or obtained from the mutant library and subjected to phenotypic screening (see Chapter 3). Figure 5.2 shows the sensitivity of these null mutants to oxidative stress. *yap1* null mutants are most sensitive to oxidative stress, followed closely by *skn7* cells, while *sod1* and *cta1* cells show moderate sensitivity.

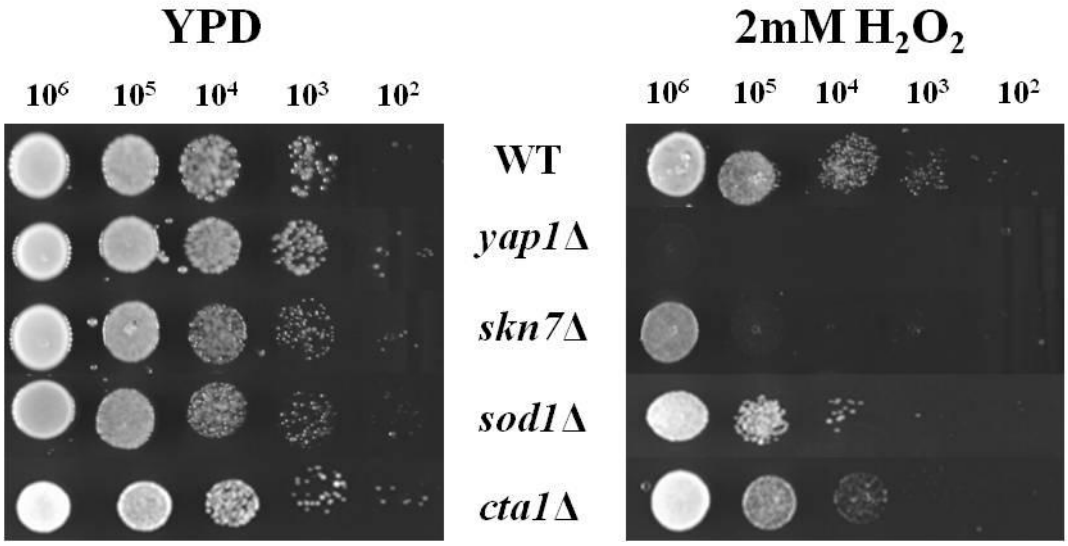


Figure 5.2: Oxidative stress sensitivity of *C. glabrata* null mutants. Overnight cultures were diluted to an OD_{600} of 0.2, tenfold serial dilutions were made and these were spotted on to solid media: either YPD (untreated control) or YPD containing 2 mM H_2O_2. Plates were incubated at 30°C for 1 day.

While growth of *yap1* cells were severely inhibited at low concentrations of H_2O_2 on solid media, these cells can grow over time under increased concentrations of H_2O_2 in liquid media, as shown in Figure 5.3. This graph also shows that *yap1* cells grow to a lower final biomass compared to wild type cells.

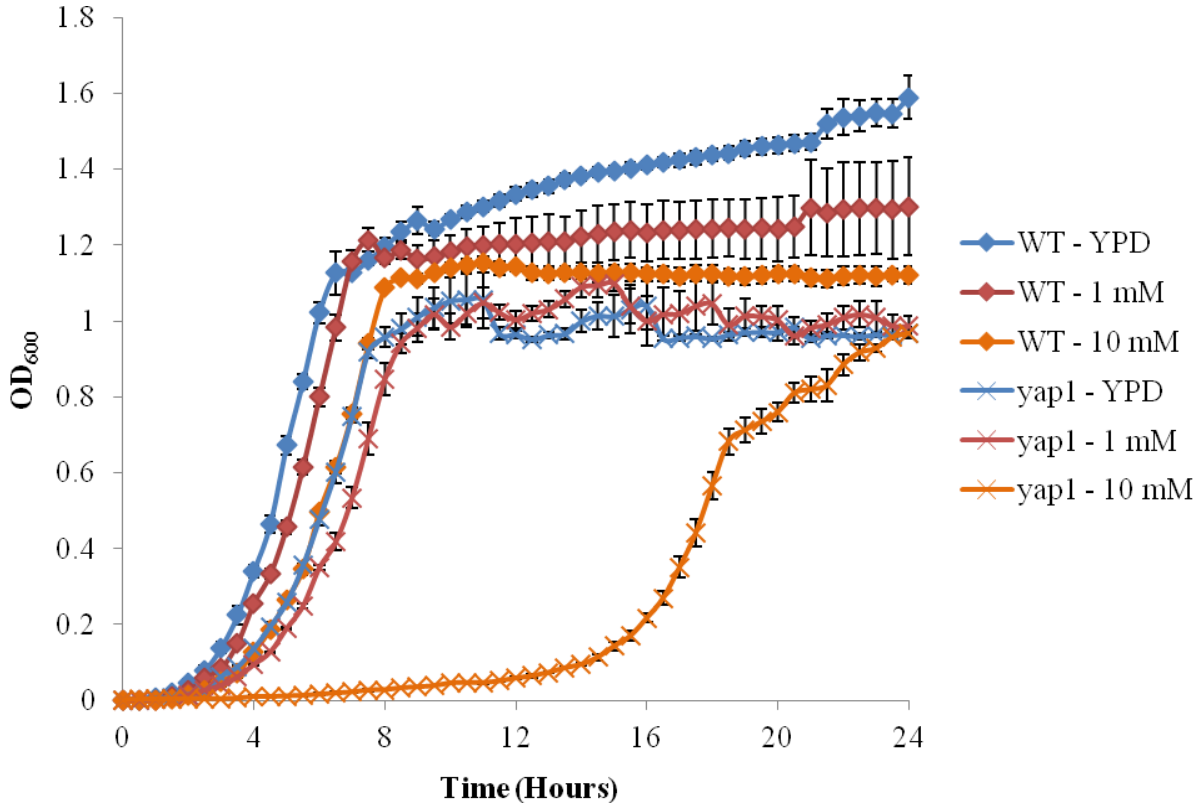


Figure 5.3: Growth of wild type and *yap1* mutants under increasing oxidative stress. Wild type *C. glabrata* and YAP1 null mutants were grown in either untreated YPD or YPD with the indicated concentration of H_2O_2 added at the start of the time course. OD_{600} measurements were taken every 30 minutes over a 24 hour timecourse. OD_{600} measurements were normalised by subtracting the background absorbance of blank media. Error bars for the standard deviation of three biological replicates are shown.

5.2.2 Phenotypic screening of *yap1*, *skn7*, *sod1* and *cta1*

Null mutants of *yap1*, *skn7*, *sod1* and *cta1* were phenotypically screened on over 50 conditions (see Chapter 3). Table 5.1 summarises the growth phenotypes found for these null mutants in *C. glabrata*.

Mutants of these oxidative stress genes are viable and all display a similar growth phenotype to the parental strain in rich media at 30°C, except *sod1* mutants. *sod1* null mutants show a slow growth phenotype and therefore a severe growth defect must be observed to be a true phenotype. *sod1* mutants were sensitive to both 16°C and 42°C, while other mutants were not sensitive to these changes in temperature. While *yap1*, *skn7* and *sod1* were all sensitive to an elevated temperature of 42°C in combination with oxidative stress, they were also sensitive to oxidative stress applied on its own. While *yap1*, *skn7* and *sod1* mutants all showed varying growth phenotypes to the different oxidative stressors, H_2O_2 and tBOOH, only a slight growth defect to the highest concentration of H_2O_2 was observed in *cta1* mutants. *sod1* was the only null mutant for which sensitivity to menadione was observed.

Null mutants of these oxidative stress genes were also observed to be sensitive to divalent cations and heavy metals. All showed varying degrees of sensitivity to $CoCl_2$. *yap1* and *sod1* mutants were sensitive to $CdCl_2$, while *skn7* and *cta1* did not show this phenotype. All mutants were observed to show varying sensitivity to $MnCl_2$, except *sod1*. *yap1*, *sod1* and *cta1* mutants were sensitive to $ZnCl_2$ however, only *yap1* mutants showed a growth defect on media containing CsCl.

Only *sod1* null mutants showed growth inhibition to the cell wall stress calcofluor white and nitrogen starvation conditions, as well as on media containing 6% ethanol and vanadate. *yap1* mutants were the only mutants to show sensitivity to arsenic compounds.

Table 5.1: Summary of phenotypes observed in the null mutants: *yap1*, *skn7*, *sod1* and *cta1*.

Phenotype	Control	Temperature		Oxidative + temperature	Oxidative stress				Divalent cations and heavy metals					Cell wall defects	Ethanol sensitivity	Nitrogen starvation	Cell cycle protein synthesis	Protein glycosylation	Protein folding defects	
Condition / **Strain**	30° C	16° C	42° C	42° C + 2 mM H_2O_2	2 mM H_2O_2	5 mM H_2O_2	5 mM tBOOH	100µM Menadione	1 mM $CoCl_2$	2 mM $CdCl_2$	8 mM $ZnCl_2$	30 mM $MnCl_2$	250 mM CsCl	1 mg/ml Calcofluor white	6 % Ethanol	No ammonium sulphate	1 µg/ml cyclohexamide	2.5 mM vanadate	1 mM Arsenic oxide	0.5 mM Meta-arsenite
Wild type	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
yap1	N	N	N	S	S	L	L	N	L	S	S	S	S	N	N	N	S	N	S	S
skn7	N	N	N	S	N	L	L	N	S	N	N	L	N	N	N	N	N	N	N	N
sod1	S*	S	S	S	S*	S	S	S	S	S	S	S*	S*	S	L	S	S*	L	S*	S*
cta1	N	N	N	N	N	S*	N	N	L	N	S	S	N	N	N	N	N	N	N	N

Labels denote: N: normal growth (grey); S*: slight growth defect (light orange); S: sensitive (orange); L: lethal (dark pink). Growth defect of 1 or 2 isolates and 2 or 3 biological replicates.

5.2.3 Phenotypic screening of YAP family null mutants

As discussed previously in the introduction, Yap1 is one of eight proteins found in the YAP (Yeast Activator Protein) family in *S. cerevisiae*. All YAP proteins are AP-1-like basic leucine zipper (bZIP) transcription factors. Table 5.2 shows the homologues identified in *C. glabrata* for these seven other YAP genes; note that no homologue was identified for Yap8 and that Yap3 has three possible homologues.

Table 5.2: Homologues of the YAP family of proteins in *C. glabrata*

YAP gene	***S. cerevisiae* standard name**	***C. glabrata* systematic name**	**Description in *S. cerevisiae***	**Null mutant available in *C. glabrata***
YAP2	*CAD2*	*CAGL0F03069g*	Involved in stress responses, iron metabolism, and pleiotropic drug resistance; controls a set of genes involved in stabilizing proteins	Yes
YAP3	*YAP3*	*CAGL0K02585g*	Basic leucine zipper (bZIP) transcription factor	No
		CAGL0M10087g	Yap3-like; not located at syntenic loci	Yes
	YPS gene	*CAGL0E01859g*	Aspartic protease, member of the yapsin family of proteases involved in cell wall growth and maintenance; attached to the plasma membrane via a (GPI) anchor	Yes
YAP4	*CIN5*	*CAGL0H08173g*	Mediates pleiotropic drug resistance and salt tolerance; nuclearly localized under oxidative stress	No
YAP5	*YAP5*	*CAGL0K08756g*	Basic leucine zipper (bZIP) iron-sensing transcription factor	Yes
YAP6	*YAP6*	*CAGL0M08800g*	Overexpression increases sodium and lithium tolerance; computational analysis suggests a role in regulation of carbohydrate metabolism	Yes
YAP7	*YAP7*	*CAGL0F01265g*	Putative basic leucine zipper (bZIP) transcription factor	Yes
YAP8	*ARR1*	-	Transcriptional activator of the basic leucine zipper (bZIP) family, required for transcription of genes involved in resistance to arsenic compounds	-

S. cerevisiae standard names and descriptions from SGD, along with their homologue in *C. glabrata* are given **[25, 29, 45]**.

Null mutants of the YAP genes available in *C. glabrata* were phenotypically screened on a wide range of phenotypic conditions (see Chapter 3). None of these YAP family null mutants were observed to be sensitive to oxidative stressors. They also displayed no phenotypes to

any other condition screened, except that of *yap7* mutants to $MnCl_2$ (Figure 12.1, p.327, Appendix III).

5.2.4 The immediate transcriptional response of *C. glabrata* to oxidative stress

Microarray analysis was performed using *C. glabrata* cells treated with oxidative stress. Using microarray data with three replicates from cells treated with 1 mM and 10 mM H_2O_2 for 15 minutes, statistical analysis using 2-way ANOVA was applied. This found that 15.6% (812 genes) of the total *C. glabrata* genome was significantly regulated upon oxidative stress treatment with 1 mM H_2O_2 (>2 fold compared to untreated cells at 15 minutes, p-value <0.05); 388 genes were up regulated, while 424 genes were down regulated. When the concentration of H_2O_2 was increased to 10 mM the number of genes significantly regulated also increased; 1505 genes (29% of the genome) were significantly regulated (>2 fold compared to untreated cells at 15 minutes, p-value <0.05), including 725 up regulated genes and 780 down regulated genes.

GO term enrichment analysis was conducted on these data sets. Results from these analyses for 1 mM H_2O_2 can be found in Table 5.3 and Table 5.4, while GO terms associated with 10 mM H_2O_2 are shown in Table 5.5 and Table 5.6.

GO terms associated with these up regulated genes include genes involved in the response to oxidative stress and the homeostasis of metal ions. Genes involved in proteolysis are also up regulated. Nearly a third of genes up regulated upon 1 mM H_2O_2 treatment are localised to the mitochondria (112/388).

GO terms associated with these down regulated genes include those involved in ribosome biogenesis, rRNA processing and the biosynthesis of sterol and ergosterol. Many genes (277 of a total of 424 genes significantly down regulated) are localised to the nucleus.

GO terms associated with those genes regulated upon treatment with 10 mM H_2O_2 include the response to oxidative stress, mitochondrion degradation and regulation of metal ions, as well as the catabolism of sugars. Genes up regulated upon treatment with an increased concentration of H_2O_2 are localised to the mitochondrion and peroxisome.

GO terms associated with these down regulated genes include those involved in ribosome biogenesis, the cell cycle and sterol and ergosterol biosynthetic processes. More than half of genes significantly down regulated upon 10 mM H_2O_2 treatment are localised to the nucleus (408/780).

Many of the genes regulated upon treatment with 1 mM H_2O_2 for 15 minutes are also regulated when *C. glabrata* is treated with 10 mM H_2O_2, as shown in the Venn diagrams (Figure 5.4). Few genes are regulated by 60 minutes under 1 mM H_2O_2 treatment and as a result this comparison is not shown. Most of the genes significantly regulated in response to 1 mM H_2O_2 are also regulated by treatment with 10 mM H_2O_2.

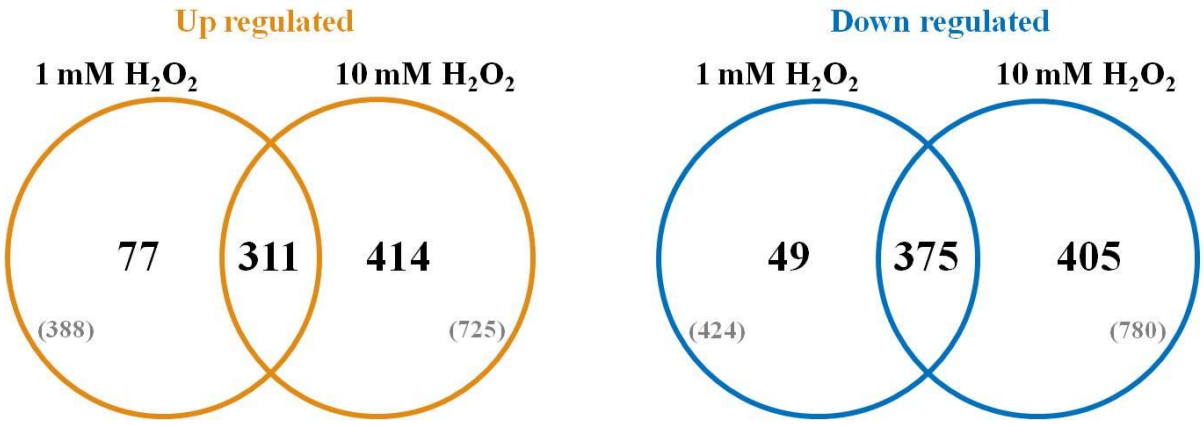


Figure 5.4: Venn diagrams comparing genes regulated by *C. glabrata* in response to 1 mM H_2O_2 and 10 mM H_2O_2. The number of genes significantly regulated by more than 2 fold upon 1 mM H_2O_2 and 10 mM H_2O_2 treatment are shown. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

Table 5.3: GO terms associated with up regulated genes under oxidative stress (1 mM H_2O_2, 15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Cellular response to oxidative stress	$7.16\ e^{-9}$	*FRT2 UGA2 GPX2 TRX3 PST2 CTA1 TRR1 GRX2 GRX4 BLM10 HSP12 TRX2 SOD2 SKN7 DOT5 SRX1 MCR1 FMP46 CCP1 AHP1 YAP1 TSA1 GAD1 GRE2 GLR1 AFT2*	26	55
Homeostasis of metal ions	$3.601\ e^{-6}$	*NFS1 CCC2 FTR1 ERV1 UTR1 NFU1 OCT1 COX17 SMF3 FRE8 AHP1 SSQ1 CCS1 HEM15 ISU2 ISU1 MMT2 ISA2*	18	98
Proteolysis	$6.72\ e^{-6}$	*PIM1 CYM1 PRB1 AFG3 DDI1 RPN12 MAS2 LAP4 OCT1 YPS1 MAS1 PRC1 PEP4 YME1*	15	74
Mitochondrion	$1.916\ e^{-13}$	*YAT1 FMP23 MRPS9 ILV6 TRX3 CDC48 LYS21 GRX2 AFG3 ALD5 ERV1 COX18 TDH3 SPG1 SOD2 MAS2 OYE2 SSQ1 CCS1 MMT2 ISA2 MRP2*	112	1072

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.4: GO terms associated with down regulated genes under oxidative stress (1 mM H_2O_2, 15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	$<1\ e^{-14}$	*UTP20 MAK5 ENP1 REI1 SPB1 KRR1 PWP2 NOP1 DBP10 TSR1 NOP14 SAS10 FAP7 NHP2 NOP6 FAL1*	132	170
rRNA processing	$<1\ e^{-14}$	*NAF1 IPI3 JJJ1 KRI1 DBP6 ESF2 DIS3 TSR4 NOP12 REX4 RRP6 BUD21 PUS7 YTM1 RRP36 NOP58 NOP4*	127	195
Nucleus	$<1\ e^{-14}$	*THG1 SLX9 UTP22 NOP7 CLB6 PPT1 UTP8 ENP2 MTR3 NSR1 ELP2 SDA1 YGR251W*	277	1965
Sterol biosynthetic process	$4.05\ e^{-5}$	*ERG11 HMG1 ERG13 ERG5 ERG2 ERG12 CYB5 MVD1 IDI1*	9	29
Ergosterol biosynthetic process	0.000342	*ERG1 ERG11 HMG1 ERG13 ERG5 ERG2 ERG12*	7	23

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.5: GO terms associated with up regulated genes under oxidative stress (10 mM H_2O_2, 15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Cellular response to oxidative stress	$1.058\ e^{-9}$	*ZTA1 GPX2 TRX3 TRR1 GRX2 GRX4 HSP12 ERV1 TRX2 GRE3 SRX1 MCR1 AHP1 TSA1 GAD1 GCY1 GLR1 SOD1 UGA2 GND1 DOT5 YJR096W MSN4 CCP1 AFT2 FRT2 PST2 CTA1 BLM10 SOD2 SKN7 FMP46 CCP1*	33	55
Mitochondrion degradation	$1.087\ e^{-6}$	*ATG8 ATG20 CIS1 ATG1 ATG7 ATG32 UTH1 ATG33 ATG17 YOR019W ATG29 ATG11 ATG13*	13	29
Homeostasis of metal ions	$5.431\ e^{-5}$	*SCO2 NFS1 YDR506C PMC1 ERV1 UTR1 SOD1 NFU1 OCT1 COX17 FRE8 AHP1 CDC25 HEM15 ISU2 ISU1 AFT2 MMT2 ISA2*	23	98
Sugar, glucoside, polyol and carboxylate catabolism	$3.457\ e^{-11}$	*TPS1 PGK1 GPM2 PSA1 NTH1 KGD2 AMS1 TDH3 ENO1 GRE3 UGP1 SDH2 TPS3 IDH1 ZWF1 CIT1 CIT3 TKL1*	30	81
Mitochondrion	$<1\ e^{-14}$	*NFU1 MDH1 CYT2 OAC1 OCT1 MCR1 GPM1 MST1 SDH2 MAS1 PUS5 UPS2 UPS1 SYM1 HSP60 DCS1 ACO1 TMA10 NIT3 ATG33*	183	1072
Peroxisome	$6.336\ e^{-7}$	*PCS60 GPD1 PEX7 PEX5 CTA1 FAA2 PNC1 STR3 POX1 PEX4 PEX28 PEX18 TES1 FOX2 PEX13 ECI1 PEX12 MLS1 SPS19 PEX15 LPX1*	21	66

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.6: GO terms associated with down regulated genes under oxidative stress (10 mM H_2O_2, 15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	$<1\ e^{-14}$	*MPP10 URB2 TOR1 MRT4 URB1 MAK11 DHR2 RRP14 UTP11 EBP2 DBP7 UTP30 DRS1 SOF1 RIX7 NOC3 RLP24 FCF2 DIP2 CBF5 ECM16 RRB1 RRP5*	140	170
Cell cycle	0.000205	*CLN3 BUD3 CIN8 CDC14 CDC20 NOP7 CLB6 SDA1 CDC6 TOR1 BUD4 CLB4 BUD8 YOX1 CLN1 SUN4 CDC31 NUD1 CLB2 CLB5*	57	316
Sterol biosynthetic process	$3.45\ e^{-5}$	*UPC2 ERG4 NCP1 ERG20 HMG1 ERG13 ERG5 ERG2 ERG12 CYB5 MVD1 IDI1*	12	29
Ergosterol biosynthetic process	0.000554	*ERG4 NCP1 ERG20 HMG1 ERG13 ERG5 ERG2 ERG12 ERG10*	9	23
Nucleus	$1.00\ e^{-14}$	*FUN30 MAK16 POP5 CLN3 ECM1 RRN6 NCL1 LSM2 YBL028C POL12 MPP10 POL31 REC107 RPA12 HAM1 LIA1 JJJ3*	408	1976

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

5.2.5 The transcriptional response of *C. glabrata* over time to oxidative stress

The expression of *C. glabrata* genes over time were analysed using hierarchical clustering methods. All genes shown to be statistically and significantly regulated in response to oxidative stress were used to create the Dendogram below. Figure 5.5 shows that while genes are regulated by 15 minutes treatment with 1 mM H_2O_2, the regulation of these genes is absent by 60 minutes. Few genes are significantly regulated by this 60 minute time point compared to untreated cells revealing that the transcriptional regulation of these cells is similar to that of an untreated growing culture of *C. glabrata*. As already shown in Figure 5.4, this Dendogram shows that the transcriptional response after 15 minutes to both 1 mM and 10 mM H_2O_2 are similar. However, unlike when *C. glabrata* is treated with 1 mM H_2O_2, 10 mM H_2O_2 treated cells continue to differentially regulate genes compared to untreated cells at the 60 minute time point. These genes are also more highly expressed at the 60 minutes time point compared to after 15 minutes treatment, for both induced and repressed transcripts.

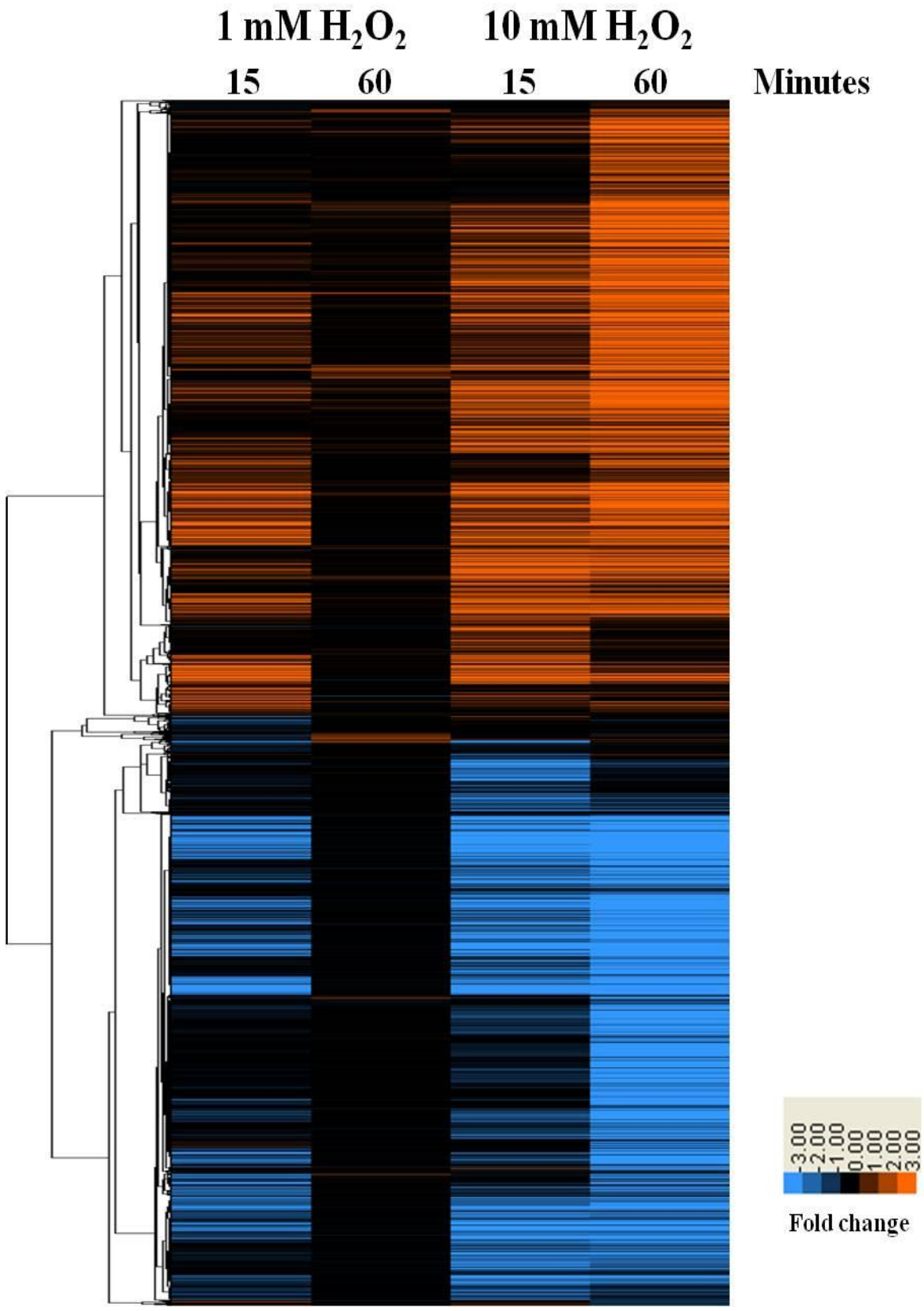


Figure 5.5: Dendogram of all statistically significant genes regulated in response to oxidative stress over time and increasing concentration of stress. Genes were clustered by hierarchical methods using centroid linkage in Cluster3 and visualised using TreeView **[141, 142]**. The fold change compared to untreated cells is shown; orange denotes up regulated genes, blue denotes down regulated genes.

5.2.6 The regulation of genes involved in oxidative stress is dose dependent in *C. glabrata*

C. glabrata genes involved in the oxidative stress response were identified from the role of their homologues in *S. cerevisiae.* When their expression over time and increasing concentration of oxidative stress (H_2O_2) was analysed, a pattern emerged. Figure 5.6.A shows the trend in expression over time and increasing concentration of H_2O_2 using 16 of the major oxidative stress genes annotated to the oxidative stress response in *S. cerevisiae* and which are also regulated in response to H_2O_2 treatment by *C. glabrata* (shown in Figure 5.6.B).

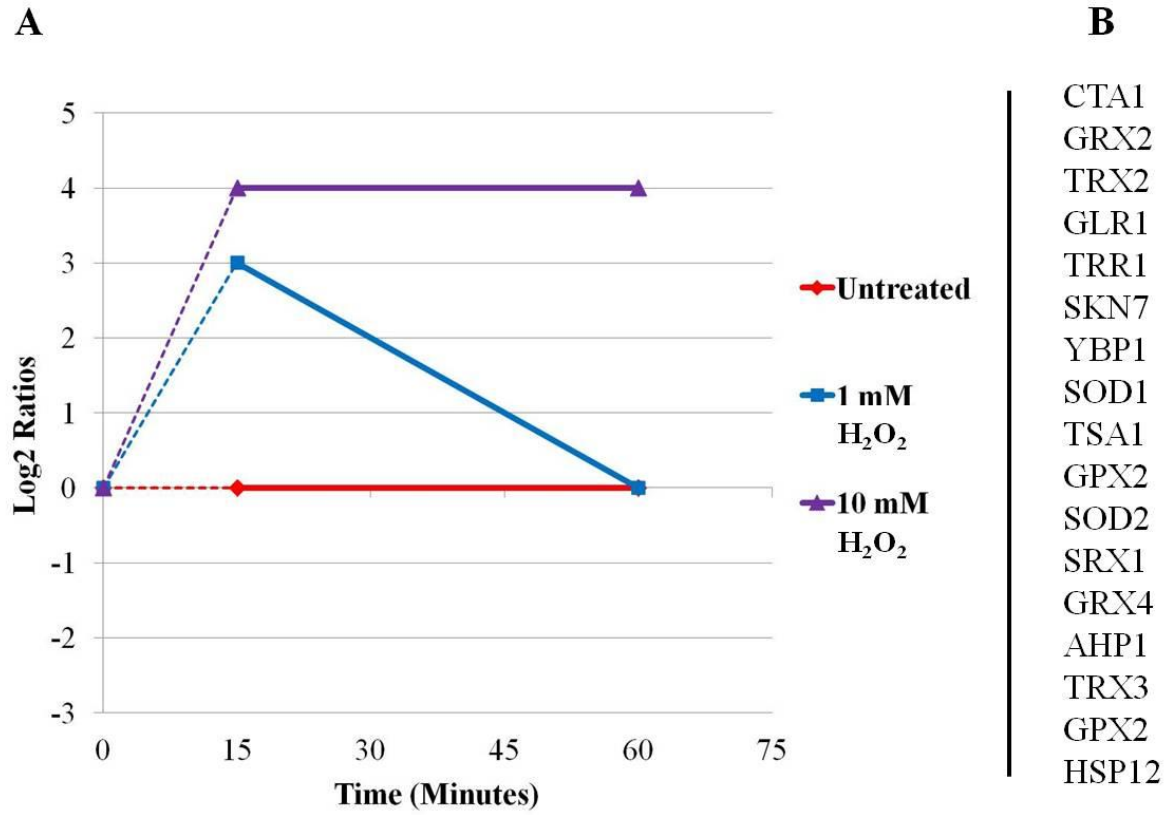


Figure 5.6: The expression trend of oxidative stress genes under oxidative stress treatment in *C. glabrata*. A: The graph shows the trend line Log_2 ratios of genes regulated upon treatment with the indicated concentration of oxidative stress; B: The major oxidative stress genes whose expression was used in A.

Using Euclidian distance metrics, other genes with a similar expression pattern as the oxidative stress genes shown in Figure 5.6 were identified. Using *TRR1* as the example gene, 69 genes were identified as shown in Table 5.7. These include genes known to be involved (in *S. cerevisiae*) in oxidative stress, metal resistance and those of unknown function as well as non-homologous genes.

19 of the 69 genes identified with this similar regulation pattern under oxidative stress treatment were found to be either non-homologous to any gene in *S. cerevisiae* or encode

proteins of unknown function. Structural predictions of these 19 genes revealed two candidates for deletion: *CAGL0F07359g* and *CAGL0L10186g*.

Table 5.7: Genes with a similar regulation pattern over time and increasing concentration of oxidative stress.

CAGL0D04840g	*YML131w*	*YCL026CB*	*GPX2*	*GRE3*	*ISA2*	*RIB4*	*RIB1*	*HSP31*
CAGL0M00132g	*YLR108c*	*YMR140w*	*YCF1*	*TSA1*	*MMT2*	*FOL3*	*NGL2*	*AHA1*
CAGL0M05401g	*YHR112c*	*YGL114w*	*GLR1*	*GRE2*	*ISU1*	*TDH3*	*HBS1*	*PLC1*
CAGL0G03993g	*YCR076c*	*YHR131c*	*SOD2*	*CCS1*	*ISU2*	*GCY1*	*SUA5*	*ERF2*
CAGL0F01815g	*YHR029c*	*YOR052c*	*TRX2*	*OYE2*	*SSQ1*	*HIS3*	*MAS1*	*MUC1*
CAGL0I08151g	*YGL117w*	*ISD11*	*GRX2*	*DUG3*	*FRE8*	*SCS7*	*DOA4*	
YBR062c	*YEL047c*	*ECM38*	*CTA1*	*UTH1*	*MRS3*	*YAH1*	*LAP4*	
YMR090w	*YDL129w*	*SRX1*	*GLO1*	*OCT1*	*SMF3*	*MET8*	*HSP78*	

For each *C. glabrata* gene identified as displaying the same regulation pattern as the oxidative stress genes shown in Figure 5.6 either the standard or systematic name of its corresponding *S. cerevisiae* homologue is shown. If it is a non-homologous gene the *C. glabrata* name is shown. Genes are coloured dependent on their function in *S. cerevisiae* (**[45]**) and arranged by decreasing number as follows: unknown function (grey); oxidative stress response (blue); metal resistance and transport (red); biosynthesis of vitamins, amino acids, cofactors and prosthetic groups (orange); transcription and translation (yellow); proteases (green); heat shock (brown); and other (purple).

CAGL0F07359g is homologous to the *S. cerevisiae* gene, *YGL117w*, which encodes a putative protein of unknown function **[25, 29, 45]**. Structural analysis of *CAGL0F07359g* revealed it contains a predicted nickel superoxide dismutase (NiSOD) domain (Personal communication, Mark Wass). SODs are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide and use metal ions as co-factors. Of those present in *S. cerevisiae* and *C. albicans*, all contain manganese or copper and zinc cores. NiSODs have not been characterised in *S. cerevisiae* or the *Candida* clade. The closest relative to *C. glabrata* in which a NiSOD has been described is in the actinobacterial genus, *Streptomyces* **[184]**.

CAGL0L10186g is homologous to the *S. cerevisiae* gene, *YOR052c*, which encodes a nuclear protein of unknown function **[48]**. Structural analysis of *CAGL0L10186g* revealed a predicted DNA-binding domain; making it a possible transcription factor (Personal communication, Mark Wass). This gene has been shown to be Yap1 dependent in *C. glabrata* in response to benomyl treatment **[110]**.

Null mutants of *CAGL0F07359g* and *CAGL0L10186g* were constructed and phenotypically screened. This revealed these null mutants were not sensitive to any condition screened, including oxidative stressors.

5.2.7 The transcriptional response of *C. glabrata* ingested by macrophages compared to the addition of exogenous oxidative stress

Microarray data by Kaur *et al*, 2007 of *C. glabrata* cells ingested by macrophages for 2 and 6 hours was used to compare to *C. glabrata* cells treated with 1 mM H_2O_2 **[2]**. The 1 mM H_2O_2 microarray dataset was used as this is closest to the reported concentration of H_2O_2 inside a macrophage (0.4 mM) **[88]**. Genes significantly regulated by *C. glabrata* cells ingested by macrophages were used to compare to genes significantly regulated by 1 mM H_2O_2 treated cells, irrespective of time. A total of 863 and 1031 genes were significantly regulated by 1 mM H_2O_2 treatment and macrophage engulfment, respectively. The Venn diagram below (Figure 5.7) shows that 121 genes are commonly regulated by *C. glabrata* in response to oxidative stress treatment and ingestion by macrophages. This equates to 14% of genes regulated by *C. glabrata* in response to 1 mM H_2O_2 treatment. Genes commonly regulated by *C. glabrata* in response to oxidative stress treatment and ingestion by macrophages can be found in Table 12.1 and Table 12.2, p.328 and p.333, Appendix III. GO term enrichment analysis was performed on these commonly regulated genes. Selected GO terms associated with these up and down regulated genes can be found in Table 5.8 and Table 5.9, respectively.

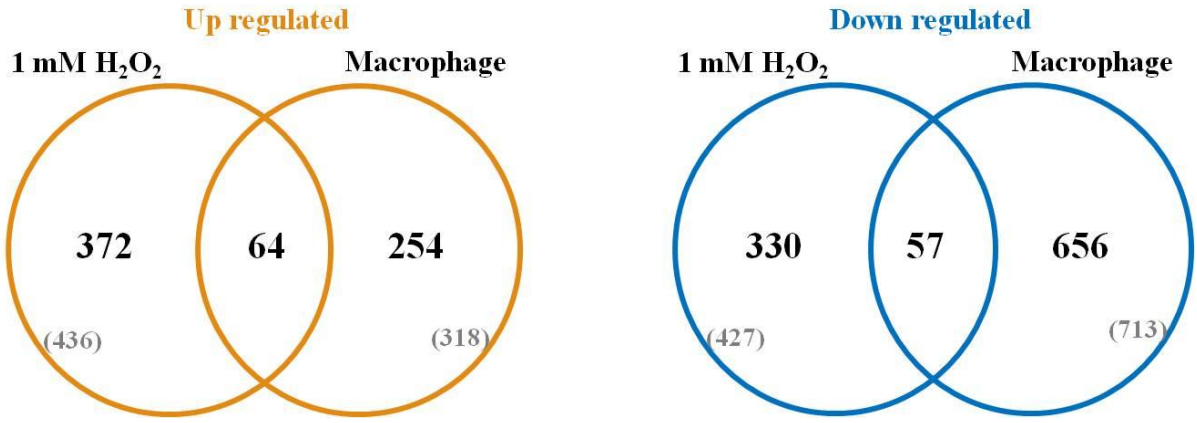


Figure 5.7: Venn diagrams comparing genes regulated by *C. glabrata* in response to oxidative stress and engulfment by macrophages. The number of genes significantly regulated by more than 2 fold upon 1 mM H_2O_2 treatment and macrophage engulfment over 2 and 6 hours are shown. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

Commonly up regulated genes are localised to the peroxisome and mitochondrion. As discussed in Kaur *et al*, 2007, YPS genes are regulated by *C. glabrata* upon macrophage engulfment and are required for virulence in macrophage models of infection **[2]**. One of the YPS genes (*CAGL0E01815g*) is up regulated in response to 1 mM H_2O_2 treatment as well as ingestion by macrophages. *CTA1*, the only catalase *C. glabrata* possesses; *SOD2*, a

mitochondrial superoxide dismutase; and *DDR48*, a DNA damage response gene are up regulated in response to oxidative stress and macrophage engulfment. The transcriptional repressor involved in pH response, *RIM101*, is also commonly up regulated and has been shown to be required for virulence in *C. albicans* **[60]**. *CAGL0F07359g*, the predicted NiSOD, identified from its regulation pattern in response to oxidative stress over time and *CAGL0L09251g*, *HAL1*, identified as constitutively up regulated over time under hyperosmotic stress conditions (see Chapter 4) are both up regulated by macrophage engulfed *C. glabrata* cells. Commonly down regulated genes are associated with DNA-directed RNA polymerase activity and sterol and ergosterol biosynthesis.

Table 5.8: GO terms associated with commonly up regulated *C. glabrata* genes in response to oxidative stress (1 mM H_2O_2) and macrophage engulfment.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Peroxisome	0.001024	*ACS1 CTA1 POX1 ECI1*	4	49
Mitochondrion	0.001053	*ACS1 LYS21 ARO3 FMP16 YSP2 ADK2 MET13 SOD2 OAC1 UTH1 ILV2 MGR3 LEU4 ESBP6 ARG8 HEM15 RDL1 FMP40 ICL2*	20	1072

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.9: GO terms associated with commonly down regulated *C. glabrata* genes in response to oxidative stress (1 mM H_2O_2) and macrophage engulfment.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
DNA-directed RNA polymerase activity	$3.17e^{-7}$	*RPB5 RPC25 RPC19 RPB8 RPA135 RPC40*	6	34
Sterol biosynthetic process	$3.72e^{-6}$	*ERG11 ERG13 ERG2 CYB5 MVD1*	5	29
Ergosterol biosynthetic process	$3.65e^{-5}$	*ERG1 ERG11 ERG13 ERG2*	4	23

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

5.2.8 Comparing the transcriptional response of *C. glabrata* to oxidative stress with that of its close relative, *S. cerevisiae*

S. cerevisiae microarray data published by Gasch *et al*, 2000, was used to investigate whether the transcriptional response to oxidative stress is well conserved between these two fungi **[1]**. The *S. cerevisiae* microarray study was conducted over a time course under oxidative stress using 0.32 mM H_2O_2. Data was used where signal intensities had been normalised and the fold change compared to untreated cells had been calculated. This found that 714 genes were up regulated by more than 2 fold by oxidative stress treatment in *S. cerevisiae*, while 755 genes were down regulated. Of the genes regulated by oxidative stress in *S. cerevisiae*, 521 genes up regulated and 564 down regulated genes had homologues in *C. glabrata*.

Liquid growth assays of the *S. cerevisiae* strain, DBY7268, upon 0.32 mM H_2O_2 were conducted and compared to *C. glabrata* ATCC 2001 cells treated with either 1 or 10 mM H_2O_2 to determine which concentration of H_2O_2 treated microarray experiments to use in the comparison analyses. Figure 5.8 shows that 0.32 mM H_2O_2 has a small affect on the growth of *S. cerevisiae* over time. This growth defect is similar to that of *C. glabrata* cells treated with 1 mM H_2O_2, while those treated with 10 mM H_2O_2 show an increased growth defect. Therefore, the 1 mM H_2O_2 treated *C. glabrata* microarray data set was used to compare to the *S. cerevisiae* 0.32 mM H_2O_2 treated microarray data.

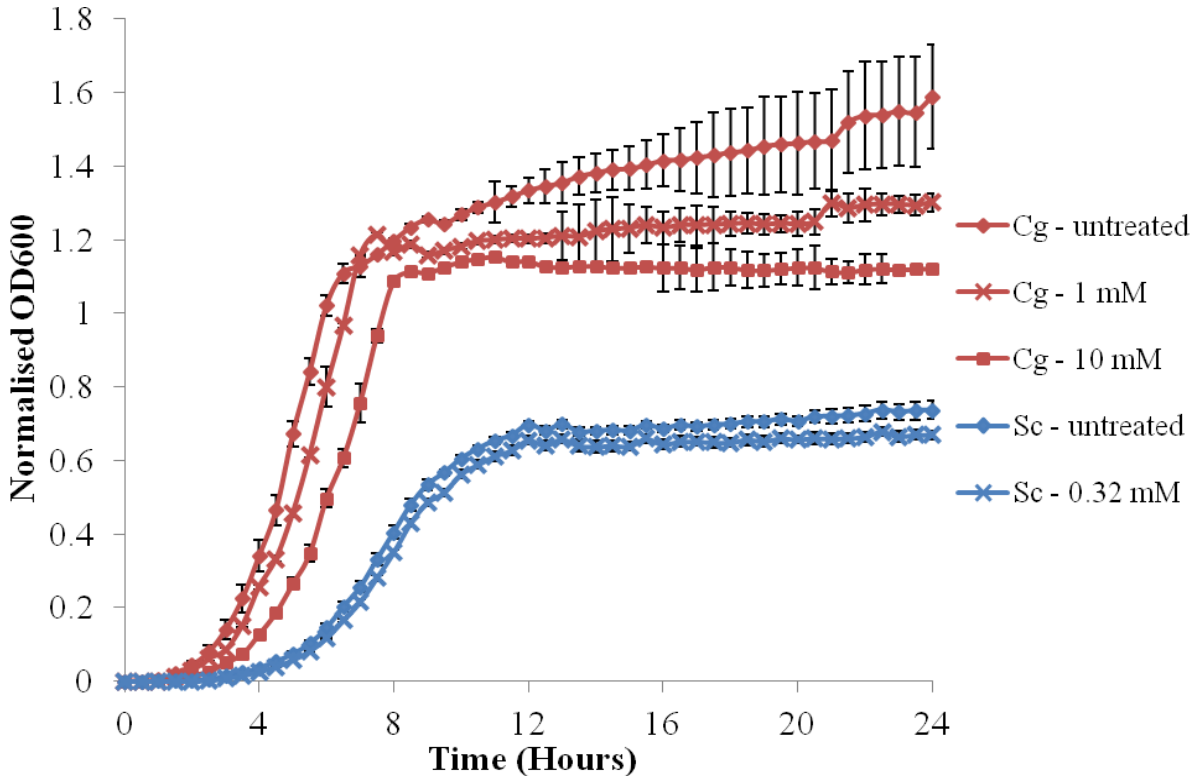


Figure 5.8: Growth of *C. glabrata* and *S. cerevisiae* under oxidative stress. *C. glabrata* ATCC 2001 (red) and *S. cerevisiae* DBY7268 (blue) were grown in either untreated YPD or YPD with the indicated concentration of H_2O_2 added at the start of the time course. OD_{600} measurements were taken every 30 minutes over a 24 hour timecourse. OD_{600} measurements were normalised by subtracting the background absorbance of blank media. Error bars for the standard deviation of three biological replicates are shown.

The number of genes up and down regulated by each organism upon oxidative stress treatment are compared in the Venn diagrams shown below (Figure 5.9). It is important to note that this comparison is between *C. glabrata* cells treated for 15 minutes with oxidative stress and *S. cerevisiae* cells treated for 20 minutes.

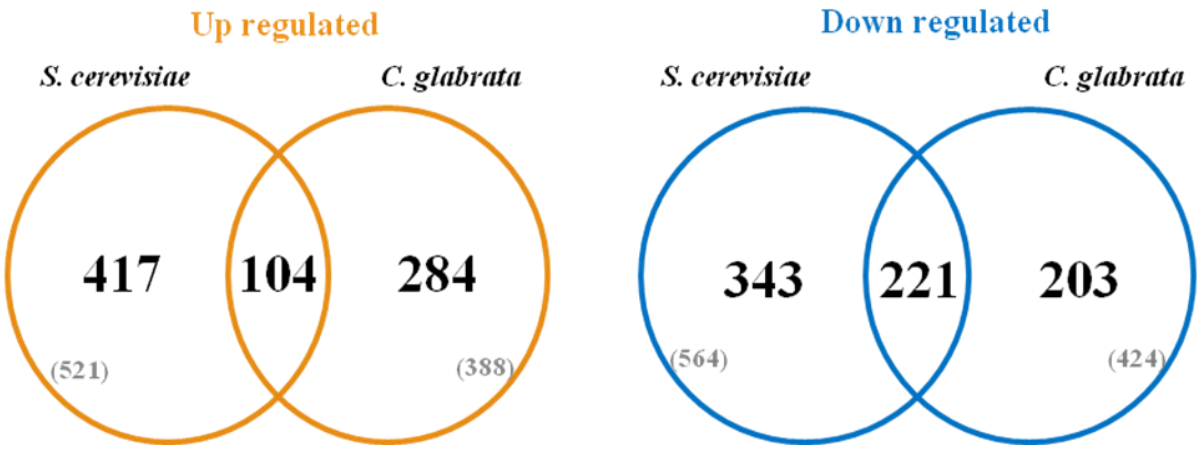


Figure 5.9: Venn diagrams comparing genes regulated under oxidative stress in *S. cerevisiae* and *C. glabrata*. The number of genes regulated by more than 2 fold under oxidative stress compared to untreated cells for each organism are shown. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets. **NB** The 15 minute time point was used for *C. glabrata*, while the 20 minute time point was used for *S. cerevisiae*, as no 15 minute time point was available for this dataset.

The response of *C. glabrata* to oxidative stress is somewhat conserved with that of *S. cerevisiae* as 52% of the *C. glabrata* genes down regulated under oxidative stress were also down regulated by *S. cerevisiae*. 26.8% of genes were observed to be up regulated in *S. cerevisiae* and *C. glabrata* upon oxidative stress treatment.

GO term enrichment analysis was conducted for the sets of genes in Figure 5.9 and can be found in Table 5.10 through Table 5.15. GO terms associated with genes up regulated in both *C. glabrata* and *S. cerevisiae* include those involved in the response to oxidative stress and oxygen radical detoxification. Genes involved in proteasomal degradation and proteolysis are also up regulated. Genes commonly down regulated in both organisms are associated with ribosome biogenesis and DNA-directed RNA polymerase activity.

GO terms associated with genes up regulated uniquely by *C. glabrata* upon oxidative stress include those involved in protein folding and the homeostasis of metal ions. Genes localised to the mitochondria are also up regulated. Genes uniquely down regulated in *C. glabrata* are associated with ribosome biogenesis and tRNA and rRNA processing. Genes involved in the biosynthesis of sterol and ergosterol are also down regulated uniquely by *C. glabrata* under oxidative stress compared to *S. cerevisiae*.

Table 5.10: GO terms associated with genes commonly up regulated by *S. cerevisiae* and *C. glabrata* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Oxidative stress response	1.90 e^{-11}	*GPX2 TRR1 GRX2 BLM10 HSP12 TRX2 SOD2 AHP1 GAD1 GRE2 GLR1*	13	55
Oxygen and radical detoxification	2.23 e^{-5}	*TRR1 TRX2 AHP1*	3	12
Proteasomal degradation (ubiquitin/proteasomal pathway)	0.000125	*DER1 CDC48 RPN3 DDI1 BLM10 OTU1 RPN12 UFD1 RPN7*	9	128
Protein processing (proteolytic)	0.000351	*DOA4 CYM1 RPN12 LAP4 MAS1 PEP4*	6	63

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.11: GO terms associated with genes commonly down regulated by *S. cerevisiae* and *C. glabrata* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	1.00 e^{-14}	*LOC1 CGR1 DBP3 NSA1 ROK1 SLX9 NOP7 UTP8 RRP36 RRS1 NOP58 NOP4 NAN1 NOP53 NIP7 DIM1*	107	170
RNA binding	1.00 e^{-14}	*NCL1 SRO9 KRR1 RRP43 DBP10 TRM8 FAL1 TRM1 NOP13 PUS4 DBP6 ESF2 NOP12 TRM11 NOP8*	55	337
DNA-directed RNA polymerase activity	9.35 e^{-13}	*RPB5 RPC53 RPC11 RPA34 RPA12 RPC37 RPC19 RPA49 RPB10 RPB8*	14	34

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.12: GO terms associated with genes uniquely up regulated by *C. glabrata* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Mitochondrion	$5.60\ e^{-12}$	*YAT1 MRPS9 ILV6 NFS1 IMG1 TRX3 SFA1 MRPL11 PNT1 RDL1 REV1 ISM1 YPL107W FMP40 MMT2 YAH1 ICL2 YME1 YMC1*	85	1072
Protein folding and stabilization	$5.13\ e^{-5}$	*SSA3 AHA1 HSP78 CAJ1 MDJ1 JAC1 FMO1 COX17 XDJ1 HSP60 SSQ1 ERO1 CCS1*	13	93
Homeostasis of metal ions	$8.95\ e^{-5}$	*NFS1 CCC2 FTR1 ERV1 UTR1 NFU1 OCT1 COX17 SMF3 SSQ1 CCS1 HEM15 MMT2*	13	98

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.13: GO terms associated with genes uniquely down regulated by *C. glabrata* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
rRNA processing	$1.00\ e^{-14}$	*UTP20 MAK5 PWP2 NOP1 RRP42 FAP7 NHP2 NOP6 FCF1 UTP6 NSA2 RAI1 UTP22 ZUO1 RRP4 NOP10 IPI1 IMP3 UTP25 NOP9*	33	195
Ribosome biogenesis	$1.11\ e^{-11}$	*UTP25 NOP9 HCA4 URB1 IMP4 RIO2 RRP12 NOG1 BMS1 RRP9 NOC4*	25	170
tRNA processing	$6.82\ e^{-10}$	*TRM7 NOP1 TRM3 TRM82 PUS2 THG1 TRM5 TAD2 TRL1 GCD14 TRZ1 TAD3*	16	80
Sterol biosynthetic process	$1.08\ e^{-6}$	*ERG11 ERG13 ERG5 ERG2 ERG12 CYB5 MVD1 IDI1*	8	29
Ergosterol biosynthetic process	0.000423	*ERG11 ERG13 ERG5 ERG2 ERG12*	5	23

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.14: GO terms associated with genes uniquely up regulated by *S. cerevisiae* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Mitochondrion	0.000185	*MDM10 MGR1 RSM10 YMR31 PHB2 MRPL6 MRP17 MBR1 MRP49 CCP1 MRPL24 RSM19 MAM3 ALD4 GRX5 MGR2*	23	170
Oxidative stress response	0.000342	*PRX1 UGA2 BLM10 SOD1 FMP46 CCP1 YAP1 TSA1 ALO1 GRX5 AFT2*	11	55
Secondary metabolism	0.000422	*COQ4 YPR1 COQ6 HMX1 ARG1*	5	12
Osmotic and salt stress response	0.00239	*CHS2 AGP2 NOP6 MYO1 RRD1 BNR1 MET22 CIN5 GRX5 RRD2*	10	59

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.15: GO terms associated with genes uniquely down regulated by *S. cerevisiae* under oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Translation	$3.71\ e^{-14}$	*RPL16B RPS3 SSB2 RPL18B WRS1 RPS19A RPS12 RPS9A RPS6A RPL5 RPL7B RPL1A RPL43A RPL11A TIF3*	50	318
Ribosomal small subunit assembly	0.000361	*RRP7 RPS14A RPS11A RPS14B RPS0B*	5	14
Mitochondrial genome maintenance	0.007022	*RRM3 MSH1 MGM101 REX2 ILV5 YHM2*	6	36

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

GO terms associated with genes up regulated uniquely by *S. cerevisiae* upon oxidative stress include those involved in secondary metabolism, the response to osmotic stress and those localised to the mitochondria. 11 genes involved in the response to oxidative stress are also uniquely up regulated by *S. cerevisiae* upon oxidative stress treatment. These include some of the major oxidative stress genes such as *YAP1* and *TSA1*. Genes uniquely down regulated in *S. cerevisiae* are associated with translation, ribosomal small subunit assembly and mitochondrial genome maintenance.

As previously discussed in the introduction, *S. cerevisiae* contains five peroxiredoxins including Tsa1 and Tsa2 (Thiol-Specific Antioxidant). *TSA1* along with ten other oxidative stress response genes are uniquely transcriptionally up regulated by *S. cerevisiae* upon oxidative stress treatment compared to *C. glabrata*. *S. cerevisiae tsa1* null mutants are sensitive to oxidative stressors, while *tsa2* mutants are not (as shown in Figure 5.10).

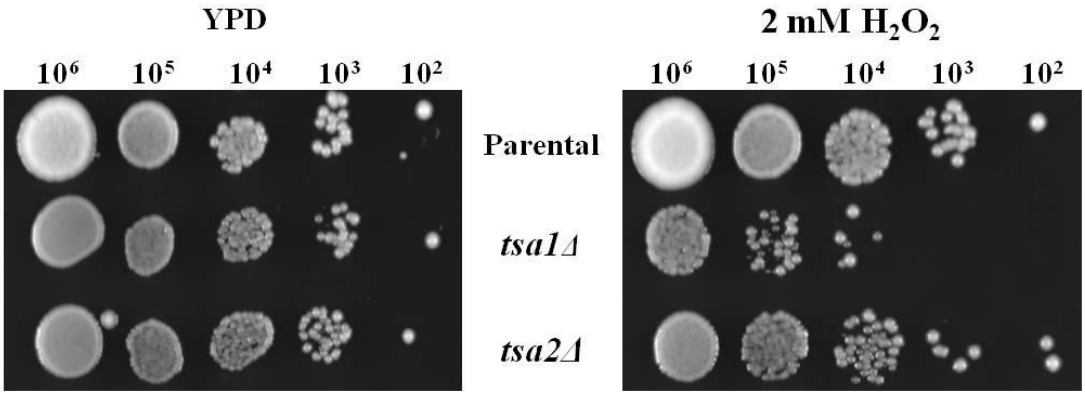


Figure 5.10: Oxidative stress sensitivity of *S. cerevisiae TSA* null mutants. Overnight cultures were diluted to an OD_{600} of 0.2, tenfold serial dilutions were made and these were spotted on to solid media: either YPD (untreated control) or YPD containing 2 mM H_2O_2. Plates were incubated at 30°C for 2 days.

Sequence comparisons of *C. glabrata* reveal two homologues of the *S. cerevisiae TSA1* gene, *CAGL0K06259g* and *CAGL0G07271g*. The DNA sequences of these genes are very similar and sequence comparisons with *S. cerevisiae* revealed these genes are both more similar to *TSA1* than *TSA2* (Figure 5.11.A). While the DNA sequences of *CAGL0K06259g* and *CAGL0G07271g* are very similar by comparing their syntenic context with *S. cerevisiae*, homology can be established. Figure 5.11.B shows that *CAGL0G07271g* is positioned at the syntenic loci of *TSA1* in *S. cerevisiae*, while *CAGL0K06259g* aligns with *TSA2*.

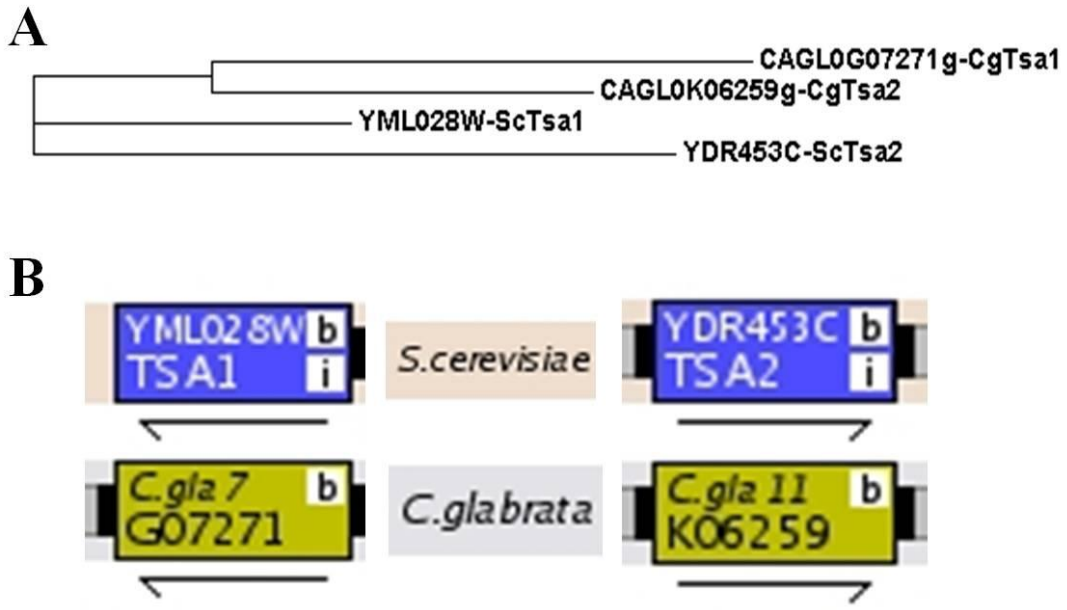


Figure 5.11: Comparison of *TSA* homologues in *C. glabrata* with *S. cerevisiae*. A. Phylogram comparing the DNA sequences of the *S. cerevisiae* and *C. glabrata TSA* genes. DNA sequences were obtained from Génolevures and SGD **[25, 45]**. Multiple sequence alignment was conducted and the phylogram created using ClustalW **[113]**. **B.** The syntenic context of the *TSA* genes of *C. glabrata* was investigated using YGOB to align these genes against *S. cerevisiae* **[29]**.

In *S. cerevisiae*, both *TSA1* and *TSA2* are transcriptionally up regulated upon 20 minutes treatment with 0.32 mM H_2O_2. However, in *C. glabrata*, only *TSA2* is transcriptionally up regulated after 15 minutes treatment with 1 mM H_2O_2.

Null mutants of *tsa1* and *tsa2*, as well as a double mutant were created in *C. glabrata* and phenotypically screened. This revealed that *C. glabrata tsa2* mutants were sensitive to H_2O_2 induced oxidative stress (as shown in Figure 5.12). *tsa1* null mutants displayed no phenotype to any condition screened. It was found that *tsa2* and the double *tsa1 tsa2* mutants were only sensitive to oxidative stress using H_2O_2 and not the similar oxidative stress compound, tBOOH. Figure 5.12 also shows that the double *tsa1tsa2* mutant is more sensitive to oxidative stress than the single *tsa2* mutant.

The protein expression of Tsa1 and Tsa2 in *C. glabrata* upon oxidative stress treatment was investigated by Western blot. The predicted protein size of Tsa1 and Tsa2 are similar; 21.5 and 21.87 kDa, respectively **[185]**. Figure 5.13 shows that Tsa protein is readily expressed by *C. glabrata* after 15 minutes treatment with 1 mM H_2O_2. Protein expression is observed in *tsa1* mutants under oxidative stress conditions however, this is absent in *tsa2* mutants. The expression of Tsa is also abolished in *tsa1tsa2* mutants. This supports the microarray data that the expression of *TSA2* and not *TSA1* is induced in *C. glabrata* upon oxidative stress treatment. The expression of Tsa2 protein is absent in *yap1* and *skn7* null mutants under

oxidative stress conditions, revealing that it's expression is dependent on both of these transcription factors in *C. glabrata*.

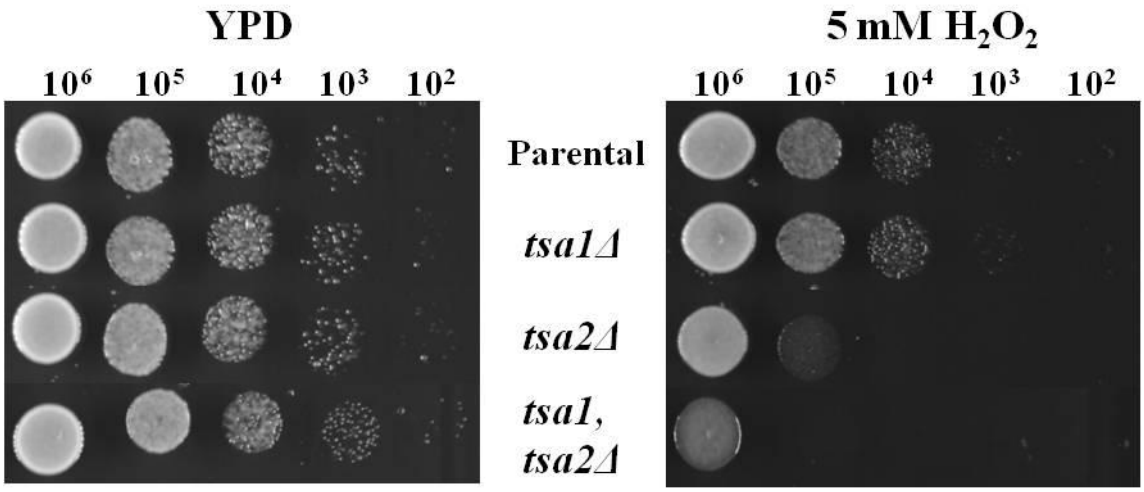


Figure 5.12: Oxidative stress sensitivity of *C. glabrata TSA* null mutants. Overnight cultures were diluted to an OD_{600} of 0.2, tenfold serial dilutions were made and these were spotted on to solid media: either YPD (untreated control) or YPD containing 5 mM H_2O_2. Plates were incubated at 30°C for 1 day.

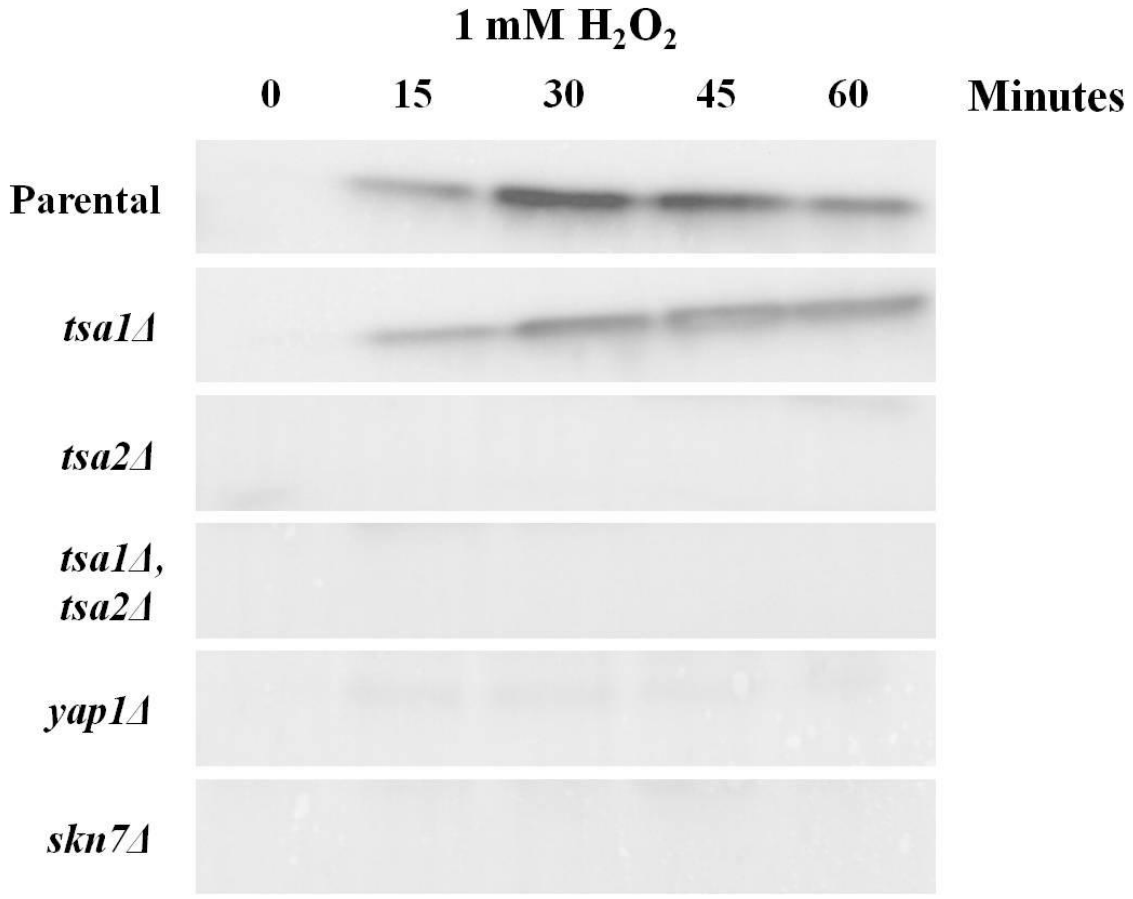


Figure 5.13: Protein expression of Tsa in *C. glabrata* under oxidative stress. Parental and the null mutant strains indicated were grown in YPD containing 1 mM H_2O_2 and cell samples were collected every 15 minutes over an hour timecourse. The same concentration of protein was run in all lanes as shown in the UV image of the SDS-PAGE gels (Figure 10.1, p.218, Appendix I). The antibody Pdrx3 was used to probe for Tsa. The above figure is representative of two biological replicate experiments.

5.2.9 Yap1 dependent transcriptional regulation in *C. glabrata* upon oxidative stress treatment

Null mutant microarray experiments under oxidative stress treatment were conducted in this study using a *C. glabrata* strain with the Yap1 encoding gene removed. The transcriptional response of wild type *C. glabrata* and *yap1* mutant cells to 15 minutes treatment with 1 mM H_2O_2 were compared. Genes significantly regulated by oxidative stress treatment in a *yap1* mutant strain were identified using an unpaired T test and those regulated by more than 2 fold (compared to untreated *yap1* cells after 15 minutes growth). This revealed a total of 303 transcripts significantly regulated in a *yap1* null mutant under oxidative stress conditions; 236 up regulated and 67 down regulated. This is significantly less than the number of genes regulated by wild type *C. glabrata* cells treated with 1 mM H_2O_2 (812 genes).

Genes regulated in response to oxidative stress treatment by wild type *C. glabrata* cells were compared to those regulated in a *yap1* mutant (as shown in Figure 5.14). This revealed that the majority of genes regulated in response to oxidative stress treatment in *C. glabrata* rely on the presence of *YAP1*.

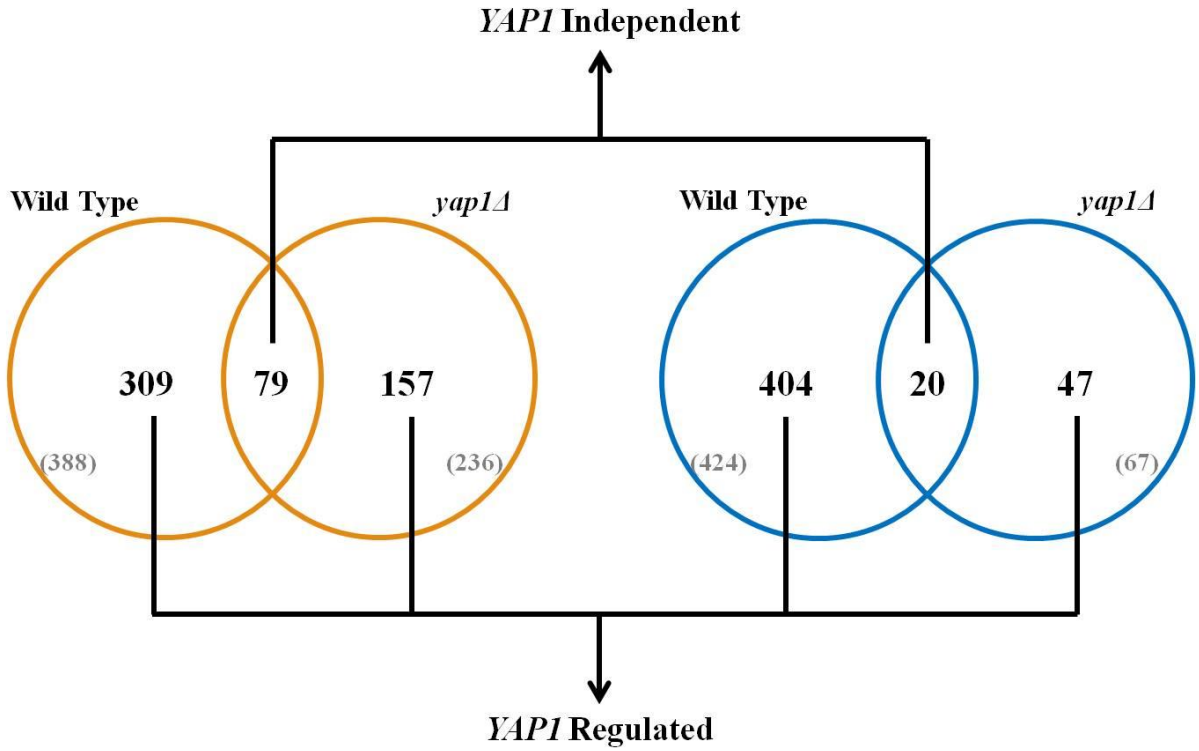


Figure 5.14: Venn diagrams comparing genes regulated under oxidative stress by wild type *C. glabrata* and *YAP1* null mutants. The number of genes significantly regulated by more than 2 fold under oxidative stress compared to untreated cells for each strain are shown. The sections of the Venn diagrams corresponding to genes regulated by *YAP1* and independently of *YAP1* are indicated. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

A total of 99 genes displayed *YAP1* independent regulation in response to oxidative stress. These include the Heat Shock Proteins; *HSP12*, *HSP31*, *HSP42*, *HSP78* and *HSP104*, as well as *RIM101*. While *CAGL0K06259g*, identified in this study as *TSA2*, is shown to be transcriptionally regulated independently of *YAP1*, its fold change is drastically reduced in a *yap1* mutant; 8.94 fold change in wild type compared to 2.11 fold change in *yap1* mutants when comparing untreated to 1 mM H_2O_2 treated cells. *FLR1*, which encodes a plasma membrane multidrug transporter, is also shown to be transcriptionally regulated independently of *YAP1* (3.6 fold change).

GO term enrichment analysis was conducted on these independently regulated genes. Those genes up regulated independently of *YAP1* are involved in the response to stress, as well as some genes of the oxidative stress response (6/55) (Table 5.16). No GO terms were found for the 20 genes independently down regulated.

The regulation of 917 genes in response to oxidative stress in *C. glabrata* is affected by the removal of *YAP1*. This includes transcripts whose regulation is abolished with the removal of *YAP1*, as well as those which are only regulated in the absence of *YAP1*. These include the most highly up regulated gene *SRX1*, which encodes a SulfiRedoXin, as well as the stress induced transcription factor *MSN4* and *YAP* family genes; *YAP3*, *YAP4* (*CIN5*) and *YAP5*. *YPS* genes were also identified as *YAP1* dependent under oxidative stress in *C. glabrata*.

GO term enrichment analysis was conducted on genes whose up regulation under oxidative stress treatment were affected in the absence of *YAP1* (Table 5.17). This revealed that the transcriptional regulation of 26 genes involved in the response to oxidative stress are dependent, whether directly or indirectly, on the presence of *YAP1* in *C. glabrata*. *YAP1* dependent genes were also associated with the homeostasis of metal ions and trehalose biosynthesis. Many of these *YAP1* regulated genes are localised to the mitochondrion and peroxisome.

GO term enrichment analysis was conducted on genes whose down regulation under oxidative stress treatment were affected by the absence of *YAP1* (Table 5.18). These genes were associated with the GO terms: ribosome biogenesis and DNA-directed RNA poloyerase activity, as well as sterol and ergosterol biosynthesis.

Table 5.16: GO terms associated with genes up regulated independently of *YAP1* in *C. glabrata* upon oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Response to stress	3.95 e^{-5}	*SSA3 HSP42 HSP78 HSP31 HSP12 GRE3 XBP1 HSP104 DDR48*	9	152
Oxidative stress response	8.69 e^{-5}	*GRX4 HSP12 GRE3 TSA1 GAD1 GCY1*	6	55

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.17: GO terms associated with genes whose up regulation is dependent on *YAP1* in *C. glabrata* upon oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Oxidative stress response	2.02 e^{-7}	*ZTA1 GPX2 TRX3 TRR1 GRX2 ERV1 GND1 MSN4 MCR1 AHP1 RCK2 AFT2 CTA1 BLM10 TRX2 SOD2 SKN7 SRX1*	26	55
Homeostasis of metal ions	1.25 e^{-5}	*SCO2 NFS1 CCC2 FTR1 TOS8 ERV1 NFU1 OCT1 COX17 SMF3 FRE8 AHP1 CDC25 SSQ1 CCS1*	19	98
Mitochondrion	1.00 e^{-14}	*TDH3 FMP43 ENO1 MTM1 YLF2 SOD2 MAS2 PUT2 FYV4*	138	1072
Peroxisome	3.00 e^{-6}	*LDH1 PEX5 CTA1 FAA2 PNC1 NPY1 STR3 POX1 PEX28 TES1 PXA2 FOX2*	16	66
Trehalose biosynthetic process	2.08 e^{-5}	*TPS1 TPS2 UGP1 PGM2 TPS3*	5	7

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 5.18: GO terms associated with genes whose down regulation is dependent on *YAP1* in *C. glabrata* upon oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	1.00 e^{-14}	*UTP20 MAK5 ENP1 REI1 SPB1 KRR1 PWP2 NOP1 DBP10 TSR1 NOP14 RRP12 NOP4 NOG1 NAN1 NOP53 NIP7 BMS1 DIM1 RRP9 RRP15 NOC4*	131	170
Sterol biosynthetic process	6.34 e^{-5}	*ERG11 HMG1 ERG13 ERG5 ERG2 ERG12 CYB5 MVD1 IDI1*	9	29
Ergosterol biosynthetic process	0.000485	*ERG1 ERG11 HMG1 ERG13 ERG5 ERG2 ERG12*	7	23
DNA-directed RNA polymerase activity	1.00 e^{-14}	*RPB5 RPC53 RPC11 RPA34 RPB8 RPA43 RPA190 RPA135 RPC40 RPO26 RPC82*	21	34

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Gasch *et al*, 2000, identified 70 genes whose regulation in *S. cerevisiae* in response to oxidative stress is dependent on the presence of *YAP1* **[1]**. Of those, 57 had homologues in *C. glabrata*. By comparing the large number of genes identified in this study to those identified in *S. cerevisiae*, 33 genes were found to be regulated by *YAP1* in response to oxidative stress in both of these closely related yeast (Table 12.3, p.337, Appendix III). These include some of the major oxidative stress genes: *CTA1*, *GSH2*, *GRX2*, *GLR1*, *SOD2*, *TRR1*, *TRR2*, *TRX2* and *GPX2*.

A total 98 genes were found to be *YAP1* dependent in *C. glabrata* in response to benomyl treatment by Lelandais *et al*, 2008 **[110]**. All of these genes had probes on our microarrays and as such were present in this microarray analyses. In response to benomyl stress and H_2O_2-induced oxidative stress, 43 genes were identified as dependent on *YAP1* in *C. glabrata* (Table 12.4, p.339, Appendix III). These include 11 genes which encode proteins of unknown function and genes known to be involved in the oxidative stress response such as *TRR1*, *TRR2*, *CCS1*, *GLR1*, *GPX2* and *GSH2*. *CRP1*, which encodes a transcription factor that activates the transcription of genes involved in stress response and *ECM4*, encoding a

glutathione transferase, are also *YAP1* dependent in *C. glabrata* under benomyl stress and oxidative stress.

As discussed in the introduction, Yap1 in *C. glabrata* contains a mutation causing a shift in binding site recognition. In an effort to identify genes directly regulated by Yap1 in *C. glabrata*, the upstream regions of all genes whose regulation was affected by the absence of *YAP1* were parsed for the exact YRE-A and YRE-O binding site motifs TTACGTAA and TTA(C/G)TAA, respectively (in collaboration with David Studholme, University of Exeter). This revealed that 11 genes contained exact YRE-A sites, 49 genes contained exact YRE-O sites and one gene (*DOT6*) contained both sites. These included *SRX1* and 11 genes which encode proteins of unknown function (Table 12.5 - Table 12.7, p.342 – p.346, Appendix III). Of the 917 genes whose regulation is affected by the removal of *YAP1* under oxidative stress conditions and the 60 which contain exact Yap1 binding sites, 8 are available in the *C. glabrata* mutant library. These were tested for sensitivity to oxidative stressors (Figure 5.15). A *yap1* mutant was included as a positive control. *aft2* mutants displayed a slight growth inhibition on 5 mM H_2O_2 plates, but this may be due to its morphology. No other mutants tested displayed oxidative stress phenotypes.

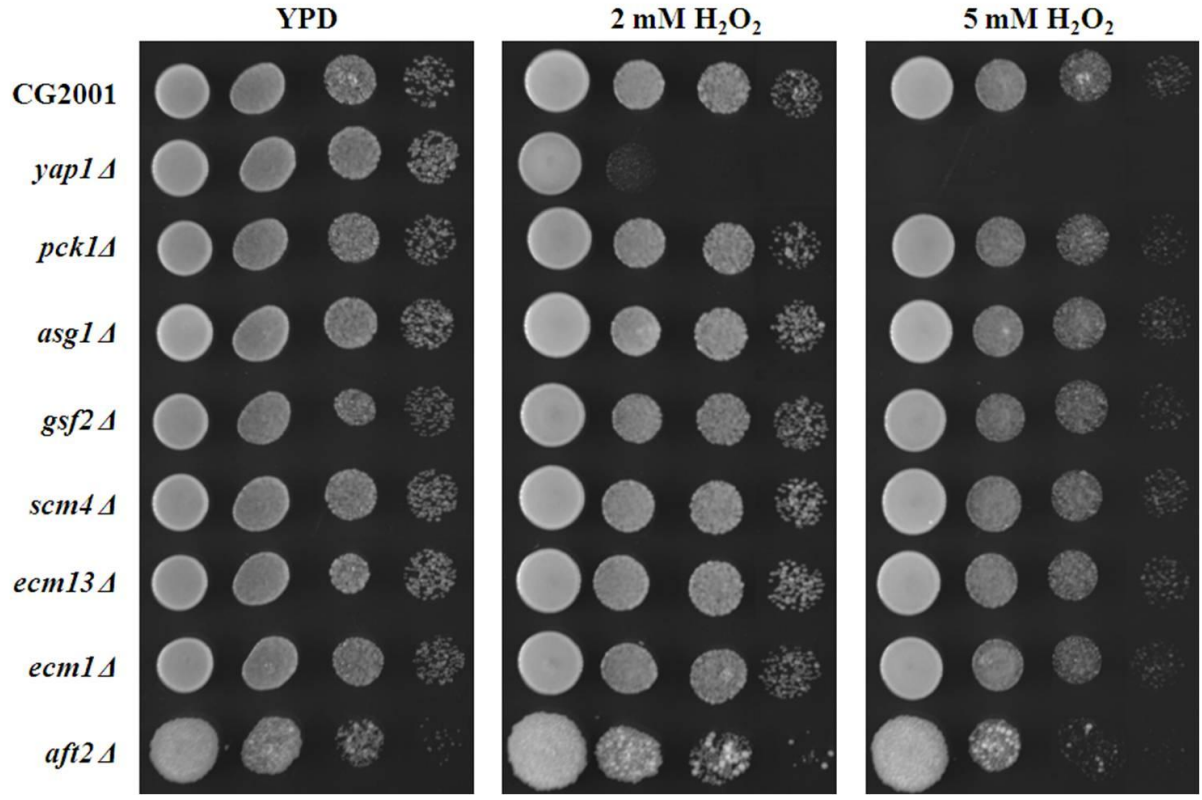


Figure 5.15: Oxidative stress phenotypes displayed by null mutants of genes regulated by *YAP1* possessing an exact Yap1 binding motif. Overnight cultures were diluted to an OD_{600} of 0.2, tenfold serial dilutions were made and these were spotted on to solid media containing the indicated stress. A *yap1* mutant was included as a positive control. Plates were incubated at 30°C for 2 days and then photographed.

5.3 Discussion

The adaptation of the pathogen *C. glabrata* to oxidative stress has been presented in this chapter and the implication of these results will be explored. Phenotypic analysis of the key players: *yap1*, *skn7*, *cta1* and *sod1*, all known to be involved in oxidative stress, revealed they are also required for resistance to other stress conditions. However, other YAP mutants were not required for oxidative stress resistance in *C. glabrata* and this was in contrast to phenotypes displayed in the corresponding mutants in *S. cerevisiae*. The transcriptional response of *C. glabrata* to oxidative stress has been investigated and the similarities and differences to that of *S. cerevisiae* will be discussed. The oxidative stress response of *C. glabrata* was compared to that of macrophage engulfed *C. glabrata* cells and the implications of these results will also be discussed. The *yap1* mutant arrays conducted in this study identified genes whose regulation under oxidative stress was affected by the loss of *YAP1* and these were compared to published *C. glabrata YAP1* dependent data under benomyl treatment. By combining these observations, the model of oxidative stress response in *C. glabrata* presented in the introduction was updated.

5.3.1 Phenotypic screening of *yap1*, *skn7*, *sod1* and *cta1* null mutants reveals functionality

Phenotypic screening revealed that as previously shown *YAP1*, *SKN7*, *SOD1* and *CTA1* are required for oxidative stress resistance in *C. glabrata* **[89, 106]**. However, unlike data published in Cuellar-Cruz *et al*, 2008, which showed *C. glabrata cta1* mutants to be highly susceptible to H_2O_2, this study showed only moderate sensitivity in *cta1* mutants **[89]**. As the strain used in this study is different to that used in this paper, secondary site mutations may be the cause of this difference in sensitivities. It is also important to note that this mutant was taken from a library of mutants made outside of our lab and taken "as is", without in depth verification of the >400 strains. Null mutants of these four major oxidative stress genes are also involved in divalent cation and heavy metal resistance, with sensitivities to $CoCl_2$, $MnCl_2$ and $ZnCl_2$. Metal ion transporters are conserved in eukaryotes and include channels, symporters, permeases and ATPases, of which *C. glabrata* possesses a total of 36 **[186]**. These transporters are responsible for the homeostasis of many divalent and heavy metals ions, which can become toxic at high concentrations. Disruption of metal ion transporters in *S. cerevisiae* results in metal ion sensitivity, therefore the phenotypes displayed by *yap1*,

cta1, *skn7* and *sod1* null mutants may indicate their involvement in metal ion transport and homeostasis **[187]**.

Liquid screens of wild type and *yap1* mutant *C. glabrata* strains revealed that not only is *YAP1* required for oxidative stress resistance but it is also required for wild type levels of growth in rich media as the final biomass of *yap1* cells was lower than that of wild type cells (Figure 5.3). This could be due to the ROS produced during normal growth as a by product of aerobic respiration **[173]**. The sensitivity of *yap1* mutants to media containing CsCl would suggest that Yap1 in *C. glabrata* is involved in caesium sensing and signalling. As previously discussed in the hyperosmotic stress chapter, CsCl treatment causes activation of the HOG and cell wall integrity pathways in *S. cerevisiae*, suggesting that Yap1 in *C. glabrata* may have roles in these pathways **[144]**. The reduced resistance of *yap1* mutants to arsenic compounds shown in this study suggests defects in the folding of actin, tubulin and other proteins **[137]**. The phenotypes of *yap1* and *sod1* mutants to $CdCl_2$ reveal their requirement for cadmium resistance and this has been shown in the corresponding *S. cerevisiae* mutants **[188, 189]**. As discussed in the introduction, metals ions are not only toxic in high concentrations but also lead to the production of ROS in the cell through the Fenton reaction **[91]**. Metal ions also cause lipid peroxidation leading to membrane damage and the oxidation of glutathione, affecting the redox state of the cell and its ability to detoxify oxidative stressors **[91, 92]**. As exposure to metal ions leads to the production of oxidative stress, this could explain the sensitivities of the oxidative stress mutants shown in this study.

As *sod1* null mutants displayed a slow growth phenotype on rich media at 30°C as well as sensitivity to 16°C, 42°C and 6 % ethanol, this would indicate the function of *SOD1* in the normal growth of *C. glabrata*. These phenotypes have been identified in a *S. cerevisiae sod1* mutant but have yet to be shown in *C. glabrata* **[190]**. *sod1* was the only null mutant for which sensitivity to menadione was observed and this is because menadione decomposition generates large amounts of superoxide, which must be converted to H_2O_2 by SODs before detoxification can proceed. This observation agrees with results published by Roetzer *et al*, 2011 **[106]**. Vanadate has been shown to cause lipid peroxidation, oxidation of glutathione and produce hydroxyl radicals through a Fenton-like reaction, similar to metal ions **[91]**. As *sod1* null mutants are also sensitive to a range of oxidative stressors and metal ions, this observation is not unexpected. The sensitivity of *sod1* mutants on media containing vanadate also reveals vacuolar defects **[138]**. The *sod1* mutant was also the only oxidative stress mutant to show growth inhibition to the cell wall stressor calcofluor white and nitrogen

starvation conditions, with sensitivity to calcofluor white suggesting defects in cell wall composition. **[136]**. This suggests that Sod1 may be associated with the cell wall; while Sod1 in *S. cerevisiae* has been localised largely to the cytosol and a small fraction in the mitochondria, the cellular localisation of Sod1 in *C. glabrata* has yet to be determined **[191]**. Six SODs have been identified in *C. albicans*, three of which are located on the cell surface and not only help to defend against oxidative attack from the host immune system but are important for virulence **[192, 193]**. Sod1 could also have a similar role in *C. glabrata* and localisation studies would be able to confirm or refute this. As many of the phenotypes displayed by *sod1* mutants in *C. glabrata* have also been identified in *S. cerevisiae*, this would suggest that Sod1 in *C. glabrata* is functionally similar to its homologue.

Phenotypic screening of these null mutants revealed that in this study, Cta1 was not as important for oxidative stress resistance as previously described and revealed that Yap1 is involved in metal ion homeostasis. This study has also shown that Sod1 has a similar function as its homologue in *S. cerevisiae* through phenotypic analyses.

5.3.2 Other YAP family members are not required for H_2O_2 resistance in *C. glabrata* and phenotypic screening reveals little functionality

Null mutants of the YAP family in *C. glabrata* (where available) were phenotypically screened (Section 5.2.3). A summary of the phenotypes presented in this study and those of the corresponding mutant in *S. cerevisiae* can be found below (Table 5.19).

While many phenotypes are shared between the *C. glabrata* and *S. cerevisiae yap1* mutants, none of the YAP family null mutants tested in this study display any of the same phenotypes as their corresponding null mutant in *S. cerevisiae*. Whereas in *S. cerevisiae*, Yap2 (Cad1) is required for resistance to H_2O_2, this is not the case for *yap2* mutants in *C. glabrata*. The implications of this will be discussed further in Section 5.3.7. As null mutants of Yap4 (Cin5) and one of the Yap3 homologues are currently unavailable in *C. glabrata*, this study was unable to compare these to phenotypes displayed in *S. cerevisiae*. *CIN5* has however been shown to be transcriptionally regulated in response to hyperosmotic and oxidative stress in this study. The sensitivity of *yap7* mutants to $MnCl_2$ has not been observed for *S. cerevisiae* mutants and the implications of this phenotype have been discussed previously in the hyperosmotic stress chapter. While this phenotype is shared with other oxidative stress response mutants such as *yap1*, *skn7* and *cta1*, *yap7* mutants do not share the other phenotypes displayed to oxidative stressors or other metals, nor is it transcriptionally

regulated in response to oxidative stress in *C. glabrata* and as such its function remains unknown.

Table 5.19: Summary of YAP family null mutant phenotypes in *C. glabrata* and *S. cerevisiae*.

***C. glabrata* systematic name**	**YAP gene**	***S. cerevisiae* standard name**	**Phenotypes in *C. glabrata***	**Phenotypes in *S. cerevisiae***	**References**
CAGL0H04631g	*YAP1*	*YAP1*	H_2O_2, tBOOH, $CoCl_2$, $CdCl_2$, $ZnCl_2$, $MnCl_2$, cyclohexamide, arsenic, CsCl	H_2O_2, $CdCl_2$, $ZnCl_2$, arsenic, cyclohexamide, menadione	**[137, 188, 194-196]**
CAGL0F03069g	*YAP2*	*CAD2*	None	H_2O_2, 16°C cyclohexamide	**[194, 197, 198]**
CAGL0K02585g		*YAP3*	N/A	NaCl, cyclohexamide, caffeine	**[197, 199, 200]**
CAGL0M10087g			None		
CAGL0E01859g	*YAP3*	*YPS gene*	None	YPS1 phenotypes: Caspofungin, Congo Red, caffeine	**[201]**
CAGL0H08173g	*YAP4*	*CIN5*	N/A	Suppresses 16°C sensitivities of other YAP nulls	**[198]**
CAGL0K08756g	*YAP5*	*YAP5*	None	16°C	**[198]**
CAGL0M08800g	*YAP6*	*YAP6*	None	None	SGD
CAGL0F01265g	*YAP7*	*YAP7*	$MnCl_2$	None	SGD
-	*YAP8*	*ARR1*	-	Arsenic	**[137]**

C. glabrata phenotypes shown are those identified in this study. Phenotypes for *S. cerevisiae* mutants were only included if tested in this study. *S. cerevisiae* standard names and descriptions from SGD, along with their homologue in *C. glabrata* are given **[25, 29, 45]**. N/A denotes where a null mutant was unavailable in *C. glabrata*. **NB** There is no identified homologue of *YAP8* in *C. glabrata*.

5.3.3 The immediate transcriptional response of *C. glabrata* to oxidative stress

Transcription profiling of *C. glabrata* revealed that many genes were statistically and significantly regulated in response to oxidative stress. As expected, many oxidative stress genes known to be involved and regulated in response to oxidative stress in *S. cerevisiae* were up regulated by *C. glabrata* upon H_2O_2 treatment. The up regulation of the major oxidative stress genes such as *CTA1*, *TRX2* and *TRR1*, to name but a few, are a means to defend against and repair the damage caused by ROS. Genes associated with proteolysis are up regulated and suggest a concerted effort to remove unwanted proteins immediately upon

stress treatment. Many genes localising with the mitochondria are induced in response to oxidative stress and this is unsurprising as ROS production occurs normally in the mitochondria. An organism would need to be able to cope with this production of ROS during growth or perish. Genes involved in the homeostasis of metal ions are also up regulated in response to oxidative stress in *C. glabrata* and this goes some way to explain the metal ion sensitivities of the oxidative stress genes tested in this study. The implications of the regulation of metal ion homeostasis genes will be discussed further in 5.3.7.

The down regulation of genes involved in ribosome biogenesis immediately upon stress suggests, as previously discussed and displayed in response to hyperosmotic stress, growth of *C. glabrata* is, without delay, ceased in response to oxidative stress. This is consistent with many other organisms in response to any stress condition applied. The down regulation of growth allows an adequate response to stress conditions and it has been shown (in this study and others) that stress response genes are not up regulated at the same time as those involved in growth **[1, 202]**. The down regulation of RNA processing and genes localised to the nucleus suggest a down regulation of transcription and the down regulation of unwanted gene products. Taken together, these observations suggest a conservation of energy with all processes geared to surviving and adapting to the stressful environment. The down regulation of ergosterol biosynthesis has been shown to be essential for oxidative and hyperosmotic stress resistance in *S. cerevisiae*, as well as virulence in *C. neoformans* **[203, 204]**. The down regulation of sterol and ergosterol biosynthesis genes in response to oxidative stress in *C. glabrata* is opposite to that which occurs in response to low oxygen conditions, such as those in the brain where *C. neoformans* infections reside, as sterol and ergosterol are induced in low oxygen conditions **[203]**. This down regulation of ergosterol is also displayed in *C. glabrata* cells responding to hyperosmotic stress in this study.

The immediate transcriptional response of *C. glabrata* after 15 minutes treatment to 1 mM and 10 mM H_2O_2 are very similar. Nearly all of the genes regulated by the lower of the two concentrations of H_2O_2 are also regulated when this is increased to 10 mM (Figure 5.4). When the concentration of oxidative stress used is increased, the number of genes statistically and significantly regulated also increased. More genes involved in the oxidative stress response and metal ion homeostasis were up regulated by 10 mM H_2O_2 treatment compared to 1 mM, resulting in more oxidative stress genes to deal with this increased threat. It is important to note that many genes involved in metal ion homeostasis have overlapping functions with those of the oxidative stress response, such as *SOD2*. Autophagy genes

involved in mitochondrial degradation, known to be important in survival of macrophage engulfed *C. glabrata*, are up regulated in response to 10 mM H_2O_2 suggesting the programmed recycling of components to sustain cellular functions and the removal of damaged organelles in times of starvation and stress **[88]**. This up regulation is only seen upon treatment with 10 mM H_2O_2 suggesting that this high concentration is very damaging to the cell. Genes associated with the peroxisome are also up regulated solely by treatment with 10 mM H_2O_2 and these have been shown to be not only important for survival in macrophages but also transiently induced upon macrophage engulfment **[88]**.

As described previously for the up regulated genes, a large proportion of genes down regulated by 10 mM H_2O_2 treatment are shared with those regulated by 1 mM H_2O_2 treatment. Similarly to those up regulated, more genes are statistically and significantly down regulated by an increased concentration of stress and more genes associated with key GO terms are down regulated. The down regulation of genes involved with ribosome biogenesis and the cell cycle both suggest a down regulation of growth and this is reflected in the increased lag time displayed by *C. glabrata* treated with 10 mM H_2O_2 (Figure 5.1). Unlike the response to increasing concentrations of NaCl presented in the hyperosmotic stress chapter, increasing concentrations of H_2O_2 display a similar response with an enhanced transcriptional response to high doses of oxidative stress.

5.3.4 The transcriptional response of *C. glabrata* over time and increasing concentration of oxidative stress

The transcriptional response of *C. glabrata* to 1 mM H_2O_2 is short and sharp with differential gene expression occurring at 15 but not 60 minutes treatment. By the 60 minute time point few genes are significantly regulated compared to untreated, therefore there is no difference between 1 mM H_2O_2 treated *C. glabrata* cells and those unstressed by 60 minutes (Figure 5.5). This reveals that similarly to hyperosmotic stress treated *C. glabrata* cells at the 60/120 minutes, the transcriptional regulation of these cells in similar to a growing population of cells. The cells have responded and adapted to the stress conditions imposed relatively quickly and this is reflected in the small effect 1 mM H_2O_2 treatment has on the growth of *C. glabrata* (Figure 5.1). Whether or not this lack of differential gene expression is present at earlier time points is unknown; microarray experiments conducted at 30 and 45 minutes treatment may shed light on this.

The transcriptional response of *C. glabrata* to 10 mM H_2O_2 is prolonged overtime, with continued differential gene regulation as well as an increased number of genes significantly up and down regulated at 60 minutes compared to 15 minutes treatment (Figure 5.5). Unlike 1 mM H_2O_2 where transcript abundance is similar to untreated cells at 60 minutes, 10 mM H_2O_2 treated cells are still responding and adapting to the imposed stress and this is reflected in the long lag time of *C. glabrata* grown in 10 mM H_2O_2 (Figure 5.1).

This pattern of gene regulation in response to oxidative stress is also observed in the regulation of known oxidative stress genes. These genes in *C. glabrata* are not regulated compared to the reference over time in untreated conditions but when treated with increasing concentrations of H_2O_2, reveal the pattern of regulation shown in Figure 5.6. An effort to identify other genes with a similar regulation trend and therefore similar function in oxidative stress was somewhat successful. Genes known in *S. cerevisiae* to be involved in oxidative stress were identified as well as those involved in metal ion homeostasis (Table 5.7). While two genes of unknown function identified for deletion revealed no phenotypes in this study, 17 other non-homologous and uncharacterised proteins remain to be investigated. Although null mutants of the putative NiSOD (*CAGL0F07359g*) and putative transcription factor (TF) (*CAGL0L10186g*) displayed no phenotypes, their involvement in the oxidative stress response of *C. glabrata* was supported by their occurrence in *C. glabrata* macrophage studies and Yap1 dependent benomyl experiments. A lack of oxidative stress phenotype means these genes are not required for resistance; other SODs present in *C. glabrata* could be making up for the lack of NiSOD and this also reveals that the putative TF is not a major regulator of oxidative stress and/or its targets overlap with other TFs in *C. glabrata*. NiSODs have not been identified in yeast previously. The closest relative of *C. glabrata* possessing a NiSOD is the actinobacteria species *Streptomyces* **[184]**. The identification of a putative Nickel containing SOD in *C. glabrata* is interesting as it is could be an example of gene transfer from bacteria to yeast. Schmidt *et al*, 2009, have already described the heterologous gene transfer of NiSOD genes from actinobacteria to other bacterial species as well as the prokaryotic green algae *Ostreococcus* spp. **[205]**. An in depth investigation would be needed to ascertain whether gene transfer had occurred in *C. glabrata*.

5.3.5 The transcriptional response of macrophage engulfed *C. glabrata* cells have some similarities as those treated with exogenous oxidative stress

When using a pathogenic organism, *in vitro* experiments are a cheap and easy way to mimic the host environment. To test the validity of these *in vitro* experiments to *ex vivo* conditions,

the transcriptional response to exogenous oxidative stress was compared to that of previously published data of macrophage engulfed *C. glabrata* **[2]**. This revealed some overlap in the transcriptional responses with the identification of peroxisome genes being regulated in both datasets. This links to the response of phagocytosed *C. glabrata* cells as described by Roetzer *et al*, 2010, which showed that phagocytosed *C. glabrata* cells increase the number of peroxisomes present, as well as inducing *CTA1* and localising its protein to the peroxisome **[88]**. *SOD2*, a mitochondrial superoxide dismutase is also transcriptionally up regulated in both macrophage engulfed and oxidative stress treated *C. glabrata* cells and could indicate the increased metabolic strain survival inside a macrophage and oxidative stress treatment causes with the increased production of ROS by the mitochondria **[173]**. Down regulated genes involved in sterol and ergosterol biosynthesis were found in both datasets, again stressing their importance in stress response, as well as conditions found inside a macrophage.

The transcriptional regulation of *C. albicans* in response to phagocytosis by macrophage cells has been shown to induce genes involved in fatty acid metabolism (peroxisomes), DNA damage repair, oxidative stress and metal ion homeostasis, as well as the down regulation of translation machinery and ribosome biogenesis genes **[87]**. Genes involved in all of these processes were identified in *C. glabrata* cells treated with oxidative stress. This would suggest that the response of *C. glabrata* to oxidative stress may also be important in a host environment.

5.3.6 The transcriptional response of *C. glabrata* to oxidative stress is similar to that of *S. cerevisiae*

As presented in 5.2.8, microarray data from 1 mM H_2O_2 treated *C. glabrata* cells were compared to 0.32 mM H_2O_2 treated *S. cerevisiae* cells. Only genes for which homologues were identified in *C. glabrata* were considered. Figure 5.9 showed there was significantly more overlap between the transcriptional responses of *C. glabrata* and *S. cerevisiae* to oxidative stress than that for hyperosmotic stress. This could be due to the different compounds used for hyperosmotic stress, while the H_2O_2 was used for oxidative stress with both organisms.

While the genes down regulated by *C. glabrata* were highly conserved with those down regulated by *S. cerevisiae* in response to oxidative stress, the up regulated genes fared worse. Of those shared between the species, 13 oxidative stress genes were present, revealing that

the oxidative stress response of *C. glabrata* is well conserved with its close relative *S. cerevisiae*. Comparisons and GO term enrichment analyses also showed that both organisms immediately down regulated growth and transcription upon oxidative stress. The implications of this have been discussed previously.

Genes uniquely up regulated by *C. glabrata* in response to oxidative stress include the homeostasis of metal ions, which will be discussed in 5.3.7, and the down regulation of sterol and ergosterol. As discussed previously, the down regulation of sterol and ergosterol in response to stress is essential for stress resistance and could partly explain the increased stress resistance seen in *C. glabrata* compared to *S. cerevisiae*. *C. glabrata* also uniquely down regulates many more ribosome biogenesis and transcription genes than *S. cerevisiae* in response to oxidative stress revealing its increased need to down regulate growth and limit unneeded transcripts and energy use, which would be more important for pathogens such as *C. glabrata*.

Unlike *S. cerevisiae*, Yap1 is not differentially transcriptionally regulated by oxidative stress in *C. glabrata*. Could the phosphorylation and/or localisation of Yap1 be more important in *C. glabrata*? The microarray data presented in this study would suggest that the transcript abundance of *YAP1* is consistently high, which could explain the lack of induction upon oxidative stress. *SOD1* is not transcriptionally regulated in *C. glabrata* at low concentrations of H_2O_2 unlike in *S. cerevisiae*; however it is up regulated at higher doses of 10 mM H_2O_2. This could be because only prolonged exposure to oxidative stress, as occurred with this higher dose, sufficiently triggers activation of *SOD1* in *C. glabrata*. It has been shown to be Yap1 independent in *C. glabrata* and this may have a part in its lack of regulation at low doses of H_2O_2 **[106]**.

Comparisons between the transcriptional responses of *C. glabrata* and *S. cerevisiae* to oxidative stress lead to the identification of *TSA2* as the functional Thiol Specific Antioxidant in *C. glabrata*. While Roezter et al, 2010, shows that both *TSA* genes are induced by oxidative stress and that this regulation is Yap1 and Skn7 dependent, the microarray experiments, null phenotypes (Figure 5.12) and protein expression assays (Figure 5.13) presented in this study confirm Saijo *et al*, 2009, observations that *CAGL0K06259g* (*TSA2*) is the functionally active, transcriptionally induced and Yap1 and Skn7 dependent *TSA* gene in *C. glabrata* **[61, 106]**. The major difference between these two yeast is the up regulation of genes involved in metal ion homeostasis in *C. glabrata*, which correlate with the metal ion sensitivities displayed by *yap1* mutants.

5.3.7 The majority of genes regulated in response to oxidative stress in *C. glabrata* rely on the presence of *YAP1*

The null mutant microarray experiments presented in this study revealed that the majority of genes regulated in response to oxidative stress in *C. glabrata* are affected by the absence of *YAP1*, whether directly or indirectly (Figure 5.14). While null mutant microarray analysis has been utilised to help identify the targets of transcriptional activators and repressors in yeast, as well as being valid only for the specific condition examined, this approach does not take into account the interactions between proteins and the effects on downstream regulation targets. The diagram below summarises the possible Yap1 interactions the null mutant microarray data has identified (Figure 5.16).

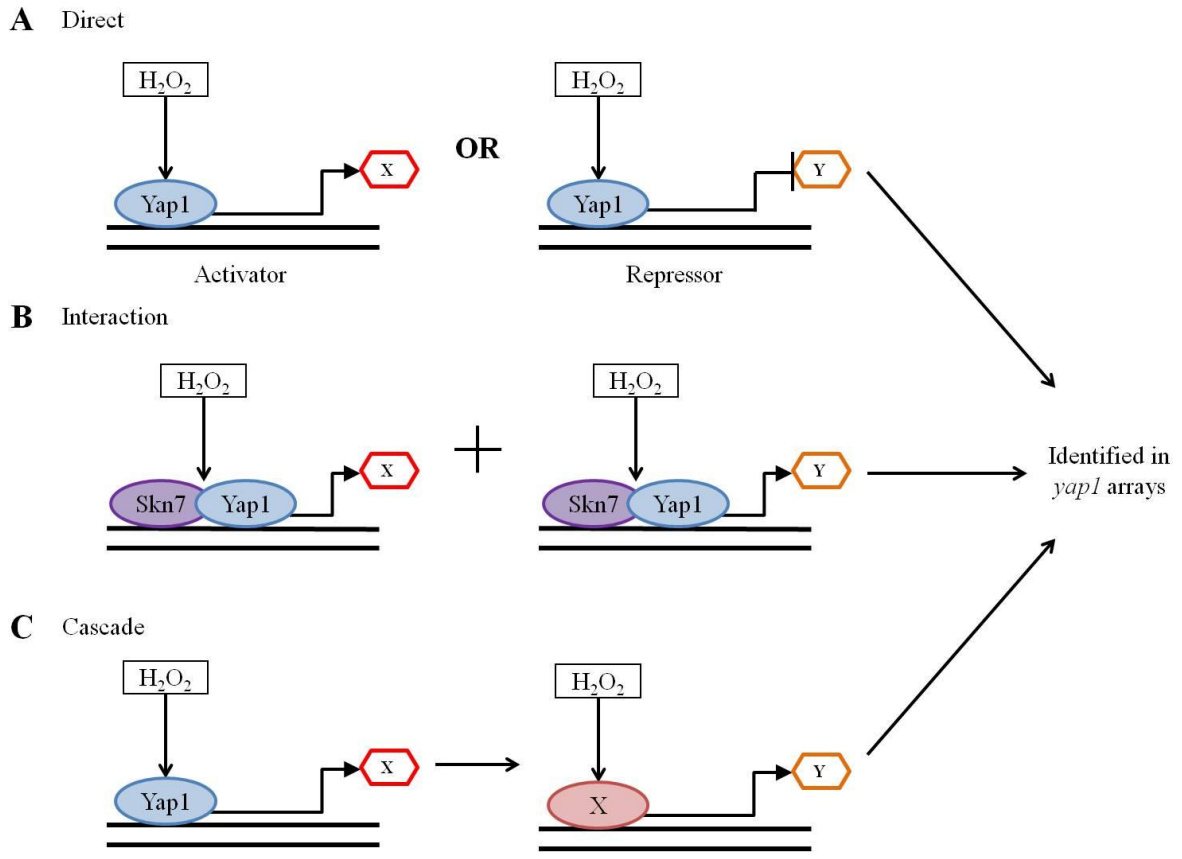


Figure 5.16: Diagram illustrating the possible Yap1 regulatory interactions identified in the null mutant microarray experiments as *YAP1* dependent. Labels denote: **A**, the direct binding of Yap1 in the upstream region of a target gene to act either as an activator or a repressor; **B**, the interaction and co-operation of Yap1 with another transcription factor (Skn7 in this example) is required for the expression of target genes; **C**, a signalling cascade involving Yap1. All these examples result in the same observation in *YAP1* null mutant transcription profiling experiments.

The microarray data presented in this study showed only 99 genes which were regulated under oxidative stress independent of *YAP1*. These include many Heat Shock Proteins, which reveal that while these are regulated in response to stress (they are also regulated by

hyperosmotic stress) their regulation does not rely on *YAP1* in *C. glabrata*. Published data in *C. glabrata* partially confirms this observation as *HSP78*, but not *HSP31* have been shown to be *YAP1* independent **[106]**. *FLR1*, which encodes a plasma membrane multidrug transporter, is also shown to be transcriptionally regulated independently of *YAP1*. This is in contrast to data published in *S. cerevisiae* and Yap1 targets identified under benomyl treatment in *C. glabrata*, where *FLR1* has been shown to be Yap1 dependent **[108, 110, 206]**. This is however in response to benomyl treatment and as the experiments presented in this study were conducted under oxidative stress conditions, this may explain this observation. As discussed in the introduction, Yap1 has been shown to be activated differently by different compounds and stresses. This could however be an error in the microarray analysis and qRT-PCR would confirm this.

As previously discussed, peroxisome genes are important for survival of phagocytosed *C. glabrata* cells with many of these genes regulated in response to oxidative stress. Null mutant transcription profiling showed that these genes are also affected by the absence of *YAP1*, linking the function of Yap1 with the adaptation of *C. glabrata* to the macrophage environment. The regulation of trehalose in response to oxidative stress was also found to be affected by the absence of *YAP1* in *C. glabrata*. While trehalose biosynthetic genes were also observed to be regulated in response to hyperosmotic stress in *C. glabrata*, whether or not this is *YAP1* dependent under hyperosmotic stress is yet to be determined. This may be a case of the absence of *YAP1* affecting a regulatory cascade, rather than directly binding to the upstream region of trehalose genes as no exact YRE-A or YRE-O binding motif was found in these genes. *YPS* genes, previously identified as induced in macrophage engulfed *C. glabrata* cells, are also *YAP1* dependent supporting data that identifies Yap1 (together with Sod1) as important in macrophage survival **[106]**. Not unsurprisingly, many known oxidative stress genes are shown to be *YAP1* dependent in this study, confirming the key regulatory role Yap1 plays in the oxidative stress response of *C. glabrata*.

This study has identified that not only are genes involved in metal ion homeostasis regulated uniquely by *C. glabrata* upon oxidative stress but that this transcriptional regulation is also affected in cells lacking *YAP1*. This observation could explain the lack of phenotypes displayed by *yap2* (*cad1*) null mutants constructed in *C. glabrata* as Cad1 in *S. cerevisiae* is required for metal and oxidative stress resistance. By connecting these observations with studies identifying the mutation and subsequent shift in preferred recognition site of *YAP1* in *C. glabrata*, this would suggest a transcriptional re-wiring of the network.

The oxidative stress responses of wild type *C. glabrata* and *S. cerevisiae* have been shown previously in this study to be similar and therefore many of the Yap1 targets identified in *C. glabrata* are also known targets in *S. cerevisiae*. Of the 57 Yap1 dependent genes identified by Gasch *et al*, 2000, 33 were identified as affected by the removal of *YAP1* in *C. glabrata* **[1]**. These include the major oxidative stress response genes suggesting this stress response and the targets of Yap1 are highly conserved between these closely related species.

As previously discussed, null mutant microarray data is unable to identify the specific binding targets of a protein and therefore further experimental techniques must be utilised. While the benomyl ChIP-chip data used in this study to compare the *C. glabrata* Yap1 dependent targets is not ideal as it is under a different stress condition, no other similar datasets for *C. glabrata* Yap1 are currently available. Therefore, this could explain why only half of the Yap1 targets identified by Lelandais *et al*, 2008, where also identified under oxidative stress in this study **[110]**. ChIP-chip experiments under oxidative stress conditions combined with the null mutant transcriptomics in this study would be needed to establish Yap1 specific dependent targets under oxidative stress.

Efforts to identify Yap1 specific targets from the null mutant arrays conducted in *C. glabrata* by parsing upstream regions for exact YRE-O or YRE-A motifs revealed that, while 60 genes fell into this category and included *SRX1* and *AFT2*, many of the main oxidative stress genes were not found to have exact binding site matches. Of the seven genes available in the *C. glabrata* mutant library and identified as *YAP1* dependent, *aft2* displayed a slight sensitivity to oxidative stress, however, no other oxidative stress phenotypes were observed (Figure 5.15). While this is disappointing, there are many more *YAP1* dependent genes identified in this study to investigate, an example being *SRX1*.

While these observations would need more experimentation and characterisation to verify, they do lead to the conclusion that Yap1 in *C. glabrata* regulates not only oxidative stress genes but is also required for metal resistance and the regulation of metal homeostasis, as well as the regulation of genes involved in macrophage survival and stress resistance.

5.3.8 An updated model of the oxidative stress response in *C. glabrata*

A model of oxidative stress response in *C. glabrata* was constructed from published data mainly from information in *S. cerevisiae*, as our knowledge of the oxidative stress response in *C. glabrata* is still in its infancy (Figure 1.4). Using the transcriptional and functional genomics analyses presented in this study, an updated model of the oxidative stress response

was created (Figure 5.17). This updated model includes the identification of: Tsa2 as the functional, transcriptionally regulated and Yap1 dependent Thio-Specific Antioxidant; Gsh2 as the transcriptionally regulated and Yap1 dependent Glutathione synthetase; and the addition of *SRX1* and *CCS1* as important oxidative stress genes. Those genes which have been identified in this study as transcriptionally regulated by oxidative stress and that transcriptional regulation is affected by the loss of Yap1 in *C. glabrata* are indicated on the figure using blue circles.

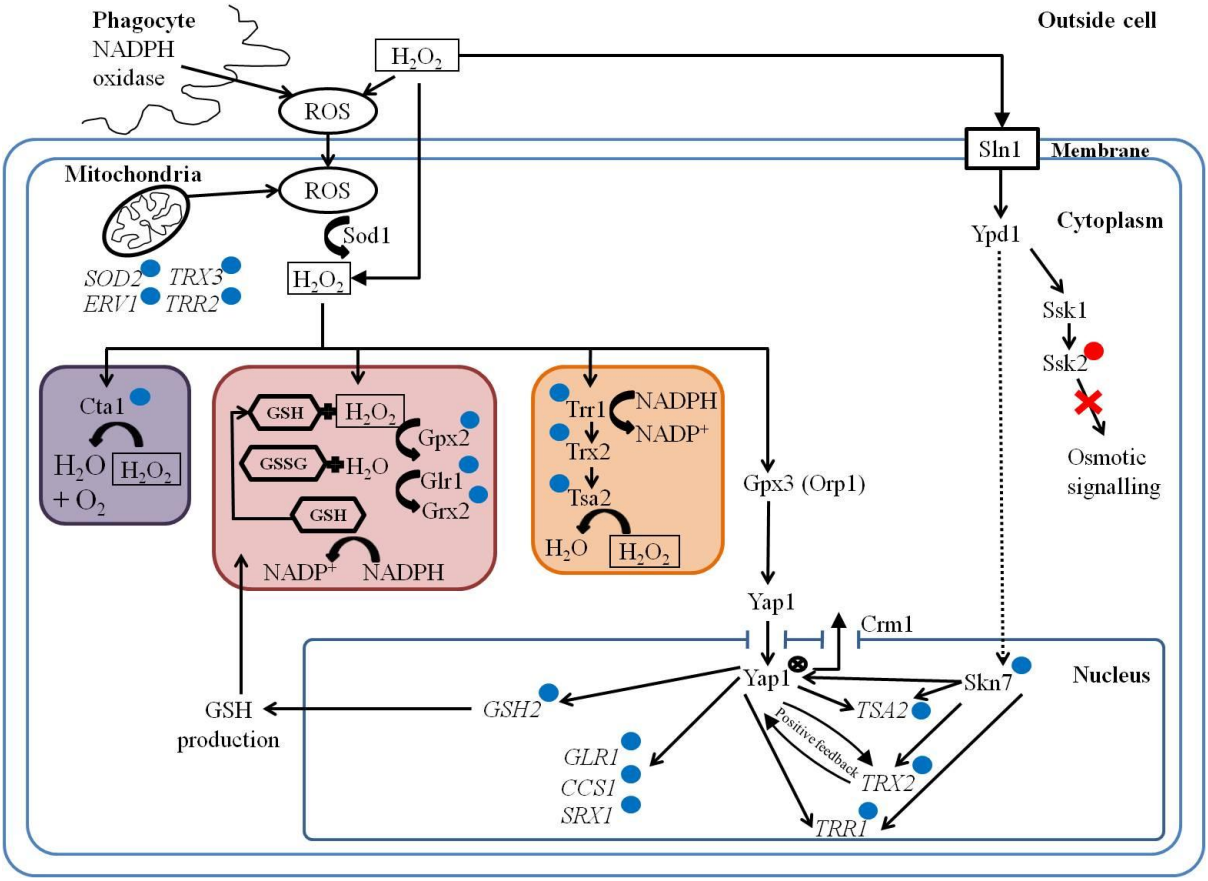


Figure 5.17: Updated model of the oxidative stress signalling pathway in *C. glabrata*. This model was made through mining the literature of *S. cerevisiae*, *C. albicans* and where possible, *C. glabrata*. It shows the production of ROS by the phagocyte and mitochondria, as well as the conversion of ROS into H_2O_2 by Sod1. The three main H_2O_2 detoxification pathways are boxed; catalase (purple), glutathione (red) and thioredoxin (orange). The two main transcription factors which regulate the oxidative stress response and some of their known targets are shown. It also shows the proposed overlap between the hyperosmotic and oxidative stress pathways through Sln1-Ypd1 to Skn7 (dashed line). The point mutation in Ssk2 in *C. glabrata* is denoted by a red circle and the red cross shows the lack of signal transduction to the hyperosmotic stress pathway. The conformational change to Yap1, which allows it to accumulate inside the nucleus, is denoted by a circle with a cross through it. Those genes which have been identified in this study as transcriptionally regulated by oxidative stress and Yap1 dependent in *C. glabrata* are indicated on the figure (blue circles). Using information from **[93-96]**.

6 Combinatorial stress adaptation in *C. glabrata*

6.1 Chapter overview

As a pathogen, *C. glabrata* would face multiple stressors simultaneously in the host environment while trying to establish an infection. The study presented thus far has discussed the adaptation of the pathogenic fungus *C. glabrata* in response to hyperosmotic and oxidative stressors applied singly. The transcriptional and functional genomics analyses in response to these two distinct stresses were investigated and have shown that while the response of *C. glabrata* to these stresses has similarities to that of its close relative, *S. cerevisiae*, some re-wiring of both the hyperosmotic and oxidative stress regulatory pathways has occurred in *C. glabrata*. One of the main aims of this thesis is to determine whether the response of *C. glabrata* to combinatorial stress is simply the addition of the two single stresses, or if a unique transcriptional response is observed.

The concentrations to use for combinatorial stress were chosen from the single stresses already investigated in Chapters 4 and 5. To ensure a moderate growth defect, growth assays using these concentrations of single stressors were conducted. The higher concentrations of 10 mM H_2O_2 with 0.5 M NaCl resulted in increased growth inhibition compared to 0.5 M NaCl alone unlike the combination of the lower dose of H_2O_2 (Figure 6.1). While a similar growth rate is observed for 0.5 M NaCl and 1 mM H_2O_2 singly and in combination, there is a decrease in the final biomass reached by cells treated with these two stressors simultaneously (Table 6.1). A more pronounced affect on the maximum biomass is observed in cells treated with the higher combination of stress therefore, 0.5 M NaCl and 1 mM H_2O_2 were used for the combinatorial stress microarray experiments. For solid plates, 0.5 M NaCl and 2 mM H_2O_2 were used, as experimentation with higher doses (Figure 1.7) resulted in complete growth inhibition.

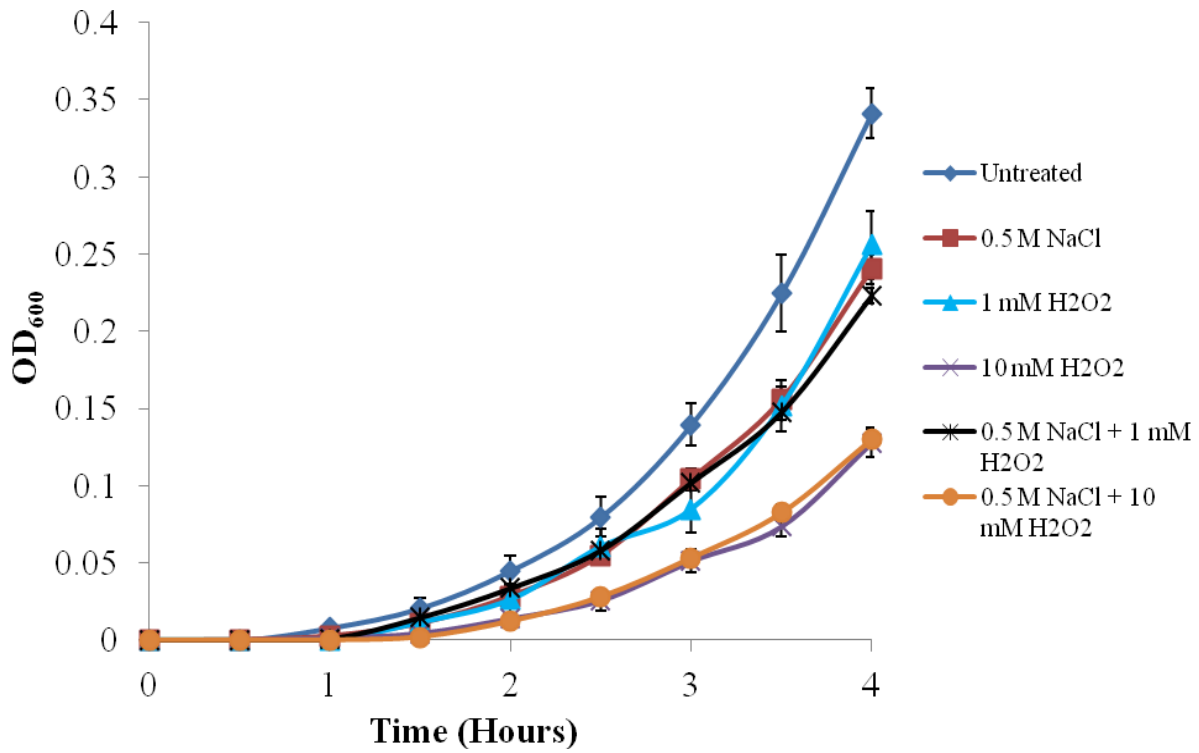


Figure 6.1: Growth of wild type *C. glabrata* to defined doses of single hyperosmotic and oxidative stress and those stresses combined. Wild type *C. glabrata* were grown in either untreated YPD or YPD containing the indicated concentration of NaCl and/or H_2O_2. OD_{600} measurements were taken every 30 minutes over a four hour timecourse. Error bars for the standard deviation of three biological replicates are shown.

Table 6.1: End point biomass of *C. glabrata* treated with single and combinatorial stress for 24 hours.

Treatment	Biomass	SD (+/-)
Untreated	1.589	0.058
0.5 M NaCl	1.311	0.100
1 mM H_2O_2	1.302	0.129
10 mM H_2O_2	1.121	0.024
0.5 M NaCl + 1 mM H_2O_2	1.160	0.095
0.5 M NaCl + 10 mM H_2O_2	1.086	0.016

The biomass measured by OD_{600} after 24 hours treatment with the indicated stress is shown along with the standard deviation of three biological replicates.

To date, there is a lack of published data on combinatorial stress; unlike hyperosmotic and oxidative stresses applied singly, no proteins involved in combinatorial stress response are known. Some proteins have been shown to be involved in both hyperosmotic and oxidative stress such as Hog1 in *C. albicans* **[165]**. Therefore, all mutants identified and discussed in the hyperosmotic and oxidative stress chapters were screened for sensitivity to combinatorial stress.

This chapter compares the transcriptional response of *C. glabrata* to the single stresses described in Chapter 4 and 5 with the aim of revealing the ESR. The ESR is a core set of genes regulated in response to any condition imposed, irrespective of the stressor and has been identified in *S. cerevisiae* and *C. glabrata* **[1, 118]**. The response of *C. glabrata* to combinatorial stress was compared to those stresses applied singly to reveal whether the response to combinatorial stress is unique or the addition of the two single stresses. As we theorise that these combinatorial stress conditions may occur inside a macrophage, the response of *C. glabrata* to combinatorial stress was compared to published microarray data of macrophage engulfed *C. glabrata* cells **[2]**.

6.2 Results

6.2.1 Phenotypic screening of *C. glabrata* null mutants to single and combinatorial stress

All 37 null mutant strains initially presented and discussed in Chapter 4 and 5 were phenotypically screened on combinatorial stress conditions. Many null mutants such as *skn7*, which are sensitive to higher doses of stress (5 mM H_2O_2), are not sensitive to the lower dose used for combinatorial stress (2 mM H_2O_2). Only those mutants for which a phenotype was observed are included in Table 6.2. The only null mutants observed with an increased growth defect on combinatorial stress conditions compared to the single stresses alone were *ste50* and *ste11*. All other null mutants which showed a phenotype on combinatorial stress also showed a similar degree of sensitivity to one of the single stresses. The growth inhibition of *ste11* and *ste50* on solid media containing either hyperosmotic or oxidative stressors singly, or both stresses simultaneously is shown in Figure 6.2.

Table 6.2: Phenotypes observed under combinatorial stress conditions

Condition / Strain	30°C	2 mM H_2O_2	0.5 M NaCl	0.5 M NaCl + 2 mM H_2O_2
Parental - CG2001	N	N	N	N
opy2	N	N	S	S
sho1	N	N	S	S
ste50	N	N	S	L
ste11	N	N	S	L
pbs2	N	N	S	S
gpd2	N	N	S	S
gpd1, gpd2	N	N	S	S
gpd2, natΔ	N	N	S	S
hog1	N	N	S	S
yap1	N	S	N	S

Labels denote: N: normal growth (grey); S: sensitive (orange); L: lethal (dark pink). Growth defect of 1 or 2 isolates and 2 or 3 biological replicates.

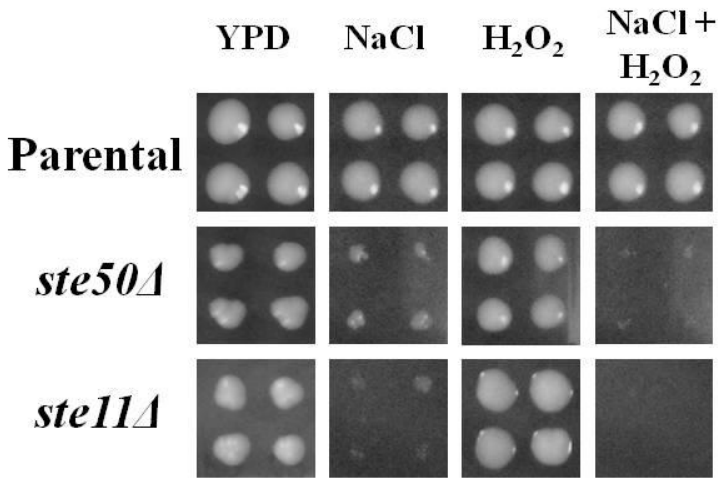


Figure 6.2: The growth of *ste11* and *ste50* mutants upon treatment with single and combinatorial hyperosmotic and oxidative stress. Each strain was spotted four times in a square on to media containing the indicated stress. Labels denote: YPD, untreated control; NaCl, 0.5 M NaCl; H_2O_2, 2 mM H_2O_2 and NaCl + H_2O_2, 0.5 M NaCl and 2 mM H_2O_2. Pictures were taken after 2 days. Representative of two technical and three biological replicates.

6.2.2 Does *C. glabrata* exhibit an Environmental Stress Response?

Before investigating the transcriptional response of *C. glabrata* to combinatorial stress, the response to hyperosmotic and oxidative stressors applied singly was compared. Those genes identified in Chapter 4 and 5 as statistically and significantly regulated in response to 15 minutes treatment with hyperosmotic and oxidative stress are compared in the Venn diagrams below (Figure 6.3). This revealed that a total of 450 genes are regulated by both hyperosmotic and oxidative stress treatment.

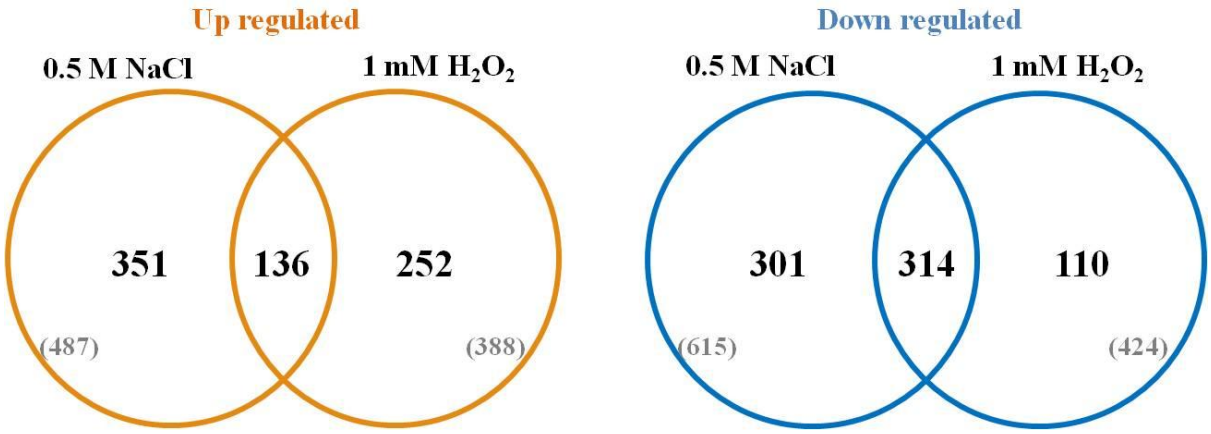


Figure 6.3: Venn diagrams comparing genes regulated under hyperosmotic and oxidative stress applied singly by *C. glabrata*. The number of genes regulated by more than 2 fold under each stress after 15 minutes treatment compared to untreated cells are shown (taken from Chapters 4 and 5). The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

GO term enrichment analysis, using the online database FunSpec (http://funspec.med.utoronto.ca/), revealed that many of the genes commonly up regulated upon hyperosmotic and oxidative stress are involved in the oxidative stress response, response to stress and protein degradation (Table 6.3).

Those genes down regulated by both hyperosmotic and oxidative stress treatment are involved in ribosome biogenesis and sterol and ergosterol biosynthesis (Table 6.4).

Table 6.3: GO terms associated with genes commonly up regulated by *C. glabrata* under hyperosmotic and oxidative stresses applied singly.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Oxidative stress response	$8.17\ e^{-7}$	*CTA1 GRX2 BLM10 HSP12 TRX2 MCR1 GAD1 GRE2 GLR1*	9	55
Response to stress	$3.16\ e^{-5}$	*SSA3 HSP42 HSP78 HSP31 HSP12 GRE3 XBP1 UTH1 HSP104 UBI4 DDR48 TIR4*	12	152
Cytoplasmic and nuclear protein degradation	$1.66\ e^{-5}$	*DOA4 PRB1 LAP4 UBI4 YPS1 PRC1 PBI2 PEP4*	8	60

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 6.4: GO terms associated with genes commonly down regulated by *C. glabrata* under hyperosmotic and oxidative stress.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	$1.00\ e^{-14}$	*UTP20 MAK5 ENP1 REI1 SPB1 KRR1 PWP2 NOP1 DBP10 TSR1 NOP14 RRP3 SSF1 NOP10 IPI1 RPF1*	126	170
Sterol biosynthetic process	0.000277	*ERG11 HMG1 ERG13 ERG2 ERG12 CYB5 MVD1*	7	29
Ergosterol biosynthetic process	0.003492	*ERG11 HMG1 ERG13 ERG2 ERG12*	5	23

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

6.2.3 The transcriptional response of *C. glabrata* to combinatorial stress

The transcriptional response of *C. glabrata* to combinatorial stress conditions was investigated. Statistical analysis using 2-way ANOVA (p-value <0.05) was applied to data with three replicates from *C. glabrata* cells treated with 1 mM H_2O_2 and 0.5 M NaCl simultaneously. This found that 35.7 % (1858 genes) of the total *C. glabrata* genome was significantly regulated immediately upon combinatorial stress treatment (>2 fold compared to untreated cells at 15 minutes, p-value <0.05); 907 genes were up regulated, while 951 genes were down regulated. Using GO terms inferred from homology with *S. cerevisiae*, GO term enrichment analysis using FunSpec (http://funspec.med.utoronto.ca/), was conducted on these regulated genes (p-value <0.01). Genes up regulated in response to combinatorial stress (compared to untreated) are involved in the response to oxidative stress, peroxisome, autophagy and trehalose biosynthesis which have all been observed in the transcriptional response of *C. glabrata* to hyperosmotic and oxidative stress applied singly in this study (Chapter 4 and 5). Genes encoding proteins which are phosphorylated or phosphorylate other proteins and many of which are involved in MAPK signalling pathways are also up regulated (Table 6.5). A similar pattern is observed in genes down regulated by combinatorial stress; down regulated genes are involved in ribosome biogenesis, sterol and ergosterol biosynthesis, the cell cycle and RNA processing (Table 6.6). Many more transcripts are regulated in response to combinatorial stress than the application of the corresponding single stresses cause (see Table 6.7).

While investigating the inferred functionality of *C. glabrata* genes separated into up and down regulated subsets is informative for most processes in which genes are either induced or repressed in response to regulatory cues, some proteins act as repressors, meaning their induction actually results in reduction in that particular cellular process. As previously described in the Introduction, many components of MAPK signalling pathways are shared, leading to cross talk between these pathways and such analysis of the regulation of each component in these pathways may be beneficial. MAPK signalling pathways and genes whose protein products require phosphorylation are regulated when both hyperosmotic stress is applied singly and combinatorial hyperosmotic and oxidative stress (Table 6.5). The MAPK signalling pathways, including the mating and filamentous growth pathways from *S. cerevisiae*, are coloured per the corresponding homologous gene expression of *C. glabrata* cells treated with combinatorial stress (Figure 6.4).

Table 6.5: GO terms associated with genes up regulated by *C. glabrata* under combinatorial stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Cellular response to oxidative stress	$1.36\ e^{-10}$	*PRX1 UGA2 ZTA1 GPX2 MXR2 TRX3 YDL124W TRR1 YPR1 GRX2 MXR1 GRX4 HSP12 ACT1 TRX2*	29	55
Protein phosphorylation	$6.06\ e^{-7}$	*PSK1 FUS3 AKL1 SPS1 KSS1 STE20 SLT2 YCK1 IKS1 IME2 YAK1 HAL5 ELM1 YPK1 NNK1 KNS1 RCK2 MEK1 SKS1 TPK2*	37	133
Peroxisome	$2.17\ e^{-12}$	*PEX22 PEX32 LDH1 PEX7 PEX5 CTA1 PEX3 FAA2 PNC1 PEX14 POX1 PEX4 PEX28 PEX18 POT1 PEX2 TES1*	31	66
Autophagy	$3.48\ e^{-7}$	*ATG8 ATG22 ATG15 ATG9 CIS1 MON1 ATG1 ATG7 NVJ1 ATG32 UTH1 ATG33 ATG23 ATG4 ATG2 ATG21*	19	45
Trehalose biosynthetic process	0.000439	*TPS1 TPS2 UGP1 PGM2 TPS3*	5	7

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 6.6: GO terms associated with genes down regulated by *C. glabrata* under combinatorial stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	1.00 e^{-14}	*TMA23 HAS1 RLP7 NOP2 IMP4 NOP15 DBP2 IPI3 RIO2 KRI1 DBP6 NOG2 CAM1 RPS9A RPS6A NOG1 NAN1 NOP53 NIP7*	143	170
Sterol biosynthetic process	0.000234	*UPC2 ERG9 ERG20 HMG1 ERG13 ERG5 ERG12 ERG8 CYB5 PDR16 MVD1 IDI1*	12	29
Ergosterol biosynthetic process	0.009062	*ERG9 ERG20 HMG1 ERG13 ERG5 ERG12 ERG8 ERG10*	8	23
Cell cycle	0.007625	*BUD3 CDC13 CIN8 BIM1 SMC1 CDC14 SMC2 ALK1 MCM6 CLB6 SWE1 CDC6 BUD4 CDC45 CLB4 BUD8 CDC5 CLN1 CLB2 CLB5*	59	316
rRNA processing	1.00 e^{-14}	*POP5 UTP20 LSM2 MAK5 ENP1 RRP7 SPB1 KRR1 RVB2 DIM1 TIF6 FHL1 MRD1 RRP9 RRP15 NOC4*	133	169

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

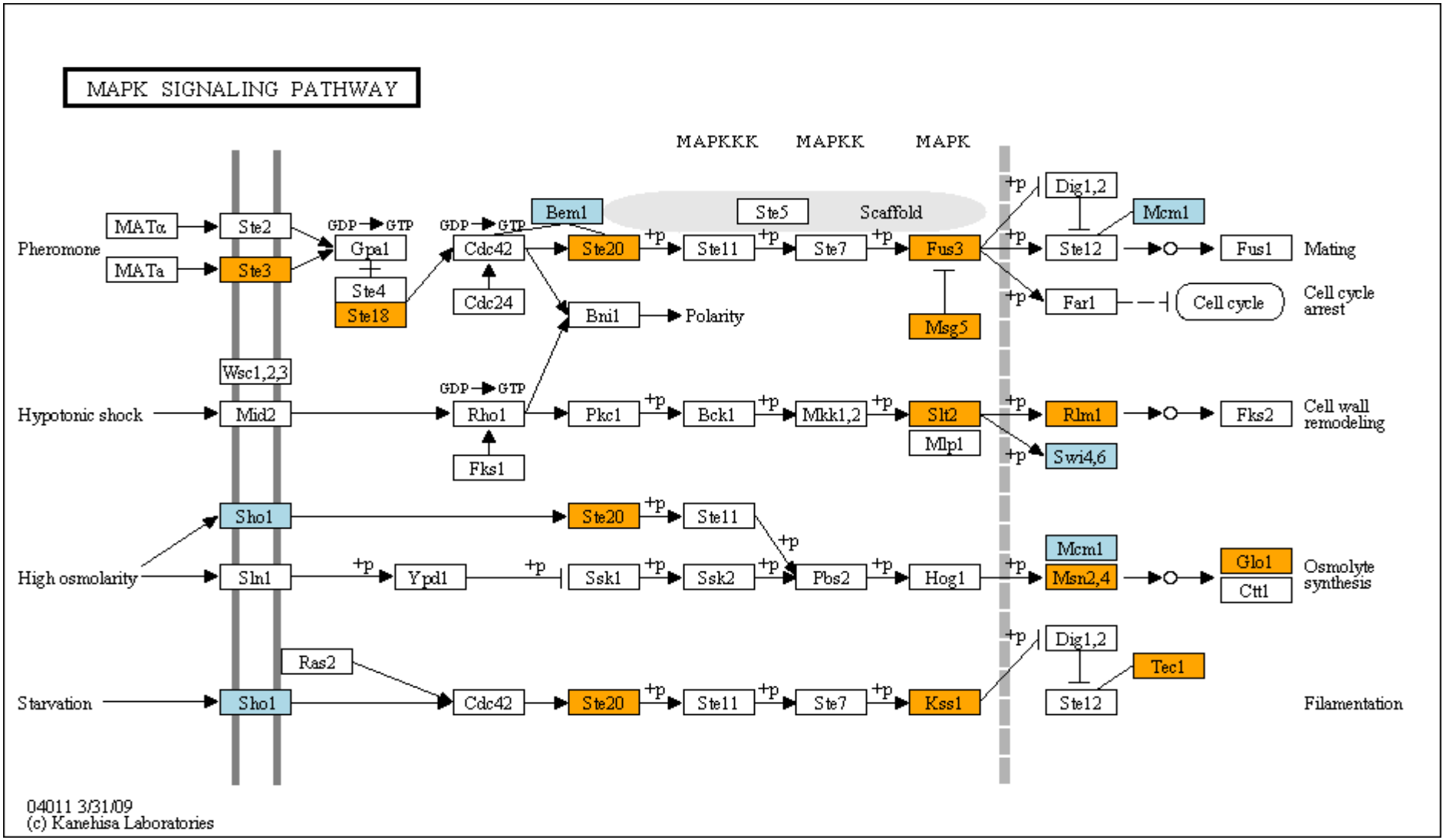


Figure 6.4: The MAPK Signalling Pathways of *S. cerevisiae* coloured as per the regulation of their homologues in *C. glabrata* under combinatorial stress treatment. *S. cerevisiae* standard names are shown. Orange denotes the corresponding homologues in *C. glabrata* which are up regulated by combinatorial stress after 15 minutes treatment, while down regulated genes are shown in blue (> 2 fold, 2-way ANOVA, p value <0.05). Diagram made using KEGG mapper – Search&Color Pathway **[207]**.

Figure 6.4 shows the cross talk between these MAPK signalling pathways which can occur. For example, *STE20* is up regulated by combinatorial stress and is also a component in three MAPK signalling pathways (pheromone, HOG and starvation). Therefore, its regulation in one pathway could have an effect on another; how does the cell control this? The simultaneous application of two stressors at the same may compound this issue for the cell. While some of the MAPKs in these signalling pathways are transcriptionally regulated, their functionality relies on their activation through phosphorylation and translocation into the nucleus and the actual protein levels and phosphorylation states of these proteins are unknown in *C. glabrata*.

The number of genes significantly (p-value <0.05, >2 fold change) up and down regulated upon combinatorial stress treatment over time are shown in Table 6.7, along with the number of genes regulated in response to the same doses of the single stresses. This shows that by 60 minutes treatment with a low dose of stress applied singly, *C. glabrata* has transcriptionally responded and adapted to the stress condition and is transcriptionally similar to untreated cells. This is in contrast to combinatorial stress treated *C. glabrata* cells which are still transcriptionally responding and adapting to the growth conditions imposed after 60 minutes treatment. This is similar to that observed when increasing the oxidative stress applied to *C. glabrata* cells resulted in a prolonged transcriptional response, although it is important to note that this is using the lower dose of 1 mM H_2O_2 (Chapter 5).

Table 6.7: Number of genes statistically and significantly regulated over time upon single and combinatorial stress by *C. glabrata* compared to untreated.

Treatment	Minutes	Up regulated	Down regulated	Total
Hyperosmotic stress	15	487	615	1102
	60	61	15	76
Oxidative stress	15	388	424	812
	60	77	3	80
Combinatorial stress	15	907	951	1858
	60	531	527	1058

The number of genes statistically and significantly up and down regulated in response to the indicated stress are shown (2-way ANOVA, p-value <0.05, >2 fold change).

Nearly all (954/1058) of the genes differentially regulated by *C. glabrata* in response to combinatorial stress treatment for 60 minutes are also regulated at the 15 minute time point. Genes regulated by 15 and 60 minutes treatment with combinatorial stress are involved in similar processes as identified for 15 minutes treatment. Those genes regulated only after 60 minutes exposure to combinatorial stress (104 genes in total) include 24 *C. glabrata* specific

genes. Of those genes with homologues in *S. cerevisiae*, GO term analysis was not able to be conducted on the up regulated genes as none satisfied a p-value cut off of 0.01. Genes down regulated uniquely after 60 minutes treatment with combinatorial stress are involved in aerobic respiration and the electron transport chain.

6.2.4 The transcriptional response of *C. glabrata* to combinatorial stress compared to single stresses

To investigate whether the transcriptional response of the human fungal pathogen *C. glabrata* to combinatorial stress involves a unique response or is simply the addition of the corresponding single stresses, genes statistically and significantly regulated by each stress were compared. A Venn diagram comparing the gene expression of *C. glabrata* to hyperosmotic, oxidative and combinatorial stress is shown in Figure 6.5. A Dendogram was constructed using genes identified from each of the results chapters and analysed using hierarchical clustering methods (Figure 6.6). These diagrams are both representative of the same data. While the Dendogram reveals genes which are regulated by all three conditions and those shared between hyperosmotic and combinatorial stress, those genes uniquely regulated by combinatorial stress are less obvious. However, use of Venn diagrams to represent this data with numbers of statistically and significantly regulated transcripts allows the identification of genes uniquely regulated by combinatorial stress alone.

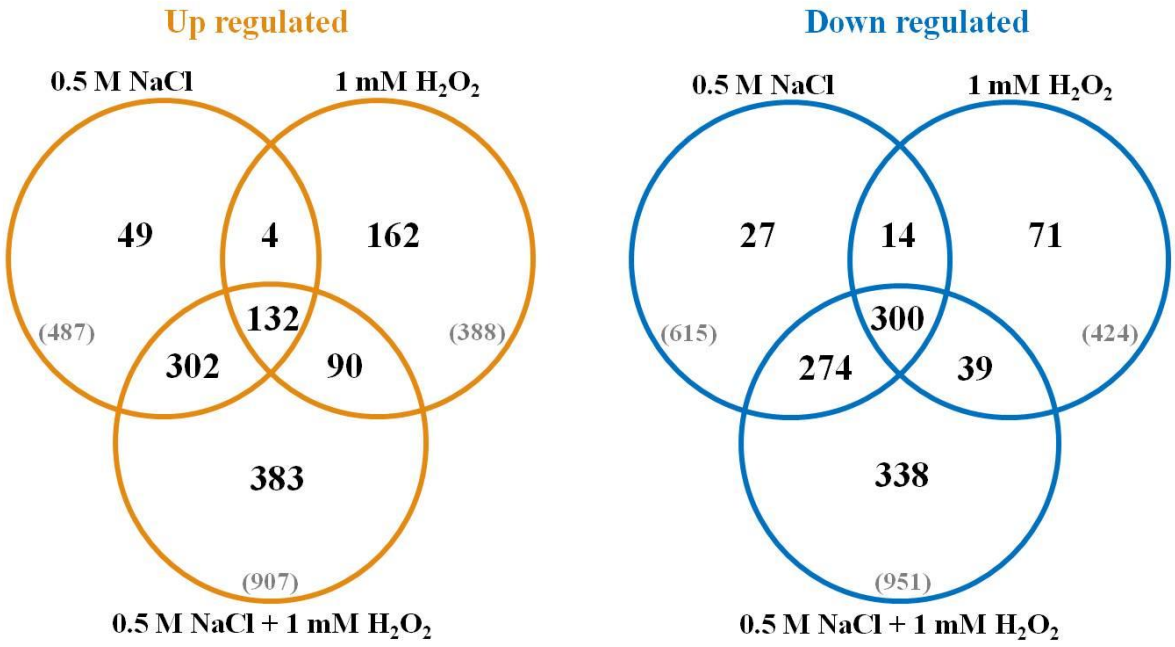


Figure 6.5: Venn diagrams comparing genes regulated under hyperosmotic, oxidative and combinatorial stress by *C. glabrata*. The number of genes regulated by more than 2 fold under each stress after 15 minutes treatment compared to untreated cells are shown (taken from Chapters 4, 5 and 6). The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

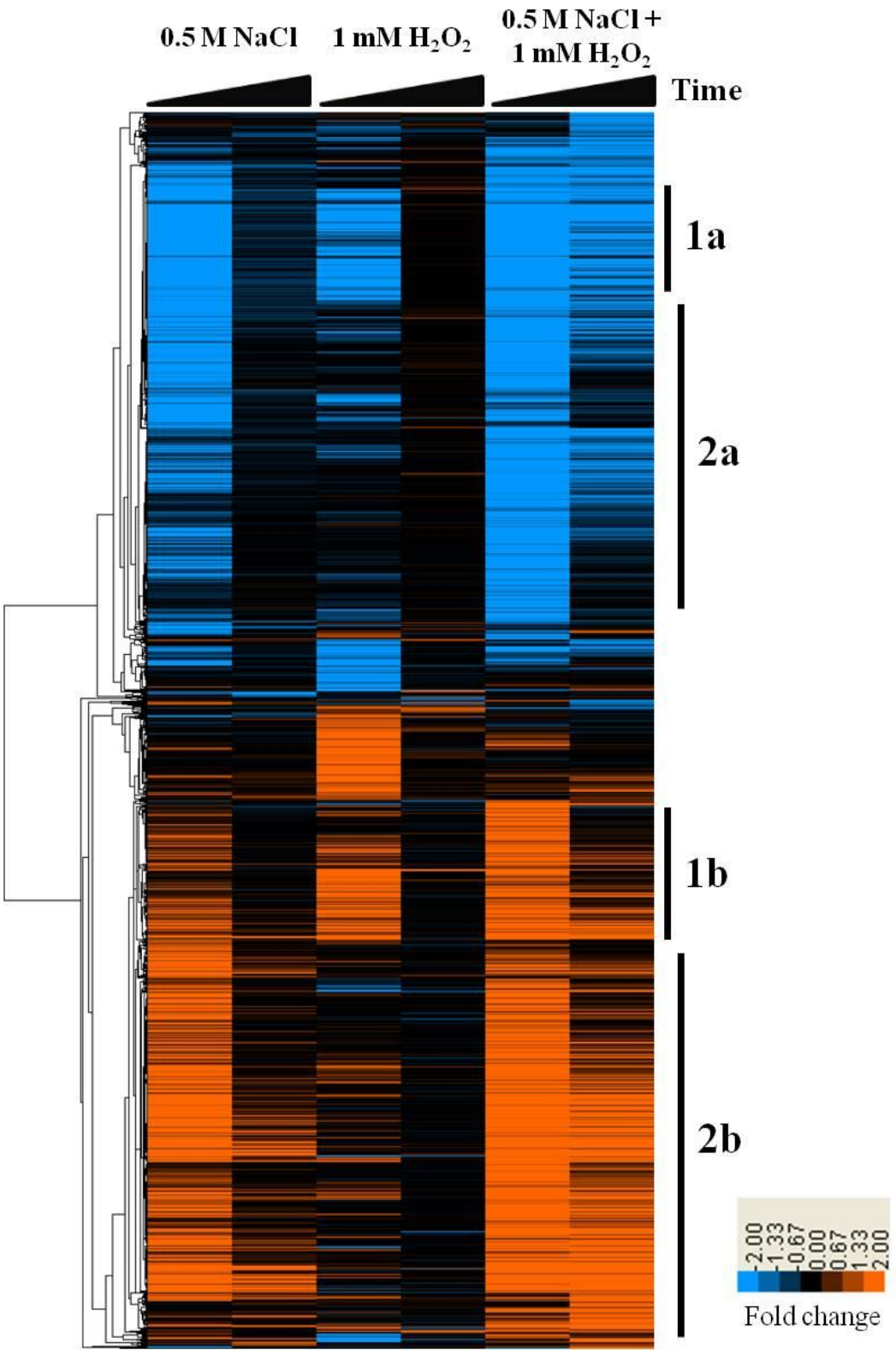


Figure 6.6: Dendogram of all significantly regulated genes in response to hyperosmotic, oxidative and combinatorial stress over time. Genes were clustered by hierarchical methods using centroid linkage in Cluster3 and visualised using TreeView **[141, 142]**. The fold change compared to untreated cells is shown; orange denotes up regulated genes, blue denotes down regulated genes. Clusters were identified and labelled as per the following: 1, common to all stresses; 2, shared between hyperosmotic and combinatorial stress; a, down regulated; b, up regulated. Very few genes are commonly regulated by hyperosmotic and oxidative stress but not combinatorial stress.

As mentioned earlier, nearly all of the genes commonly regulated by *C. glabrata* in response to both hyperosmotic and oxidative stressors applied singly are also regulated by combinatorial stress conditions, with very few genes (18) shared by only single hyperosmotic and oxidative stress (Figure 6.5). The GO terms associated with these genes were described in 6.2.2 and were identified as part of the ESR of *C. glabrata*, with the induction of oxidative stress response genes, heat shock proteins and protein degradation while ribosome biogenesis and sterol and ergosterol biosynthesis was repressed.

A smaller number of transcripts were regulated by both single oxidative stress and combinatorial stress (129), with more genes regulated uniquely by single oxidative stress compared to combinatorial stress (233). No GO terms satisfied a <0.01 p-value cut off for those genes down regulated solely by oxidative stress (71 genes) or shared between oxidative and combinatorial stress (39). Those genes up regulated only by oxidative stress were involved in amino acid biosynthesis and the proteasome (Table 13.1, p.347, Appendix IV). Genes up regulated by both oxidative stress and combinatorial stress are involved in the oxidative stress response and the homeostasis of metal ions, both of which has been thoroughly discussed in Chapter 5 (Table 13.2, p.347, Appendix IV).

Many genes (576 genes in total) are shared between hyperosmotic and combinatorial stress, as shown in both the Dendogram and Venn diagram in Figure 6.5 and Figure 6.6. The shared genes up regulated by both combinatorial and hyperosmotic stress are involved in phosphorylation and the biosynthesis of trehalose and proline (Table 6.8). GO terms associated with those transcripts commonly down regulated by combinatorial stress and hyperosmotic stress revealed little biological information with roles in large and ambiguous biological processes.

Table 6.8: GO terms associated with genes up regulated by *C. glabrata* under combinatorial and single hyperosmotic stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Phosphorylation	0.001753	*PSK1 CDC19 AKL1 GLK1 PRO1 ADK2 HXK2 KSS1 DBF2 TDA10 SLT2 IME2 HAL5 RCK2 NPR1 CMK2 MEK1*	18	206
Trehalose biosynthetic process	$2.12\ e^{-6}$	*TPS1 TPS2 UGP1 PGM2 TPS3*	5	7
Biosynthesis of proline	0.004827	*PRO1 PRO2*	2	3

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Figure 6.5 shows that of the 1858 genes transcriptionally regulated by *C. glabrata* in response to combinatorial stress, 721 genes are regulated uniquely by this stress and not observed in *C. glabrata* cells treated with the corresponding single stresses; 383 transcripts are up regulated and 338 transcripts are down regulated. Genes uniquely up regulated by combinatorial stress are involved in protein phosphorylation, sporulation and autophagy (Table 13.3, p. 348, Appendix IV). These include *FUS3* and *STE20* which both encode phosphorylatable proteins involved in pseudohyphal/invasive growth and mating in *S. cerevisiae* **[208]**. Sporulation is a step in meiosis, the process by which the budding yeast *S. cerevisiae* reproduces sexually. Genes involved in sporulation are up regulated uniquely by combinatorial stress treatment, which is interesting as a mating cycle for *C. glabrata* has not been observed. On closer inspection, other genes whose products are involved in meiosis in *S. cerevisiae* are also significantly regulated by *C. glabrata* in response to combinatorial stress (Figure 6.7). Figure 6.8 shows the regulation of *C. glabrata* genes involved in the peroxisome (as inferred from homology with *S. cerevisiae*) in response to combinatorial stress as many of these transcripts are induced uniquely by combinatorial stress conditions.

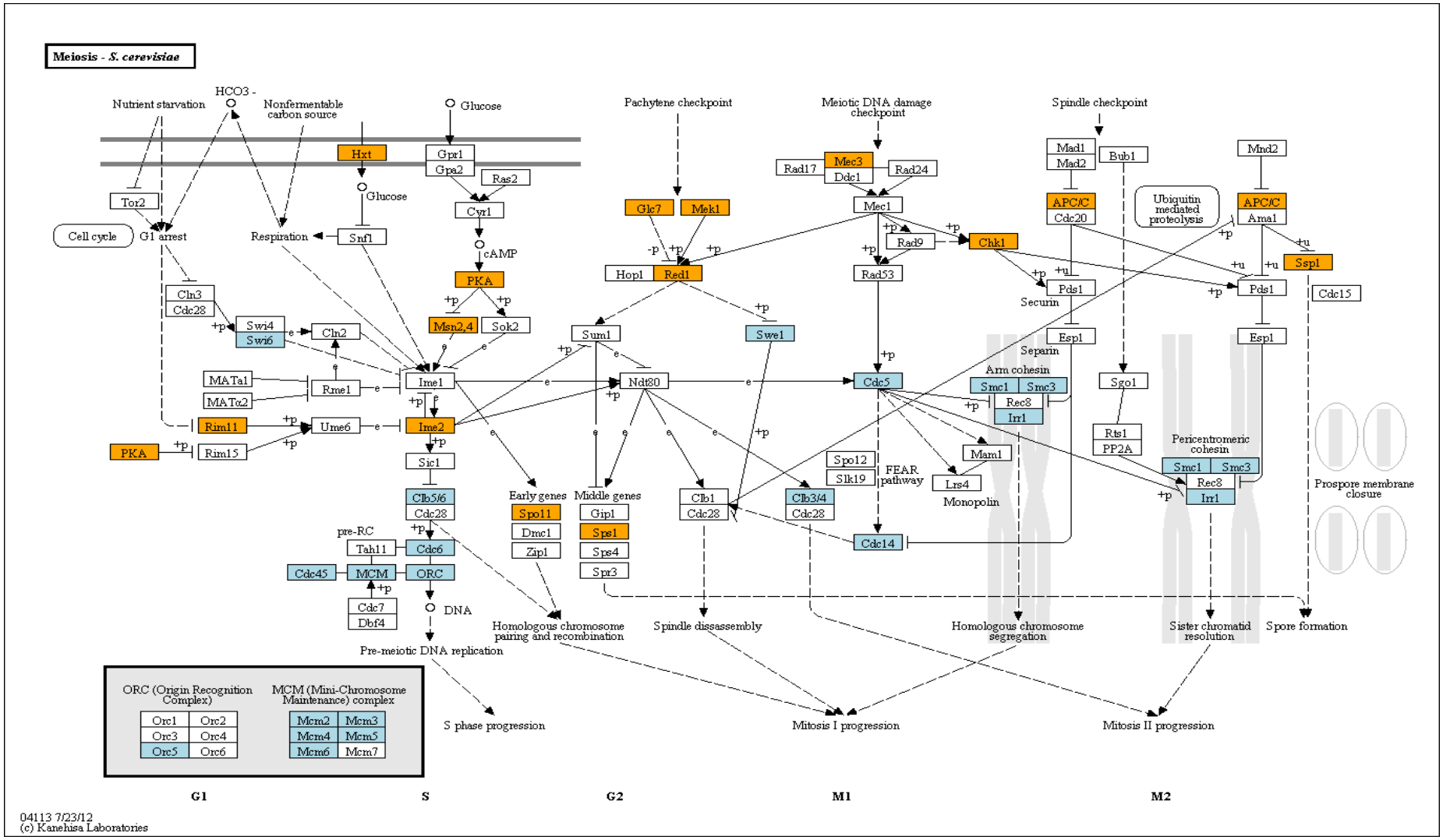


Figure 6.7: The Meiosis pathway of *S. cerevisiae* coloured as per the regulation of their homologues in *C. glabrata* under combinatorial stress treatment. *S. cerevisiae* standard names are shown. Orange denotes the corresponding homologues in *C. glabrata* which are up regulated by combinatorial stress after 15 minutes treatment, while down regulated genes are shown in blue (> 2 fold, 2-way ANOVA, p value <0.05). Diagram made using KEGG mapper – Search&Color Pathway **[207]**.

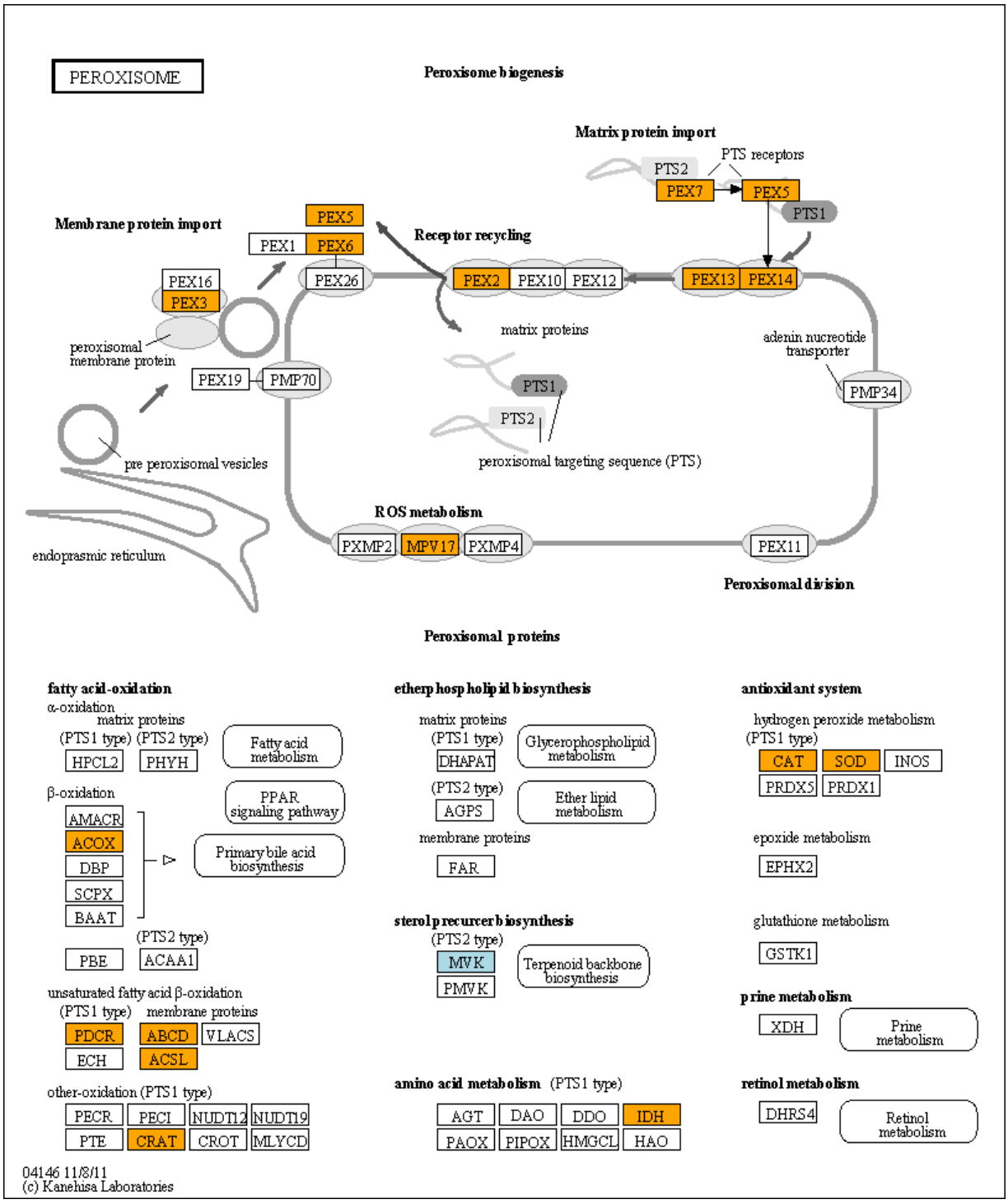


Figure 6.8: The peroxisome of *S. cerevisiae* coloured as per the regulation of their homologues in *C. glabrata* under combinatorial stress treatment. *S. cerevisiae* standard names are shown. Orange denotes the corresponding homologues in *C. glabrata* which are up regulated by combinatorial stress after 15 minutes treatment, while down regulated genes are shown in blue (> 2 fold, 2-way ANOVA, p value <0.05). Diagram made using KEGG mapper – Search&Color Pathway **[207]**.

There are 21 genes up regulated uniquely by combinatorial stress, whose proteins have sequence-specific DNA binding domains suggesting transcription factor activity. These include *TEC1*, *AFT2* and *PDR1* which all encode known transcription factors in *S. cerevisiae* and are involved in pseudohyphal growth, iron homeostasis and multidrug resistance, respectively **[45]**. *YAP1*, encoding one of the main oxidative stress transcription factors, and *YAP3*, also a member of the YAP family, are up regulated uniquely by *C. glabrata* in response to combinatorial stress. There are also 338 genes which are uniquely down regulated in response to combinatorial stress in *C. glabrata*. These genes are involved in translation and the cell cycle (Table 13.4, p.348, Appendix IV). Of the 721 genes uniquely regulated by combinatorial stress treatment, 122 are *C. glabrata* specific genes with no homologue identified from *S. cerevisiae*.

Comparisons between the transcriptional response of *C. glabrata* to single and combinatorial stress have shown many genes regulated uniquely by combinatorial stress indicating that this response is not simply the addition of the single stressors. These uniquely regulated genes are involved in important cellular processes: mating, cell cycle and many genes whose products are phosphorylated suggesting the involvement of MAPK signalling pathways in the unique regulation of the combinatorial stress response.

The majority of bioinformatic analysis conducted in this study use inferred functionality from the corresponding *S. cerevisiae* gene. While this is informative, especially for organisms such as *C. glabrata* with little functional annotation, these analysis techniques automatically disregard those genes which either have no function in *S. cerevisiae*, or for which a homologue has not been identified. These *C. glabrata* specific genes may hold the key as to why *C. glabrata* is more stress resistant, as well as how it has diverged to become a human pathogen. There are 122 non-homologous or functionally unknown genes which are significantly up and down regulated uniquely by *C. glabrata* in response to 15 minutes treatment with combinatorial stress (Table 13.7, p.357, Appendix IV). To try and assign biological function to some of these genes, structural predictions were made on those which are available in the *C. glabrata* null mutant library (Table 6.9). Only one gene displayed high confidence predictions, revealing this gene may be a putative transcription factor with signal transduction activity. Homology with *S. cerevisiae* suggests that the other two genes (*CAGL0E04620g* and *CAGL0F06545g)* encode a GPI-linked protein and a protein involved in activation of Rim101, respectively. These null mutants were phenotypically screened under

single and combinatorial stress conditions (Figure 6.9). None of these null mutants displayed sensitivity to single or combinatorial hyperosmotic and oxidative stressors.

Table 6.9: Predicted domains of those genes uniquely regulated by combinatorial stress and available in the *C. glabrata* null mutant library.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Systematic Name**	**Description** [a]	**Predicted Domains** [b]
CAGL0E04620g	*ECM33*	YBR078W	GPI-anchored protein of unknown function, has a possible role in apical bud growth; phosphorylated in mitochondria	-
CAGL0F06545g	*RIM9*	YMR063W	Protein of unknown function, involved in the proteolytic activation of Rim101p in response to alkaline pH; has similarity to *A. nidulans* PalI; putative membrane protein	-
CAGL0L03674g	*GSM1*	YJL103C	Putative zinc cluster protein of unknown function; proposed to be involved in the regulation of energy metabolism, based on patterns of expression and sequence analysis	Zinc binding domain; fungal transcriptional regulatory protein; PAS domain; signal transduction

[a] Descriptions are taken from SGD **[45]**. [b] Predicted domains, Personal Communication from Mark Wass, Imperial College London.

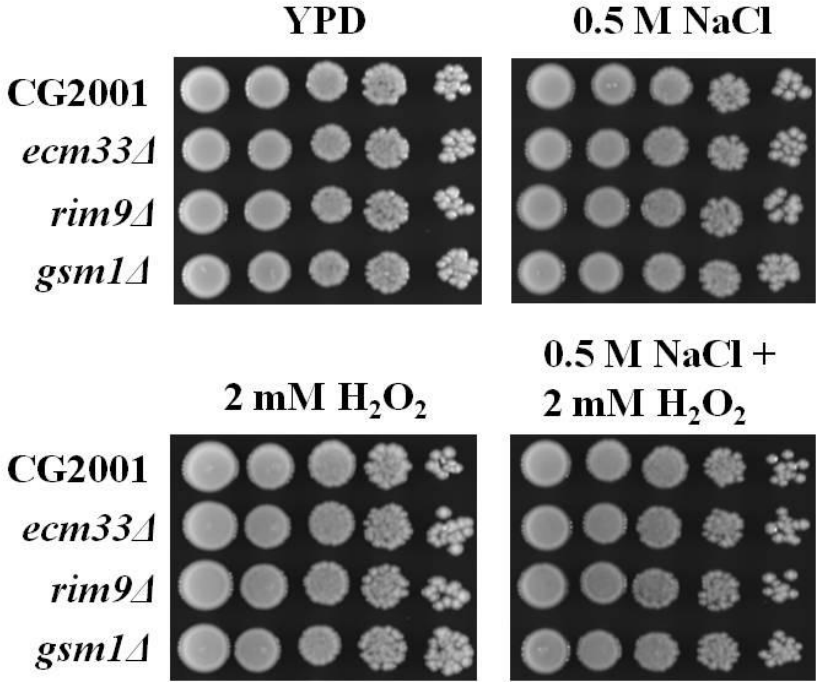


Figure 6.9: Phenotypic screening of null mutants of genes uniquely regulated by combinatorial stress. Overnight cultures were diluted to an OD_{600} of 0.2, tenfold serial dilutions were made and these were spotted on to solid media containing the indicated stress. Plates were incubated at 30°C for 2 days then photographed.

6.2.5 Combinatorial stress response compared to macrophage engulfment

Microarray data by Kaur *et al*, 2007, of *C. glabrata* cells ingested by macrophages for 2 and 6 hours was used to compare to *C. glabrata* cells treated simultaneously with 1 mM H_2O_2 and 0.5 M NaCl **[2]**. This macrophage dataset was also used to compare to genes uniquely regulated by combinatorial stress in *C. glabrata*. The Venn diagrams below (Figure 6.10) show that a total of 358 transcripts are regulated by combinatorial stress conditions and when *C. glabrata* is engulfed by macrophages. This equates to 35.8% of genes up regulated and 34.2% of down regulated genes by macrophage engulfed *C. glabrata* cells. These include genes identified and discussed in Chapter 5 as regulated by oxidative stress and macrophage engulfment such as *CTA1*, *SOD2* and *RIM101*. *RLM1*, which encodes a transcription factor involved in cell wall integrity that is activated by the MAPK Slt2, and *RCK2*, which encodes a kinase involved in hyperosmotic and oxidative stress, are both up regulated by *C. glabrata* in response to combinatorial stress and macrophage engulfment. Genes involved in fatty acid metabolism and transport are also up regulated (Table 6.10). Genes down regulated (Table 6.11) in response to macrophage engulfment and combinatorial stress are involved in translation, ribosome biogenesis and RNA binding, which have all been identified and discussed in Chapters 4 and 5. Genes involved in sterol biosynthesis were also down regulated, but not uniquely regulated by combinatorial stress conditions as these were identified in oxidative stress treated *C. glabrata* cells. The down regulation of genes involved in cellular cell wall organisation were also observed.

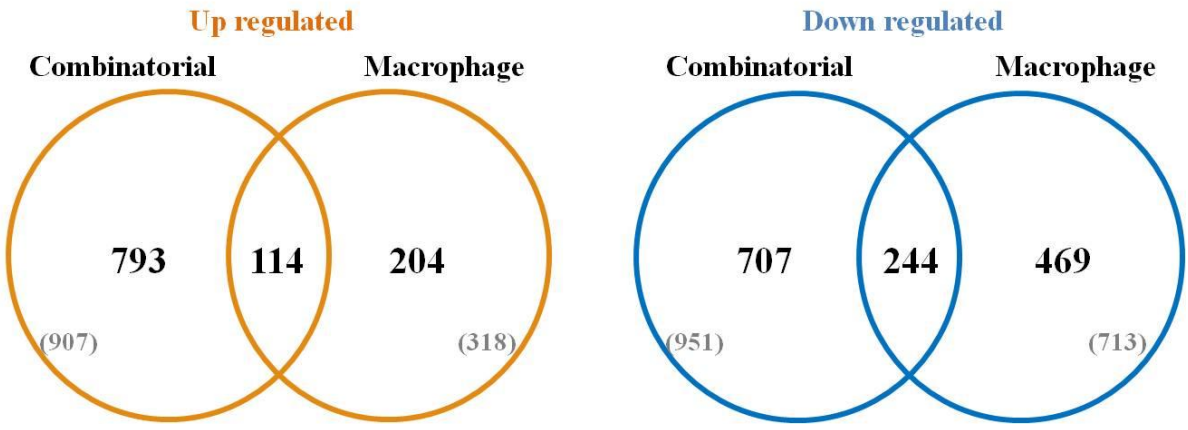


Figure 6.10: Venn diagrams comparing genes regulated by *C. glabrata* under combinatorial stress and macrophage engulfment. The number of genes regulated by more than 2 fold under each condition compared to untreated cells are shown. The number of up regulated genes are shown in the diagram on the left, while the number of down regulated genes are shown on the right. The total number of genes in each section are shown in brackets.

Table 6.10: GO terms associated with genes up regulated by *C. glabrata* under combinatorial stress treatment and macrophage engulfment.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Fatty acid metabolic process	$1.89\ e^{-7}$	*YAT1 FAA2 YAT2 POX1 POT1 FOX2 ECI1*	7	28
Fatty acid transport	0.000744	*PXA2 PXA1*	2	3
Oxidative stress response	0.00171	*UGA2 CTA1 BLM10 SOD2 GAD1*	5	55

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 6.11: GO terms associated with genes down regulated by *C. glabrata* under combinatorial stress treatment and macrophage engulfment.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Translation	$1.00\ e^{-14}$	*EFB1 RBG1 ILS1 RPL32 GRS1 SUP45 RPS9B SSZ1 RPL20A TIF11 RPL9B CDC60 RPL7B SUI3 TIF6*	82	318
Ribosome biogenesis	0.001009	*RPS9B NOP1 NHP2 SNU13 ARB1 RPS2 RPL40B RPL8B RPS0B EMG1 RPP0 RPS15*	15	170
Sterol biosynthetic process	0.003227	*ERG9 ERG13 CYB5 PDR16 MVD1*	5	29
Cellular cell wall organization	0.004186	*CHS2 ECM33 UTR2 SCW4 CIS3 EXG1 GAS1 SUN4 SRL1*	9	89
RNA binding	$7.99\ e^{-8}$	*EMG1 RPL6B RPS18B TIF11 PUB1 RPL16B RNH201 RPS15 CDC33*	33	337

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Of those genes identified as commonly regulated by combinatorial stress and macrophage engulfment (358 genes), 122 transcripts are regulated uniquely by combinatorial stress and are therefore not regulated in response to stresses applied singly (27 genes induced, 95 genes repressed) (Table 13.5 and Table 13.6, p. 349- p.351, Appendix IV). Many of the transcripts up regulated uniquely by combinatorial stress and macrophage engulfment are localised to the peroxisome and are either involved in fatty acid metabolism or the import of fatty acids into the peroxisome. *PDR12*, which encodes a multi-drug transporter and *CAGL0E01837g*, which has been identified as a member of the expanded YPS gene cluster found in *C. glabrata*, were also up regulated **[2]**. Ribosome biogenesis and translation are both common themes among genes down regulated in response to stress and this is again observed in those transcripts which are down regulated uniquely by combinatorial stress and macrophage engulfment. Genes involved in cellular cell wall organisation are also down regulated showing that this is unique to combinatorial stress. There are 10 *C. glabrata* specific genes regulated in this group (2 up regulated and 8 down regulated) and these have been discussed in 6.2.4.

These observations demonstrate that the conditions found inside a macrophage elicit a similar transcriptional response from *C. glabrata* as observed for combinatorial stress treatment.

6.3 Discussion

This chapter has presented the transcriptional adaptation over time of the human fungal pathogen *C. glabrata* to combinatorial stress, the simultaneous application of hyperosmotic and oxidative stressors. The implications of these results will now be discussed. As genes involved in combinatorial stress response have not been identified in any yeast, phenotypic screening was conducted on all null mutants examined in the preceding single stress chapters and revealed only two null mutants displayed increased sensitivity to combinatorial stress conditions. The transcriptional response of *S. cerevisiae* to combinatorial stress has not been investigated therefore any similarities with that of *C. glabrata* could not be explored. An ESR, a set of genes regulated irrespective of stress treatment, has been identified previously in *C. glabrata* and this was investigated in this study **[118]**. The transcriptional response of *C. glabrata* to single and combinatorial stressors were compared and revealed that there is a unique response to combinatorial stress, not observed under single stress treatment. Those genes regulated uniquely by combinatorial stress conditions could point towards the mechanism behind this combinatorial stress response and will now be discussed. Through this project, the response of *C. glabrata* to combinatorial stress was found to be similar to that of macrophage engulfed *C. glabrata* cells, confirming our theory that the host setting elicits combinatorial stress conditions.

6.3.1 Genes required for resistance to combinatorial stress are involved in many distinct stress responses

Combinatorial stress response has not been widely investigated in yeast and as such, no genes have been specifically identified as being involved in combinatorial stress response. The main aim of this project was to investigate whether the response of *C. glabrata* to combinatorial stress was unique or could be explained by simply combining the responses of stressors applied singly. All null mutants previously investigated in the single stress chapters were screened for increased sensitivity to combinatorial hyperosmotic and oxidative stress, compared to those stressors applied singly. Only two null mutants displayed increased sensitivity to combinatorial stress: *ste11* and *ste50*. *STE11* and *STE50* encode proteins involved in the HOG pathway, a MAPK signalling pathway discussed in Chapter 4. The MAPK signalling pathways of *S. cerevisiae* have been extensively studied and it is known that many components are involved in more than one pathway (for examples see Figure 6.4). As shown in the hyperosmotic stress chapter (Chapter 4), *ste11* and *ste50* are required for

resistance to many distinct stressors including: temperature shifts, hyperosmotic stress, heavy metals, cell wall stressors and defects in protein glycosylation, protein synthesis and protein folding. While many of these phenotypes are also displayed by other null mutants of the HOG pathway in *C. glabrata* (Table 4.2), *ste11* and *ste50* mutants displayed the most severe growth inhibition to many conditions. The sensitivity of *ste11* and *ste50* mutants implies that combinatorial stress leads to crosstalk between the signalling pathways, as described in the introduction.

6.3.2 *C. glabrata* does exhibit an ESR

The transcriptional response of *C. glabrata* to hyperosmotic and oxidative stressors applied singly has been investigated and described in Chapters 4 and 5. In many fungi, *S. cerevisiae* included, a core set of genes have been identified as regulated in response to non-optimum conditions, regardless of the stress imposed **[1, 165]**. To investigate these previous findings, genes identified as statistically and significantly regulated by *C. glabrata* upon single stress treatment were compared in Figure 6.3. This revealed that 450 genes were differentially regulated by *C. glabrata* in response to both stresses applied singly compared to untreated cells. ESR genes induced by stress in this study were found to be involved in (using inferred functionality from homology with *S. cerevisiae*) the response to stress, including the induction of genes encoding heat shock proteins and the response to oxidative stress. The observation of nine genes involved in oxidative stress being regulated in response to hyperosmotic stress conditions by *C. glabrata* has been discussed previously in Chapter 4, as well as the possible roles of heat shock genes. Genes involved in protein degradation were also up regulated in response to stress conditions, suggesting the removal of unneeded proteins is important when adapting to any stress. As observed throughout this study, genes involved in growth are consistently down regulated immediately in response to non-optimal conditions. This observation is not an artefact of the response of cells to a new environment as the experimental design used in this study compares stress treated cells to untreated (YPD) cells which have also been moved to new media, hence, any response to this would be removed during data analysis.

The number of genes identified in this study as part of the ESR of *C. glabrata* is lower than the number published by Roetzer *et al*, 2008, where they showed a total of 1050 genes regulated irrespective of the stress imposed **[118]**. While the thesis presented here used only two stressors to identify genes involved in the ESR, this paper compared the transcriptional response of *C. glabrata* to four distinct stressors: hyperosmotic stress; oxidative stress;

glucose starvation and heat shock, and this may explain the large difference in the total number of genes observed. Although a smaller number of ESR genes were observed in this study, they are involved and associated with similar biological functions and GO terms as presented in the aforementioned paper.

These observations suggest that *C. glabrata* does possess an ESR as displayed by other fungi, mainly associated with the repression of growth and non-essential processes to conserve energy and the induction of genes involved in protein folding, the degradation of unwanted proteins and removal of ROS. While a few oxidative stress genes were regulated by hyperosmotic stress, the transcriptional responses of *C. glabrata* to these single stressors are largely distinct, as the major genes involved in each stress response, inferred from homology with *S. cerevisiae*, are only regulated by that specific stress.

6.3.3 The transcriptional response of *C. glabrata* to combinatorial stress

This chapter has explored the transcriptional response of *C. glabrata* to the simultaneous addition of hyperosmotic and oxidative stressors (0.5 M NaCl and 1 mM H_2O_2). These were the same concentrations used in Chapters 4 and 5 to investigate the response of this fungus to stressors applied singly and liquid growth assays found that, at these doses, these stressors had a similar affect on growth when applied singly or in combination (Figure 6.1).

Genes found to be up regulated in response to combinatorial hyperosmotic and oxidative stress treatment were revealed to be involved in oxidative stress and the biosynthesis of trehalose, as well as localised to the peroxisome and involved in autophagy. All of these GO terms were observed in genes up regulated by *C. glabrata* cells treated with single oxidative and hyperosmotic stress and so their functional implications have been discussed previously in Chapters 4 and 5. Many genes up regulated by combinatorial stress encode proteins which require phosphorylation for their activation, including *FUS3*, *STE20* and *SLT2*. This indicates that MAPK signalling pathways are regulated by combinatorial stress conditions. While some of these genes are regulated by *C. glabrata* in response to hyperosmotic stress, many are unique to combinatorial stress and the implications of this will be discussed herein.

In response to combinatorial stress treatment, *C. glabrata* immediately down regulates genes involved in ribosome biogenesis, the cell cycle and RNA processing suggesting a repression of growth and transcription, perhaps to conserve energy. Genes involved in sterol and ergosterol biosynthesis are also down regulated upon combinatorial stress treatment, which as discussed in Chapters 4 and 5, are known to be essential for oxidative and hyperosmotic

stress resistance in *S. cerevisiae* and must be repressed to effectively survive these stress conditions **[204]**.

As discussed in the Introduction, it has been reported that the formation of new chromosomes by *C. glabrata* is used as a virulence mechanism, with clinical isolates displaying chromosome re-arrangement as well as extra chromosomes **[27]**. The process of aneuploidy (extra chromosomes compared to wild type) increases transcript abundance and this would be reflected in transcriptional analyses. While this can have detrimental effects to the cell, it has been shown that dosage compensation occurs at the level of protein synthesis and therefore an increase in transcript levels does not always result in increased protein levels **[209]**. While the duration of combinatorial stress treatment of *C. glabrata* investigated in this thesis is unlikely to be long enough for aneuploidy to occur, the transcriptional response is similar to that displayed by aneuploidy *S. cerevisiae* cells, with the up regulation of stress response genes including that of oxidative stress and the down regulation of those responsible for ribosome biogenesis **[210]**. Whether this is because a loss or gain in chromosome number leads to stress conditions, in turn activating stress responses would need further investigation.

As seen with higher doses of oxidative stress (10 mM compared to 1mM H_2O_2), presented in Chapter 5, *C. glabrata* displays a prolonged transcriptional response to combinatorial stress over time. Unlike in response to hyperosmotic and oxidative stress applied singly, many transcripts are differentially regulated after 60 minutes treatment (Table 6.7). Most of these are the same genes significantly regulated after 15 minutes treatment suggesting a prolonged activation of the signalling and regulatory pathways involved. This may be because of cross talk between signalling pathways, as discussed in the Introduction and it may take longer for the cell to interpret the correct response to combinatorial stress than single stress conditions. Another possibility is that as many signalling pathways rely on the phosphorylation and de-phosphorylation of MAPKs in order to control transcriptional activity and under combinatorial stress conditions, protein phosphatases are not induced, regulatory components stay activated longer. This may also be a rate limiting step of protein phosphatases, where there are simply not enough of these proteins to de-phosphorylate all the signalling components required.

Most of the genes regulated uniquely by 60 minutes combinatorial stress treatment compared to only 15 minutes are down regulated and are involved in electron transport chain and aerobic respiration. This implies a repression of processes to generate energy by *C. glabrata* in response to prolonged combinatorial stress and could be a way to conserve energy under

these extended stress conditions. Unlike in the single oxidative stress, where prolonged transcriptional regulation resulted in an increased lag time in liquid growth assays, combinatorial stress conditions displayed no difference in the lag time or growth rate compared to single stresses (Figure 6.1). However, combinatorial stress (0.5 M NaCl and 1mM H_2O_2) did result in a lower final biomass suggesting more energy is used to adequately respond and survive combinatorial stress conditions.

This study has shown that in response to combinatorial stress, *C. glabrata* elicits a prolonged transcriptional response, with the majority of genes regulated at 15 minutes, also regulated after 60 minutes. Genes involved in MAPK signalling pathways are regulated, indicating their involvement, as well as those involved in mating. This transcriptional response is similar to that seen by aneuploidy cells of *S. cerevisiae* and whether chromosomal re-arrangement occurs in response to combinatorial stress in *C. glabrata* is an interesting possibility.

6.3.4 The response of *C. glabrata* to combinatorial stress is unique and not simply the addition of two single stresses

One of the main aims of this study was to investigate whether the transcriptional response of *C. glabrata* to combinatorial stress could be explained by simply combining the response to stressors applied singly or whether a unique response would be observed. The results presented in this chapter and compared to those from the corresponding single stresses showed that while some genes are regulated in response to all of the three stress conditions imposed, a set of 721 genes are regulated uniquely by combinatorial stress conditions. As shown in Figure 6.5, nearly all of the genes regulated commonly by *C. glabrata* in response to single stressors are also regulated by combinatorial stress treatment and therefore their functional implications have already been discussed in Chapter 4 and 5. Experimentation has shown that each single stress has its own distinct response with a set of genes regulated either uniquely by that single stress or shared with combinatorial stress. A higher number of differentially regulated genes are shared between hyperosmotic and combinatorial stress than oxidative and combinatorial stress. These induced genes are involved in trehalose and proline biosynthesis which have been discussed previously; trehalose has been shown to be important for stress response in *S. cerevisiae*, while the regulation of proline in response to hyperosmotic stress is novel to *C. glabrata*. Genes which encode phosphorylatable proteins are also up regulated by hyperosmotic and combinatorial stress, but not oxidative stress and

this suggests that the response to hyperosmotic stress is heavily controlled by MAPK pathways, such as the HOG pathway, in which many components require phosphorylation.

It has been shown that many genes are regulated uniquely by combinatorial stress treatment and are not regulated by the corresponding stress when applied alone. Genes up regulated uniquely by combinatorial stress are localised to peroxisomes, involved in autophagy and are those whose protein products require phosphorylation. As discussed in the oxidative stress chapter (Chapter 5), peroxisomes are important in the survival of *C. glabrata* when engulfed by macrophages and their observed regulation could suggest that combinatorial stress conditions are similar to that found inside a macrophage **[88]**. The induction of genes involved in autophagy suggest the degradation of un-needed proteins and organelles; a process key to surviving stress and/or starvation conditions to conserve nutrients and relocate this energy to essential processes.

The observed over-representation of genes which encode phosphorylatable proteins regulated uniquely by combinatorial stress suggests the involvement of MAPK signalling pathways in this unique response by *C. glabrata*. Genes encoding products with DNA binding activity and therefore possible transcription factor activity are also uniquely up regulated by combinatorial stress and along with these MAPK pathways could be the regulators of the combinatorial stress response. These include homologous genes which encode the MAPK Fus3 and the MAPKKKK Ste20, as well as the transcription factor Tec1 which are involved in filamentation and mating in *S. cerevisiae* **[208]**. As discussed in the introduction, *C. glabrata* only forms pseudohyphae under nitrogen starvation conditions and has not been shown to mate although homologous components of the mating pathway are present **[33, 35]**. Ste20 is not required for nitrogen starvation induced pseudohypae formation in *C. glabrata*, but is essential for cell wall integrity and virulence **[147]**. While the phosphorylation state of those involved in these pathways are unknown, this up regulation suggests that filamentation may occur as a result of combinatorial stress. While this has not been specifically tested for in this study, *C. glabrata* cells grown on combinatorial stress conditions have not been observed to form psuedohypae (Data not shown). Phenotypic screening of the *C. glabrata* mutant library as well as the null mutants constructed in this study on nitrogen starvation media would be informative to ascertain their involvement. Genes with inferred functionality from *S. cerevisiae* involved in sporulation are also regulated uniquely by *C. glabrata* in response to combinatorial stress. This is interesting as *C. glabrata* has not been shown to go through a sexual cycle, even though it has retained nearly all the homologous mating genes from *S.*

cerevisiae. Could combinatorial stress trigger sporulation/mating in *C. glabrata*? Could this be happening in the host environment too? A more thorough investigation would be needed to ascertain this.

PDR1, which encodes a transcription factor in *C. glabrata*, and the multidrug transporter encoded by *PDR12*, are up regulated uniquely by combinatorial stress. This transcription factor regulates drug resistance and its targets, *PDR12* being one of them, have been shown to enhance the virulence of drug resistant *C. glabrata* isolates **[24]**. The expression of *PDR1* has been shown to be higher in azole resistant *C. glabrata* clinical isolates compared to susceptible isolates and this would suggest that combinatorial stress mimics the host environment and elicits a similar response from *C. glabrata* **[211]**. *AFT2* also encodes a transcription factor and is involved in iron homeostasis and oxidative stress in *S. cerevisiae* **[212]**. Iron homeostasis has been shown to be important for bacterial pathogens and human diseases which cause iron overload correlate with an increase in infections **[57, 213]**. The unique up regulation of *AFT2* in response to combinatorial stress could be part of the response to oxidative stress but could also point towards the mechanisms behind this unique response to combinatorial stress. While phenotypic screening of null mutants on media containing additional iron did not result in any phenotypes, screening on low iron media, although not conducted here, could help to explore this observation.

Those genes down regulated uniquely by *C. glabrata* in response to combinatorial stress treatment were found to be involved in the cell cycle suggesting a cessation in cell growth. In response to stress, particularly oxidative stress, there is a need to repair damaged DNA and proteins before growth can continue, with DNA damage check points built into the cell cycle. While the addition of both stressors simultaneously did not cause a decrease in growth rate or lag time compared to those stressors applied singly, there was an observed reduction in the final maximum biomass (Figure 6.1 and Table 6.1). Genes involved in translation were also down regulated by combinatorial stress, again suggesting a repression of non-essential protein production and this would help to conserve energy for cell survival under stress.

Of those genes uniquely regulated by combinatorial stress in *C. glabrata*, 122 had no homology or an unknown function in *S. cerevisiae*. Of these uniquely regulated transcripts, three had interesting predicted domains and functions, as well as being available in the *C. glabrata* null mutant library (*ECM33*, *RIM9* and *GSM1*) (Table 6.9). Null mutants of these genes were screened for sensitivity to single and combinatorial stresses; unfortunately, none displayed any growth inhibition (Figure 6.9). This showed that although these genes are

regulated by *C. glabrata* in response to combinatorial stress, they are not required for resistance.

This study has demonstrated that the response of *C. glabrata* to combinatorial hyperosmotic and oxidative stressors is unique and not simply the addition of two single stresses. The transcription factors regulated may be what are controlling the unique response observed by *C. glabrata* to combinatorial stress conditions. While the phosphorylation states of the proteins regulated in response to combinatorial stress are unknown, their observed regulation implies their pathways involvement in combinatorial stress.

6.3.5 Combinatorial stress elicits a similar transcriptional response from *C. glabrata* as macrophage engulfment

As has been shown for oxidative stress and previously discussed in Chapter 5, the transcriptional response of *C. glabrata* to oxidative stress has many similarities to that of *C. glabrata* cells engulfed by macrophages. As described in section 6.3.2, there are many genes which are regulated in response to both oxidative stress applied singly and combinatorial stress. These included *CTA1*, *SOD2* and *RIM101* which were also identified as being regulated by *C. glabrata* in response to macrophage engulfment in this chapter, as well as Chapter 5, and as such their functional implications have been discussed previously.

It was ascertained that 358 genes are regulated by both combinatorial stress and macrophage engulfment, an increase from comparisons made in the single stress chapters (Figure 6.10). This would suggest that combinatorial stress treatment more closely mimics the host environment than single stressors, one of the original theories of this study. It is important to note that while some of these genes were also regulated by hyperosmotic and oxidative stress applied singly, 122 genes were uniquely regulated by combinatorial stress and also found to be regulated by macrophage engulfment (Table 13.5 and Table 13.6, p.349 – p.351, Appendix IV).

As with all other stress conditions investigated in this thesis, genes down regulated upon treatment with combinatorial stress and engulfment by macrophages were associated with ribosome biogenesis, RNA binding and other RNA processes and translation. These have all been discussed in Chapter 4-6 in relation to their functional implications; the repression of growth as well as transcription and translation may help conserve energy under stress conditions by limiting the production of unneeded RNA and protein products. These processes have also been identified in genes repressed by phagocytosed *C. albicans* **[87]**. The

down regulation of genes involved in cell wall organisation is observed in macrophage engulfed cells and is also found to be unique to combinatorial stress. This also implies a down regulation of growth under these conditions.

RCK2, which encodes a kinase involved in the hyperosmotic and oxidative stress response of *S. cerevisiae* through phosphorylation by Hog1, is up regulated uniquely by *C. glabrata* in response to combinatorial stress conditions **[164, 214]**. The regulation of *RCK2* in response to combinatorial stress and macrophage engulfment demonstrates that both hyperosmotic and oxidative stress occur inside a macrophage. As a kinase, the regulatory role of Rck2 is governed by its phosphorylation state and although this is unknown in this study, its transcriptional induction would suggest its involvement in the response to these two environments. It would be informative to investigate the phosphorylation of Rck2 in response to single and combinatorial hyperosmotic and oxidative stress, particularly as Hog1 in *C. glabrata* may not be phosphorylated by oxidative stress, as it is in *S. cerevisiae* and *C. albicans* **[70, 114, 164]**.

Those genes uniquely induced by combinatorial stress and also found to be up regulated by the engulfment of *C. glabrata* cells by macrophages were involved in fatty acid metabolism and transport, with the majority of these genes localised to the peroxisome. Fatty acids can be used as an energy source during starvation conditions, as the peroxisome breaks down long chain fatty acids, producing large amounts of ATP. Starvation conditions would be present inside a macrophage as the *C. glabrata* cell would have limited access to nutrients. As discussed previously in Chapter 5, the induction of genes involved in fatty acid metabolism has also been identified in phagocytosed *C. albicans* cells, again linking the response of *C. glabrata* to combinatorial hyperosmotic and oxidative stress to the response to the host environment **[87]**.

The transcriptional response of *C. glabrata* to combinatorial stress is more similar to that of macrophage engulfed cells than the response to single stresses, and this includes genes uniquely regulated by combinatorial stress. This shows that combinatorial stress conditions are found in a host setting and while investigating single stresses are informative, multiple stressors applied simultaneously are more likely to reveal biological insights relevant to a pathogen. Genes involved in the metabolism of fatty acids by the peroxisome are also regulated in response to combinatorial stress and macrophage engulfment revealing the nutrient limited conditions imposed and this links to the regulation of genes involved in the filamentation pathway of *S. cerevisiae*.

7 Discussion

C. glabrata is an opportunistic fungal pathogen with an increasing incidence and innate resistance to antifungal drug treatment. Evolutionarily, *C. glabrata* is more closely related to the model yeast *S. cerevisiae* than *C. albicans* and other *Candida* species **[26]**. As such, much of the biological information on functionality, processes and pathways are inferred from *S. cerevisiae*. While this is informative, as few studies have been conducted in *C. glabrata* compared to the model yeast, any gene which does not have a homologue or has unknown functions in *S. cerevisiae* are removed from much of the analysis. While this study has made a concerted effort to annotate these *C. glabrata* specific genes, much more work needs to be done; these genes may hold clues as to how *C. glabrata* evolved to become a human pathogen while its closest relatives are non-pathogenic.

Little is known about the virulence mechanisms of *C. glabrata*; while it does not produce hyphae, proteins involved in stress response have been shown to be required for virulence **[147]**. Stress responses have been shown to be important for bacterial pathogens in adapting to the host environment including iron deprivation, pH, hyperosmotic stress and the oxidative stress found inside macrophages of the human immune system **[56, 57, 62]**. We theorised that these stresses would not occur singly in a host setting, but simultaneously and in combination. Very few studies have been undertaken to investigate the response to more than one stress condition, even though this would be more relevant in a host setting.

To investigate the response of *C. glabrata* to combinatorial stress, the response to stressors applied singly were first examined. Would the response to combinatorial stress simply be the addition of two single stresses? Transcription profiling over time, under hyperosmotic and oxidative stressors applied alone and in combination were conducted. The analysis of gene regulation data has played a fundamental role in understanding the molecular biology of many species, from humans to yeast. The transcriptome of the model yeast *S. cerevisiae* has been extensively studied and while this is informative for the *C. glabrata* community as it is so closely related, only the transcriptional analysis of *C. glabrata* can give specific insights into the gene regulation of this pathogen. While there are very few microarray analysis studies of *C. glabrata* compared to *S. cerevisiae* for example, all have added to the molecular knowledge of this pathogen. With advances in technology and reductions in cost in the last few years, RNAseq would be more appropriate in measuring the abundance of transcripts if this project were to be undertaken today. Conducting qRT-PCR experiments on a select

number of genes under the same conditions, and potentially from the same RNA samples used for these microarray experiments, would have been informative to validate the transcriptional data. It is important to note that transcript abundance does not always equal protein levels inside the cell as post translational modifications and protein degradation can limit protein abundance. Therefore, combining the transcriptional analysis presented here with proteome data under the same conditions would help equate the changes in transcript levels with actual levels of the corresponding protein.

Null mutants were created by targeted gene deletion (using the NAT disruption cassette) of genes identified from the literature and the microarray analysis conducted in this study. These mutants, along with those available in the small but growing library of *C. glabrata* null mutants, were phenotypically screened to elucidate their function. This approach has been used extensively in *S. cerevisiae* to screen the YKO library, revealing functionality and the processes in which the genes are involved. In this study, null mutants were screened using a pinning robot (RoToR, Singer Instruments) on approximately 50 conditions, many of which have been tested on *S. cerevisiae* null mutants. Future work includes screening these null mutants on more phenotypic conditions such as pH, iron starvation and anaerobic environments, as well as testing in animal models of infection. Animal studies using mice and the nematode, *Caenorhabditis elegans*, have been conducted in *C. glabrata* and other *Candida* spp. and could be used to test the affect of these null mutants on virulence (as reviewed in **[215]**). Competitive growth assays by microarray could also be conducted using the null mutants constructed in this study as all contain unique barcodes as used in the *S. cerevisiae* YKO library.

The first stress response investigated was that of hyperosmotic stress; the increased concentration of solutes outside a cell. Sodium chloride was chosen to elicit this stress on *C. glabrata* cells instead of sorbitol, as NaCl incorporates the cationic and hyperosmotic stress shown to found inside macrophages of the human immune system, whereas sorbitol can be used by many fungi including *C. glabrata* as an alternative carbon source **[64]**. Growth assays were conducted and three concentrations of NaCl were defined **[71]**. Microarray experiments were conducted using these concentrations to examine the transcriptional regulation of *C. glabrata* in response to hyperosmotic stress. It was found that compared to 0.1 M or 2 M NaCl, 0.5 M NaCl treatment elicited the transcriptional regulation of homologous genes known to be involved in hyperosmotic stress in *S. cerevisiae*. As such, 0.5 M NaCl was chosen to be investigated further. Microarray analysis showed that the majority

of genes were regulated immediately upon stress treatment at the 15 minute time point; results from the 5 minute time point suggest this is too early for signals to be transduced and for transcription to take place. Analysis of gene expression over time showed few genes statistically and significantly regulated after 60 and 120 minutes treatment. This suggested that by these later time points *C. glabrata* has responded and adapted to the stressful environment as transcript abundance is similar to that of a growing culture of cells.

Comparisons with *S. cerevisiae* showed that the transcriptional response to hyperosmotic stress was not similar to that observed in this study by *C. glabrata*. While some key genes involved in hyperosmotic stress were regulated by both yeast, such as *CIN5* and *HSP12*, which encode a transcription factor and a small heat shock protein, some were regulated only by *S. cerevisiae*. *GPD1* encodes a glycerol dehydrogenase, shown to be crucial for hyperosmotic stress resistance in *S. cerevisiae* and involved in the biosynthesis of the osmolyte, glycerol **[77]**. While *GPD1* is up regulated in response to hyperosmotic stress by *S. cerevisiae*, its paralogue *GPD2* is up regulated by *C. glabrata*. The null mutants *gpd1* and *gpd2* were constructed in *C. glabrata* and phenotypically screened. This revealed that in *C. glabrata* it is *GPD2* which is required for hyperosmotic stress resistance, not *GPD1* as is the case for *S. cerevisiae*. Phenotypic screening also revealed that *GPD2* is involved in other stress responses and may have roles in calcium signalling and be required for caesium resistance, which has been observed to be Hog1 dependent in *S. cerevisiae* **[160]**. Glycerol accumulation assays of these null mutants, as well as qRT-PCR experiments to validate these results would be preferential. Comparisons of the transcriptional regulation of these closely related yeast in response to hyperosmotic stressors also revealed the up regulation of genes involved in proline synthesis and transport by *C. glabrata* alone. Proline has been shown to accumulate in plants in response to a wide range of conditions and act as a osmolyte and antioxidant **[172]**. Studies have shown that supplementing growth media with proline increases the hyperosmotic stress resistance of *C. glabrata* **[81]**. Whether this proline regulation is *HOG1* dependent in *C. glabrata* is currently unknown and further investigation would be needed to ascertain this. Over expression studies of the genes responsible for proline biosynthesis and transport in *C. glabrata* under hyperosmotic stress would be the next step in exploring these observations.

The HOG pathway has been extensively studied in *S. cerevisiae* and many components of this MAPK signalling pathway have homologues in *C. glabrata*. Null mutants of HOG pathway components were either constructed in the course of this study or obtained from the

C. glabrata mutant library. These null mutants were phenotypically screened revealing their functions in metal resistance and cell wall integrity; many phenotypes which have not been observed for their corresponding *S. cerevisiae* homologues. Using a functional genomics approach has helped identified and confirm the roles of *OPY2*, *STE20α* and *MSB2* in *C. glabrata*. A homologue of *OPY2*, which encodes an osmo-sensor in *S. cerevisiae*, has not been previously identified in *C. glabrata* and the phenotypic screening results presented in this study showed that this gene is required for resistance to not only hyperosmotic stress but also metal ions and cell wall stressors. While a functional homologue of *STE20* in *C. glabrata* had been identified previously, sequence searches revealed a possible paralogue, denoted in this study as *STE20β*. *ste20β* mutants were not observed to have any phenotypes, unlike *ste20α* cells which display many and varied phenotypes similar to other HOG pathway mutants. The phenotypic screening results of *msb2* mutants were surprising to say the least. Msb2 has been shown to act together with Sho1 as an osmo-sensor and be required for hyperosmotic stress resistance in *S. cerevisiae*. *msb2* mutants in *C. glabrata* were not required for hyperosmotic stress resistance, but did display sensitivity to metal chlorides and cell wall stressors. *HKR1*, a paralogue of *MSB2*, has been identified and as this null mutant is available in the *C. glabrata* mutant library, phenotypic screening would help to reveal its function. As many null mutants of the HOG pathway tested in this study display sensitivity to other stresses, experiments by Western blot to explore the phosphorylation of Hog1 in wild type and mutant strains of *C. glabrata* under different stress conditions would be informative. HOG pathway null mutants were sensitive to caesium chloride and it has been shown in *S. cerevisiae* that the response to this metal stress is partially dependent on Hog1 **[160]**. Microarray experiments using null mutants of the HOG pathway in response to a range of stressors, especially the MAPK Hog1, would also help elucidate the transcriptional role of the HOG pathway in *C. glabrata*.

The null mutants *hsp12* and *hal1* were specifically constructed due to their transcriptional up regulation by *C. glabrata* in response to hyperosmotic stress. While the sequence of *HSP12* in *C. glabrata* is very similar to that of *HSP12* in *S. cerevisiae*, the corresponding *HAL1* genes show little sequence similarity. *hal1* mutants in both organisms do not display any phenotypes, however over-expression of *HAL1* in *S. cerevisiae* increases hyperosmotic stress resistance and therefore performing over-expression studies of *HAL1* in *C. glabrata* would help to confirm or refute its inferred function **[183]**. While *HSP12* is regulated by hyperosmotic stress in *C. glabrata*, a *hsp12* mutant does not display any phenotypes, unlike

hsp12 mutants in *S. cerevisiae* **[168]**. As their sequences are conserved, it can be assumed that their function is also conserved which suggests that either a paralogue or other heat shock proteins are functionally similar and render *HSP12* in *C. glabrata* redundant.

While the HOG pathway of *C. glabrata* was assumed to be similar to that of *S. cerevisiae*, recent studies have shown that the Sln1 branch of the HOG pathway in the sequenced strain, ATCC 2001, contains a mutation in *SSK2* resulting in a truncated protein product and rendering this branch non-functional **[70]**. Through the course of this study, null mutants of HOG pathway components were constructed using the type strain, ATCC 2001 and another common lab strain BG2. BG2 does not possess this mutation in *SSK2* and therefore both branches of the HOG pathway are functional. As previously demonstrated by Gregori *et al*, 2007, null mutants constructed in a BG2 background do not display sensitivities to hyperosmotic, metal or cell wall stressors, unlike the same null mutants made in an ATCC 2001 background. The phenotypic screening conducted in this study revealed that these two lab strains, commonly inter-changed, were more different than just a single point mutation in *SSK2*. These findings also reiterate how important choosing the right strain can be when investigating any organism.

The functional genomics and transcript profiling presented in this study has shown a re-wiring of the HOG pathway in *C. glabrata* compared to its close relative, *S. cerevisiae*. The regulation of genes involved in a range of stress responses, as well as the phenotypes displayed by HOG pathway null mutants has revealed the involvement and overlap between different MAPK signalling pathways in *C. glabrata*. As *ypd1* mutants were successfully constructed in a BG2 strain of *C. glabrata* and the same null mutant has proven to be inviable in *S. cerevisiae* unless also made in a strain lacking either *PBS2* or *HOG1*, a re-wiring of the Sln1 branch of the HOG pathway has occurred in *C. glabrata*. While construction of *sln1* mutants in a BG2 background was unsuccessful, *sln1* mutants created in an ATCC 2001 background were sensitive to the cell wall stressor SDS suggesting its novel function in the cell wall integrity pathway.

In the course of studying the transcriptional response of *C. glabrata*, genes involved in oxidative stress such as *CTA1* and *TRX2* were up regulated by hyperosmotic stress conditions. Phenotypic screening of HOG pathway mutants on oxidative stressors revealed no sensitivities and therefore the involvement, if any, of the HOG pathway on oxidative stress resistance in *C. glabrata* was deemed insignificant or redundant. This is in contrast to the role of Hog1 in hyperosmotic and oxidative stress response in *C. albicans* **[114]**.

Much of the biological information gleaned from these *C. glabrata* microarrays are inferred from homology with *S. cerevisiae*. As many genes either have no homologue or homology with a gene of unknown function in *S. cerevisiae*, those genes regulated in response to hyperosmotic stress and those which are *C. glabrata* specific were investigated. Structural predictions and bioinformatical analysis helped to identify a homologue of *HOR7* in *C. glabrata*, as well as possible kinases and transcription factors regulated in response to hyperosmotic stress.

As a pathogen that is engulfed by macrophages of the human immune system, *C. glabrata* would encounter oxidative stress through the ROS produced by the host to avoid infection. In a similar fashion as to when investigating hyperosmotic stress, three defined doses of hydrogen peroxide were determined to investigate the response of *C. glabrata* to oxidative stress (1, 10 and 100 mM H_2O_2) **[71]**. Functional genomics analysis of the major genes involved in oxidative stress revealed their functionality in *C. glabrata*. While differing degrees of sensitivity were observed to those published for *cta1* mutants in *C. glabrata*, this may be due to strain differences. The phenotypes displayed by *sod1* mutants were very similar to that of *sod1* mutants in *S. cerevisiae*, suggesting they have similar functions in both yeast. *yap1* mutants displayed many phenotypes to a range of compounds and stress conditions including oxidative stress, heavy metals, arsenic and cyclohexamide.

Transcript profiling of *C. glabrata* using these concentrations of oxidative stress showed that many genes involved in oxidative stress (as inferred from *S. cerevisiae*) were significantly regulated. When the gene expression of *C. glabrata* was investigated over time and increasing concentration of oxidative stress, a pattern of regulation emerged with high concentrations of oxidative stress prolonging the regulation of oxidative stress genes. Other genes which had a similar regulation pattern were identified, including genes whose homology with *S. cerevisiae* were dubious and whose functions were unknown. A possible transcription factor and a NiSOD were identified using structural predictions. As a DNA binding domain was predicted for the TF, over-expression studies and ChIP-Chip experiments would form the further work to investigate this gene's function. The NiSOD gene identified in *C. glabrata* is a candidate for gene transfer between bacteria and fungi as the closest organism known to possess Ni-binding SODs is *Streptomyces coelicolor* and has been shown to be an example of heterologous gene transfer in bacteria **[184, 205]**. While the null mutants of these genes constructed and phenotypically screened did not display any phenotypes in this study and therefore their functions remain unknown, other genes were

identified and are yet to be investigated. The down regulation of sterol and ergosterol biosynthesis in response to oxidative stress was observed and has been shown to be required for stress resistance and virulence in other fungal pathogens **[203, 204]**.

Comparisons of the oxidative stress response of *C. glabrata* conducted in this study and previously published data from its close relative *S. cerevisiae* revealed the transcriptional adaptation of these yeasts to be somewhat similar. Many genes associated with oxidative stress in *S. cerevisiae* were regulated by both organisms, along with the shared down regulation of genes involved in ribosome biogenesis suggesting a cessation of growth. These comparisons identified the transcriptional regulation of a homologue of *TSA2* by *C. glabrata* in response to oxidative stress rather than *TSA1*, the paralogue regulated by *S. cerevisiae*. Null mutants of these genes constructed in *C. glabrata* revealed differing phenotypes to the corresponding null mutants in *S. cerevisiae,* suggesting these genes had switched functions in *C. glabrata*. The induction of Tsa2 rather than Tsa1 in *C. glabrata* was confirmed by Western blot, where removal of *TSA2* resulted in no protein band, while protein was still present in *tsa1* mutants. The induction of Tsa2 was also revealed, by Western blot, to be dependent on *YAP1* and *SKN7* in *C. glabrata*. The up regulation and *SKN7* dependency of *TSA2* in *C. glabrata* has been shown previously using qRT-PCR **[61]**. The *yap1* microarray experiments under oxidative stress treatment conducted in this study also show that the removal of *YAP1* leads to a reduction in *TSA2* transcriptional regulation (8.94 fold change in wild type compared to 2.11 fold change in *yap1* cells).

As the environment inside a macrophage has been reported to present oxidative stress to pathogens such as *C. glabrata*, the transcriptional response of *C. glabrata* to exogenous oxidative stress was compared to published data from macrophage engulfed *C. glabrata* cells **[2]**. This revealed that many genes regulated by oxidative stress were also regulated by macrophage engulfment including YPS adhesin genes identified in *C. glabrata* as required for virulence **[2]**.

Transcript profiling using a *C. glabrata* strain lacking *YAP1* under oxidative stress treatment revealed that nearly all of the genes statistically and significantly regulated by wild type *C. glabrata* cells are affected by the removal of *YAP1*. These could include genes regulated directly by Yap1 either as an activator or a repressor, through co-operation with another transcription factor such as Skn7 or part of a regulatory cascade. The un-translated region upstream of genes affected by the removal of *YAP1* were parsed for Yap1 binding motifs. Those which possessed exact YRE-A and YRE-O motifs were considered to Yap1 dependent

and those available in the *C. glabrata* null mutant library were phenotypically screened. Quantification using qRT-PCR of the genes identified as dependent on *YAP1* under oxidative stress, along with ChIP-chip studies of Yap1 and Skn7 binding sites in *C. glabrata* would help to validate these observations. As Yap1 is phosphorylated upon oxidative stress in *S. cerevisiae*, its phosphorylation state in *C. glabrata* in response to the conditions in which phenotypes were observed would be informative. Due to the number of genes whose expression was affected by the removal of *YAP1* in *C. glabrata*, other YAP genes were investigated as their functions overlap in *S. cerevisiae*. Null mutants of these YAP genes in *C. glabrata* were obtained and phenotypic screening revealed little functional similarity to their corresponding *S. cerevisiae* homologue as well as their redundancy in oxidative stress resistance in *C. glabrata*. This observation, along with the large transcriptional impact of removing *YAP1* and the regulation of metal resistance genes by *C. glabrata* suggested that Yap1 may have taken over the role of other YAP genes, specifically Yap2 (Cad1).

After investigating the response of *C. glabrata* to hyperosmotic and oxidative stressors applied singly, the transcriptional adaptation of this pathogen was examined in response to the simultaneous application of these two distinct stresses. The concentrations of hyperosmotic and oxidative stress used were chosen to stress but not kill the *C. glabrata* cells, as very high doses of stressors had been observed to limit transcriptional regulation over time as well as result in cell death.

All of the null mutants phenotypically screened in this study were also tested on combinatorial stress conditions and their growth was scored against the corresponding single stresses. Utilising this functional genomics approach revealed that *STE11* and *STE50* are required for combinatorial stress resistance. As the proteins they encode are shared between many MAPK pathways in *S. cerevisiae*, the transcript profiling under combinatorial stress revealed the up regulation of components and downstream targets of these pathways and *ste11* and *ste50* mutants in *C. glabrata* display many diverse phenotypes, this suggested that combinatorial stress involved many other signalling pathways than just hyperosmotic or oxidative stress. Further work includes liquid growth assays using a 96 well plate reader and screening all available null mutants constructed in *C. glabrata* under combinatorial stress conditions.

As observed in *S. cerevisiae*, the ESR is triggered when any non-optimum condition is applied. Studies have shown that an ESR also exists in *C. glabrata*, controlled by the stress response transcription factors Msn2/4 **[118]**. The transcriptional response of *C. glabrata* to

hyperosmotic and oxidative stressors applied singly were compared to investigate these previous findings. The analysis conducted in this study revealed that *C. glabrata* does display an ESR, although the number of genes included in this response was smaller than those identified by Roetzer *et al*, 2008 **[118]**. This may be because only two stresses were used in this thesis compared to the four stressors in this publication.

Analysis of the transcript profiling conducted in this study revealed that a large number of genes were statistically and significantly regulated in response to combinatorial stress and many were regulated uniquely by combinatorial stress as their regulation was not observed in response to single stressors. GO term enrichment analysis of these genes, inferred from their homology with *S. cerevisiae*, revealed their involvement in MAPK signalling pathways, specifically the filamentation/starvation pathway. This is of note as *C. glabrata* not does form hyphae, only pseudohypae under nitrogen starvation conditions **[33]**. Genes with homology to mating genes in *S. cerevisiae* were also regulated by *C. glabrata* in response to combinatorial stress. This is also of note as *C. glabrata* has yet to display a mating cycle even though it possesses homologues of many mating genes in *S. cerevisiae* **[35]**. These raise the question as to whether combinatorial stress triggers mating or filamentation in *C. glabrata* and as there are indicators that combinatorial stress occurs in the host, whether these processes are also occurring in the host environment.

The transcriptional response of the human pathogen *C. glabrata* to combinatorial stress was compared to that of *C. glabrata* cells engulfed by macrophages. This revealed that the transcriptional response of *C. glabrata* to macrophage engulfment was more similar to combinatorial stress treated cells than those where stressors were applied singly. This included many genes which were regulated uniquely by combinatorial stress. Similar processes were regulated by *C. glabrata* in response to combinatorial stress as have been identified in phagocytosed *C. albicans* cells, including fatty acid metabolism, oxidative stress and repression of translation **[87]**. These observations suggest that our hypothesis was correct; combinatorial stress conditions occur in the host environment and the transcriptional adaptation of *C. glabrata* to these conditions are similar.

It was hoped that the transcript profiling conducted in this study would be used to bioinformatically model the response of *C. glabrata* to environmental stresses using a Dynamic Bayesian Network approach. This was attempted by our collaborators but their methods proved unsuccessful. It is hoped that the timecourse microarray data produced in this study will be analysed further to benefit the *C. glabrata* community.

This study has presented the transcriptional response of *C. glabrata* to hyperosmotic and oxidative stress, singly and in combination by the simultaneous application of both stresses. The response of *C. glabrata* to combinatorial stress has not been investigated before and therefore provides novel insights into this area of research. Through this study, 20 targeted null mutants have been constructed, adding to the growing *C. glabrata* mutant library: a source for the *C. glabrata* community. This thesis has shown that the response to combinatorial stress cannot simply be explained by combining the response to single stressors. It has also shown that if the use of a host environment such as mouse or macrophage models of infections is unfeasible, combinatorial stress conditions are more similar to the host environment than the application of a single stress alone. The transcript profiling and functional genomics analyses presented in this study have helped build on our knowledge of the response of *C. glabrata* to these environmental stress conditions.

8 References

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9 Poster, Oral and Written Publications

The work presented in this thesis has been presented in/at the following conferences and publications:

1.7 Poster Presentations

- Bristish Mycological Society's Annual Scientific Meeting, University of Exeter, UK September 2011
- Wellcome Trust Functional Genomics and Systems Biology, Cambridge, UK December 2011
- FEBS Human Fungal Pathogens, Nice, France May 2011. Awarded a Youth Travel Fund fellowship
- American Society of Microbiology Candida and Candidiasis, Miami, USA March 2010
- MMEMS workshop, Evolution of Stress Responses, University of Aberdeen, UK September 2009
- Dynamics in Systems Biology, University of Aberdeen, UK, September 2009

1.8 Oral Presentations

- The transcriptional response of the fungal pathogen *Candida glabrata* to combinatorial stress, April 2012 at the American Society of Microbiology's Candida and Candidiasis Conference, San Francisco, USA. Awarded an ASM Student Travel Grant.

1.9 Written Publications

- Kaloriti D, Tillmann A, Cook E, Jacobsen M, You T, Lenardon M, Ames L, Barahona M, Chandrasekaran K, Coghill G, Goodman D, Gow NA, Grebogi C, Ho HL, Ingram P, McDonagh A, Moura AP, Pang W, Puttnam M, Radmaneshfar E, Romano MC, Silk D, Stark J, Stumpf M, Thiel M, Thorne T, Usher J, Yin Z, Haynes K, Brown AJ. (2012) Combinatorial stresses kill pathogenic *Candida* species. Medical Mycology.

10 Appendix I

Table 10.1: Primers used in this study to construct null mutants.

S. cerevisiae **Standard Name**	***C. glabrata*** **Systematic Name**	***S. cerevisiae*** **barcodes used**	**Primer Name**	**Primer Sequence**
PBS2	*CAGL0L05632g*	*YJL128C*	-600-F	GTGGGCCGGGCAAAACAGAGG
			-500-F	GTCTCAGCTGTCACTGA
			-1-NAT-R	GCGTCGACCTGCAGCGTACGTCCATCTGTTCGTCTGCAAGCACGGCGCGCCTAGCAGCGGCGCACCCTCTCCATTGCCTC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGGGCATCCTAACAATACTGCAGTCAGCGGCCGCATCCCTGCCCTGCATTGCATAAAGGTGG
			+500-R	GCCCTTCTAGTACAACCG
			+600-R	GCTGATCAACTTCCATCAG
			-Int-F	CAGCAGGATACGGAAGGAAC
			-Int-R	ACCTCTTGAGGAAAGTG
SLN1	*CAGL0H06567g*	*YIL147C*	-600-F	CCACTGAGAGACCTTTCC
			-500-F	CCAACACATACACTCTCTC
			-1-NAT-R	GCGTCGACCTGCAGCGTACGCGTGTCCCATCGAGCTGCATACACGGCGCGCCTAGCAGCGGGCTGATTCTGTGGAATCC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCAAGATAGGCTAACAGTCGCGTCAGCGGCCGCATCCCTGCCTTGTTCATTATCCAGGG
			+500-R	GCTTTCTAGGTATGCTC
			+600-R	CTGACTCCTGCTCTTTGTC
			-Int-F	GCCGAAGGCAGAGGCCTG
			-Int-R	AGAGAACCGTCTGTGGC
SHO1	*CAGL0G03597g*	*YER118C*	-600-F	CCCTCATATCTCGTGGC
			-500-F	GGATCAGATGGAGCTACTC
			-1-NAT-R	GCGTCGACCTGCAGCGTACGGCCTCTGGTGCGTTAGCATTCACGGCGCGCCTAGCAGCGGCCTCTCCTGCCTGACATAAC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGGGGCAGCATAAACACTTCCAGTCAGCGGCCGCATCCCTGCCAACGATACCGAAGCCTAG
			+500-R	CTCCGGTGTCGTCGTCT

			+600-R	TGCTAACCCAAAGGGTG
			-Int-F	GGGTCGACTCTTTCTCAC
			-Int-R	AGCCATTGTCTAGTCCC
GPD2	*CAGL0C05137g*	*YOL059W*	-600-F	CCACAGGATCACACGTAC
			-500-F	GGCTCCACTGGACAACTG
			-1-NAT-R	GCGTCGACCTGCAGCGTACGCTCCAGTCTGCTGCGGATATCACGGCGCGCCTAGCAGCGGCCTGGCTAACCGAACAAAC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCCGTCCATCTAATGTATGAGGTCAGCGGCCGCATCCCTGCGCTGGCCAATAAGTACATC
			+500-R	CCGGACAATAGTCGATC
			+600-R	TCTCGGCTCTCATTGCC
			-Int-F	GTGGTGCCCTATCGGGTGC
			-Int-R	GGACCTTGTGGTCGACG
SOD1	*CAGL0C04741g*	*YJR104C*	-600-F	CGTACCCGCAGTGTGAGC
			-500-F	CTGCGAACAGCTGCCAC
			-1-NAT-R	GCGTCGACCTGCAGCGTACGCATAGGATTACAAATGCGGCCACGGCGCGCCTAGCAGCGGCAGCAACAGCTTTAACC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGGACGCGAGTCGATGATACCTGTCAGCGGCCGCATCCCTGCCCAACTAACTACCACCAGC
			+500-R	GGTGGGACTTGCACCAC
			+600-R	CCAGCTTCGTTGCCGGTAG
			-Int-F	CCGCTGGCCCTCACTTC
			-Int-R	AGGTAGGGCCGATCAGC
YPD1	*CAGL0K04961g*	*YDL235C*	-600-F	GGGGTCATCGGCAGCCT
			-500-F	CAAGGTCTCAGTGGATG
			-1-NAT-R	GCGTCGACCTGCAGCGTACGTGGTATTGTTCATAAGGGCCCACGGCGCGCCTAGCAGCGGGCCAAGCATTGATTTGC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGGCTACTTCGCAACCTGTTTAGTCAGCGGCCGCATCCCTGCCAGAAGCATGATGCGACC
			+500-R	ATCTCTGGCGAGGGCTCC
			+600-R	GCACGGCGTATGATTAGG
			-Int-F	CGTGAACCAACTACCAC
			-Int-R	GGGTATGGCACGACCCT
TSA1	*CAGL0G07271g*	*YML028W*	-600-F	CCTGTCTCGAGCATGTGG
			-500-F	CGCTGTCAATCCTGCAGAG

			-1-NAT-R	GCGTCGACCTGCAGCGTACGGATCCG TATGTCGAATGGCTCACGGCGCGCCT AGCAGCGGGAGCGACCATTCTTGCT
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGACTCGG TTTGCTCGAGTACAGTCAGCGGCCGC ATCCCTGCGCAGCTAGTCTAGTCCG
			+500-R	CTGCAAGATGTAGAGGTGAG
			+600-R	GGGGACTTCTCCTCGTG
			-Int-F	CGCCTCCACCGACTCCG
			-Int-R	GGGTCGATGATGAACAGGCC
GPD1	*CAGL0K01683g*	*YDL022W*	-600-F	CGGGAAGTATATGTGCG
			-500-F	TGCAGGCCCTCTGGATGC
			-int-F	CACCAGTTCTTGCCCCG
			-int-R	CGTCCTTTCCTTCACCTCTG
			+500-R	AGTGGGATTCCAGTCAC
			+600-R	GCGTCGACATGGCTGCGT
			-1-NAT-R	GCGTCGACCTGCAGCGTACGGCCCAA TTAGTACATATCCGCACGGCGCGCCT AGCAGCGGCAGCAGCGGAGTTAGAC ATG
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCGCATT CAGTACATTTACCGGTCAGCGGCCGC ATCCCTGCGGAATGCATCGCTGATTG AG
HAL1	*CAGL0L09251g*	*YPR005C*	-600-F	GGGATCGATACGTGCTTT
			-500-F	GCACACACAAATCCAATTCC
			-int-F	CAAAGATCGCAACGGGAG
			-int-R	AGCATCGAGCTCTGTAGC
			+500-R	GCTCGTGTATCATGAGATTCG
			+600-R	CAGTTTGCTGGGTGGTAG
			1-NAT-R	GCGTCGACCTGCAGCGTACGCGGGTG ACTGTCGCAATCTACACGGCGCGCCT AGCAGCGGGACATGGTAGTGAACAG G
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGTTATAT GGCCGCACCCGATGGTCAGCGGCCGC ATCCCTGCCTACCTCAAGTGCTGGAC A
HSP12	*CAGL0J04202g*	*YFL014W*	-600-F	CATGGTGTGCTGGGAAAC
			-500-F	AGGTATAGTGCCTCACTC
			-int-F	CTTCTCTGACAAGCTAAACG
			-int-R	GTATTCGACGGCATCGTT
			+500-R	GGCATTGAGAAGTGCAGA
			+600-R	TGGGCTCTGTTATAGGTG

			-1-NAT-R	GCGTCGACCTGCAGCGTACGCTGCCCTTATAGAAGTGTAGCACGGCGCGCCTAGCAGCGGCTACCAGCGTCAGACATTG
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCACCCAGACCGATTAAGGAGGTCAGCGGCCGCATCCCTGCAAGTCTGTTCACGGTGGTG
MSB2	*CAGL0F08833g*	*YGR014W*	-600-F	CCCGAACAAAGGGACCC
			-500-F	GGGCATCCTCTGTAGGAGCC
			-int-F	GCGTCATATCGTCTGAGC
			-int-R	GGGCACATCAACGCTGC
			+500-R	TGGAAACAATGTGGCAC
			+600-R	AGTCCGACGTTCATGCCC
			-1-NAT-R	GCGTCGACCTGCAGCGTACGAATAGGACTTAACCCGCCAGCACGGCGCGCCTAGCAGCGGCTCAAACGCGCAATAGTGC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCCCTCTTAGAAACGACGTAAGTCAGCGGCCGCATCCCTGCGGCTGCTAGCCAATGAG
OPY2	*CAGL0D01276g*	*YPR075C*	-600-F	CCTAACGGTTGCTCCTGT
			-500-F	TGCAAGAAGCAGAGCTGTC
			-int-F	GACACATGCCCTTACAC
			-int-R	GTTTGACCATTCCGCAG
			+500-R	GCTGTTACTGTGCACTC
			+600-R	TGGCTGCAGGTGTTTCT
			-1-NAT-R	GCGTCGACCTGCAGCGTACGGAATATGGCAGTGGTCCCAACACGGCGCGCCTAGCAGCGGTCTGTAGACTCCTCTGC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCGACTACCCTAATGTGTTTCGTCAGCGGCCGCATCCCTGCGCTGACACCTAGAGATC
TSA2	*CAGL0K06259g*	*YDR453C*	-600-F	CAGAGAGCAACGCACTTCC
			-500-F	GTCCGTACGTCATCGCTC
			-int-F	AAGCCTTGGACGCTCAAG
			-int-R	GGAAACCTTCGACCAATCTC
			+500-R	CCCTTCGATAAGAGTGTGGG
			+600-R	GTTCAAGTTTCCGGGTCCG
			-1-NAT-R	GCGTCGACCTGCAGCGTACGACAGTGTAGAAAGGTAGCCTCACGGCGCGCCTAGCAGCGGCCCAAGCTCATCGCTAGACG
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGCGCCCTACGAAATGGTATAAGTCAGCGGCCGC

				ATCCCTGCCGCTCGTCTTCATATCCAG
-	*CAGL0L10186g* (TF)	*YOR052C*	-600-F	GCCTGTTGAGAACAGTCAGT
			-500-F	TGATGTGGTGAGCTCTGA
			-1-NAT-R	GCGTCGACCTGCAGCGTACGTGAGAC ATTGTGCAAATCGGCACGGCGCGCCT AGCAGCGGCACACACTGAGTTCTCCC TA
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGATGCAG TCGTCACGTCTCGTGTCAGCGGCCGC ATCCCTGCCTATTCCCAGTGTTGCCA
			+500-R	CTGTCTCTGTCAGAGTCA
			+600-R	AAACGGTCAC ACTCATGCAC
			-int-F	GAAAGGGACGCCTGAGAG
			-int-R	GCGTCTGTTCAGAGGCC
-	*CAGL0F07259g* (NiSOD)	*YGL117W*	-600-F	GCCCTTCGCTACATATC
			-500-F	ACCGTCAACTCGGCCCA
			-1-NAT-R	GCGTCGACCTGCAGCGTACGTGGAAG GGTTTCATTGTCCCCACGGCGCGCCT AGCAGCGGGTCGTTAAAGTGGGCCTG
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGGTCATA GCCGACCCTTAGGTGTCAGCGGCCGC ATCCCTGCGGCAGCGTAAGTTACGGG
			+500-F	CTATCTCATCAGAGCCTC
			+600-F	GAGAGAACTCTAGTGTGGG
			-int-F	GGTTGGAGACCGTGCAC
			-int-R	CGACATCTGCCAGGTCG
*STE20*β	*CAGL0M10153g*	*YHL007C*	-600-F	CGGCTGAGCTATTGGTG
			-500-F	CTATCAGAGTCCACATA
			-1-NAT-R	GCGTCGACCTGCAGCGTACGTGCTAG GTTGTCTGTGCCCACACGGCGCGCCT AGCAGCGGCAGCGGACATGACTATA GC
			+1-NAT-F	CGACGGTGTCGGTCTCGTAGAGGTAG ATCCCATCAGCCAGGTCAGCGGCCGC ATCCCTGCGCTTCTGTTGAATCAGGG
			+500-R	GCCAGTGGGATGTTGAAAG
			+600-R	GAGTTGGTTGCGATGGCTCC
			-int-F	CCGTCCGAAGGCCACTCG
			-int-R	CGGGTTGGAACTTCCAC

Primers were made using the published genome sequence data from Génolevures. *S. cerevisiae* barcodes were taken from the YKO library (Open Biosystems).

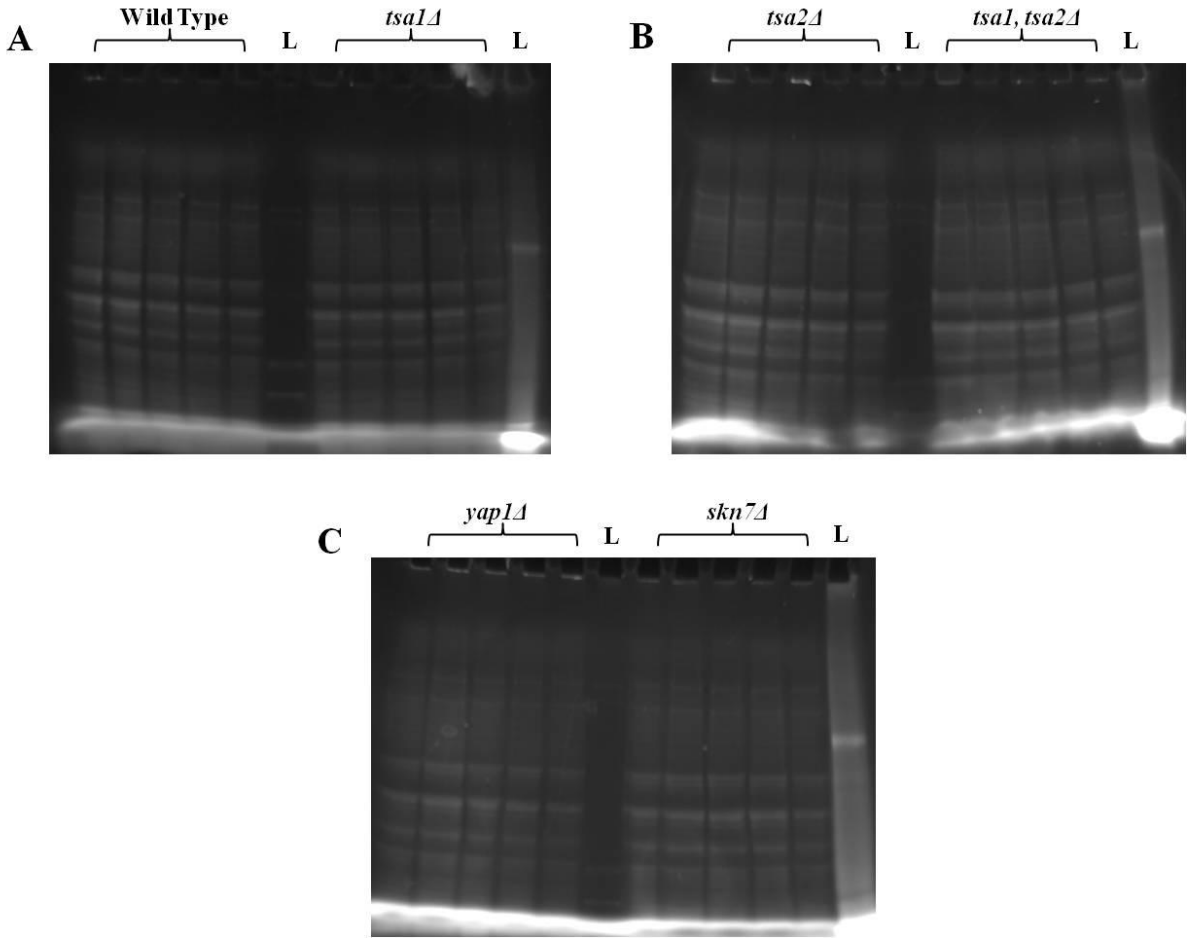

Figure 10.1: UV images of SDS-PAGE gels. UV exposed images were taken using a G Box from Syngene to check for similar loading of protein samples into each well. Samples were loaded as per Western blot figure in Chapter 5. L denotes protein ladder. **NB**. Two different ladders were used in this experiment.

Table 10.2: Primers used to sequence pMP10 clones.

Primer Name	Sequence
F1	GGTGGAGGGAACATCGTT
F2	TGCGAGATGATCCCGCA
R1	CAGTGGTGTGATGGTCGT
F3	GAGTCATCCGCTAGGTGG
R2	TATGCGGCATCAGAGCAG
F4	TGTAGCGGCGCATTAAGC

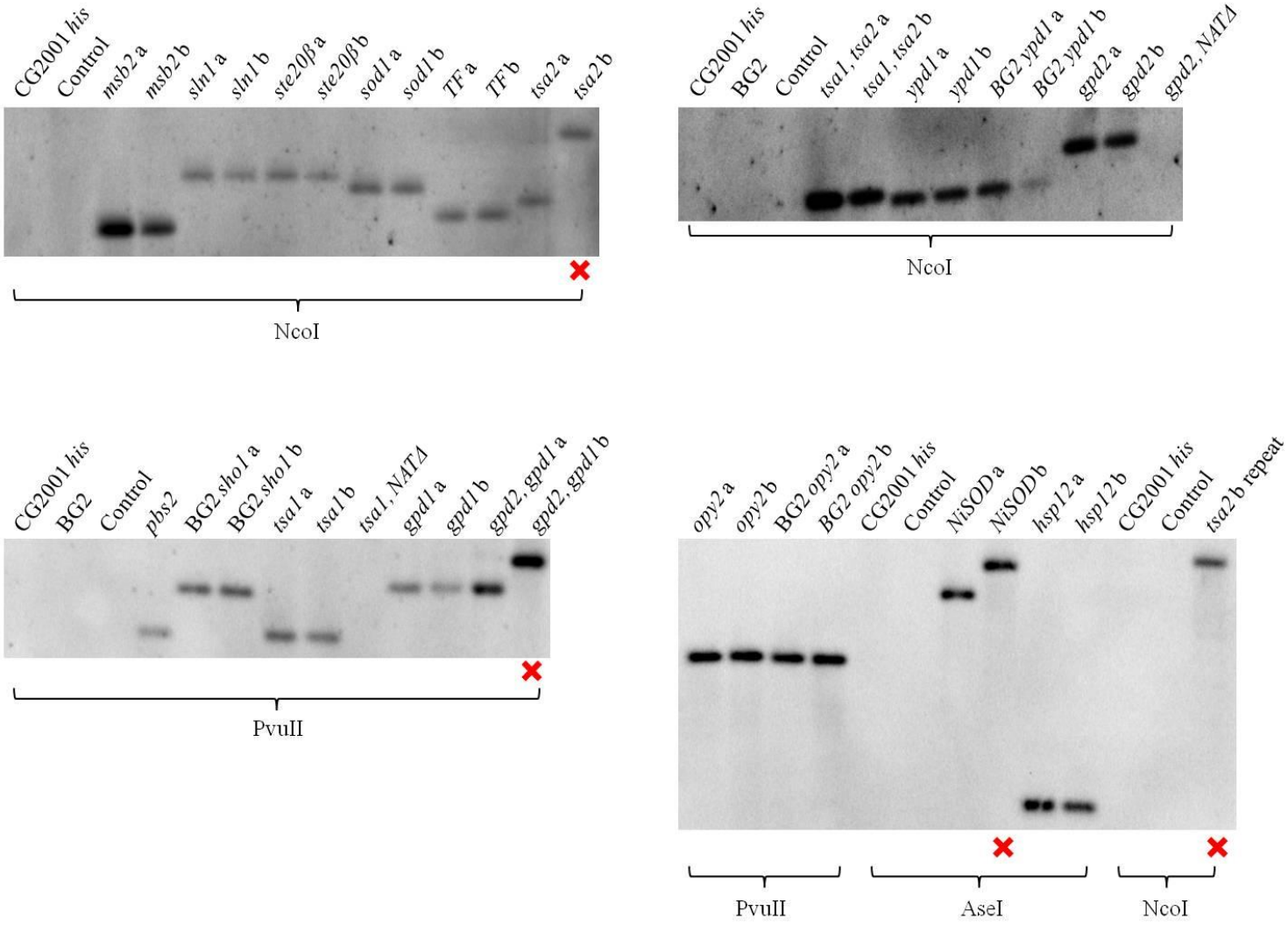

Figure 10.2: Null mutants constructed in this study probed for the NAT cassette by Southern blot. A no DNA control and parental strain was run for each restriction enzyme used (as indicated). Those marked with a red X are an incorrect size. NB. Although an incorrect size DNA fragment was observed, each pair of null mutant isolates displayed the same phenotypes.

11 Appendix II

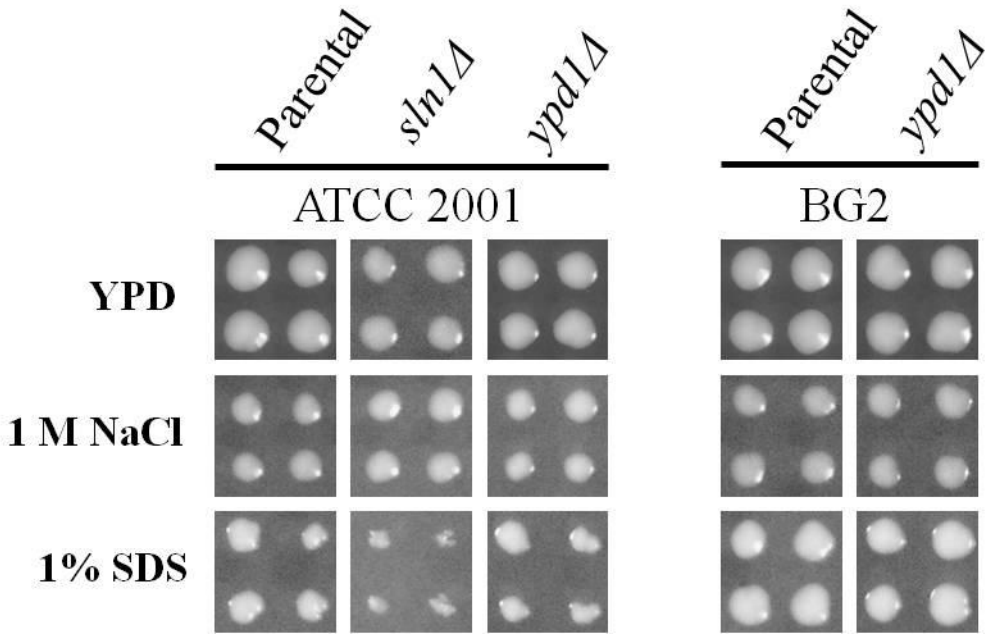

Figure 11.1: Sensitivities of *sln1* and *ypd1* mutants in different *C. glabrata* background strains. Each strain was spotted four times in a square on to media containing the indicated stress. NB. An *sln1* mutant was unsuccessful in a BG2 strain. Pictures were taken after 2 days. Representative of two technical and three biological replicates.

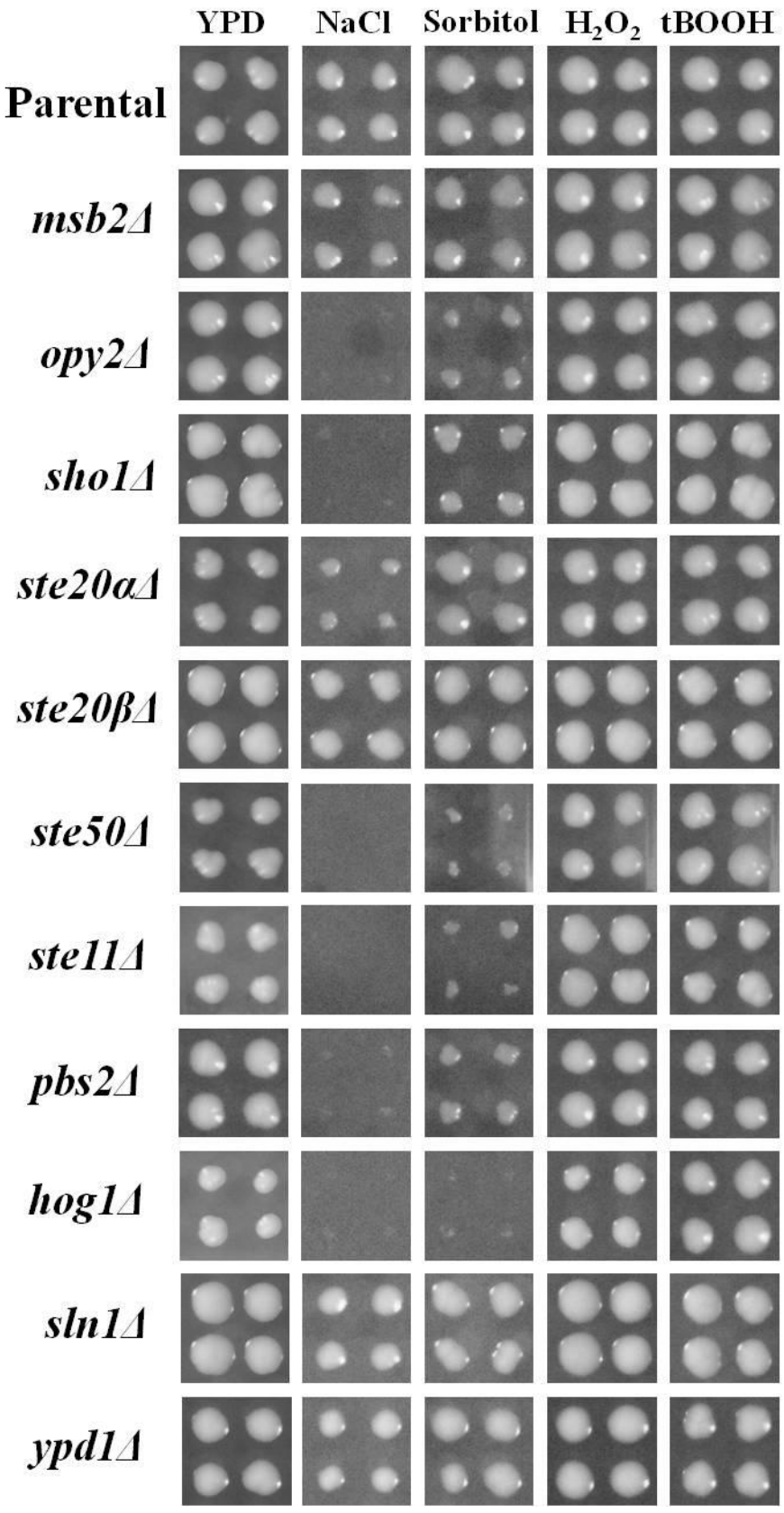

Figure 11.2: Nulls mutants of the HOG pathway do not show oxidative stress sensitivity. Each strain was spotted four times in a square on to media containing the indicated stress. Pictures were taken after 2 days. Representative of two technical and three biological replicates.

Table 11.1: Genes up regulated by *C. glabrata* and *S. cerevisiae* upon hyperosmotic stress treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0I10010g	*YGR142W*	*BTN2*	v-SNARE binding protein that facilitates specific protein retrieval from a late endosome to the Golgi; modulates arginine uptake, possible role in mediating pH homeostasis between the vacuole and plasma membrane H(+)-ATPase
CAGL0J04158g	*YOR220W*	*RCN2*	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and is induced in response to the DNA-damaging agent MMS; phosphorylated in response to alpha factor
CAGL0L04598g	*YLR270W*	*DCS1*	Non-essential hydrolase involved in mRNA decapping, may function in a feedback mechanism to regulate deadenylation, contains pyrophosphatase activity and a HIT (histidine triad) motif; interacts with neutral trehalase Nth1p
CAGL0E06512g	*YKL142W*	*MRP8*	Protein of unknown function; undergoes sumoylation; transcription induced under cell wall stress; protein levels are reduced under anaerobic conditions; originally thought to be a mitochondrial ribosomal protein based on sequence analysis
CAGL0D00352g	*YKL188C*	*PXA2*	Subunit of a heterodimeric peroxisomal ATP-binding cassette transporter complex (Pxa1p-Pxa2p), required for import of long-chain fatty acids into peroxisomes; similarity to human adrenoleukodystrophy transporter and ALD-related proteins
CAGL0L06006g	*YGL180W*	*ATG1*	Protein ser/thr kinase required for vesicle formation in autophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway; structurally required for phagophore assembly site formation; during autophagy forms a complex with Atg13p and Atg17p
CAGL0J01331g	*YMR090W*		
CAGL0L07656g	*YML004C*	*GLO1*	Monomeric glyoxalase I, catalyzes the detoxification of methylglyoxal (a by-product of glycolysis) via condensation with glutathione to produce S-D-lactoylglutathione; expression regulated by methylglyoxal levels and osmotic stress
CAGL0A01694g	*YGL036W*		
CAGL0L07480g	*YBR066C*	*NRG2*	Transcriptional repressor that mediates glucose repression and negatively regulates filamentous growth; has similarity to Nrg1p
CAGL0C04191g	*YBR006W*	*UGA2*	Succinate semialdehyde dehydrogenase involved in the utilization of gamma-aminobutyrate (GABA) as a nitrogen source; part of the 4-aminobutyrate and glutamate degradation pathways; localized to the cytoplasm
CAGL0C04543g	*YJR096W*		
CAGL0G03289g	*YBL075C*	*SSA3*	ATPase involved in protein folding and the response to stress; plays a role in SRP-dependent cotranslational protein-membrane targeting and translocation; member of the heat shock protein 70 (HSP70) family; localized to the cytoplasm

CAGL0I05874g	*YIL053W*	*RHR2*	Constitutively expressed isoform of DL-glycerol-3-phosphatase; involved in glycerol biosynthesis, induced in response to both anaerobic and, along with the Hor2p/Gpp2p isoform, osmotic stress
CAGL0M13761g	*YMR302C*	*YME2*	Integral inner mitochondrial membrane protein with a role in maintaining mitochondrial nucleoid structure and number; mutants exhibit an increased rate of mitochondrial DNA escape; shows some sequence similarity to exonucleases
CAGL0G02563g	*YKR098C*	*UBP11*	Ubiquitin-specific protease that cleaves ubiquitin from ubiquitinated proteins
CAGL0G05269g	*YDR070C*	*FMP16*	Putative protein of unknown function; proposed to be involved in responding to conditions of stress; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0J08272g	*YNL274C*	*GOR1*	Glyoxylate reductase; null mutation results in increased biomass after diauxic shift; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0F05071g	*YLR284C*	*ECI1*	Peroxisomal delta3,delta2-enoyl-CoA isomerase, hexameric protein that converts 3-hexenoyl-CoA to trans-2-hexenoyl-CoA, essential for the beta-oxidation of unsaturated fatty acids, oleate-induced
CAGL0H08173g	*YOR028C*	*CIN5*	Basic leucine zipper (bZIP) transcription factor of the yAP-1 family, mediates pleiotropic drug resistance and salt tolerance; nuclearly localized under oxidative stress and sequestered in the cytoplasm by Lot6p under reducing conditions
CAGL0M12474g	*YIL055C*		
CAGL0A01716g	*YGL037C*	*PNC1*	Nicotinamidase that converts nicotinamide to nicotinic acid as part of the NAD(+) salvage pathway, required for life span extension by calorie restriction; PNC1 expression responds to all known stimuli that extend replicative life span
CAGL0M04191g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0M08822g	*YDR258C*	*HSP78*	Oligomeric mitochondrial matrix chaperone that cooperates with Ssc1p in mitochondrial thermotolerance after heat shock; able to prevent the aggregation of misfolded proteins as well as resolubilize protein aggregates
CAGL0G03773g	*YLL023C*		
CAGL0G02717g	*YIL099W*	*SGA1*	Intracellular sporulation-specific glucoamylase involved in glycogen degradation; induced during starvation of a/a diploids late in sporulation, but dispensable for sporulation
CAGL0L01925g	*YKL035W*	*UGP1*	UDP-glucose pyrophosphorylase (UGPase), catalyses the reversible formation of UDP-Glc from glucose 1-phosphate and UTP, involved in a wide variety of metabolic pathways, expression modulated by Pho85p through Pho4p
CAGL0I01100g	*YOR120W*	*GCY1*	Putative NADP(+) coupled glycerol dehydrogenase, proposed to be involved in an alternative pathway for glycerol catabolism; member of the aldo-keto reductase

			(AKR) family
CAGL0E06424g	*YKL150W*	*MCR1*	Mitochondrial NADH-cytochrome b5 reductase, involved in ergosterol biosynthesis
CAGL0D06006g	*YJR052W*	*RAD7*	Protein that recognizes and binds damaged DNA in an ATP-dependent manner (with Rad16p) during nucleotide excision repair; subunit of Nucleotide Excision Repair Factor 4 (NEF4) and the Elongin-Cullin-Socs (ECS) ligase complex
CAGL0I07865g	*YOL084W*	*PHM7*	Protein of unknown function, expression is regulated by phosphate levels; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery and vacuole
CAGL0M13255g	*YKL065C*	*YET1*	Endoplasmic reticulum transmembrane protein; may interact with ribosomes, based on co-purification experiments; homolog of human BAP31 protein
CAGL0J04202g	*YFL014W*	*HSP12*	Plasma membrane localized protein that protects membranes from desiccation; induced by heat shock, oxidative stress, osmostress, stationary phase entry, glucose depletion, oleate and alcohol; regulated by the HOG and Ras-Pka pathways
CAGL0J05962g	*YNL155W*		
CAGL0H08261g	*YOR019W*		
CAGL0E01815g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0K04631g	*YGR067C*		
CAGL0E00803g	*YDR171W*	*HSP42*	Small heat shock protein (sHSP) with chaperone activity; forms barrel-shaped oligomers that suppress unfolded protein aggregation; involved in cytoskeleton reorganization after heat shock
CAGL0K12958g	*YML131W*		
CAGL0H09130g	*YKL201C*	*MNN4*	Putative positive regulator of mannosylphosphate transferase (Mnn6p), involved in mannosylphosphorylation of N-linked oligosaccharides; expression increases in late-logarithmic and stationary growth phases
CAGL0K10164g	*YDR077W*	*SED1*	Major stress-induced structural GPI-cell wall glycoprotein in stationary-phase cells, associates with translating ribosomes, possible role in mitochondrial genome maintenance; ORF contains two distinct variable minisatellites
CAGL0A00341g	*YGL010W*		
CAGL0K05813g	*YDR513W*	*GRX2*	Cytoplasmic glutaredoxin, thioltransferase, glutathione-dependent disulfide oxidoreductase involved in maintaining redox state of target proteins, also exhibits glutathione peroxidase activity, expression induced in response to stress
CAGL0B00726g	*YCL040W*	*GLK1*	Glucokinase, catalyzes the phosphorylation of glucose at C6 in the first irreversible step of glucose metabolism; one of three glucose phosphorylating enzymes; expression regulated by non-fermentable carbon sources
CAGL0F07513g	*YKL093W*	*MBR1*	Protein involved in mitochondrial functions and stress response; overexpression suppresses growth defects of

			hap2, hap3, and hap4 mutants
CAGL0G05335g	*YDR074W*	*TPS2*	Phosphatase subunit of the trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate trehalose; expression is induced by stress conditions and repressed by the Ras-cAMP pathway
CAGL0G02915g	*YGR086C*	*PIL1*	Primary component of eisosomes, which are large immobile cell cortex structures associated with endocytosis; null mutants show activation of Pkc1p/Ypk1p stress resistance pathways; detected in phosphorylated state in mitochondria
CAGL0J11308g	*YNL183C*	*NPR1*	Protein kinase that stabilizes several plasma membrane amino acid transporters by antagonizing their ubiquitin-mediated degradation
CAGL0K03421g	*YMR105C*	*PGM2*	Phosphoglucomutase, catalyzes the conversion from glucose-1-phosphate to glucose-6-phosphate, which is a key step in hexose metabolism; functions as the acceptor for a Glc-phosphotransferase
CAGL0G06886g	*YJL017W*		
CAGL0E02981g	*YGR149W*		
CAGL0H08844g	*YMR173W*	*DDR48*	DNA damage-responsive protein, expression is increased in response to heat-shock stress or treatments that produce DNA lesions; contains multiple repeats of the amino acid sequence NNNDSYGS
CAGL0K06105g	*YLR267W*	*BOP2*	Protein of unknown function
CAGL0D04026g	*YGR019W*	*UGA1*	Gamma-aminobutyrate (GABA) transaminase (4-aminobutyrate aminotransferase) involved in the 4-aminobutyrate and glutamate degradation pathways; required for normal oxidative stress tolerance and nitrogen utilization
CAGL0F07579g	*YKL096W*	*CWP1*	Cell wall mannoprotein, linked to a beta-1,3- and beta-1,6-glucan heteropolymer through a phosphodiester bond; involved in cell wall organization; required for propionic acid resistance
CAGL0G04455g	*YJL106W*	*IME2*	Serine/threonine protein kinase involved in activation of meiosis, associates with Ime1p and mediates its stability, activates Ndt80p; IME2 expression is positively regulated by Ime1p
CAGL0D03564g	*YKL193C*	*SDS22*	Conserved nuclear regulatory subunit of Glc7p type 1 protein serine-threonine phosphatase (PP1), functions positively with Glc7p to promote dephosphorylation of nuclear substrates required for chromosome transmission during mitosis
CAGL0M04675g	*YOR285W*	*RDL1*	Protein of unknown function, localized to the mitochondrial outer membrane
CAGL0G01738g	*YGR086C*	*PIL1*	Primary component of eisosomes, which are large immobile cell cortex structures associated with endocytosis; null mutants show activation of Pkc1p/Ypk1p stress resistance pathways; detected in phosphorylated state in mitochondria
CAGL0M06963g	*YNR034W*	*SOL1*	Protein with a possible role in tRNA export; shows similarity to 6-phosphogluconolactonase non-catalytic domains but does not exhibit this enzymatic activity;

			homologous to Sol2p, Sol3p, and Sol4p
CAGL0A01826g	*YHR096C*	*HXT5*	Hexose transporter with moderate affinity for glucose, induced in the presence of non-fermentable carbon sources, induced by a decrease in growth rate, contains an extended N-terminal domain relative to other HXTs
CAGL0I10582g	*YGR127W*		
CAGL0K01353g	*YDL046W*	*NPC2*	Functional homolog of human NPC2/He1, which is a cholesterol-binding protein whose deficiency causes Niemann-Pick type C2 disease involving retention of cholesterol in lysosomes
CAGL0L04378g	*YOR161C*	*PNS1*	Protein of unknown function; has similarity to Torpedo californica tCTL1p, which is postulated to be a choline transporter, neither null mutation nor overexpression affects choline transport
CAGL0E01881g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0F06061g	*YMR041C*	*ARA2*	NAD-dependent arabinose dehydrogenase, involved in biosynthesis of dehydro-D-arabinono-1,4-lactone; similar to plant L-galactose dehydrogenase
CAGL0H02585g	*YMR250W*	*GAD1*	Glutamate decarboxylase, converts glutamate into gamma-aminobutyric acid (GABA) during glutamate catabolism; involved in response to oxidative stress
CAGL0A03102g	*YDR380W*	*ARO10*	Phenylpyruvate decarboxylase, catalyzes decarboxylation of phenylpyruvate to phenylacetaldehyde, which is the first specific step in the Ehrlich pathway
CAGL0C01793g	*YBR230C*	*OM14*	Integral mitochondrial outer membrane protein; abundance is decreased in cells grown in glucose relative to other carbon sources; appears to contain 3 alpha-helical transmembrane segments; ORF encodes a 97-basepair intron
CAGL0F04807g	*YIL136W*	*OM45*	Protein of unknown function, major constituent of the mitochondrial outer membrane; located on the outer (cytosolic) face of the outer membrane
CAGL0I10494g	*YPR149W*	*NCE102*	Protein of unknown function; contains transmembrane domains; involved in secretion of proteins that lack classical secretory signal sequences; component of the detergent-insoluble glycolipid-enriched complexes (DIGs)
CAGL0K08536g	*YKL103C*	*LAP4*	Vacuolar aminopeptidase yscI; zinc metalloproteinase that belongs to the peptidase family M18; often used as a marker protein in studies of autophagy and cytosol to vacuole targeting (CVT) pathway
CAGL0C00275g	*YDR533C*	*HSP31*	Possible chaperone and cysteine protease with similarity to E. coli Hsp31; member of the DJ-1/ThiJ/PfpI superfamily, which includes human DJ-1 involved in Parkinson's disease; exists as a dimer and contains a putative metal-binding site
CAGL0F03113g	*YDR425W*	*SNX41*	Sorting nexin, involved in the retrieval of late-Golgi SNAREs from the post-Golgi endosome to the trans-Golgi network; interacts with Snx4p
CAGL0B02563g	*YML128C*	*MSC1*	Protein of unknown function; mutant is defective in directing meiotic recombination events to homologous chromatids; the authentic, non-tagged protein is detected in

			highly purified mitochondria and is phosphorylated
CAGL0J04004g	*YOR228C*		
CAGL0E05170g	*YOL151W*	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase); stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway
CAGL0B01100g	*YLR178C*	*TFS1*	Protein that interacts with and inhibits carboxypeptidase Y and Ira2p; phosphatidylethanolamine-binding protein (PEBP) family member; targets to vacuolar membranes during stationary phase; acetylated by NatB N-terminal acetyltransferase
CAGL0C02321g	*YER037W*	*PHM8*	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p
CAGL0C03113g	*YLR270W*	*DCS1*	Non-essential hydrolase involved in mRNA decapping, may function in a feedback mechanism to regulate deadenylation, contains pyrophosphatase activity and a HIT (histidine triad) motif; interacts with neutral trehalase Nth1p
CAGL0G02739g	*YIL101C*	*XBP1*	Transcriptional repressor that binds to promoter sequences of the cyclin genes, CYS3, and SMF2; expression is induced by stress or starvation during mitosis, and late in meiosis; member of the Swi4p/Mbp1p family; potential Cdc28p substrate
CAGL0M08426g	*YJL163C*		
CAGL0G05247g	*YDR069C*	*DOA4*	Ubiquitin isopeptidase, required for recycling ubiquitin from proteasome-bound ubiquitinated intermediates, acts at the late endosome/prevacuolar compartment to recover ubiquitin from ubiquitinated membrane proteins en route to the vacuole
CAGL0M12793g	*YER079W*		
CAGL0H02101g	*YHR087W*	*RTC3*	Protein of unknown function involved in RNA metabolism; has structural similarity to SBDS, the human protein mutated in Shwachman-Diamond Syndrome (the yeast SBDS ortholog = SDO1); null mutation suppresses cdc13-1 temperature sensitivity
CAGL0M09713g	*YMR152W*	*YIM1*	Protein of unknown function; null mutant displays sensitivity to DNA damaging agents; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0C04323g	*YDR001C*	*NTH1*	Neutral trehalase, degrades trehalose; required for thermotolerance and may mediate resistance to other cellular stresses; may be phosphorylated by Cdc28p
CAGL0I01122g	*YHR104W*	*GRE3*	Aldose reductase involved in methylglyoxal, d-xylose, arabinose, and galactose metabolism; stress induced (osmotic, ionic, oxidative, heat shock, starvation and heavy metals); regulated by the HOG pathway
CAGL0M02211g	*YPL154C*	*PEP4*	Vacuolar aspartyl protease (proteinase A), required for the posttranslational precursor maturation of vacuolar proteinases; important for protein turnover after oxidative damage; synthesized as a zymogen, self-activates
CAGL0J09812g	*YBR126C*	*TPS1*	Synthase subunit of trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the

			storage carbohydrate trehalose; also found in a monomeric form; expression is induced by the stress response and repressed by the Ras-cAMP pathway
CAGL0J09394g	*YDL124W*		
CAGL0E01859g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0J03674g	*YOR245C*	*DGA1*	Diacylglycerol acyltransferase, catalyzes the terminal step of triacylglycerol (TAG) formation, acylates diacylglycerol using acyl-CoA as an acyl donor, localized to lipid particles
CAGL0H10120g	*YBR056W*		
CAGL0I01012g	*YOR036W*	*PEP12*	Target membrane receptor (t-SNARE) for vesicular intermediates traveling between the Golgi apparatus and the vacuole; controls entry of biosynthetic, endocytic, and retrograde traffic into the prevacuolar compartment; syntaxin
CAGL0M04763g	*YOR289W*		
CAGL0M06545g	*YKL124W*	*SSH4*	Specificity factor required for Rsp5p-dependent ubiquitination and sorting of cargo proteins at the multivesicular body; identified as a high-copy suppressor of a SHR3 deletion, increasing steady-state levels of amino acid permeases
CAGL0L05720g	*YJL132W*		
CAGL0F04719g	*YLR258W*	*GSY2*	Glycogen synthase, similar to Gsy1p; expression induced by glucose limitation, nitrogen starvation, heat shock, and stationary phase; activity regulated by cAMP-dependent, Snf1p and Pho85p kinases as well as by the Gac1p-Glc7p phosphatase
CAGL0A02816g	*YDR368W*	*YPR1*	NADPH-dependent aldo-keto reductase, reduces multiple substrates including 2-methylbutyraldehyde and D,L-glyceraldehyde, expression is induced by osmotic and oxidative stress; functionally redundant with other aldo-keto reductases
CAGL0G03883g	*YLL026W*	*HSP104*	Heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70) to refold and reactivate previously denatured, aggregated proteins; responsive to stresses including: heat, ethanol, and sodium arsenite; involved in [PSI+] propagation
CAGL0K02519g	*YMR081C*	*ISF1*	Serine-rich, hydrophilic protein with similarity to Mbr1p; overexpression suppresses growth defects of hap2, hap3, and hap4 mutants; expression is under glucose control; cotranscribed with NAM7 in a cyp1 mutant
CAGL0G04433g	*YJL108C*	*PRM10*	Pheromone-regulated protein, proposed to be involved in mating; predicted to have 5 transmembrane segments; induced by treatment with 8-methoxypsoralen and UVA irradiation
CAGL0K05137g	*YPR026W*	*ATH1*	Acid trehalase required for utilization of extracellular trehalose
CAGL0M02013g	*YPL166W*	*ATG29*	Autophagy-specific protein that is required for recruitment of other ATG proteins to the pre-autophagosomal structure (PAS); interacts with Atg17p and localizas to the PAS in a manner interdependent with Atg17p and Cis1p; not

			conserved
CAGL0E05984g	*YMR173W*	*DDR48*	DNA damage-responsive protein, expression is increased in response to heat-shock stress or treatments that produce DNA lesions; contains multiple repeats of the amino acid sequence NNNDSYGS
CAGL0M10439g	*YDR001C*	*NTH1*	Neutral trehalase, degrades trehalose; required for thermotolerance and may mediate resistance to other cellular stresses; may be phosphorylated by Cdc28p
CAGL0J10846g	*YHR071W*	*PCL5*	Cyclin, interacts with and phosphorylated by Pho85p cyclin-dependent kinase (Cdk), induced by Gcn4p at level of transcription, specifically required for Gcn4p degradation, may be sensor of cellular protein biosynthetic capacity
CAGL0B03817g	*YJR008W*		

Table 11.2: Genes down regulated by *C. glabrata* and *S. cerevisiae* upon hyperosmotic stress treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0F05115g	*YLR413W*		
CAGL0L10890g	*YOR272W*	*YTM1*	Constituent of 66S pre-ribosomal particles, forms a complex with Nop7p and Erb1p that is required for maturation of the large ribosomal subunit; has seven C-terminal WD repeats
CAGL0F02849g	*YDR412W*	*RRP17*	Component of the pre-60S pre-ribosomal particle; required for cell viability under standard (aerobic) conditions but not under anaerobic conditions
CAGL0K08360g	*YKR056W*	*TRM2*	tRNA methyltransferase, 5-methylates the uridine residue at position 54 of tRNAs and may also have a role in tRNA stabilization or maturation; endo-exonuclease with a role in DNA repair
CAGL0J11352g	*YNL186W*	*UBP10*	Ubiquitin-specific protease that deubiquitinates ubiquitin-protein moieties; may regulate silencing by acting on Sir4p; involved in posttranscriptionally regulating Gap1p and possibly other transporters; primarily located in the nucleus
CAGL0G03575g	*YER117W*	*RPL23B*	Protein component of the large (60S) ribosomal subunit, identical to Rpl23Ap and has similarity to E. coli L14 and rat L23 ribosomal proteins
CAGL0L11638g	*YDR365C*	*ESF1*	Nucleolar protein involved in pre-rRNA processing; depletion causes severely decreased 18S rRNA levels
CAGL0G04411g	*YJL109C*	*UTP10*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0G08041g	*YDR091C*	*RLI1*	Essential iron-sulfur protein required for ribosome biogenesis and translation initiation; facilitates binding of a multifactor complex (MFC) of translation initiation factors to the small ribosomal subunit; predicted ABC family ATPase
CAGL0F08129g	*YGR245C*	*SDA1*	Highly conserved nuclear protein required for actin cytoskeleton organization and passage through Start, plays

			a critical role in G1 events, binds Nap1p, also involved in 60S ribosome biogenesis
CAGL0K11748g	*YDR025W*	*RPS11A*	Protein component of the small (40S) ribosomal subunit; identical to Rps11Bp and has similarity to E. coli S17 and rat S11 ribosomal proteins
CAGL0H03773g	*YNL002C*	*RLP7*	Nucleolar protein with similarity to large ribosomal subunit L7 proteins; constituent of 66S pre-ribosomal particles; plays an essential role in processing of precursors to the large ribosomal subunit RNAs
CAGL0E02937g	*YGL029W*	*CGR1*	Protein involved in nucleolar integrity and processing of the pre-rRNA for the 60S ribosome subunit; transcript is induced in response to cytotoxic stress but not genotoxic stress
CAGL0D02706g	*YHR197W*	*RIX1*	Essential component of the Rix1 complex (Rix1p, Ipi1p, Ipi3p) that is required for processing of ITS2 sequences from 35S pre-rRNA; Rix1 complex associates with Mdn1p in pre-60S ribosomal particles
CAGL0I02398g	*YHR170W*	*NMD3*	Protein involved in nuclear export of the large ribosomal subunit; acts as a Crm1p-dependent adapter protein for export of nascent ribosomal subunits through the nuclear pore complex
CAGL0I03630g	*YDL150W*	*RPC53*	RNA polymerase III subunit C53
CAGL0E02673g	*YOR004W*	*UTP23*	Essential nucleolar protein that is a component of the SSU (small subunit) processome involved in 40S ribosomal subunit biogenesis; has homology to PINc domain protein Fcf1p, although the PINc domain of Utp23p is not required for function
CAGL0G04631g	*YDR492W*	*IZH1*	Membrane protein involved in zinc ion homeostasis, member of the four-protein IZH family; transcription is regulated directly by Zap1p, expression induced by zinc deficiency and fatty acids; deletion increases sensitivity to elevated zinc
CAGL0J11286g	*YNL182C*	*IPI3*	Essential component of the Rix1 complex (Rix1p, Ipi1p, Ipi3p) that is required for processing of ITS2 sequences from 35S pre-rRNA; highly conserved and contains WD40 motifs; Rix1 complex associates with Mdn1p in pre-60S ribosomal particles
CAGL0J10912g	*YHR065C*	*RRP3*	Protein involved in rRNA processing; required for maturation of the 35S primary transcript of pre-rRNA and for cleavage leading to mature 18S rRNA; homologous to eIF-4a, which is a DEAD box RNA-dependent ATPase with helicase activity
CAGL0M02409g	*YPL146C*	*NOP53*	Nucleolar protein; involved in biogenesis of the 60S subunit of the ribosome; interacts with rRNA processing factors Cbf5p and Nop2p; null mutant is viable but growth is severely impaired
CAGL0F07403g	*YGL111W*	*NSA1*	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis
CAGL0J00891g	*YKL143W*	*LTV1*	Component of the GSE complex, which is required for proper sorting of amino acid permease Gap1p; required for ribosomal small subunit export from nucleus; required for growth at low temperature
CAGL0G04851g	*YLR372W*	*SUR4*	Elongase, involved in fatty acid and sphingolipid

			biosynthesis; synthesizes very long chain 20-26-carbon fatty acids from C18-CoA primers; involved in regulation of sphingolipid biosynthesis
CAGL0D02838g	*YLL034C*	*RIX7*	Putative ATPase of the AAA family, required for export of pre-ribosomal large subunits from the nucleus; distributed between the nucleolus, nucleoplasm, and nuclear periphery depending on growth conditions
CAGL0D05500g	*YGR187C*	*HGH1*	Nonessential protein of unknown function; predicted to be involved in ribosome biogenesis; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; similar to mammalian BRP16 (Brain protein 16)
CAGL0F04103g	*YBL028C*		
CAGL0G03443g	*YER110C*	*KAP123*	Karyopherin beta, mediates nuclear import of ribosomal proteins prior to assembly into ribosomes and import of histones H3 and H4; localizes to the nuclear pore, nucleus, and cytoplasm; exhibits genetic interactions with RAI1
CAGL0I10560g	*YGR128C*	*UTP8*	Nucleolar protein required for export of tRNAs from the nucleus; also copurifies with the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0F07139g	*YGL120C*	*PRP43*	RNA helicase in the DEAH-box family, functions in both RNA polymerase I and polymerase II transcript metabolism, involved in release of the lariat-intron from the spliceosome
CAGL0I01606g	*YJR041C*	*URB2*	Nucleolar protein required for normal metabolism of the rRNA primary transcript, proposed to be involved in ribosome biogenesis
CAGL0L02871g	*YOR206W*	*NOC2*	Protein that forms a nucleolar complex with Mak21p that binds to 90S and 66S pre-ribosomes, as well as a nuclear complex with Noc3p that binds to 66S pre-ribosomes; both complexes mediate intranuclear transport of ribosomal precursors
CAGL0M10241g	*YKL006W*	*RPL14A*	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl14Bp and has similarity to rat L14 ribosomal protein; rpl14a csh5 double null mutant exhibits synthetic slow growth
CAGL0F02541g	*YDR398W*	*UTP5*	Subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit
CAGL0G01364g	*YBR121C*	*GRS1*	Cytoplasmic and mitochondrial glycyl-tRNA synthase that ligates glycine to the cognate anticodon bearing tRNA; transcription termination factor that may interact with the 3'-end of pre-mRNA to promote 3'-end formation
CAGL0J10890g	*YHR066W*	*SSF1*	Constituent of 66S pre-ribosomal particles, required for ribosomal large subunit maturation; functionally redundant with Ssf2p; member of the Brix family
CAGL0I03344g	*YDL167C*	*NRP1*	Putative RNA binding protein of unknown function; localizes to stress granules induced by glucose deprivation; predicted to be involved in ribosome biogenesis
CAGL0L05500g	*YJL122W*	*ALB1*	Shuttling pre-60S factor; involved in the biogenesis of ribosomal large subunit; interacts directly with Arx1p; responsible for Tif6p recycling defects in absence of Rei1p

CAGL0D02090g	*YMR116C*	*ASC1*	G-protein beta subunit and guanine nucleotide dissociation inhibitor for Gpa2p; ortholog of RACK1 that inhibits translation; core component of the small (40S) ribosomal subunit; represses Gcn4p in the absence of amino acid starvation
CAGL0I07931g	*YOL080C*	*REX4*	Putative RNA exonuclease possibly involved in pre-rRNA processing and ribosome assembly
CAGL0D01716g	*YBR088C*	*POL30*	Proliferating cell nuclear antigen (PCNA), functions as the sliding clamp for DNA polymerase delta; may function as a docking site for other proteins required for mitotic and meiotic chromosomal DNA replication and for DNA repair
CAGL0C00737g	*YLR222C*	*UTP13*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0G00154g	*YGR285C*	*ZUO1*	Cytosolic ribosome-associated chaperone that acts, together with Ssz1p and the Ssb proteins, as a chaperone for nascent polypeptide chains; contains a DnaJ domain and functions as a J-protein partner for Ssb1p and Ssb2p
CAGL0L02849g	*YOR207C*	*RET1*	Second-largest subunit of RNA polymerase III, which is responsible for the transcription of tRNA and 5S RNA genes, and other low molecular weight RNAs
CAGL0K10010g	*YDR083W*	*RRP8*	Nucleolar protein involved in rRNA processing, pre-rRNA cleavage at site A2; also involved in telomere maintenance; mutation is synthetically lethal with a gar1 mutation
CAGL0E01991g	*YOL121C*	*RPS19A*	Protein component of the small (40S) ribosomal subunit, required for assembly and maturation of pre-40 S particles; mutations in human RPS19 are associated with Diamond Blackfan anemia; nearly identical to Rps19Bp
CAGL0M09845g	*YLR409C*	*UTP21*	Subunit of U3-containing 90S preribosome and Small Subunit (SSU) processome complexes involved in production of 18S rRNA and assembly of small ribosomal subunit; synthetic defect with STI1 Hsp90 cochaperone; human homolog linked to glaucoma
CAGL0F02563g	*YDR399W*	*HPT1*	Dimeric hypoxanthine-guanine phosphoribosyltransferase, catalyzes the formation of both inosine monophosphate and guanosine monophosphate; mutations in the human homolog HPRT1 can cause Lesch-Nyhan syndrome and Kelley-Seegmiller syndrome
CAGL0M03905g	*YNL308C*	*KRI1*	Essential nucleolar protein required for 40S ribosome biogenesis; physically and functionally interacts with Krr1p
CAGL0M04829g	*YMR229C*	*RRP5*	RNA binding protein with preference for single stranded tracts of U's involved in synthesis of both 18S and 5.8S rRNAs; component of both the ribosomal small subunit (SSU) processosome and the 90S preribosome
CAGL0D00836g	*YDL063C*		
CAGL0F02299g	*YFR001W*	*LOC1*	Nuclear protein involved in asymmetric localization of ASH1 mRNA; binds double-stranded RNA in vitro; constituent of 66S pre-ribosomal particles
CAGL0M07227g	*YHR196W*	*UTP9*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in

			processing of pre-18S rRNA
CAGL0K01089g	*YGR081C*	*SLX9*	Protein required for pre-rRNA processing; associated with the 90S pre-ribosome and 43S small ribosomal subunit precursor; interacts with U3 snoRNA; deletion mutant has synthetic fitness defect with an sgs1 deletion mutant
CAGL0G01078g	*YGR034W*	*RPL26B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl26Ap and has similarity to E. coli L24 and rat L26 ribosomal proteins; binds to 5.8S rRNA
CAGL0I03608g	*YDL153C*	*SAS10*	Essential subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit; disrupts silencing when overproduced
CAGL0K01859g	*YDL014W*	*NOP1*	Nucleolar protein, component of the small subunit processome complex, which is required for processing of pre-18S rRNA; has similarity to mammalian fibrillarin
CAGL0A02189g	*YDR341C*		
CAGL0C04565g	*YJR097W*	*JJJ3*	Protein of unknown function, contains a J-domain, which is a region with homology to the E. coli DnaJ protein
CAGL0H05709g	*YPL093W*	*NOG1*	Putative GTPase that associates with free 60S ribosomal subunits in the nucleolus and is required for 60S ribosomal subunit biogenesis; constituent of 66S pre-ribosomal particles; member of the ODN family of nucleolar G-proteins
CAGL0L12672g	*YPL043W*	*NOP4*	Nucleolar protein, essential for processing and maturation of 27S pre-rRNA and large ribosomal subunit biogenesis; constituent of 66S pre-ribosomal particles; contains four RNA recognition motifs (RRMs)
CAGL0J08844g	*YDL148C*	*NOP14*	Nucleolar protein, forms a complex with Noc4p that mediates maturation and nuclear export of 40S ribosomal subunits; also present in the small subunit processome complex, which is required for processing of pre-18S rRNA
CAGL0J10010g	*YNL062C*	*GCD10*	Subunit of tRNA (1-methyladenosine) methyltransferase with Gcd14p, required for the modification of the adenine at position 58 in tRNAs, especially tRNAi-Met; first identified as a negative regulator of GCN4 expression
CAGL0G01991g	*YOR056C*	*NOB1*	Essential nuclear protein involved in proteasome maturation and synthesis of 40S ribosomal subunits; required for cleavage of the 20S pre-rRNA to generate the mature 18S rRNA
CAGL0A04015g	*YLR197W*	*NOP56*	Essential evolutionarily-conserved nucleolar protein component of the box C/D snoRNP complexes that direct 2'-O-methylation of pre-rRNA during its maturation; overexpression causes spindle orientation defects
CAGL0L03872g	*YNL113W*	*RPC19*	RNA polymerase subunit, common to RNA polymerases I and III
CAGL0E03245g	*YGR159C*	*NSR1*	Nucleolar protein that binds nuclear localization sequences, required for pre-rRNA processing and ribosome biogenesis
CAGL0G10043g	*YPR187W*	*RPO26*	RNA polymerase subunit ABC23, common to RNA polymerases I, II, and III; part of central core; similar to bacterial omega subunit

CAGL0J03718g	*YOR243C*	*PUS7*	Pseudouridine synthase, catalyzes pseudouridylation at position 35 in U2 snRNA, position 50 in 5S rRNA, position 13 in cytoplasmic tRNAs, and position 35 in pre-tRNA(Tyr); conserved in archaea, vertebrates, and some bacteria
CAGL0M06523g	*YBR191W*	*RPL21A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl21Bp and has similarity to rat L21 ribosomal protein
CAGL0G03091g	*YGR094W*	*VAS1*	Mitochondrial and cytoplasmic valyl-tRNA synthetase
CAGL0K03861g	*YMR131C*	*RRB1*	Essential nuclear protein involved in early steps of ribosome biogenesis; physically interacts with the ribosomal protein Rpl3p
CAGL0J01848g	*YPR010C*	*RPA135*	RNA polymerase I subunit A135
CAGL0E01573g	*YNL313C*		
CAGL0E05016g	*YGL148W*	*ARO2*	Bifunctional chorismate synthase and flavin reductase, catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP) to form chorismate, which is a precursor to aromatic amino acids
CAGL0M07678g	*YMR014W*	*BUD22*	Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern
CAGL0H01023g	*YDR279W*	*RNH202*	Ribonuclease H2 subunit, required for RNase H2 activity; related to human AGS2 that causes Aicardi-Goutieres syndrome
CAGL0M10197g	*YKL009W*	*MRT4*	Protein involved in mRNA turnover and ribosome assembly, localizes to the nucleolus
CAGL0L10164g	*YOR051C*		
CAGL0F00561g	*YJR063W*	*RPA12*	RNA polymerase I subunit A12.2; contains two zinc binding domains, and the N terminal domain is responsible for anchoring to the RNA pol I complex
CAGL0L03047g	*YKR024C*	*DBP7*	Putative ATP-dependent RNA helicase of the DEAD-box family involved in ribosomal biogenesis; essential for growth under anaerobic conditions
CAGL0H09724g	*YER006W*	*NUG1*	GTPase that associates with nuclear 60S pre-ribosomes, required for export of 60S ribosomal subunits from the nucleus
CAGL0B00352g	*YCL059C*	*KRR1*	Essential nucleolar protein required for the synthesis of 18S rRNA and for the assembly of 40S ribosomal subunit
CAGL0E02585g	*YOR001W*	*RRP6*	Nuclear exosome exonuclease component; has 3'-5' exonuclease activity; involved in RNA processing, maturation, surveillance, degradation, tethering, and export; has similarity to E. coli RNase D and to human PM-Sc1 100 (EXOSC10)
CAGL0J04070g	*YOR224C*	*RPB8*	RNA polymerase subunit ABC14.5, common to RNA polymerases I, II, and III
CAGL0K09328g	*YCR053W*	*THR4*	Threonine synthase, conserved protein that catalyzes formation of threonine from 0-phosphohomoserine; expression is regulated by the GCN4-mediated general amino acid control pathway
CAGL0M12144g	*YAL035W*	*FUN12*	GTPase, required for general translation initiation by promoting Met-tRNAiMet binding to ribosomes and

			ribosomal subunit joining; homolog of bacterial IF2
CAGL0I09790g	*YOR310C*	*NOP58*	Protein involved in pre-rRNA processing, 18S rRNA synthesis, and snoRNA synthesis; component of the small subunit processome complex, which is required for processing of pre-18S rRNA
CAGL0A00231g	*YGL021W*	*ALK1*	Protein kinase; accumulation and phosphorylation are periodic during the cell cycle; phosphorylated in response to DNA damage; contains characteristic motifs for degradation via the APC pathway; similar to Alk2p and to mammalian haspins
CAGL0J00957g	*YLR009W*	*RLP24*	Essential protein with similarity to Rpl24Ap and Rpl24Bp, associated with pre-60S ribosomal subunits and required for ribosomal large subunit biogenesis
CAGL0H03377g	*YGL078C*	*DBP3*	Putative ATP-dependent RNA helicase of the DEAD-box family involved in ribosomal biogenesis
CAGL0J11154g	*YNL175C*	*NOP13*	Nucleolar protein found in preribosomal complexes; contains an RNA recognition motif (RRM)
CAGL0L06754g	*YBR143C*	*SUP45*	Polypeptide release factor (eRF1) in translation termination; mutant form acts as a recessive omnipotent suppressor; methylated by Mtq2p-Trm112p in ternary complex eRF1-eRF3-GTP; mutation of methylation site confers resistance to zymocin
CAGL0H02057g	*YHR089C*	*GAR1*	Protein component of the H/ACA snoRNP pseudouridylase complex, involved in the modification and cleavage of the 18S pre-rRNA
CAGL0M00946g	*YLR435W*	*TSR2*	Protein with a potential role in pre-rRNA processing
CAGL0B03553g	*YEL055C*	*POL5*	DNA Polymerase phi; has sequence similarity to the human MybBP1A and weak sequence similarity to B-type DNA polymerases, not required for chromosomal DNA replication; required for the synthesis of rRNA
CAGL0H07975g	*YDL051W*	*LHP1*	RNA binding protein required for maturation of tRNA and U6 snRNA precursors; acts as a molecular chaperone for RNAs transcribed by polymerase III; homologous to human La (SS-B) autoantigen
CAGL0L10516g	*YKR081C*	*RPF2*	Essential protein involved in the processing of pre-rRNA and the assembly of the 60S ribosomal subunit; interacts with ribosomal protein L11; localizes predominantly to the nucleolus; constituent of 66S pre-ribosomal particles
CAGL0J09966g	*YNL064C*	*YDJ1*	Protein chaperone involved in regulation of the HSP90 and HSP70 functions; involved in protein translocation across membranes; member of the DnaJ family
CAGL0J05698g	*YNL151C*	*RPC31*	RNA polymerase III subunit C31; contains HMG-like C-terminal domain
CAGL0B04125g	*YPR110C*	*RPC40*	RNA polymerase subunit, common to RNA polymerase I and III
CAGL0M01430g	*YDR324C*	*UTP4*	Subunit of U3-containing 90S preribosome and Small Subunit (SSU) processome complexes involved in production of 18S rRNA and assembly of small ribosomal subunit; member of t-Utp subcomplex involved with transcription of 35S rRNA transcript
CAGL0D05588g	*YLL011W*	*SOF1*	Essential protein required for biogenesis of 40S (small) ribosomal subunit; has similarity to the beta subunit of

			trimeric G-proteins and the splicing factor Prp4p
CAGL0E02013g	*YNL301C*	*RPL18B*	Protein component of the large (60S) ribosomal subunit, identical to Rpl18Ap and has similarity to rat L18 ribosomal protein
CAGL0J01155g	*YLR003C*	*CMS1*	Subunit of U3-containing 90S preribosome processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit; overexpression rescues supressor mutant of mcm10; null mutant is viable
CAGL0M04719g	*YOR287C*		
CAGL0L08976g	*YFL002C*	*SPB4*	Putative ATP-dependent RNA helicase, nucleolar protein required for synthesis of 60S ribosomal subunits at a late step in the pathway; sediments with 66S pre-ribosomes in sucrose gradients
CAGL0J01045g	*YJL033W*	*HCA4*	Putative nucleolar DEAD box RNA helicase; high-copy number suppression of a U14 snoRNA processing mutant suggests an involvement in 18S rRNA synthesis
CAGL0C01639g	*YBR247C*	*ENP1*	Protein associated with U3 and U14 snoRNAs, required for pre-rRNA processing and 40S ribosomal subunit synthesis; localized in the nucleus and concentrated in the nucleolus
CAGL0F03927g	*YMR217W*	*GUA1*	GMP synthase, an enzyme that catalyzes the second step in the biosynthesis of GMP from inosine 5'-phosphate (IMP); transcription is not subject to regulation by guanine but is negatively regulated by nutrient starvation
CAGL0B00484g	*YCL054W*	*SPB1*	AdoMet-dependent methyltransferase involved in rRNA processing and 60S ribosomal subunit maturation; methylates G2922 in the tRNA docking site of the large subunit rRNA and in the absence of snR52, U2921; suppressor of PAB1 mutants
CAGL0J09746g	*YDL201W*	*TRM8*	Subunit of a tRNA methyltransferase complex composed of Trm8p and Trm82p that catalyzes 7-methylguanosine modification of tRNA
CAGL0G00924g	*YLR336C*	*SGD1*	Essential nuclear protein with a possible role in the osmoregulatory glycerol response; interacts with phospholipase C (Plc1p); putative homolog of human NOM1 which is implicated in acute myeloid leukemia
CAGL0I08547g	*YER156C*		
CAGL0L00671g	*YER056C*	*FCY2*	Purine-cytosine permease, mediates purine (adenine, guanine, and hypoxanthine) and cytosine accumulation
CAGL0E05478g	*YOR340C*	*RPA43*	RNA polymerase I subunit A43
CAGL0G07843g	*YGR103W*	*NOP7*	Component of several different pre-ribosomal particles; forms a complex with Ytm1p and Erb1p that is required for maturation of the large ribosomal subunit; required for exit from G₀ and the initiation of cell proliferation
CAGL0H01419g	*YDR299W*	*BFR2*	Essential protein that is a component of 90S preribosomes; may be involved in rRNA processing; multicopy suppressor of sensitivity to Brefeldin A; expression is induced during lag phase and also by cold shock
CAGL0L06314g	*YDR101C*	*ARX1*	Shuttling pre-60S factor; involved in the biogenesis of ribosomal large subunit biogenesis; interacts directly with Alb1; responsible for Tif6 recycling defects in absence of

			Rei1; associated with the ribosomal export complex
CAGL0L07832g	*YCR016W*		
CAGL0F08459g	*YGR264C*	*MES1*	Methionyl-tRNA synthetase, forms a complex with glutamyl-tRNA synthetase (Gus1p) and Arc1p, which increases the catalytic efficiency of both tRNA synthetases; also has a role in nuclear export of tRNAs
CAGL0M13893g	*YMR309C*	*NIP1*	eIF3c subunit of the eukaryotic translation initiation factor 3 (eIF3), involved in the assembly of preinitiation complex and start codon selection
CAGL0M01804g	*YBR079C*	*RPG1*	eIF3a subunit of the core complex of translation initiation factor 3 (eIF3), essential for translation; part of a subcomplex (Prt1p-Rpg1p-Nip1p) that stimulates binding of mRNA and tRNA(i)Met to ribosomes
CAGL0M06941g	*YNL022C*		
CAGL0J10252g	*YNL075W*	*IMP4*	Component of the SSU processome, which is required for pre-18S rRNA processing; interacts with Mpp10p; member of a superfamily of proteins that contain a sigma(70)-like motif and associate with RNAs
CAGL0M09977g	*YLR401C*	*DUS3*	Dihydrouridine synthase, member of a widespread family of conserved proteins including Smm1p, Dus1p, and Dus4p; contains a consensus oleate response element (ORE) in its promoter region
CAGL0C01441g	*YPL183C*	*RTT10*	Cytoplasmic protein with a role in regulation of Ty1 transposition
CAGL0J07744g	*YNL247W*		
CAGL0L03806g	*YNL110C*	*NOP15*	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis; localizes to both nucleolus and cytoplasm
CAGL0A02673g	*YDR361C*	*BCP1*	Essential protein involved in nuclear export of Mss4p, which is a lipid kinase that generates phosphatidylinositol 4,5-biphosphate and plays a role in actin cytoskeleton organization and vesicular transport
CAGL0H04675g	*YDR429C*	*TIF35*	eIF3g subunit of the core complex of translation initiation factor 3 (eIF3), which is essential for translation
CAGL0M05159g	*YMR239C*	*RNT1*	RNAase III; involved in rDNA transcription and rRNA processing; also cleaves a stem-loop structure at the 3' end of U2 snRNA to ensure formation of the correct U2 3' end
CAGL0A01958g	*YOL022C*	*TSR4*	Cytoplasmic protein of unknown function; essential gene in S288C background, while tsr4 null mutations in a CEN.PK2 background confer a reduced growth rate
CAGL0J00473g	*YHR052W*	*CIC1*	Essential protein that interacts with proteasome components and has a potential role in proteasome substrate specificity; also copurifies with 66S pre-ribosomal particles
CAGL0E05764g	*YPL212C*	*PUS1*	tRNA:pseudouridine synthase, introduces pseudouridines at positions 26-28, 34-36, 65, and 67 of tRNA; nuclear protein that appears to be involved in tRNA export; also acts on U2 snRNA
CAGL0I01826g	*YHR148W*	*IMP3*	Component of the SSU processome, which is required for pre-18S rRNA processing, essential protein that interacts with Mpp10p and mediates interactions of Imp4p and

			Mpp10p with U3 snoRNA
CAGL0D05874g	*YJR002W*	*MPP10*	Component of the SSU processome and 90S preribosome, required for pre-18S rRNA processing, interacts with and controls the stability of Imp3p and Imp4p, essential for viability; similar to human Mpp10p
CAGL0L00341g	*YKL172W*	*EBP2*	Essential protein required for the maturation of 25S rRNA and 60S ribosomal subunit assembly, localizes to the nucleolus; constituent of 66S pre-ribosomal particles
CAGL0A03652g	*YBR142W*	*MAK5*	Essential nucleolar protein, putative DEAD-box RNA helicase required for maintenance of M1 dsRNA virus; involved in biogenesis of large (60S) ribosomal subunits
CAGL0E00979g	*YDR165W*	*TRM82*	Subunit of a tRNA methyltransferase complex composed of Trm8p and Trm82p that catalyzes 7-methylguanosine modification of tRNA
CAGL0M04279g	*YLR129W*	*DIP2*	Nucleolar protein, specifically associated with the U3 snoRNA, part of the large ribonucleoprotein complex known as the small subunit (SSU) processome, required for 18S rRNA biogenesis, part of the active pre-rRNA processing complex
CAGL0M13519g	*YMR290C*	*HAS1*	ATP-dependent RNA helicase; localizes to both the nuclear periphery and nucleolus; highly enriched in nuclear pore complex fractions; constituent of 66S pre-ribosomal particles
CAGL0J10032g	*YNL061W*	*NOP2*	Probable RNA m(5)C methyltransferase, essential for processing and maturation of 27S pre-rRNA and large ribosomal subunit biogenesis; localized to the nucleolus; constituent of 66S pre-ribosomal particles
CAGL0K04741g	*YNL209W*	*SSB2*	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p; may be involved in the folding of newly-synthesized polypeptide chains; member of the HSP70 family; homolog of SSB1
CAGL0B04169g	*YPR112C*	*MRD1*	Essential conserved protein that is part of the 90S preribosome; required for production of 18S rRNA and small ribosomal subunit; contains five consensus RNA-binding domains
CAGL0L04026g	*YNL119W*	*NCS2*	Protein required for thiolation of the uridine at the wobble position of Lys(UUU) and Glu(UUC) tRNAs; has a role in urmylation and in invasive and pseudohyphal growth; inhibits replication of Brome mosaic virus in S. cerevisiae
CAGL0M01496g	*YDR395W*	*SXM1*	Nuclear transport factor (karyopherin) involved in protein transport between the cytoplasm and nucleoplasm; similar to Nmd5p, Cse1p, Lph2p, and the human cellular apoptosis susceptibility protein, CAS1
CAGL0J05412g	*YGL099W*	*LSG1*	Putative GTPase involved in 60S ribosomal subunit biogenesis; required for the release of Nmd3p from 60S subunits in the cytoplasm
CAGL0C01331g	*YIL110W*	*MNI1*	Putative S-adenosylmethionine-dependent methyltransferase of the seven beta-strand family; deletion mutant exhibits a weak vacuolar protein sorting defect, enhanced resistance to caspofungin, and is synthetically lethal with MEN mutants
CAGL0G03311g	*YBL076C*	*ILS1*	Cytoplasmic isoleucine-tRNA synthetase, target of the G1-

			specific inhibitor reveromycin A
CAGL0J03344g	*YER082C*	*UTP7*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0M06567g	*YDR021W*	*FAL1*	Nucleolar protein required for maturation of 18S rRNA, member of the eIF4A subfamily of DEAD-box ATP-dependent RNA helicases
CAGL0E01463g	*YOL139C*	*CDC33*	Cytoplasmic mRNA cap binding protein and translation initiation factor eIF4E; the eIF4E-cap complex is responsible for mediating cap-dependent mRNA translation via interactions with translation initiation factor eIF4G (Tif4631p or Tif4632p)
CAGL0F00407g	*YJR070C*	*LIA1*	Deoxyhypusine hydroxylase, a HEAT-repeat containing metalloenzyme that catalyzes hypusine formation; binds to and is required for the modification of Hyp2p (eIF5A); complements S. pombe mmd1 mutants defective in mitochondrial positioning
CAGL0K09614g	*YNL132W*	*KRE33*	Essential protein of unknown function; heterozygous mutant shows haploinsufficiency in K1 killer toxin resistance
CAGL0M01826g	*YBR078W*	*ECM33*	GPI-anchored protein of unknown function, has a possible role in apical bud growth; GPI-anchoring on the plasma membrane crucial to function; phosphorylated in mitochondria; similar to Sps2p and Pst1p
CAGL0B01188g	*YLR183C*	*TOS4*	Forkhead Associated domain containing protein and putative transcription factor found associated with chromatin; target of SBF transcription factor; expression is periodic and peaks in G1; similar to PLM2
CAGL0K06457g	*YDR465C*	*RMT2*	Arginine methyltransferase; ribosomal protein L12 is a substrate
CAGL0K01991g	*YBL024W*	*NCL1*	S-adenosyl-L-methionine-dependent tRNA: m5C-methyltransferase, methylates cytosine to m5C at several positions in tRNAs and intron-containing pre-tRNAs; similar to Nop2p and human proliferation associated nucleolar protein p120
CAGL0G05940g	*YNL162W*	*RPL42A*	Protein component of the large (60S) ribosomal subunit, identical to Rpl42Bp and has similarity to rat L44 ribosomal protein
CAGL0F01925g	*YLR051C*	*FCF2*	Essential nucleolar protein involved in the early steps of 35S rRNA processing; interacts with Faf1p; member of a transcriptionally co-regulated set of genes called the RRB regulon
CAGL0H02189g	*YMR269W*	*TMA23*	Nucleolar protein of unknown function implicated in ribosome biogenesis; TMA23 may be a fungal-specific gene as no homologs have been yet identified in higher eukaryotes
CAGL0I07975g	*YOL077C*	*BRX1*	Nucleolar protein, constituent of 66S pre-ribosomal particles; depletion leads to defects in rRNA processing and a block in the assembly of large ribosomal subunits; possesses a sigma(70)-like RNA-binding motif
CAGL0I04730g	*YBR034C*	*HMT1*	Nuclear SAM-dependent mono- and asymmetric arginine dimethylating methyltransferase that modifies hnRNPs, including Npl3p and Hrp1p, thus facilitating nuclear

			export of these proteins; required for viability of npl3 mutants
CAGL0G06248g	*YAL025C*	*MAK16*	Essential nuclear protein, constituent of 66S pre-ribosomal particles; required for maturation of 25S and 5.8S rRNAs; required for maintenance of M1 satellite double-stranded RNA of the L-A virus
CAGL0H02431g	*YMR259C*		
CAGL0G02409g	*YKR092C*	*SRP40*	Nucleolar, serine-rich protein with a role in preribosome assembly or transport; may function as a chaperone of small nucleolar ribonucleoprotein particles (snoRNPs); immunologically and structurally to rat Nopp140
CAGL0F04433g	*YBL039C*	*URA7*	Major CTP synthase isozyme (see also URA8), catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to UTP, forming CTP, the final step in de novo biosynthesis of pyrimidines; involved in phospholipid biosynthesis
CAGL0M02805g	*YPL126W*	*NAN1*	U3 snoRNP protein, component of the small (ribosomal) subunit (SSU) processosome containing U3 snoRNA; required for the biogenesis of18S rRNA
CAGL0K09460g	*YOR145C*	*PNO1*	Essential nucleolar protein required for pre-18S rRNA processing, interacts with Dim1p, an 18S rRNA dimethyltransferase, and also with Nob1p, which is involved in proteasome biogenesis; contains a KH domain
CAGL0H02079g	*YHR088W*	*RPF1*	Nucleolar protein involved in the assembly and export of the large ribosomal subunit; constituent of 66S pre-ribosomal particles; contains a sigma(70)-like motif, which is thought to bind RNA
CAGL0E03069g	*YGR145W*	*ENP2*	Essential nucleolar protein of unknown function; contains WD repeats, interacts with Mpp10p and Bfr2p, and has homology to Spb1p
CAGL0F07645g	*YKL099C*	*UTP11*	Subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit
CAGL0I08393g	*YPR163C*	*TIF3*	Translation initiation factor eIF-4B, has RNA annealing activity; contains an RNA recognition motif and binds to single-stranded RNA
CAGL0G08778g	*YIL127C*	*RRT14*	Putative protein of unknown function; identified in a screen for mutants with decreased levels of rDNA transcription; green fluorescent protein (GFP)-fusion protein localizes to the nucleolus; predicted to be involved in ribosome biogenesis
CAGL0I07799g	*YBR154C*	*RPB5*	RNA polymerase subunit ABC27, common to RNA polymerases I, II, and III; contacts DNA and affects transactivation
CAGL0D03674g	*YPL226W*	*NEW1*	ATP binding cassette family member; Asn/Gln-rich rich region supports [NU+] prion formation and susceptibility to [PSI+] prion induction; homologous to mRNA export factor from S. pombe and similar protein from C. albicans
CAGL0E05676g	*YPL207W*	*TYW1*	Protein required for the synthesis of wybutosine, a modified guanosine found at the 3'-position adjacent to the anticodon of phenylalanine tRNA which supports reading frame maintenance by stabilizing codon-anticodon interactions

CAGL0B01881g	*YDR120C*	*TRM1*	tRNA methyltransferase; two forms of the protein are made by alternative translation starts; localizes to both the nucleus and mitochondrion to produce the modified base N2,N2-dimethylguanosine in tRNAs in both compartments
CAGL0J09922g	*YNL066W*	*SUN4*	Cell wall protein related to glucanases, possibly involved in cell wall septation; member of the SUN family
CAGL0G00484g	*YGR272C*		
CAGL0H03685g	*YLR175W*	*CBF5*	Pseudouridine synthase catalytic subunit of box H/ACA small nucleolar ribonucleoprotein particles (snoRNPs), acts on both large and small rRNAs and on snRNA U2; mutations in human ortholog dyskerin cause the disorder dyskeratosis congenita
CAGL0J11066g	*YLR002C*	*NOC3*	Protein that forms a nuclear complex with Noc2p that binds to 66S ribosomal precursors to mediate their intranuclear transport; also binds to chromatin to promote the association of DNA replication factors and replication initiation
CAGL0F04499g	*YBL042C*	*FUI1*	High affinity uridine permease, localizes to the plasma membrane; also mediates low but significant transport of the cytotoxic nucleoside analog 5-fluorouridine; not involved in uracil transport
CAGL0G00264g	*YGR280C*	*PXR1*	Essential protein involved in rRNA and snoRNA maturation; competes with TLC1 RNA for binding to Est2p, suggesting a role in negative regulation of telomerase; human homolog inhibits telomerase; contains a G-patch RNA interacting domain
CAGL0K04587g	*YLR367W*	*RPS22B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps22Ap and has similarity to E. coli S8 and rat S15a ribosomal proteins
CAGL0I02926g	*YDR496C*	*PUF6*	Pumilio-homology domain protein that binds ASH1 mRNA at PUF consensus sequences in the 3' UTR and represses its translation, resulting in proper asymmetric localization of ASH1 mRNA
CAGL0J05984g	*YNL141W*	*AAH1*	Adenine deaminase (adenine aminohydrolase), converts adenine to hypoxanthine; involved in purine salvage; transcriptionally regulated by nutrient levels and growth phase; Aah1p degraded upon entry into quiescence via SCF and the proteasome
CAGL0E01925g	*YOL124C*	*TRM11*	Catalytic subunit of an adoMet-dependent tRNA methyltransferase complex (Trm11p-Trm112p), required for the methylation of the guanosine nucleotide at position 10 (m2G10) in tRNAs; contains a THUMP domain and a methyltransferase domain
CAGL0C05379g	*YNL209W*	*SSB2*	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p; may be involved in the folding of newly-synthesized polypeptide chains; member of the HSP70 family; homolog of SSB1
CAGL0C00715g	*YLR221C*	*RSA3*	Protein with a likely role in ribosomal maturation, required for accumulation of wild-type levels of large (60S) ribosomal subunits; binds to the helicase Dbp6p in pre-60S ribosomal particles in the nucleolus
CAGL0D03124g	*YLL008W*	*DRS1*	Nucleolar DEAD-box protein required for ribosome

			assembly and function, including synthesis of 60S ribosomal subunits; constituent of 66S pre-ribosomal particles
CAGL0H02937g	*YJL069C*	*UTP18*	Possible U3 snoRNP protein involved in maturation of pre-18S rRNA, based on computational analysis of large-scale protein-protein interaction data
CAGL0K02541g	*YHL011C*	*PRS3*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes
CAGL0L04950g	*YMR049C*	*ERB1*	Constituent of 66S pre-ribosomal particles, forms a complex with Nop7p and Ytm1p that is required for maturation of the large ribosomal subunit; required for maturation of the 25S and 5.8S ribosomal RNAs; homologous to mammalian Bop1
CAGL0I07579g	*YOL097C*	*WRS1*	Cytoplasmic tryptophanyl-tRNA synthetase, aminoacylates tryptophanyl-tRNA
CAGL0M12122g	*YAL036C*	*RBG1*	Member of the DRG family of GTP-binding proteins; associates with translating ribosomes; interacts with Tma46p, Ygr250cp, Gir2p and Yap1p via two-hybrid
CAGL0I10670g	*YPR144C*	*NOC4*	Nucleolar protein, forms a complex with Nop14p that mediates maturation and nuclear export of 40S ribosomal subunits
CAGL0L11594g	*YOR119C*	*RIO1*	Essential serine kinase involved in cell cycle progression and processing of the 20S pre-rRNA into mature 18S rRNA
CAGL0E06534g	*YJL010C*	*NOP9*	Essential subunit of U3-containing 90S preribosome involved in production of 18S rRNA and assembly of small ribosomal subunit; also part of pre-40S ribosome and required for its export into cytoplasm; binds RNA and contains pumilio domain
CAGL0H06985g	*YML093W*	*UTP14*	Subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit
CAGL0J07766g	*YNL248C*	*RPA49*	RNA polymerase I subunit A49
CAGL0F01023g	*YOL041C*	*NOP12*	Nucleolar protein involved in pre-25S rRNA processing and biogenesis of large 60S ribosomal subunit; contains an RNA recognition motif (RRM); binds to Ebp2; similar to Nop13p and Nsr1p
CAGL0J08679g	*YOR091W*	*TMA46*	Protein of unknown function that associates with ribosomes; interacts with GTPase Rbg1p
CAGL0F04983g	*YLR276C*	*DBP9*	ATP-dependent RNA helicase of the DEAD-box family involved in biogenesis of the 60S ribosomal subunit
CAGL0J01265g	*YMR093W*	*UTP15*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0I06006g	*YJL148W*	*RPA34*	RNA polymerase I subunit A34.5
CAGL0D05016g	*YPR143W*	*RRP15*	Nucleolar protein, constituent of pre-60S ribosomal particles; required for proper processing of the 27S pre-rRNA at the A3 and B1 sites to yield mature 5.8S and 25S rRNAs

CAGL0K02233g	*YER126C*	*NSA2*	Protein constituent of 66S pre-ribosomal particles, contributes to processing of the 27S pre-rRNA

Table 11.3: Genes up regulated uniquely by *S. cerevisiae* upon hyperosmotic stress treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0B00968g	*YCL027W*	*FUS1*	Membrane protein localized to the shmoo tip, required for cell fusion; expression regulated by mating pheromone; proposed to coordinate signaling, fusion, and polarization events required for fusion; potential Cdc28p substrate
CAGL0I08195g	*YER020W*	*GPA2*	Nucleotide binding alpha subunit of the heterotrimeric G protein that interacts with the receptor Gpr1p, has signaling role in response to nutrients; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery
CAGL0J09614g	*YDL208W*	*NHP2*	Nuclear protein related to mammalian high mobility group (HMG) proteins, essential for function of H/ACA-type snoRNPs, which are involved in 18S rRNA processing
CAGL0C00539g	*YBR132C*	*AGP2*	High affinity polyamine permease, preferentially uses spermidine over putrescine; expression is down-regulated by osmotic stress; plasma membrane carnitine transporter, also functions as a low-affinity amino acid permease
CAGL0K03905g	*YMR133W*	*REC114*	Protein involved in early stages of meiotic recombination; possibly involved in the coordination of recombination and meiotic division; mutations lead to premature initiation of the first meiotic division
CAGL0I01474g	*YJR046W*	*TAH11*	DNA replication licensing factor, required for pre-replication complex assembly
CAGL0K03509g	*YMR110C*	*HFD1*	Putative fatty aldehyde dehydrogenase, located in the mitochondrial outer membrane and also in lipid particles; has similarity to human fatty aldehyde dehydrogenase (FALDH) which is implicated in Sjogren-Larsson syndrome
CAGL0L02651g	*YOR219C*	*STE13*	Dipeptidyl aminopeptidase, Golgi integral membrane protein that cleaves on the carboxyl side of repeating -X-Ala- sequences, required for maturation of alpha factor, transcription is induced by a-factor
CAGL0K06369g	*YDR460W*	*TFB3*	Subunit of TFIIH and nucleotide excision repair factor 3 complexes, involved in transcription initiation, required for nucleotide excision repair; ring finger protein similar to mammalian CAK and TFIIH subunit
CAGL0K01727g	*YDL020C*	*RPN4*	Transcription factor that stimulates expression of proteasome genes; Rpn4p levels are in turn regulated by the 26S proteasome in a negative feedback control mechanism; RPN4 is transcriptionally regulated by various stress responses
CAGL0G01540g	*YNL036W*	*NCE103*	Carbonic anhydrase; poorly transcribed under aerobic conditions and at an undetectable level under anaerobic conditions; involved in non-classical protein export pathway
CAGL0I06765g	*YDR069C*	*DOA4*	Ubiquitin isopeptidase, required for recycling ubiquitin

			from proteasome-bound ubiquitinated intermediates, acts at the late endosome/prevacuolar compartment to recover ubiquitin from ubiquitinated membrane proteins en route to the vacuole
CAGL0K08734g	*YIR014W*		
CAGL0D03916g	*YHR034C*	*PIH1*	Protein of unresolved function; may function in protein folding and/or rRNA processing, interacts with a chaperone (Hsp82p), two chromatin remodeling factors (Rvb1p, Rvb2p) and two rRNA processing factors (Rrp43p, Nop58p)
CAGL0L02827g	*YOR208W*	*PTP2*	Phosphotyrosine-specific protein phosphatase involved in the inactivation of mitogen-activated protein kinase (MAPK) during osmolarity sensing; dephosporylates Hog1p MAPK and regulates its localization; localized to the nucleus
CAGL0G05984g	*YHR139C*	*SPS100*	Protein required for spore wall maturation; expressed during sporulation; may be a component of the spore wall; expression also induced in cells treated with the mycotoxin patulin
CAGL0F00891g	*YOL047C*		
CAGL0I07161g	*YOR141C*	*ARP8*	Nuclear actin-related protein involved in chromatin remodeling, component of chromatin-remodeling enzyme complexes
CAGL0K10318g	*YDR379W*	*RGA2*	GTPase-activating protein for the polarity-establishment protein Cdc42p; implicated in control of septin organization, pheromone response, and haploid invasive growth; regulated by Pho85p and Cdc28p
CAGL0F07909g	*YOL089C*	*HAL9*	Putative transcription factor containing a zinc finger; overexpression increases salt tolerance through increased expression of the ENA1 (Na+/Li+ extrusion pump) gene while gene disruption decreases both salt tolerance and ENA1 expression
CAGL0M11682g	*YLR108C*		
CAGL0L03377g	*YJL089W*	*SIP4*	C6 zinc cluster transcriptional activator that binds to the carbon source-responsive element (CSRE) of gluconeogenic genes; involved in the positive regulation of gluconeogenesis; regulated by Snf1p protein kinase; localized to the nucleus
CAGL0J05082g	*YJL057C*	*IKS1*	Putative serine/threonine kinase; expression is induced during mild heat stress; deletion mutants are hypersensitive to copper sulphate and resistant to sorbate; interacts with an N-terminal fragment of Sst2p
CAGL0I01672g	*YJR036C*	*HUL4*	Protein with similarity to hect domain E3 ubiquitin-protein ligases, not essential for viability
CAGL0F08195g	*YGR249W*	*MGA1*	Protein similar to heat shock transcription factor; multicopy suppressor of pseudohyphal growth defects of ammonium permease mutants
CAGL0F08063g	*YGR241C*	*YAP1802*	Protein involved in clathrin cage assembly; binds Pan1p and clathrin; homologous to Yap1801p, member of the AP180 protein family
CAGL0M07249g	*YPL060W*	*LPE10*	Mitochondrial inner membrane magnesium transporter, involved in maintenance of magnesium concentrations

			inside mitochondria; indirectly affects splicing of group II introns; functionally and structurally related to Mrs2p
CAGL0H01639g	*YDR523C*	*SPS1*	Putative protein serine/threonine kinase expressed at the end of meiosis and localized to the prospore membrane, required for correct localization of enzymes involved in spore wall synthesis
CAGL0F05445g	*YDR202C*	*RAV2*	Subunit of RAVE (Rav1p, Rav2p, Skp1p), a complex that associates with the V1 domain of the vacuolar membrane (H+)-ATPase (V-ATPase) and promotes assembly and reassembly of the holoenzyme
CAGL0E06116g	*YPL230W*	*USV1*	Putative transcription factor containing a C2H2 zinc finger; mutation affects transcriptional regulation of genes involved in growth on non-fermentable carbon sources, response to salt stress and cell wall biosynthesis
CAGL0A01089g	*YPL272C*		
CAGL0A02431g	*YDR349C*	*YPS7*	Putative GPI-anchored aspartic protease, located in the cytoplasm and endoplasmic reticulum
CAGL0J00803g	*YJL042W*	*MHP1*	Microtubule-associated protein involved in assembly and stabilization of microtubules; overproduction results in cell cycle arrest at G2 phase; similar to Drosophila protein MAP and to mammalian MAP4 proteins
CAGL0F05929g	*YMR034C*		
CAGL0I09526g	*YGL128C*	*CWC23*	Component of a complex containing Cef1p, putatively involved in pre-mRNA splicing; has similarity to E. coli DnaJ and other DnaJ-like proteins and to S. pombe Cwf23p
CAGL0H06545g	*YIL146C*	*ATG32*	Mitochondrial-anchored transmembrane receptor that interacts with the autophagy adaptor protein, Atg11p, and is essential for mitophagy, the selective vacuolar degradation of mitochondria in response to starvation
CAGL0I01518g	*YJR044C*	*VPS55*	Late endosomal protein involved in late endosome to vacuole trafficking; functional homolog of human obesity receptor gene-related protein (OB-RGRP)
CAGL0C02299g	*YER038C*	*KRE29*	Essential subunit of the Mms21-Smc5-Smc6 complex, required for growth and DNA repair; heterozygous mutant shows haploinsufficiency in K1 killer toxin resistance
CAGL0C02673g	*YLR392C*		
CAGL0K02959g	*YHR004C*	*NEM1*	Probable catalytic subunit of Nem1p-Spo7p phosphatase holoenzyme; regulates nuclear growth by controlling phospholipid biosynthesis, required for normal nuclear envelope morphology and sporulation; homolog of the human protein Dullard
CAGL0H09636g	*YER010C*		
CAGL0I06094g	*YJL155C*	*FBP26*	Fructose-2,6-bisphosphatase, required for glucose metabolism
CAGL0M04741g	*YOR288C*	*MPD1*	Member of the protein disulfide isomerase (PDI) family; interacts with and inhibits the chaperone activity of Cne1p; MPD1 overexpression in a pdi1 null mutant suppresses defects in Pdi1p functions such as carboxypeptidase Y maturation
CAGL0J05368g	*YGL098W*	*USE1*	Essential SNARE protein localized to the ER, involved in

			retrograde traffic from the Golgi to the ER; forms a complex with the SNAREs Sec22p, Sec20p and Ufe1p
CAGL0H09152g	*YJR059W*	*PTK2*	Putative serine/threonine protein kinase involved in regulation of ion transport across plasma membrane; enhances spermine uptake
CAGL0I06270g	*YJL166W*	*QCR8*	Subunit 8 of ubiquinol cytochrome-c reductase complex, which is a component of the mitochondrial inner membrane electron transport chain; oriented facing the intermembrane space; expression is regulated by Abf1p and Cpf1p
CAGL0L04686g	*YGR110W*	*CLD1*	Mitochondrial cardiolipin-specific phospholipase; functions upstream of Taz1p to generate monolyso-cardiolipin; transcription increases upon genotoxic stress; involved in restricting Ty1 transposition; has homology to mammalian CGI-58
CAGL0D02882g	*YLR006C*	*SSK1*	Cytoplasmic response regulator, part of a two-component signal transducer that mediates osmosensing via a phosphorelay mechanism; dephosphorylated form is degraded by the ubiquitin-proteasome system; potential Cdc28p substrate
CAGL0J00385g	*YHR049W*	*FSH1*	Putative serine hydrolase that localizes to both the nucleus and cytoplasm; sequence is similar to S. cerevisiae Fsh2p and Fsh3p and the human candidate tumor suppressor OVCA2
CAGL0B03113g	*YLR352W*		
CAGL0I10725g	*YGR122W*		
CAGL0M01562g	*YDR391C*		
CAGL0C04895g	*YJR110W*	*YMR1*	Phosphatidylinositol 3-phosphate (PI3P) phosphatase; involved in various protein sorting pathways, including CVT targeting and endosome to vacuole transport; has similarity to the conserved myotubularin dual specificity phosphatase family
CAGL0K04455g	*YGR059W*	*SPR3*	Sporulation-specific homolog of the yeast CDC3/10/11/12 family of bud neck microfilament genes; septin protein involved in sporulation; regulated by ABFI
CAGL0J11528g	*YNL193W*		
CAGL0F07601g	*YKL096W*	*CWP1*	Cell wall mannoprotein, linked to a beta-1,3- and beta-1,6-glucan heteropolymer through a phosphodiester bond; involved in cell wall organization; required for propionic acid resistance
CAGL0M08382g	*YKL167C*	*MRP49*	Mitochondrial ribosomal protein of the large subunit, not essential for mitochondrial translation
CAGL0M09493g	*YMR160W*		
CAGL0L03674g	*YJL103C*	*GSM1*	Putative zinc cluster protein of unknown function; proposed to be involved in the regulation of energy metabolism, based on patterns of expression and sequence analysis
CAGL0K08844g	*YHL021C*	*AIM17*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; null mutant displays reduced frequency of mitochondrial genome loss

CAGL0F06325g	*YMR053C*	*STB2*	Protein that interacts with Sin3p in a two-hybrid assay and is part of a large protein complex with Sin3p and Stb1p
CAGL0G05566g	*YDL222C*	*FMP45*	Integral membrane protein localized to mitochondria (untagged protein); required for sporulation and maintaining sphingolipid content; has sequence similarity to SUR7 and YNL194C
CAGL0E05280g	*YOL151W*	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase); stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway
CAGL0H10054g	*YBR053C*		
CAGL0M07359g	*YPL054W*	*LEE1*	Zinc-finger protein of unknown function
CAGL0J04224g	*YFL016C*	*MDJ1*	Co-chaperone that stimulates the ATPase activity of the HSP70 protein Ssc1p; involved in protein folding/refolding in the mitochodrial matrix; required for proteolysis of misfolded proteins; member of the HSP40 (DnaJ) family of chaperones
CAGL0J01463g	*YKL096W*	*CWP1*	Cell wall mannoprotein, linked to a beta-1,3- and beta-1,6-glucan heteropolymer through a phosphodiester bond; involved in cell wall organization; required for propionic acid resistance
CAGL0J00517g	*YHR031C*	*RRM3*	DNA helicase involved in rDNA replication and Ty1 transposition; relieves replication fork pauses at telomeric regions; structurally and functionally related to Pif1p
CAGL0A02134g	*YHR017W*	*YSC83*	Non-essential mitochondrial protein of unknown function; mRNA induced during meiosis, peaking between mid to late prophase of meiosis I; similar to S. douglasii YSD83
CAGL0M07315g	*YPL057C*	*SUR1*	Probable catalytic subunit of a mannosylinositol phosphorylceramide (MIPC) synthase, forms a complex with probable regulatory subunit Csg2p; function in sphingolipid biosynthesis is overlapping with that of Csh1p
CAGL0J00561g	*YHR029C*	*YHI9*	Protein of unknown function; null mutant is defective in unfolded protein response; possibly involved in a membrane regulation metabolic pathway; member of the PhzF superfamily, though most likely not involved in phenazine production
CAGL0M13277g	*YKL067W*	*YNK1*	Nucleoside diphosphate kinase, catalyzes the transfer of gamma phosphates from nucleoside triphosphates, usually ATP, to nucleoside diphosphates by a mechanism that involves formation of an autophosphorylated enzyme intermediate
CAGL0K05995g	*YLR260W*	*LCB5*	Minor sphingoid long-chain base kinase, paralog of Lcb4p responsible for few percent of the total activity, possibly involved in synthesis of long-chain base phosphates, which function as signaling molecules
CAGL0F08085g	*YGR243W*	*FMP43*	Putative protein of unknown function; expression regulated by osmotic and alkaline stresses; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0K10824g	*YLR149C*		
CAGL0H01177g	*YDR284C*	*DPP1*	Diacylglycerol pyrophosphate (DGPP) phosphatase, zinc-

			regulated vacuolar membrane-associated lipid phosphatase, dephosphorylates DGPP to phosphatidate (PA) and Pi, then PA to diacylglycerol; involved in lipid signaling and cell metabolism
CAGL0K12078g	*YDR043C*	*NRG1*	Transcriptional repressor that recruits the Cyc8p-Tup1p complex to promoters; mediates glucose repression and negatively regulates a variety of processes including filamentous growth and alkaline pH response
CAGL0M13541g	*YMR291W*	*TDA1*	Putative kinase of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus; YMR291W is not an essential gene
CAGL0L10186g	*YOR052C*		
CAGL0L05962g	*YGL178W*	*MPT5*	Member of the Puf family of RNA-binding proteins; binds to mRNAs encoding chromatin modifiers and spindle pole body components; involved in longevity, maintenance of cell wall integrity, and sensitivity to and recovery from pheromone arrest
CAGL0M05445g	*YBR203W*	*COS111*	Protein required for resistance to the antifungal drug ciclopirox olamine; not related to the subtelomerically-encoded COS family; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0H07535g	*YBR160W*	*CDC28*	Catalytic subunit of the main cell cycle cyclin-dependent kinase (CDK); alternately associates with G1 cyclins (CLNs) and G2/M cyclins (CLBs) which direct the CDK to specific substrates
CAGL0J02156g	*YIL001W*		
CAGL0J03080g	*YER067W*	*RGI1*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus; YER067W is not an essential gene; protein abundance is increased upon intracellular iron depletion
CAGL0J09592g	*YDL209C*	*CWC2*	Member of the NineTeen Complex (NTC) that contains Prp19p and stabilizes U6 snRNA in catalytic forms of the spliceosome containing U2, U5, and U6 snRNAs; binds directly to U6 snRNA; similar to S. pombe Cwf2
CAGL0D02684g	*YIL144W*	*TID3*	Component of the evolutionarily conserved kinetochore-associated Ndc80 complex (Ndc80p-Nuf2p-Spc24p-Spc25p); conserved coiled-coil protein involved in chromosome segregation, spindle checkpoint activity, kinetochore assembly and clustering
CAGL0K04301g	*YGR052W*	*FMP48*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; induced by treatment with 8-methoxypsoralen and UVA irradiation
CAGL0H09966g	*YBR047W*	*FMP23*	Putative protein of unknown function; proposed to be involved in iron or copper homeostatis; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0L09889g	*YKL025C*	*PAN3*	Essential subunit of the Pan2p-Pan3p poly(A)-ribonuclease complex, which acts to control poly(A) tail length and regulate the stoichiometry and activity of postreplication repair complexes
CAGL0K05115g	*YPR025C*	*CCL1*	Cyclin associated with protein kinase Kin28p, which is the

			TFIIH-associated carboxy-terminal domain (CTD) kinase involved in transcription initiation at RNA polymerase II promoters
CAGL0E01111g	*YDR159W*	*SAC3*	Nuclear pore-associated protein, forms a complex with Thp1p that is involved in transcription and in mRNA export from the nucleus
CAGL0A04433g	*YBL064C*	*PRX1*	Mitochondrial peroxiredoxin (1-Cys Prx) with thioredoxin peroxidase activity, has a role in reduction of hydroperoxides; reactivation requires Trr2p and glutathione; induced during respiratory growth and oxidative stress; phosphorylated
CAGL0A03806g	*YOR298W*	*MUM3*	Protein of unknown function involved in the organization of the outer spore wall layers; has similarity to the tafazzins superfamily of acyltransferases
CAGL0K11319g	*YDR249C*		
CAGL0M11330g	*YOR292C*		
CAGL0F00605g	*YCL040W*	*GLK1*	Glucokinase, catalyzes the phosphorylation of glucose at C6 in the first irreversible step of glucose metabolism; one of three glucose phosphorylating enzymes; expression regulated by non-fermentable carbon sources
CAGL0K01815g	*YDL017W*	*CDC7*	DDK (Dbf4-dependent kinase) catalytic subunit required for firing origins and replication fork progression in mitosis through phosphorylation of Mcm2-7p complexes and Cdc45p; kinase activity correlates with cyclical DBF4 expression
CAGL0M02101g	*YPL159C*	*PET20*	Mitochondrial protein, required for respiratory growth under some conditions and for stability of the mitochondrial genome
CAGL0F07975g	*YGR237C*		
CAGL0C01903g	*YBL084C*	*CDC27*	Subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C), which is a ubiquitin-protein ligase required for degradation of anaphase inhibitors, including mitotic cyclins, during the metaphase/anaphase transition
CAGL0L08448g	*YPR149W*	*NCE102*	Protein of unknown function; contains transmembrane domains; involved in secretion of proteins that lack classical secretory signal sequences; component of the detergent-insoluble glycolipid-enriched complexes (DIGs)
CAGL0L00715g	*YIL048W*	*NEO1*	Putative aminophospholipid translocase (flippase) involved in endocytosis and vacuolar biogenesis; localizes to endosomes and the Golgi aparatus
CAGL0C03850g	*YIL010W*	*DOT5*	Nuclear thiol peroxidase which functions as an alkyl-hydroperoxide reductase during post-diauxic growth
CAGL0M14025g	*YMR315W*		
CAGL0J04488g	*YLR417W*	*VPS36*	Component of the ESCRT-II complex; contains the GLUE (GRAM Like Ubiquitin binding in EAP45) domain which is involved in interactions with ESCRT-I and ubiquitin-dependent sorting of proteins into the endosome
CAGL0G09152g	*YPL196W*	*OXR1*	Protein of unknown function required for normal levels of resistance to oxidative damage, null mutants are sensitive to hydrogen peroxide; member of a conserved family of proteins found in eukaryotes but not in prokaryotes

CAGL0H07623g	*YGL250W*	*RMR1*	Protein required for meiotic recombination and gene conversion; null mutant displays reduced PIS1 expression and growth defects on non-fermentable carbon sources and minimal media; GFP-fusion protein localizes to both cytoplasm and nucleus
CAGL0K04323g	*YGR053C*		
CAGL0H07557g	*YGL254W*	*FZF1*	Transcription factor involved in sulfite metabolism, sole identified regulatory target is SSU1, overexpression suppresses sulfite-sensitivity of many unrelated mutants due to hyperactivation of SSU1, contains five zinc fingers
CAGL0K04279g	*YGR049W*	*SCM4*	Potential regulatory effector of CDC4 function, suppresses a temperature-sensitive allele of CDC4, tripartite protein structure in which a charged region separates two uncharged domains, not essential for mitosis or meiosis
CAGL0K10186g	*YDR076W*	*RAD55*	Protein that stimulates strand exchange by stabilizing the binding of Rad51p to single-stranded DNA; involved in the recombinational repair of double-strand breaks in DNA during vegetative growth and meiosis; forms heterodimer with Rad57p
CAGL0G05720g	*YNL183C*	*NPR1*	Protein kinase that stabilizes several plasma membrane amino acid transporters by antagonizing their ubiquitin-mediated degradation
CAGL0G04609g	*YOL100W*	*PKH2*	Serine/threonine protein kinase involved in sphingolipid-mediated signaling pathway that controls endocytosis; activates Ypk1p and Ykr2p, components of signaling cascade required for maintenance of cell wall integrity; redundant with Pkh1p
CAGL0J08459g	*YDR505C*	*PSP1*	Asn and gln rich protein of unknown function; high-copy suppressor of POL1 (DNA polymerase alpha) and partial suppressor of CDC2 (polymerase delta) and CDC6 (pre-RC loading factor) mutations; overexpression results in growth inhibition
CAGL0I02420g	*YHR171W*	*ATG7*	Autophagy-related protein and dual specificity member of the E1 family of ubiquitin-activating enzymes; mediates the conjugation of Atg12p with Atg5p and Atg8p with phosphatidylethanolamine, required steps in autophagosome formation
CAGL0J02464g	*YIL031W*	*ULP2*	Peptidase that deconjugates Smt3/SUMO-1 peptides from proteins, plays a role in chromosome cohesion at centromeric regions and recovery from checkpoint arrest induced by DNA damage or DNA replication defects; potential Cdc28p substrate
CAGL0A03300g	*YGL192W*	*IME4*	Probable mRNA N6-adenosine methyltransferase required for entry into meiosis; transcribed in diploid cells; haploids repress IME4 transcription via production of antisense IME4 transcripts; antisense transcription is repressed in diploids
CAGL0A02475g	*YDR350C*	*ATP22*	Mitochondrial inner membrane protein required for assembly of the F0 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis
CAGL0G02035g	*YOR061W*	*CKA2*	Alpha' catalytic subunit of casein kinase 2, a Ser/Thr protein kinase with roles in cell growth and proliferation; the holoenzyme also contains CKA1, CKB1 and CKB2,

			the many substrates include transcription factors and all RNA polymerases
CAGL0L07634g	*YML002W*		
CAGL0E03267g	*YGR161C*	*RTS3*	Putative component of the protein phosphatase type 2A complex
CAGL0M09108g	*YJR091C*	*JSN1*	Member of the Puf family of RNA-binding proteins, interacts with mRNAs encoding membrane-associated proteins; involved in localizing the Arp2/3 complex to mitochondria; overexpression causes increased sensitivity to benomyl
CAGL0E01727g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0I04554g	*YBR014C*	*GRX7*	Cis-golgi localized monothiol glutaredoxin; more similar in activity to dithiol than other monothiol glutaredoxins; involved in the oxidative stress response; does not bind metal ions; functional overlap with GRX6
CAGL0J11506g	*YNL192W*	*CHS1*	Chitin synthase I, requires activation from zymogenic form in order to catalyze the transfer of N-acetylglucosamine (GlcNAc) to chitin; required for repairing the chitin septum during cytokinesis; transcription activated by mating factor
CAGL0E00891g	*YDR169C*	*STB3*	Ribosomal RNA processing element (RRPE)-binding protein involved in the induction of non-ribosomal protein growth genes by glucose; binds Sin3p in a two-hybrid assay
CAGL0I07755g	*YOL089C*	*HAL9*	Putative transcription factor containing a zinc finger; overexpression increases salt tolerance through increased expression of the ENA1 (Na+/Li+ extrusion pump) gene while gene disruption decreases both salt tolerance and ENA1 expression
CAGL0L01947g	*YKL034W*	*TUL1*	Golgi-localized RING-finger ubiquitin ligase (E3), involved in ubiquitinating and sorting membrane proteins that contain polar transmembrane domains to multivesicular bodies for delivery to the vacuole for quality control purposes
CAGL0A02530g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress
CAGL0E01419g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0F09163g	*YER182W*	*FMP10*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0J07634g	*YNL242W*	*ATG2*	Peripheral membrane protein required for vesicle formation during autophagy, pexophagy, and the cytoplasm-to-vaucole targeting (Cvt) pathway; involved in Atg9p cycling between the phagophore assembly site and mitochondria
CAGL0M03113g	*YJL085W*	*EXO70*	Subunit of the exocyst complex (Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, and Exo84p), which directs secretory vesicles to active sites of exocytosis; acts with Sec3p in membrane targeting of the exocyst via

			PI(4,5)P2 binding
CAGL0F08745g	*YLR327C*	*TMA10*	Protein of unknown function that associates with ribosomes
CAGL0E04510g	*YOR022C*		
CAGL0J00253g	*YGR023W*	*MTL1*	Protein with both structural and functional similarity to Mid2p, which is a plasma membrane sensor required for cell integrity signaling during pheromone-induced morphogenesis; suppresses rgd1 null mutations
CAGL0B03201g	*YKR011C*		
CAGL0I09878g	*YOR317W*	*FAA1*	Long chain fatty acyl-CoA synthetase with a preference for C12:0-C16:0 fatty acids; involved in the activation of imported fatty acids; localized to both lipid particles and mitochondrial outer membrane; essential for stationary phase
CAGL0I01364g	*YJR050W*	*ISY1*	Member of NineTeen Complex (NTC) that contains Prp19p and stabilizes U6 snRNA in catalytic forms of spliceosome containing U2, U5, and U6 snRNAs, interacts with Prp16p to modulate splicing fidelity; isy1 syf2 cells have defective spindles
CAGL0F06039g	*YMR039C*	*SUB1*	Transcriptional coactivator, facilitates elongation through factors that modify RNAP II; role in peroxide resistance involving Rad2p; role in the hyperosmotic stress response through polymerase recruitment at RNAP II and RNAP III genes
CAGL0K01683g	*YDL022W*	*GPD1*	NAD-dependent glycerol-3-phosphate dehydrogenase, key enzyme of glycerol synthesis, essential for growth under osmotic stress; expression regulated by high-osmolarity glycerol response pathway; homolog of Gpd2p
CAGL0G03861g	*YGR046W*	*TAM41*	Mitochondrial protein involved in protein import into the mitochondrial matrix; maintains the functional integrity of the TIM23 protein translocator complex; viability of null mutant is strain-dependent; mRNA is targeted to the bud
CAGL0J03014g	*YIL056W*	*VHR1*	Transcriptional activator, required for the vitamin H-responsive element (VHRE) mediated induction of VHT1 (Vitamin H transporter) and BIO5 (biotin biosynthesis intermediate transporter) in response to low biotin concentrations
CAGL0F07007g	*YGL059W*	*PKP2*	Mitochondrial protein kinase that negatively regulates activity of the pyruvate dehydrogenase complex by phosphorylating the ser-133 residue of the Pda1p subunit; acts in concert with kinase Pkp1p and phosphatases Ptc5p and Ptc6p
CAGL0M02002g	*YPL165C*	*SET6*	SET domain protein of unknown function; deletion heterozygote is sensitive to compounds that target ergosterol biosynthesis, may be involved in compound availability
CAGL0I10516g	*YGR130C*		
CAGL0A03058g	*YDR379W*	*RGA2*	GTPase-activating protein for the polarity-establishment protein Cdc42p; implicated in control of septin organization, pheromone response, and haploid invasive growth; regulated by Pho85p and Cdc28p
CAGL0H03575g	*YNL012W*	*SPO1*	Meiosis-specific prospore protein; required for meiotic

			spindle pole body duplication and separation; required to produce bending force necessary for proper prospore membrane assembly during sporulation; has similarity to phospholipase B
CAGL0M08096g	*YOR208W*	*PTP2*	Phosphotyrosine-specific protein phosphatase involved in the inactivation of mitogen-activated protein kinase (MAPK) during osmolarity sensing; dephosporylates Hog1p MAPK and regulates its localization; localized to the nucleus
CAGL0K04213g	*YGR042W*		
CAGL0M06347g	*YBR183W*	*YPC1*	Alkaline ceramidase that also has reverse (CoA-independent) ceramide synthase activity, catalyzes both breakdown and synthesis of phytoceramide; overexpression confers fumonisin B1 resistance
CAGL0H05137g	*YPL061W*	*ALD6*	Cytosolic aldehyde dehydrogenase, activated by Mg2+ and utilizes NADP+ as the preferred coenzyme; required for conversion of acetaldehyde to acetate; constitutively expressed; locates to the mitochondrial outer surface upon oxidative stress
CAGL0E01793g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0L00693g	*YIL049W*	*DFG10*	Protein of unknown function, involved in filamentous growth
CAGL0B01529g	*YDR142C*	*PEX7*	Peroxisomal signal receptor for the N-terminal nonapeptide signal (PTS2) of peroxisomal matrix proteins; WD repeat protein; defects in human homolog cause lethal rhizomelic chondrodysplasia punctata (RCDP)
CAGL0I00924g	*YDR272W*	*GLO2*	Cytoplasmic glyoxalase II, catalyzes the hydrolysis of S-D-lactoylglutathione into glutathione and D-lactate
CAGL0M11660g	*YIL053W*	*RHR2*	Constitutively expressed isoform of DL-glycerol-3-phosphatase; involved in glycerol biosynthesis, induced in response to both anaerobic and, along with the Hor2p/Gpp2p isoform, osmotic stress
CAGL0K04565g	*YGR065C*	*VHT1*	High-affinity plasma membrane H+-biotin (vitamin H) symporter; mutation results in fatty acid auxotrophy; 12 transmembrane domain containing major facilitator subfamily member; mRNA levels negatively regulated by iron deprivation and biotin
CAGL0H03619g	*YNL011C*		
CAGL0I06028g	*YJL149W*	*DAS1*	Putative SCF ubiquitin ligase F-box protein; interacts physically with both Cdc53p and Skp1 and genetically with CDC34; similar to putative F-box protein YDR131C
CAGL0F03223g	*YDR436W*	*PPZ2*	Serine/threonine protein phosphatase Z, isoform of Ppz1p; involved in regulation of potassium transport, which affects osmotic stability, cell cycle progression, and halotolerance
CAGL0I06644g	*YDR077W*	*SED1*	Major stress-induced structural GPI-cell wall glycoprotein in stationary-phase cells, associates with translating ribosomes, possible role in mitochondrial genome maintenance; ORF contains two distinct variable minisatellites
CAGL0I07513g	*YOL100W*	*PKH2*	Serine/threonine protein kinase involved in sphingolipid-

			mediated signaling pathway that controls endocytosis; activates Ypk1p and Ykr2p, components of signaling cascade required for maintenance of cell wall integrity; redundant with Pkh1p
CAGL0E04708g	*YDR058C*	*TGL2*	Protein with lipolytic activity towards triacylglycerols and diacylglycerols when expressed in E. coli; role in yeast lipid degradation is unclear
CAGL0D06688g	*YOR374W*	*ALD4*	Mitochondrial aldehyde dehydrogenase, required for growth on ethanol and conversion of acetaldehyde to acetate; phosphorylated; activity is K+ dependent; utilizes NADP+ or NAD+ equally as coenzymes; expression is glucose repressed
CAGL0G04169g	*YDR306C*		
CAGL0M05621g	*YPL057C*	*SUR1*	Probable catalytic subunit of a mannosylinositol phosphorylceramide (MIPC) synthase, forms a complex with probable regulatory subunit Csg2p; function in sphingolipid biosynthesis is overlapping with that of Csh1p
CAGL0I03190g	*YEL024W*	*RIP1*	Ubiquinol-cytochrome-c reductase, a Rieske iron-sulfur protein of the mitochondrial cytochrome bc1 complex; transfers electrons from ubiquinol to cytochrome c1 during respiration
CAGL0K04609g	*YGR065C*	*VHT1*	High-affinity plasma membrane H+-biotin (vitamin H) symporter; mutation results in fatty acid auxotrophy; 12 transmembrane domain containing major facilitator subfamily member; mRNA levels negatively regulated by iron deprivation and biotin
CAGL0M12430g	*YIL053W*	*RHR2*	Constitutively expressed isoform of DL-glycerol-3-phosphatase; involved in glycerol biosynthesis, induced in response to both anaerobic and, along with the Hor2p/Gpp2p isoform, osmotic stress
CAGL0I01166g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress
CAGL0A02970g	*YDR374C*		
CAGL0M08536g	*YKL162C*		
CAGL0B02673g	*YDR254W*	*CHL4*	Outer kinetochore protein required for chromosome stability, interacts with kinetochore proteins Ctf19p, Ctf3p, and Iml3p; exhibits a two-hybrid interaction with Mif2p; association with CEN DNA requires Ctf19p
CAGL0K05863g	*YGL160W*	*AIM14*	Putative protein of with similarity to iron/copper reductases (FRE1-8), possibly involved in iron homeostasis; may interact with ribosomes; null mutant displays elevated frequency of mitochondrial genome loss
CAGL0F02651g	*YDR403W*	*DIT1*	Sporulation-specific enzyme required for spore wall maturation, involved in the production of a soluble LL-dityrosine-containing precursor of the spore wall; transcripts accumulate at the time of prospore enclosure
CAGL0K07480g	*YMR105C*	*PGM2*	Phosphoglucomutase, catalyzes the conversion from glucose-1-phosphate to glucose-6-phosphate, which is a key step in hexose metabolism; functions as the acceptor for a Glc-phosphotransferase

CAGL0D02112g	*YKL134C*	*OCT1*	Mitochondrial intermediate peptidase, cleaves N-terminal residues of a subset of proteins upon import, after their cleavage by mitochondrial processing peptidase (Mas1p-Mas2p); may contribute to mitochondrial iron homeostasis
CAGL0G01430g	*YNL045W*	*LAP2*	Leucyl aminopeptidase yscIV (leukotriene A4 hydrolase) with epoxide hydrolase activity, metalloenzyme containing one zinc atom; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus
CAGL0G02805g	*YIL106W*	*MOB1*	Component of the mitotic exit network; associates with and is required for the activation and Cdc15p-dependent phosphorylation of the Dbf2p kinase; required for cytokinesis and cell separation; component of the CCR4 transcriptional complex
CAGL0M03399g	*YEL012W*	*UBC8*	Ubiquitin-conjugating enzyme that negatively regulates gluconeogenesis by mediating the glucose-induced ubiquitination of fructose-1,6-bisphosphatase (FBPase); cytoplasmic enzyme that catalyzes the ubiquitination of histones in vitro
CAGL0H02717g	*YLR165C*	*PUS5*	Pseudouridine synthase, catalyzes only the formation of pseudouridine (Psi)-2819 in mitochondrial 21S rRNA; not essential for viability
CAGL0L11374g	*YML070W*	*DAK1*	Dihydroxyacetone kinase, required for detoxification of dihydroxyacetone (DHA); involved in stress adaptation
CAGL0A04697g	*YFR049W*	*YMR31*	Mitochondrial ribosomal protein of the small subunit, has similarity to human mitochondrial ribosomal protein MRP-S36
CAGL0G06028g	*YHR137W*	*ARO9*	Aromatic aminotransferase II, catalyzes the first step of tryptophan, phenylalanine, and tyrosine catabolism
CAGL0G08107g	*YDR096W*	*GIS1*	JmjC domain-containing histone demethylase; transcription factor involved in the expression of genes during nutrient limitation; also involved in the negative regulation of DPP1 and PHR1
CAGL0F07029g	*YGL125W*	*MET13*	Major isozyme of methylenetetrahydrofolate reductase, catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in the methionine biosynthesis pathway
CAGL0J06050g	*YNL160W*	*YGP1*	Cell wall-related secretory glycoprotein; induced by nutrient deprivation-associated growth arrest and upon entry into stationary phase; may be involved in adaptation prior to stationary phase entry; has similarity to Sps100p
CAGL0E02299g	*YOL013C*	*HRD1*	Ubiquitin-protein ligase required for endoplasmic reticulum-associated degradation (ERAD) of misfolded proteins; genetically linked to the unfolded protein response (UPR); regulated through association with Hrd3p; contains an H2 ring finger
CAGL0K11275g	*YDR247W*	*VHS1*	Cytoplasmic serine/threonine protein kinase; identified as a high-copy suppressor of the synthetic lethality of a sis2 sit4 double mutant, suggesting a role in G1/S phase progression; homolog of Sks1p
CAGL0M03377g	*YEL011W*	*GLC3*	Glycogen branching enzyme, involved in glycogen accumulation; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm in a punctate pattern

CAGL0M03157g	*YJL083W*	*TAX4*	EH domain-containing protein involved in regulating phosphatidylinositol 4,5-bisphosphate levels and autophagy; Irs4p and Tax4p bind and activate the PtdIns phosphatase Inp51p; Irs4p and Tax4p are involved in localizing Atg17p to the PAS
CAGL0F08217g	*YGR250C*		
CAGL0G02651g	*YIL097W*	*FYV10*	Protein of unknown function, required for survival upon exposure to K1 killer toxin; involved in proteasome-dependent catabolite inactivation of FBPase; contains CTLH domain; plays role in anti-apoptosis
CAGL0F05995g	*YMR037C*	*MSN2*	Transcriptional activator related to Msn4p; activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress response elements of responsive genes, inducing gene expression
CAGL0L04708g	*YGR111W*		
CAGL0K07766g	*YBR114W*	*RAD16*	Protein that recognizes and binds damaged DNA in an ATP-dependent manner (with Rad7p) during nucleotide excision repair; subunit of Nucleotide Excision Repair Factor 4 (NEF4) and the Elongin-Cullin-Socs (ECS) ligase complex
CAGL0L12914g	*YMR031C*		
CAGL0M08954g	*YOR019W*		
CAGL0H07645g	*YGL249W*	*ZIP2*	Meiosis-specific protein involved in normal synaptonemal complex formation and pairing between homologous chromosomes during meiosis
CAGL0L00583g	*YPL230W*	*USV1*	Putative transcription factor containing a C2H2 zinc finger; mutation affects transcriptional regulation of genes involved in growth on non-fermentable carbon sources, response to salt stress and cell wall biosynthesis
CAGL0H09108g	*YPL260W*		
CAGL0K02387g	*YGL171W*	*ROK1*	ATP-dependent RNA helicase of the DEAD box family; required for 18S rRNA synthesis
CAGL0C01815g	*YBL086C*		

Table 11.4: Genes down regulated uniquely by *S. cerevisiae* upon hyperosmotic stress treatment.

***C. glabrata* Systematic Name**	***S. cerevisiae* Systematic Name**	***S. cerevisiae* Standard Name**	***S. cerevisiae* Description**
CAGL0C03025g	*YCR052W*	*RSC6*	Component of the RSC chromatin remodeling complex; essential for mitotic growth; homolog of SWI/SNF subunit Swp73p
CAGL0G00902g	*YLR335W*	*NUP2*	Nucleoporin involved in nucleocytoplasmic transport, binds to either the nucleoplasmic or cytoplasmic faces of the nuclear pore complex depending on Ran-GTP levels; also has a role in chromatin organization
CAGL0F08547g	*YAL003W*	*EFB1*	Translation elongation factor 1 beta; stimulates nucleotide exchange to regenerate EF-1 alpha-GTP for the next elongation cycle; part of the EF-1 complex, which facilitates binding of aminoacyl-tRNA to the ribosomal A site
CAGL0K02409g	*YPR020W*	*ATP20*	Subunit g of the mitochondrial F1F0 ATP synthase; reversibly phosphorylated on two residues; unphosphorylated form is required for dimerization of the ATP synthase complex
CAGL0J11792g	*YML043C*	*RRN11*	Component of the core factor (CF) rDNA transcription factor complex; CF is required for transcription of 35S rRNA genes by RNA polymerase I and is composed of Rrn6p, Rrn7p, and Rrn11p
CAGL0F01573g	*YLR067C*	*PET309*	Specific translational activator for the COX1 mRNA, also influences stability of intron-containing COX1 primary transcripts; localizes to the mitochondrial inner membrane; contains seven pentatricopeptide repeats (PPRs)
CAGL0L03740g	*YOR095C*	*RKI1*	Ribose-5-phosphate ketol-isomerase, catalyzes the interconversion of ribose 5-phosphate and ribulose 5-phosphate in the pentose phosphate pathway; participates in pyridoxine biosynthesis
CAGL0D04708g	*YPR124W*	*CTR1*	High-affinity copper transporter of the plasma membrane, mediates nearly all copper uptake under low copper conditions; transcriptionally induced at low copper levels and degraded at high copper levels
CAGL0G06358g	*YOR327C*	*SNC2*	Vesicle membrane receptor protein (v-SNARE) involved in the fusion between Golgi-derived secretory vesicles with the plasma membrane; member of the synaptobrevin/VAMP family of R-type v-SNARE proteins
CAGL0H05511g	*YPL081W*	*RPS9A*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins
CAGL0I09856g	*YOR315W*	*SFG1*	Nuclear protein, putative transcription factor required for growth of superficial pseudohyphae (which do not invade the agar substrate) but not for invasive pseudohyphal growth; may act together with Phd1p; potential Cdc28p substrate
CAGL0J00869g	*YKL144C*	*RPC25*	RNA polymerase III subunit C25, required for transcription initiation; forms a heterodimer with Rpc17p; paralog of Rpb7p
CAGL0A01584g	*YGL032C*	*AGA2*	Adhesion subunit of a-agglutinin of a-cells, C-terminal

			sequence acts as a ligand for alpha-agglutinin (Sag1p) during agglutination, modified with O-linked oligomannosyl chains, linked to anchorage subunit Aga1p via two disulfide bonds
CAGL0C03355g	*YNR054C*	*ESF2*	Essential nucleolar protein involved in pre-18S rRNA processing; binds to RNA and stimulates ATPase activity of Dbp8; involved in assembly of the small subunit (SSU) processome
CAGL0M09757g	*YLR287C*		
CAGL0E05258g	*YOR327C*	*SNC2*	Vesicle membrane receptor protein (v-SNARE) involved in the fusion between Golgi-derived secretory vesicles with the plasma membrane; member of the synaptobrevin/VAMP family of R-type v-SNARE proteins
CAGL0F05709g	*YDR184C*	*ATC1*	Nuclear protein, possibly involved in regulation of cation stress responses and/or in the establishment of bipolar budding pattern
CAGL0L02035g	*YKL029C*	*MAE1*	Mitochondrial malic enzyme, catalyzes the oxidative decarboxylation of malate to pyruvate, which is a key intermediate in sugar metabolism and a precursor for synthesis of several amino acids
CAGL0K05357g	*YPR035W*	*GLN1*	Glutamine synthetase (GS), synthesizes glutamine from glutamate and ammonia; with Glt1p, forms the secondary pathway for glutamate biosynthesis from ammonia; expression regulated by nitrogen source and by amino acid limitation
CAGL0F01551g	*YLR068W*	*FYV7*	Essential protein required for maturation of 18S rRNA; required for survival upon exposure to K1 killer toxin
CAGL0A03168g	*YDR382W*	*RPP2B*	Ribosomal protein P2 beta, a component of the ribosomal stalk, which is involved in the interaction between translational elongation factors and the ribosome; regulates the accumulation of P1 (Rpp1Ap and Rpp1Bp) in the cytoplasm
CAGL0L00517g	*YMR185W*		
CAGL0B02475g	*YML123C*	*PHO84*	High-affinity inorganic phosphate (Pi) transporter and low-affinity manganese transporter; regulated by Pho4p and Spt7p; mutation confers resistance to arsenate; exit from the ER during maturation requires Pho86p
CAGL0I07491g	*YDR492W*	*IZH1*	Membrane protein involved in zinc ion homeostasis, member of the four-protein IZH family; transcription is regulated directly by Zap1p, expression induced by zinc deficiency and fatty acids; deletion increases sensitivity to elevated zinc
CAGL0J02354g	*YIL018W*	*RPL2B*	Protein component of the large (60S) ribosomal subunit, identical to Rpl2Ap and has similarity to E. coli L2 and rat L8 ribosomal proteins; expression is upregulated at low temperatures
CAGL0E03938g	*YLL045C*	*RPL8B*	Ribosomal protein L4 of the large (60S) ribosomal subunit, nearly identical to Rpl8Ap and has similarity to rat L7a ribosomal protein; mutation results in decreased amounts of free 60S subunits
CAGL0G08668g	*YNL066W*	*SUN4*	Cell wall protein related to glucanases, possibly involved in cell wall septation; member of the SUN family

CAGL0M06501g	*YBR189W*	*RPS9B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Ap and has similarity to E. coli S4 and rat S9 ribosomal proteins
CAGL0M05137g	*YGL097W*	*SRM1*	Nucleotide exchange factor for Gsp1p, localizes to the nucleus, required for nucleocytoplasmic trafficking of macromolecules; suppressor of the pheromone response pathway; potentially phosphorylated by Cdc28p
CAGL0M08030g	*YJR032W*	*CPR7*	Peptidyl-prolyl cis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds to Hsp82p and contributes to chaperone activity
CAGL0M13849g	*YMR307W*	*GAS1*	Beta-1,3-glucanosyltransferase, required for cell wall assembly and also has a role in transcriptional silencing; localizes to the cell surface via a glycosylphosphatidylinositol (GPI) anchor; also found at the nuclear periphery
CAGL0J02310g	*YIL016W*	*SNL1*	Protein of unknown function proposed to be involved in nuclear pore complex biogenesis and maintenance as well as protein folding; has similarity to the mammalian BAG-1 protein
CAGL0K12210g	*YBR104W*	*YMC2*	Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis; member of the mitochondrial carrier (MCF) family; has similarity with Ymc1p
CAGL0L03564g	*YJL097W*	*PHS1*	Essential 3-hydroxyacyl-CoA dehydratase of the ER membrane, involved in elongation of very long-chain fatty acids; evolutionarily conserved, similar to mammalian PTPLA and PTPLB; involved in sphingolipid biosynthesis and protein trafficking
CAGL0K00429g	*YJR072C*	*NPA3*	Essential, conserved, cytoplasmic ATPase; phosphorylated by the Pcl1p-Pho85p kinase complex
CAGL0L08734g	*YPL013C*	*MRPS16*	Mitochondrial ribosomal protein of the small subunit
CAGL0J11418g	*YNL188W*	*KAR1*	Essential protein involved in karyogamy during mating and in spindle pole body duplication during mitosis, localizes to the half-bridge of the spindle pole body, interacts with Spc72p during karyogamy, also interacts with Cdc31p
CAGL0G09515g	*YLR300W*	*EXG1*	Major exo-1,3-beta-glucanase of the cell wall, involved in cell wall beta-glucan assembly; exists as three differentially glycosylated isoenzymes
CAGL0L09647g	*YIR012W*	*SQT1*	Essential protein involved in a late step of 60S ribosomal subunit assembly or modification; contains multiple WD repeats; interacts with Qsr1p in a two-hybrid assay
CAGL0M02497g	*YOR234C*	*RPL33B*	Ribosomal protein L37 of the large (60S) ribosomal subunit, nearly identical to Rpl33Ap and has similarity to rat L35a; rpl33b null mutant exhibits normal growth while rpl33a rpl33b double null mutant is inviable
CAGL0A03366g	*YMR120C*	*ADE17*	Enzyme of 'de novo' purine biosynthesis containing both 5-aminoimidazole-4-carboxamide ribonucleotide transformylase and inosine monophosphate cyclohydrolase activities, isozyme of Ade16p; ade16 ade17 mutants require adenine and histidine

CAGL0K12276g	*YBR106W*	*PHO88*	Probable membrane protein, involved in phosphate transport; pho88 pho86 double null mutant exhibits enhanced synthesis of repressible acid phosphatase at high inorganic phosphate concentrations
CAGL0H04983g	*YDL055C*	*PSA1*	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase), synthesizes GDP-mannose from GTP and mannose-1-phosphate in cell wall biosynthesis; required for normal cell wall structure
CAGL0G02079g	*YOR063W*	*RPL3*	Protein component of the large (60S) ribosomal subunit, has similarity to E. coli L3 and rat L3 ribosomal proteins; involved in the replication and maintenance of killer double stranded RNA virus
CAGL0I00792g	*YDL083C*	*RPS16B*	Protein component of the small (40S) ribosomal subunit; identical to Rps16Ap and has similarity to E. coli S9 and rat S16 ribosomal proteins
CAGL0I09636g	*YMR239C*	*RNT1*	RNAase III; involved in rDNA transcription and rRNA processing; also cleaves a stem-loop structure at the 3' end of U2 snRNA to ensure formation of the correct U2 3' end
CAGL0E04070g	*YHL039W*		
CAGL0J00715g	*YOR101W*	*RAS1*	GTPase involved in G-protein signaling in the adenylate cyclase activating pathway, plays a role in cell proliferation; localized to the plasma membrane; homolog of mammalian RAS proto-oncogenes
CAGL0J03146g	*YER070W*	*RNR1*	One of two large regulatory subunits of ribonucleotide-diphosphate reductase; the RNR complex catalyzes rate-limiting step in dNTP synthesis, regulated by DNA replication and DNA damage checkpoint pathways via localization of small subunits
CAGL0G05027g	*YDR064W*	*RPS13*	Protein component of the small (40S) ribosomal subunit; has similarity to E. coli S15 and rat S13 ribosomal proteins
CAGL0J04334g	*YBL018C*	*POP8*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends
CAGL0J04026g	*YOR227W*	*HER1*	Protein of unknown function required for proliferation or remodeling of the ER that is caused by overexpression of Hmg2p; may interact with ribosomes, based on co-purification experiments
CAGL0M02959g	*YMR277W*	*FCP1*	Carboxy-terminal domain (CTD) phosphatase, essential for dephosphorylation of the repeated C-terminal domain of the RNA polymerase II large subunit (Rpo21p)
CAGL0M02849g	*YLR048W*	*RPS0B*	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps0Ap; required for maturation of 18S rRNA along with Rps0Ap; deletion of either RPS0 gene reduces growth rate, deletion of both genes is lethal
CAGL0F02937g	*YEL054C*	*RPL12A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl12Bp; rpl12a rpl12b double mutant exhibits slow growth and slow translation; has similarity to E. coli L11 and rat L12 ribosomal proteins
CAGL0E06292g	*YOR101W*	*RAS1*	GTPase involved in G-protein signaling in the adenylate cyclase activating pathway, plays a role in cell proliferation; localized to the plasma membrane; homolog of mammalian RAS proto-oncogenes

CAGL0I06138g	*YJL157C*	*FAR1*	Cyclin-dependent kinase inhibitor that mediates cell cycle arrest in response to pheromone; also forms a complex with Cdc24p, Ste4p, and Ste18p that may specify the direction of polarized growth during mating; potential Cdc28p substrate
CAGL0J05346g	*YGL097W*	*SRM1*	Nucleotide exchange factor for Gsp1p, localizes to the nucleus, required for nucleocytoplasmic trafficking of macromolecules; suppressor of the pheromone response pathway; potentially phosphorylated by Cdc28p
CAGL0G08129g	*YDR097C*	*MSH6*	Protein required for mismatch repair in mitosis and meiosis, forms a complex with Msh2p to repair both single-base & insertion-deletion mispairs; potentially phosphorylated by Cdc28p
CAGL0D00462g	*YKL185W*	*ASH1*	Zinc-finger inhibitor of HO transcription; mRNA is localized and translated in the distal tip of anaphase cells, resulting in accumulation of Ash1p in daughter cell nuclei and inhibition of HO expression; potential Cdc28p substrate
CAGL0L05412g	*YJL115W*	*ASF1*	Nucleosome assembly factor, involved in chromatin assembly and disassembly, anti-silencing protein that causes derepression of silent loci when overexpressed; plays a role in regulating Ty1 transposition
CAGL0A04037g	*YLR196W*	*PWP1*	Protein with WD-40 repeats involved in rRNA processing; associates with trans-acting ribosome biogenesis factors; similar to beta-transducin superfamily
CAGL0I00484g	*YLR300W*	*EXG1*	Major exo-1,3-beta-glucanase of the cell wall, involved in cell wall beta-glucan assembly; exists as three differentially glycosylated isoenzymes
CAGL0H10098g	*YBR055C*	*PRP6*	Splicing factor, component of the U4/U6-U5 snRNP complex
CAGL0B01203g	*YLR185W*	*RPL37A*	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl37Bp and to rat L37 ribosomal protein
CAGL0I06721g	*YER146W*	*LSM5*	Lsm (Like Sm) protein; part of heteroheptameric complexes (Lsm2p-7p and either Lsm1p or 8p): cytoplasmic Lsm1p complex involved in mRNA decay; nuclear Lsm8p complex part of U6 snRNP and possibly involved in processing tRNA, snoRNA, and rRNA
CAGL0F06347g	*YMR054W*	*STV1*	Subunit a of the vacuolar-ATPase V0 domain, one of two isoforms (Stv1p and Vph1p); Stv1p is located in V-ATPase complexes of the Golgi and endosomes while Vph1p is located in V-ATPase complexes of the vacuole
CAGL0L11462g	*YLR448W*	*RPL6B*	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl6Ap and to rat L6 ribosomal protein; binds to 5.8S rRNA
CAGL0A01760g	*YDR341C*		
CAGL0F01749g	*YLR058C*	*SHM2*	Cytosolic serine hydroxymethyltransferase, converts serine to glycine plus 5,10 methylenetetrahydrofolate; major isoform involved in generating precursors for purine, pyrimidine, amino acid, and lipid biosynthesis
CAGL0G01210g	*YLR351C*	*NIT3*	Nit protein, one of two proteins in S. cerevisiae with similarity to the Nit domain of NitFhit from fly and worm and to the mouse and human Nit protein which interacts with the Fhit tumor suppressor; nitrilase superfamily

			member
CAGL0D01034g	*YDL055C*	*PSA1*	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase), synthesizes GDP-mannose from GTP and mannose-1-phosphate in cell wall biosynthesis; required for normal cell wall structure
CAGL0L06886g	*YMR142C*	*RPL13B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Ap; not essential for viability; has similarity to rat L13 ribosomal protein
CAGL0F01793g	*YLR056W*	*ERG3*	C-5 sterol desaturase, catalyzes the introduction of a C-5(6) double bond into episterol, a precursor in ergosterol biosynthesis; mutants are viable, but cannot grow on non-fermentable carbon sources
CAGL0K01705g	*YDL021W*	*GPM2*	Homolog of Gpm1p phosphoglycerate mutase, which converts 3-phosphoglycerate to 2-phosphoglycerate in glycolysis; may be non-functional derivative of a gene duplication event
CAGL0J07238g	*YOR369C*	*RPS12*	Protein component of the small (40S) ribosomal subunit; has similarity to rat ribosomal protein S12
CAGL0L02475g	*YEL009C*	*GCN4*	Basic leucine zipper (bZIP) transcriptional activator of amino acid biosynthetic genes in response to amino acid starvation; expression is tightly regulated at both the transcriptional and translational levels
CAGL0M01694g	*YBR084W*	*MIS1*	Mitochondrial C1-tetrahydrofolate synthase, involved in interconversion between different oxidation states of tetrahydrofolate (THF); provides activities of formyl-THF synthetase, methenyl-THF cyclohydrolase, and methylene-THF dehydrogenase
CAGL0F07073g	*YGL123W*	*RPS2*	Protein component of the small (40S) subunit, essential for control of translational accuracy; phosphorylation by C-terminal domain kinase I (CTDK-I) enhances translational accuracy; similar to E. coli S5 and rat S2 ribosomal proteins
CAGL0G05049g	*YDR063W*	*AIM7*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus; null mutant is viable and displays elevated frequency of mitochondrial genome loss
CAGL0J04180g	*YFL013C*	*IES1*	Subunit of the INO80 chromatin remodeling complex
CAGL0H07491g	*YBR158W*	*AMN1*	Protein required for daughter cell separation, multiple mitotic checkpoints, and chromosome stability; contains 12 degenerate leucine-rich repeat motifs; expression is induced by the Mitotic Exit Network (MEN)
CAGL0K05555g	*YOR078W*	*BUD21*	Component of small ribosomal subunit (SSU) processosome that contains U3 snoRNA; originally isolated as bud-site selection mutant that displays a random budding pattern
CAGL0K02211g	*YER127W*	*LCP5*	Essential protein involved in maturation of 18S rRNA; depletion leads to inhibited pre-rRNA processing and reduced polysome levels; localizes primarily to the nucleolus
CAGL0A02365g	*YDR346C*	*SVF1*	Protein with a potential role in cell survival pathways, required for the diauxic growth shift; expression in mammalian cells increases survival under conditions inducing apoptosis

CAGL0F00715g	*YAL025C*	*MAK16*	Essential nuclear protein, constituent of 66S pre-ribosomal particles; required for maturation of 25S and 5.8S rRNAs; required for maintenance of M1 satellite double-stranded RNA of the L-A virus
CAGL0K07007g	*YBR238C*		
CAGL0H00462g	*YJR123W*	*RPS5*	Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins
CAGL0A01562g	*YGR148C*	*RPL24B*	Ribosomal protein L30 of the large (60S) ribosomal subunit, nearly identical to Rpl24Ap and has similarity to rat L24 ribosomal protein; not essential for translation but may be required for normal translation rate
CAGL0B00748g	*YCL039W*	*GID7*	Protein of unknown function, involved in proteasome-dependent catabolite inactivation of fructose-1,6-bisphosphatase; contains six WD40 repeats; computational analysis suggests that Gid7p and Moh1p have similar functions
CAGL0E04620g	*YBR078W*	*ECM33*	GPI-anchored protein of unknown function, has a possible role in apical bud growth; GPI-anchoring on the plasma membrane crucial to function; phosphorylated in mitochondria; similar to Sps2p and Pst1p
CAGL0H00440g	*YJR124C*		
CAGL0M06303g	*YPL090C*	*RPS6A*	Protein component of the small (40S) ribosomal subunit; identical to Rps6Bp and has similarity to rat S6 ribosomal protein
CAGL0M00748g	*YLR443W*	*ECM7*	Non-essential putative integral membrane protein; mutant has cell wall defects; transcription is induced under conditions of zinc deficiency
CAGL0I00418g	*YGL055W*	*OLE1*	Delta(9) fatty acid desaturase, required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria
CAGL0F07469g	*YBR238C*		
CAGL0K03729g	*YMR125W*	*STO1*	Large subunit of the nuclear mRNA cap-binding protein complex, interacts with Npl3p to carry nuclear poly(A)+ mRNA to cytoplasm; also involved in nuclear mRNA degradation and telomere maintenance; orthologous to mammalian CBP80
CAGL0H05643g	*YPL090C*	*RPS6A*	Protein component of the small (40S) ribosomal subunit; identical to Rps6Bp and has similarity to rat S6 ribosomal protein
CAGL0I05126g	*YER086W*	*ILV1*	Threonine deaminase, catalyzes the first step in isoleucine biosynthesis; expression is under general amino acid control; ILV1 locus exhibits highly positioned nucleosomes whose organization is independent of known ILV1 regulation
CAGL0M01474g	*YDR397C*	*NCB2*	Subunit of a heterodimeric NC2 transcription regulator complex with Bur6p; complex binds to TBP and can repress transcription by preventing preinitiation complex assembly or stimulate activated transcription; homologous to human NC2beta
CAGL0K07293g	*YMR246W*	*FAA4*	Long chain fatty acyl-CoA synthetase, regulates protein

			modification during growth in the presence of ethanol, functions to incorporate palmitic acid into phospholipids and neutral lipids
CAGL0J03652g	*YPL160W*	*CDC60*	Cytosolic leucyl tRNA synthetase, ligates leucine to the appropriate tRNA
CAGL0D02530g	*YNL327W*	*EGT2*	Glycosylphosphatidylinositol (GPI)-anchored cell wall endoglucanase required for proper cell separation after cytokinesis, expression is activated by Swi5p and tightly regulated in a cell cycle-dependent manner
CAGL0I04994g	*YER091C*	*MET6*	Cobalamin-independent methionine synthase, involved in methionine biosynthesis and regeneration; requires a minimum of two glutamates on the methyltetrahydrofolate substrate, similar to bacterial metE homologs
CAGL0I06336g	*YJL208C*	*NUC1*	Major mitochondrial nuclease, has RNAse and DNA endo- and exonucleolytic activities; has roles in mitochondrial recombination, apoptosis and maintenance of polyploidy
CAGL0H08866g	*YMR172W*	*HOT1*	Transcription factor required for the transient induction of glycerol biosynthetic genes GPD1 and GPP2 in response to high osmolarity; targets Hog1p to osmostress responsive promoters; has similarity to Msn1p and Gcr1p
CAGL0G07227g	*YML026C*	*RPS18B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps18Ap and has similarity to E. coli S13 and rat S18 ribosomal proteins
CAGL0I00814g	*YDL081C*	*RPP1A*	Ribosomal stalk protein P1 alpha, involved in the interaction between translational elongation factors and the ribosome; accumulation of P1 in the cytoplasm is regulated by phosphorylation and interaction with the P2 stalk component
CAGL0A03388g	*YMR121C*	*RPL15B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl15Ap and has similarity to rat L15 ribosomal protein; binds to 5.8 S rRNA
CAGL0J11220g	*YNL178W*	*RPS3*	Protein component of the small (40S) ribosomal subunit, has apurinic/apyrimidinic (AP) endonuclease activity; essential for viability; has similarity to E. coli S3 and rat S3 ribosomal proteins
CAGL0F01045g	*YOL040C*	*RPS15*	Protein component of the small (40S) ribosomal subunit; has similarity to E. coli S19 and rat S15 ribosomal proteins
CAGL0J10076g	*YNL058C*		
CAGL0C02189g	*YER043C*	*SAH1*	S-adenosyl-L-homocysteine hydrolase, catabolizes S-adenosyl-L-homocysteine which is formed after donation of the activated methyl group of S-adenosyl-L-methionine (AdoMet) to an acceptor
CAGL0C01919g	*YNL145W*	*MFA2*	Mating pheromone a-factor, made by a cells; interacts with alpha cells to induce cell cycle arrest and other responses leading to mating; biogenesis involves C-terminal modification, N-terminal proteolysis, and export; also encoded by MFA1
CAGL0K07414g	*YMR242C*	*RPL20A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl20Bp and has similarity to rat L18a ribosomal protein
CAGL0F02431g	*YJL200C*	*ACO2*	Putative mitochondrial aconitase isozyme; similarity to Aco1p, an aconitase required for the TCA cycle;

			expression induced during growth on glucose, by amino acid starvation via Gcn4p, and repressed on ethanol
CAGL0I10648g	*YGR124W*	*ASN2*	Asparagine synthetase, isozyme of Asn1p; catalyzes the synthesis of L-asparagine from L-aspartate in the asparagine biosynthetic pathway
CAGL0B04257g	*YBR031W*	*RPL4A*	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl4Bp and has similarity to E. coli L4 and rat L4 ribosomal proteins

Table 11.5: Genes up regulated uniquely by *C. glabrata* upon hyperosmotic stress treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0J11550g	*YNL195C*		
CAGL0A02145g	*YHR016C*	*YSC84*	Actin-binding protein involved in bundling of actin filaments and endocytosis of actin cortical patches; activity stimulated by Las17p; contains SH3 domain similar to Rvs167p
CAGL0H03289g	*YGL082W*		
CAGL0C05137g	*YOL059W*	*GPD2*	NAD-dependent glycerol 3-phosphate dehydrogenase, homolog of Gpd1p, expression is controlled by an oxygen-independent signaling pathway required to regulate metabolism under anoxic conditions; located in cytosol and mitochondria
CAGL0M06259g	*YBR179C*	*FZO1*	Mitofusin, mitochondrial integral membrane protein involved in mitochondrial fusion and mitochondrial genome maintenance; contains N-terminal GTPase domain; targeted for destruction by cytosolic components of the ubiquitin-proteasome system
CAGL0H05621g	*YPL089C*	*RLM1*	MADS-box transcription factor, component of the protein kinase C-mediated MAP kinase pathway involved in the maintenance of cell integrity; phosphorylated and activated by the MAP-kinase Slt2p
CAGL0L10582g	*YMR196W*		
CAGL0B00946g	*YCL028W*	*RNQ1*	[PIN(+)] prion, an infectious protein conformation that is generally an ordered protein aggregate
CAGL0I06182g	*YKL164C*	*PIR1*	O-glycosylated protein required for cell wall stability; attached to the cell wall via beta-1,3-glucan; mediates mitochondrial translocation of Apn1p; expression regulated by the cell integrity pathway and by Swi5p during the cell cycle
CAGL0L04664g	*YOR176W*	*HEM15*	Ferrochelatase, a mitochondrial inner membrane protein, catalyzes the insertion of ferrous iron into protoporphyrin IX, the eighth and final step in the heme biosynthetic pathway
CAGL0M09339g	*YBL107C*		
CAGL0H00528g	*YJR119C*	*JHD2*	JmjC domain family histone demethylase specific for H3-K4 (histone H3 Lys4); removes methyl groups specifically added by Set1p methyltransferase; protein levels regulated by Not4p (E3 ubiquitin ligase)

			polyubiquitin-mediated degradation
CAGL0G08712g	*YIL125W*	*KGD1*	Component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes a key step in the tricarboxylic acid (TCA) cycle, the oxidative decarboxylation of alpha-ketoglutarate to form succinyl-CoA
CAGL0L11902g	*YER170W*	*ADK2*	Mitochondrial adenylate kinase, catalyzes the reversible synthesis of GTP and AMP from GDP and ADP; may serve as a back-up for synthesizing GTP or ADP depending on metabolic conditions; 3' sequence of ADK2 varies with strain background
CAGL0I00506g	*YLR299W*	*ECM38*	Gamma-glutamyltranspeptidase, major glutathione-degrading enzyme; involved in detoxification of electrophilic xenobiotics; expression induced mainly by nitrogen starvation
CAGL0L11440g	*YML072C*	*TCB3*	Lipid-binding protein, localized to the bud via specific mRNA transport; non-tagged protein detected in a phosphorylated state in mitochondria; GFP-fusion protein localizes to the cell periphery; C-termini of Tcb1p, Tcb2p and Tcb3p interact
CAGL0G06314g	*YAL028W*	*FRT2*	Tail-anchored endoplasmic reticulum membrane protein, interacts with homolog Frt1p but is not a substrate of calcineurin (unlike Frt1p), promotes growth in conditions of high Na+, alkaline pH, or cell wall stress; potential Cdc28p substrate
CAGL0J03256g	*YER075C*	*PTP3*	Phosphotyrosine-specific protein phosphatase involved in the inactivation of mitogen-activated protein kinase (MAPK) during osmolarity sensing; dephosporylates Hog1p MAPK and regulates its localization; localized to the cytoplasm
CAGL0M13783g	*YMR304W*	*UBP15*	Ubiquitin-specific protease that may play a role in ubiquitin precursor processing
CAGL0L12012g	*YER175C*	*TMT1*	Trans-aconitate methyltransferase, cytosolic enzyme that catalyzes the methyl esterification of 3-isopropylmalate, an intermediate of the leucine biosynthetic pathway, and trans-aconitate, which inhibits the citric acid cycle
CAGL0F07117g	*YGL121C*	*GPG1*	Proposed gamma subunit of the heterotrimeric G protein that interacts with the receptor Gpr1p; involved in regulation of pseudohyphal growth; requires Gpb1p or Gpb2p to interact with Gpa2p; overproduction causes prion curing
CAGL0M12859g	*YIL075C*	*RPN2*	Subunit of the 26S proteasome, substrate of the N-acetyltransferase Nat1p
CAGL0G03619g	*YER119C*	*AVT6*	Vacuolar amino acid transporter, exports aspartate and glutamate from the vacuole; member of a family of seven S. cerevisiae genes (AVT1-7) related to vesicular GABA-glycine transporters
CAGL0D06512g	*YLR310C*	*CDC25*	Membrane bound guanine nucleotide exchange factor (GEF or GDP-release factor); indirectly regulates adenylate cyclase through activation of Ras1p and Ras2p by stimulating the exchange of GDP for GTP; required for progression through G1
CAGL0C00968g	*YOL155C*	*HPF1*	Haze-protective mannoprotein that reduces the particle

			size of aggregated proteins in white wines
CAGL0F04895g	*YPR160W*	*GPH1*	Non-essential glycogen phosphorylase required for the mobilization of glycogen, activity is regulated by cyclic AMP-mediated phosphorylation, expression is regulated by stress-response elements and by the HOG MAP kinase pathway
CAGL0I03652g	*YDL149W*	*ATG9*	Transmembrane protein involved in forming Cvt and autophagic vesicles; cycles between the phagophore assembly site (PAS) and other cytosolic punctate structures, not found in autophagosomes; may be involved in membrane delivery to the PAS
CAGL0B03465g	*YLR251W*	*SYM1*	Protein required for ethanol metabolism; induced by heat shock and localized to the inner mitochondrial membrane; homologous to mammalian peroxisomal membrane protein Mpv17
CAGL0J07194g	*YAL055W*	*PEX22*	Putative peroxisomal membrane protein required for import of peroxisomal proteins, functionally complements a Pichia pastoris pex22 mutation
CAGL0I08151g	*YALI0E33297G*		
CAGL0G07062g	*YML020W*		
CAGL0J10494g	*YML022W*	*APT1*	Adenine phosphoribosyltransferase, catalyzes the formation of AMP from adenine and 5-phosphoribosylpyrophosphate; involved in the salvage pathway of purine nucleotide biosynthesis
CAGL0L01705g	*YKL047W*	*ANR2*	Putative protein of unknown function, predicted to be palmitoylated; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
CAGL0K03459g	*YMR107W*	*SPG4*	Protein required for survival at high temperature during stationary phase; not required for growth on nonfermentable carbon sources
CAGL0M12551g	*YIL057C*	*RGI2*	Putative protein of unknown function; expression induced under carbon limitation and repressed under high glucose
CAGL0K04169g	*YGR040W*	*KSS1*	Mitogen-activated protein kinase (MAPK) involved in signal transduction pathways that control filamentous growth and pheromone response; the KSS1 gene is nonfunctional in S288C strains and functional in W303 strains
CAGL0M02981g	*YMR278W*	*PGM3*	Phosphoglucomutase, catalyzes interconversion of glucose-1-phosphate and glucose-6-phospate; transcription induced in response to stress; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus; non-essential
CAGL0L10208g	*YKR072C*	*SIS2*	Negative regulatory subunit of the protein phosphatase 1 Ppz1p; involved in ion homeostasis and cell cycle progression
CAGL0K06677g	*YBR213W*	*MET8*	Bifunctional dehydrogenase and ferrochelatase, involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis
CAGL0M11242g	*YMR226C*	*TMA29*	NADP(+)-dependent dehydrogenase; acts on serine, L-

			allo-threonine, and other 3-hydroxy acids; green fluorescent protein fusion protein localizes to the cytoplasm and nucleus; may interact with ribosomes, based on co-purification experiments
CAGL0I04620g	*YBR042C*	*CST26*	Protein required for incorporation of stearic acid into phosphatidylinositol; affects chromosome stability when overexpressed
CAGL0C04785g	*YJR115W*		
CAGL0L06270g	*YER177W*	*BMH1*	14-3-3 protein, major isoform; controls proteome at post-transcriptional level, binds proteins and DNA, involved in regulation of many processes including exocytosis, vesicle transport, Ras/MAPK signaling, and rapamycin-sensitive signaling
CAGL0J07612g	*YNL241C*	*ZWF1*	Glucose-6-phosphate dehydrogenase (G6PD), catalyzes the first step of the pentose phosphate pathway; involved in adapting to oxidatve stress; homolog of the human G6PD which is deficient in patients with hemolytic anemia
CAGL0H02563g	*NA*		
CAGL0I02046g	*YPR127W*		
CAGL0B03619g	*YEL060C*	*PRB1*	Vacuolar proteinase B (yscB), a serine protease of the subtilisin family; involved in protein degradation in the vacuole and required for full protein degradation during sporulation
CAGL0K04763g	*NORBH*		
CAGL0B03509g	*YLR248W*	*RCK2*	Protein kinase involved in the response to oxidative and osmotic stress; identified as suppressor of S. pombe cell cycle checkpoint mutations
CAGL0B03289g	*YBR281C*	*DUG2*	Probable di- and tri-peptidase; forms a complex with Dug1p and Dug3p to degrade glutathione (GSH) and other peptides containing a gamma-glu-X bond in an alternative pathway to GSH degradation by gamma-glutamyl transpeptidase (Ecm38p)
CAGL0K11209g	*YDR244W*	*PEX5*	Peroxisomal membrane signal receptor for the C-terminal tripeptide signal sequence (PTS1) of peroxisomal matrix proteins, required for peroxisomal matrix protein import; also proposed to have PTS1-receptor independent functions
CAGL0K05687g	*YHR179W*	*OYE2*	Widely conserved NADPH oxidoreductase containing flavin mononucleotide (FMN), homologous to Oye3p with slight differences in ligand binding and catalytic properties; may be involved in sterol metabolism
CAGL0E05918g	*YPL219W*	*PCL8*	Cyclin, interacts with Pho85p cyclin-dependent kinase (Cdk) to phosphorylate and regulate glycogen synthase, also activates Pho85p for Glc8p phosphorylation
CAGL0K05775g	*YDR511W*	*ACN9*	Protein of the mitochondrial intermembrane space, required for acetate utilization and gluconeogenesis; has orthologs in higher eukaryotes
CAGL0K04719g	*YNL208W*		
CAGL0G03531g	*YER115C*	*SPR6*	Protein of unknown function, expressed during sporulation; not required for sporulation, but gene exhibits genetic interactions with other genes required

			for sporulation
CAGL0A04829g	*YGL253W*	*HXK2*	Hexokinase isoenzyme 2 that catalyzes phosphorylation of glucose in the cytosol; predominant hexokinase during growth on glucose; functions in the nucleus to repress expression of HXK1 and GLK1 and to induce expression of its own gene
CAGL0D02420g	*YLR119W*	*SRN2*	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; suppressor of rna1-1 mutation; may be involved in RNA export from nucleus
CAGL0G07645g	*NA*		
CAGL0L04312g	*YOR157C*	*PUP1*	Beta 2 subunit of the 20S proteasome; endopeptidase with trypsin-like activity that cleaves after basic residues; synthesized as a proprotein before being proteolytically processed for assembly into 20S particle; human homolog is subunit Z
CAGL0L06248g	*YBR085C-A*		
CAGL0J05324g	*YJL068C*		
CAGL0J06820g	*YPL123C*	*RNY1*	Vacuolar RNase of the T(2) family, relocalizes to the cytosol where it cleaves tRNAs upon oxidative or stationary phase stress; promotes apoptosis under stress conditions and this function is independent of its catalytic activity
CAGL0D05104g			
CAGL0K04939g	*YNL217W*		
CAGL0K10868g	*YDR256C*	*CTA1*	Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1p) during fatty acid beta-oxidation
CAGL0J11616g	*NORBH*		
CAGL0B03685g	*YCR004C*	*YCP4*	Protein of unknown function, has sequence and structural similarity to flavodoxins; predicted to be palmitoylated; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0I04202g	*YGL164C*	*YRB30*	RanGTP-binding protein, inhibits RanGAP1 (Rna1p)-mediated GTP hydrolysis of RanGTP (Gsp1p); shares similarity to proteins in other fungi but not in higher eukaryotes
CAGL0M05951g	*YKR049C*	*FMP46*	Putative redox protein containing a thioredoxin fold; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0M09405g	*YBL103C*	*RTG3*	Basic helix-loop-helix-leucine zipper (bHLH/Zip) transcription factor that forms a complex with another bHLH/Zip protein, Rtg1p, to activate the retrograde (RTG) and TOR pathways
CAGL0H06259g	*YAL017W*	*PSK1*	One of two (see also PSK2) PAS domain containing S/T protein kinases; coordinately regulates protein synthesis and carbohydrate metabolism and storage in response to a unknown metabolite that reflects nutritional status
CAGL0L07744g	*YCR011C*	*ADP1*	Putative ATP-dependent permease of the ABC transporter family of proteins

CAGL0C05489g	*YDL234C*	*GYP7*	GTPase-activating protein for yeast Rab family members including: Ypt7p (most effective), Ypt1p, Ypt31p, and Ypt32p (in vitro); involved in vesicle mediated protein trafficking
CAGL0E00649g	*YCR079W*	*PTC6*	Mitochondrial protein phosphatase of type 2C with similarity to mammalian PP1Ks; involved in mitophagy; null mutant is sensitive to rapamycin and has decreased phosphorylation of the Pda1 subunit of pyruvate dehydrogenase
CAGL0C03674g	*YNR040W*		
CAGL0D05434g	*YPR065W*	*ROX1*	Heme-dependent repressor of hypoxic genes; contains an HMG domain that is responsible for DNA bending activity
CAGL0B00704g	*YCL043C*	*PDI1*	Protein disulfide isomerase, multifunctional protein resident in the endoplasmic reticulum lumen, essential for the formation of disulfide bonds in secretory and cell-surface proteins, unscrambles non-native disulfide bonds
CAGL0F06919g	*YIR035C*		
CAGL0C01771g	*YBR241C*		
CAGL0M02915g	*NORBH*		
CAGL0K00891g	*YGR205W*	*TDA10*	ATP-binding protein of unknown function; crystal structure resembles that of E.coli pantothenate kinase and other small kinases
CAGL0J05390g	*NORBH*		
CAGL0F06875g	*YIR034C*	*LYS1*	Saccharopine dehydrogenase (NAD+, L-lysine-forming), catalyzes the conversion of saccharopine to L-lysine, which is the final step in the lysine biosynthesis pathway
CAGL0J09306g	*YDL128W*	*VCX1*	Vacuolar H+/Ca2+ exchanger involved in control of cytosolic Ca2+ concentration; has similarity to sodium/calcium exchangers, including the bovine Na+/Ca2+,K+ antiporter
CAGL0D00704g	*YDL072C*	*YET3*	Protein of unknown function; YET3 null mutant decreases the level of secreted invertase; homolog of human BAP31 protein
CAGL0E03003g	*YGR147C*	*NAT2*	Protein with an apparent role in acetylation of N-terminal methionine residues
CAGL0I06072g	*YJL154C*	*VPS35*	Endosomal subunit of membrane-associated retromer complex required for retrograde transport; receptor that recognizes retrieval signals on cargo proteins, forms subcomplex with Vps26p and Vps29p that selects cargo proteins for retrieval
CAGL0B02431g	*YML120C*	*NDI1*	NADH:ubiquinone oxidoreductase, transfers electrons from NADH to ubiquinone in the respiratory chain but does not pump protons, in contrast to the higher eukaryotic multisubunit respiratory complex I; phosphorylated; homolog of human AMID
CAGL0C05269g	*YOL065C*	*INP54*	Phosphatidylinositol 4,5-bisphosphate 5-phosphatase with a role in secretion, localizes to the endoplasmic reticulum via the C-terminal tail; lacks the Sac1 domain and proline-rich region found in the other 3 INP proteins
CAGL0K11990g	*YBR059C*	*AKL1*	Ser-Thr protein kinase, member (with Ark1p and Prk1p)

			of the Ark kinase family; involved in endocytosis and actin cytoskeleton organization
CAGL0H02541g	*YMR252C*		
CAGL0I05610g	*YNR014W*		
CAGL0J11176g	*YNL176C*	*TDA7*	Cell cycle-regulated gene of unknown function, promoter bound by Fkh2p
CAGL0F05973g	*YMR036C*	*MIH1*	Protein tyrosine phosphatase involved in cell cycle control; regulates the phosphorylation state of Cdc28p; homolog of S. pombe cdc25
CAGL0H04323g	*YJR125C*	*ENT3*	Protein containing an N-terminal epsin-like domain involved in clathrin recruitment and traffic between the Golgi and endosomes; associates with the clathrin adaptor Gga2p
CAGL0D01254g	*NORBH*		
CAGL0D03894g	*YDR300C*	*PRO1*	Gamma-glutamyl kinase, catalyzes the first step in proline biosynthesis
CAGL0D03938g	*YHR035W*		
CAGL0D02244g	*YOR351C*	*MEK1*	Meiosis-specific serine/threonine protein kinase, functions in meiotic checkpoint, promotes recombination between homologous chromosomes by suppressing double strand break repair between sister chromatids
CAGL0G03047g	*YGR092W*	*DBF2*	Ser/Thr kinase involved in transcription and stress response; functions as part of a network of genes in exit from mitosis; localization is cell cycle regulated; activated by Cdc15p during the exit from mitosis
CAGL0G01166g	*YLR348C*	*DIC1*	Mitochondrial dicarboxylate carrier, integral membrane protein, catalyzes a dicarboxylate-phosphate exchange across the inner mitochondrial membrane, transports cytoplasmic dicarboxylates into the mitochondrial matrix
CAGL0I02134g	*YHR160C*	*PEX18*	Peroxin required for targeting of peroxisomal matrix proteins containing PTS2; interacts with Pex7p; partially redundant with Pex21p
CAGL0J07084g	*YPL113C*		
CAGL0J04114g	*YOR222W*	*ODC2*	Mitochondrial inner membrane transporter, exports 2-oxoadipate and 2-oxoglutarate from the mitochondrial matrix to the cytosol for use in lysine and glutamate biosynthesis and in lysine catabolism
CAGL0E05610g	*YAL038W*	*CDC19*	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration
CAGL0D01474g	*YBR108W*	*AIM3*	Protein interacting with Rvs167p; null mutant is viable and displays elevated frequency of mitochondrial genome loss
CAGL0M13189g	*YKL062W*	*MSN4*	Transcriptional activator related to Msn2p; activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress response elements of responsive genes, inducing gene expression
CAGL0L10494g	*YOR070C*	*GYP1*	Cis-golgi GTPase-activating protein (GAP) for the Rab family members Ypt1p (in vivo) and for Ypt1p, Sec4p, Ypt7p, and Ypt51p (in vitro); involved in vesicle

			docking and fusion
CAGL0F08261g	*YGR254W*	*ENO1*	Enolase I, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is repressed in response to glucose
CAGL0J09350g	*YDL126C*	*CDC48*	ATPase in ER, nuclear membrane and cytosol with homology to mammalian p97; in a complex with Npl4p and Ufd1p participates in retrotranslocation of ubiquitinated proteins from the ER into the cytosol for degradation by the proteasome
CAGL0J06468g	*YMR261C*	*TPS3*	Regulatory subunit of trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate trehalose; expression is induced by stress conditions and repressed by the Ras-cAMP pathway
CAGL0G07623g	*YBR286W*	*APE3*	Vacuolar aminopeptidase Y, processed to mature form by Prb1p
CAGL0G09977g	*YPR184W*	*GDB1*	Glycogen debranching enzyme containing glucanotranferase and alpha-1,6-amyloglucosidase activities, required for glycogen degradation; phosphorylated in mitochondria
CAGL0I01276g	*YHR112C*		
CAGL0J08481g	*YDR506C*		
CAGL0L07986g	*YCR026C*	*NPP1*	Nucleotide pyrophosphatase/phosphodiesterase family member; mediates extracellular nucleotide phosphate hydrolysis along with Npp2p and Pho5p; activity and expression enhanced during conditions of phosphate starvation
CAGL0H04983g	*YDL055C*	*PSA1*	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase), synthesizes GDP-mannose from GTP and mannose-1-phosphate in cell wall biosynthesis; required for normal cell wall structure
CAGL0M13981g	*YMR313C*	*TGL3*	Triacylglycerol lipase of the lipid particle, responsible for all the TAG lipase activity of the lipid particle; contains the consensus sequence motif GXSXG, which is found in lipolytic enzymes; required with Tgl4p for timely bud formation
CAGL0A01606g	*YGL033W*	*HOP2*	Meiosis-specific protein that localizes to chromosomes, preventing synapsis between nonhomologous chromosomes and ensuring synapsis between homologs; complexes with Mnd1p to promote homolog pairing and meiotic double-strand break repair
CAGL0K12782g	*YFL044C*	*OTU1*	Deubiquitylation enzyme that binds to the chaperone-ATPase Cdc48p; may contribute to regulation of protein degradation by deubiquitylating substrates that have been ubiquitylated by Ufd2p; member of the Ovarian Tumor (OTU) family
CAGL0M09581g	*YBL099W*	*ATP1*	Alpha subunit of the F1 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis; phosphorylated
CAGL0K09218g	*YCR061W*		

CAGL0K09526g	*YDR394W*	*RPT3*	One of six ATPases of the 19S regulatory particle of the 26S proteasome involved in the degradation of ubiquitinated substrates; substrate of N-acetyltransferase B
CAGL0G08932g	*YOL018C*	*TLG2*	Syntaxin-like t-SNARE that forms a complex with Tlg1p and Vti1p and mediates fusion of endosome-derived vesicles with the late Golgi; binds Vps45p, which prevents Tlg2p degradation and also facilitates t-SNARE complex formation
CAGL0E05148g	*YGL156W*	*AMS1*	Vacuolar alpha mannosidase, involved in free oligosaccharide (fOS) degradation; delivered to the vacuole in a novel pathway separate from the secretory pathway
CAGL0D00264g	*YOR373W*	*NUD1*	Component of the spindle pole body outer plaque, required for exit from mitosis
CAGL0L08888g	*YPL006W*	*NCR1*	Vacuolar membrane protein that transits through the biosynthetic vacuolar protein sorting pathway, involved in sphingolipid metabolism; glycoprotein and functional orthologue of human Niemann Pick C1 (NPC1) protein
CAGL0A01892g	*NORBH*		
CAGL0L00957g	*YER048C*	*CAJ1*	Nuclear type II J heat shock protein of the E. coli dnaJ family, contains a leucine zipper-like motif, binds to non-native substrates for presentation to Ssa3p, may function during protein translocation, assembly and disassembly
CAGL0A04147g	*YLR191W*	*PEX13*	Integral peroxisomal membrane required for the translocation of peroxisomal matrix proteins, interacts with the PTS1 signal recognition factor Pex5p and the PTS2 signal recognition factor Pex7p, forms a complex with Pex14p and Pex17p
CAGL0K08910g	*YOR259C*	*RPT4*	One of six ATPases of the 19S regulatory particle of the 26S proteasome involved in the degradation of ubiquitinated substrates; required for spindle pole body duplication; localized mainly to the nucleus throughout the cell cycle
CAGL0A03410g	*NORBH*		
CAGL0M09207g	*YJR086W*	*STE18*	G protein gamma subunit, forms a dimer with Ste4p to activate the mating signaling pathway, forms a heterotrimer with Gpa1p and Ste4p to dampen signaling; C-terminus is palmitoylated and farnesylated, which are required for normal signaling
CAGL0K11858g	*YDR032C*	*PST2*	Protein with similarity to members of a family of flavodoxin-like proteins; induced by oxidative stress in a Yap1p dependent manner; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0L05434g	*YKR042W*	*UTH1*	Mitochondrial outer membrane and cell wall localized SUN family member required for mitochondrial autophagy; involved in the oxidative stress response, life span during starvation, mitochondrial biogenesis, and cell death
CAGL0E05082g	*YGL153W*	*PEX14*	Peroxisomal membrane peroxin that is a central component of the peroxisomal protein import machinery;

			interacts with both PTS1 (Pex5p) and PTS2 (Pex7p), peroxisomal matrix protein signal recognition factors and membrane receptor Pex13p
CAGL0D01782g	*YGL197W*	*MDS3*	Protein with an N-terminal kelch-like domain, putative negative regulator of early meiotic gene expression; required, with Pmd1p, for growth under alkaline conditions
CAGL0B01727g	*YDR109C*		
CAGL0J04026g	*YOR227W*	*HER1*	Protein of unknown function required for proliferation or remodeling of the ER that is caused by overexpression of Hmg2p; may interact with ribosomes, based on co-purification experiments
CAGL0M11000g	*YNR034W-A*		
CAGL0J01397g	*YMR087W*		
CAGL0K09702g	*YNL134C*		
CAGL0D04972g	*YPR140W*	*TAZ1*	Lyso-phosphatidylcholine acyltransferase, required for normal phospholipid content of mitochondrial membranes; may remodel acyl groups of cardiolipin in the inner membrane; human ortholog tafazzin is implicated in Barth syndrome
CAGL0K08932g	*YOR258W*	*HNT3*	Member of the third branch of the histidine triad (HIT) superfamily of nucleotide-binding proteins; similar to Aprataxin, a Hint related protein that is mutated in individuals with ataxia with oculomotor apraxia
CAGL0G05830g	*YHR146W*	*CRP1*	Protein that binds to cruciform DNA structures
CAGL0F00693g	*YOR323C*	*PRO2*	Gamma-glutamyl phosphate reductase, catalyzes the second step in proline biosynthesis
CAGL0A02024g	*YOL025W*	*LAG2*	Protein involved in the determination of longevity and also in the negative regulation of SCF E3-ubiquitin ligase function; LAG2 is preferentially expressed in young cells; overexpression extends the mean and maximum life span of cells
CAGL0K11594g	*YKL119C*	*VPH2*	Integral membrane protein required for vacuolar H+-ATPase (V-ATPase) function, although not an actual component of the V-ATPase complex; functions in the assembly of the V-ATPase; localized to the endoplasmic reticulum (ER)
CAGL0F07777g	*YMR170C*	*ALD2*	Cytoplasmic aldehyde dehydrogenase, involved in ethanol oxidation and beta-alanine biosynthesis; uses NAD+ as the preferred coenzyme; expression is stress induced and glucose repressed; very similar to Ald3p
CAGL0L11044g	*YOR280C*	*FSH3*	Putative serine hydrolase; likely target of Cyc8p-Tup1p-Rfx1p transcriptional regulation; sequence is similar to S. cerevisiae Fsh1p and Fsh2p and the human candidate tumor suppressor OVCA2
CAGL0G05544g	*NORBH*		
CAGL0M13101g	*YKL051W*	*SFK1*	Plasma membrane protein that may act together with or upstream of Stt4p to generate normal levels of the essential phospholipid PI4P, at least partially mediates proper localization of Stt4p to the plasma membrane
CAGL0A01243g	*YCR098C*	*GIT1*	Plasma membrane permease, mediates uptake of

			glycerophosphoinositol and glycerophosphocholine as sources of the nutrients inositol and phosphate; expression and transport rate are regulated by phosphate and inositol availability
CAGL0F00869g	*YOL048C*	*RRT8*	Putative protein of unknown function; identified in a screen for mutants with increased levels of rDNA transcription; green fluorescent protein (GFP)-fusion protein localizes to lipid particles
CAGL0D02464g	*YNL325C*	*FIG4*	Phosphatidylinositol 3,5-bisphosphate (PtdIns[3,5]P) phosphatase; required for efficient mating and response to osmotic shock; physically associates with and regulated by Vac14p; contains a SAC1-like domain
CAGL0C00451g	*YBR137W*		
CAGL0K05247g	*YBL101C*	*ECM21*	Protein involved in regulating the endocytosis of plasma membrane proteins; identified as a substrate for ubiquitination by Rsp5p and deubiquitination by Ubp2p; promoter contains several Gcn4p binding elements
CAGL0K08228g	*YKR051W*		
CAGL0I07249g	*YDR389W*	*SAC7*	GTPase activating protein (GAP) for Rho1p, involved in signaling to the actin cytoskeleton, null mutations suppress tor2 mutations and temperature sensitive mutations in actin; potential Cdc28p substrate
CAGL0D00990g	*YDL057W*		
CAGL0E06006g	*YPL224C*	*MMT2*	Putative metal transporter involved in mitochondrial iron accumulation; closely related to Mmt1p
CAGL0M03839g	*YNL305C*		
CAGL0H09944g	*YBR046C*	*ZTA1*	NADPH-dependent quinone reductase, GFP-tagged protein localizes to the cytoplasm and nucleus; has similarity to E. coli quinone oxidoreductase and to human zeta-crystallin
CAGL0L07502g	*YOR010C*	*TIR2*	Putative cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; transcription is induced by cold shock and anaerobiosis
CAGL0M08206g	*YJL171C*		
CAGL0K00803g	*YGR209C*	*TRX2*	Cytoplasmic thioredoxin isoenzyme of the thioredoxin system which protects cells against oxidative and reductive stress, forms LMA1 complex with Pbi2p, acts as a cofactor for Tsa1p, required for ER-Golgi transport and vacuole inheritance
CAGL0M11902g	*YAL034C*	*FUN19*	Non-essential protein of unknown function; expression induced in response to heat stress
CAGL0G03553g	*YER116C*	*SLX8*	Subunit of the Slx5-Slx8 SUMO-targeted ubiquitin ligase (STUbL) complex; stimulated by prior attachment of SUMO to the substrate; contains a C-terminal RING domain
CAGL0F04213g	*YBL030C*	*PET9*	Major ADP/ATP carrier of the mitochondrial inner membrane, exchanges cytosolic ADP for mitochondrially synthesized ATP; phosphorylated; required for viability in many common lab strains carrying a mutation in the polymorphic SAL1 gene
CAGL0J02904g	*YER054C*	*GIP2*	Putative regulatory subunit of the protein phosphatase

			Glc7p, involved in glycogen metabolism; contains a conserved motif (GVNK motif) that is also found in Gac1p, Pig1p, and Pig2p
CAGL0F02717g	*NORBH*		
CAGL0F04741g	*YOL016C*	*CMK2*	Calmodulin-dependent protein kinase; may play a role in stress response, many CA++/calmodulan dependent phosphorylation substrates demonstrated in vitro, amino acid sequence similar to Cmk1p and mammalian Cam Kinase II
CAGL0J02002g	*YIL006W*	*YIA6*	Mitochondrial NAD+ transporter, involved in the transport of NAD+ into the mitochondria (see also YEA6); member of the mitochondrial carrier subfamily; disputed role as a pyruvate transporter; has putative mouse and human orthologs
CAGL0L02079g	*YBR291C*	*CTP1*	Mitochondrial inner membrane citrate transporter, member of the mitochondrial carrier family
CAGL0F06941g	*YGL062W*	*PYC1*	Pyruvate carboxylase isoform, cytoplasmic enzyme that converts pyruvate to oxaloacetate; highly similar to isoform Pyc2p but differentially regulated; mutations in the human homolog are associated with lactic acidosis
CAGL0B01243g	*YCR040W*	*MATALPHA1*	Transcriptional co-activator involved in regulation of mating-type-specific gene expression; targets the transcription factor Mcm1p to the promoters of alpha-specific genes; one of two genes encoded by the MATalpha mating type cassette
CAGL0I00330g	*YGL126W*	*SCS3*	Protein required for inositol prototrophy, identified as an ortholog of the FIT family of proteins involved in triglyceride droplet biosynthesis; disputed role in the synthesis of inositol phospholipids from inositol
CAGL0L06864g	*YMR140W*	*SIP5*	Protein of unknown function; interacts with both the Reg1p/Glc7p phosphatase and the Snf1p kinase
CAGL0M09669g	*YMR154C*	*RIM13*	Calpain-like cysteine protease involved in proteolytic activation of Rim101p in response to alkaline pH; has similarity to A. nidulans palB
CAGL0L03938g	*YNL115C*		
CAGL0F04191g	*YBL029CA*		
CAGL0I07887g	*NORBH*		
CAGL0M00550g	*YJR130C*	*STR2*	Cystathionine gamma-synthase, converts cysteine into cystathionine
CAGL0H04037g	*YOR178C*	*GAC1*	Regulatory subunit for Glc7p type-1 protein phosphatase (PP1), tethers Glc7p to Gsy2p glycogen synthase, binds Hsf1p heat shock transcription factor, required for induction of some HSF-regulated genes under heat shock
CAGL0I02794g	*YOR114W*		
CAGL0H08888g	*YPL221W*	*FLC1*	Putative FAD transporter; required for uptake of FAD into endoplasmic reticulum; involved in cell wall maintenance
CAGL0K04037g	*NORBH*		
CAGL0M11792g	*YAL031C*	*GIP4*	Cytoplasmic Glc7-interacting protein whose overexpression relocalizes Glc7p from the nucleus and prevents chromosome segregation; potential Cdc28p

			substrate
CAGL0I09482g	*YBR273C*	*UBX7*	UBX (ubiquitin regulatory X) domain-containing protein that interacts with Cdc48p
CAGL0B04059g	*YJR019C*	*TES1*	Peroxisomal acyl-CoA thioesterase likely to be involved in fatty acid oxidation rather than fatty acid synthesis; conserved protein also found in human peroxisomes; TES1 mRNA levels increase during growth on fatty acids
CAGL0G02101g	*YKR076W*	*ECM4*	Omega class glutathione transferase; not essential; similar to Ygr154cp; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
CAGL0J08976g	*YLR218C*	*COA4*	Protein that localizes to the mitochondrial intermembrane space via the Mia40p-Erv1p system; mutants exhibit glycogen storage defects and growth defects on a non-fermentable carbon source; contains twin cysteine-x9-cysteine motifs
CAGL0E05192g	*YPL088W*		
CAGL0I04180g	*NORBH*		
CAGL0C04455g	*YDL004W*	*ATP16*	Delta subunit of the central stalk of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis; phosphorylated
CAGL0L12254g	*YLR089C*	*ALT1*	Alanine transaminase (glutamic pyruvic transaminase); involved in alanine biosynthetic and catabolic processes; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0E04548g	*YOR020W-A*		
CAGL0L09273g	*YPR006C*	*ICL2*	2-methylisocitrate lyase of the mitochondrial matrix, functions in the methylcitrate cycle to catalyze the conversion of 2-methylisocitrate to succinate and pyruvate; ICL2 transcription is repressed by glucose and induced by ethanol
CAGL0H08129g	*YOR030W*	*DFG16*	Probable multiple transmembrane protein, involved in diploid invasive and pseudohyphal growth upon nitrogen starvation; required for accumulation of processed Rim101p
CAGL0G06446g	*NORBH*		
CAGL0G02849g	*YPL186C*	*UIP4*	Protein that interacts with Ulp1p, a Ubl (ubiquitin-like protein)-specific protease for Smt3p protein conjugates; detected in a phosphorylated state in the mitochondrial outer membrane; also detected in ER and nuclear envelope
CAGL0L03916g	*YOR113W*	*AZF1*	Zinc-finger transcription factor, involved in induction of CLN3 transcription in response to glucose; genetic and physical interactions indicate a possible role in mitochondrial transcription or genome maintenance
CAGL0M05467g	*YBR204C*		
CAGL0J04268g	*YBL015W*	*ACH1*	Protein with CoA transferase activity, particularly for CoASH transfer from succinyl-CoA to acetate; has minor acetyl-CoA-hydrolase activity; phosphorylated; required for acetate utilization and for diploid pseudohyphal growth

CAGL0J01870g	*YGL167C*	*PMR1*	High affinity Ca2+/Mn2+ P-type ATPase required for Ca2+ and Mn2+ transport into Golgi; involved in Ca2+ dependent protein sorting and processing; mutations in human homolog ATP2C1 cause acantholytic skin condition Hailey-Hailey disease
CAGL0H00781g	*YPL247C*		
CAGL0M13651g	*YMR297W*	*PRC1*	Vacuolar carboxypeptidase Y (proteinase C), broad-specificity C-terminal exopeptidase involved in non-specific protein degradation in the vacuole; member of the serine carboxypeptidase family
CAGL0G06006g	*YHR138C*		
CAGL0B01595g	*NORBH*		
CAGL0I10054g	*YGR143W*	*SKN1*	Protein involved in sphingolipid biosynthesis; type II membrane protein with similarity to Kre6p
CAGL0H03113g	*YGL090W*	*LIF1*	Component of the DNA ligase IV complex that mediates nonhomologous end joining in DNA double-strand break repair; physically interacts with Dnl4p and Nej1p; homologous to mammalian XRCC4 protein
CAGL0H01837g	*NORBH*		
CAGL0K09900g	*YOR358W*	*HAP5*	Subunit of the heme-activated, glucose-repressed Hap2/3/4/5 CCAAT-binding complex, a transcriptional activator and global regulator of respiratory gene expression; required for assembly and DNA binding activity of the complex
CAGL0L02717g	*YOR215C*	*AIM41*	Putative protein of unknown function; the authentic protein is detected in highly purified mitochondria in high-throughput studies; null mutant displays reduced frequency of mitochondrial genome loss
CAGL0F01265g	*YOL028C*	*YAP7*	Putative basic leucine zipper (bZIP) transcription factor
CAGL0M09647g	*YMR155W*		
CAGL0B04763g	*YCL005W*	*LDB16*	Protein of unknown function; null mutants have decreased net negative cell surface charge; GFP-fusion protein expression is induced in response to the DNA-damaging agent MMS; native protein is detected in purified mitochondria
CAGL0H04785g	*YML013W*	*UBX2*	Protein involved in ER-associated protein degradation; proposed to coordinate the assembly of proteins involved in ERAD; contains a UBX (ubiquitin regulatory X) domain and a ubiquitin-associated (UBA) domain
CAGL0H00506g	*YJR121W*	*ATP2*	Beta subunit of the F1 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis; phosphorylated
CAGL0H07337g	*NORBH*		
CAGL0H03311g	*NORBH*		
CAGL0M06325g	*YPL089C*	*RLM1*	MADS-box transcription factor, component of the protein kinase C-mediated MAP kinase pathway involved in the maintenance of cell integrity; phosphorylated and activated by the MAP-kinase Slt2p
CAGL0C05027g	*YAR035W*	*YAT1*	Outer mitochondrial carnitine acetyltransferase, minor ethanol-inducible enzyme involved in transport of

			activated acyl groups from the cytoplasm into the mitochondrial matrix; phosphorylated
CAGL0J11770g	*YMR008C*	*PLB1*	Phospholipase B (lysophospholipase) involved in lipid metabolism, required for deacylation of phosphatidylcholine and phosphatidylethanolamine but not phosphatidylinositol
CAGL0K01133g	*YGR080W*	*TWF1*	Twinfilin, highly conserved actin monomer-sequestering protein involved in regulation of the cortical actin cytoskeleton, composed of two cofilin-like regions, localizes actin monomers to sites of rapid filament assembly
CAGL0K03663g	*NA*		
CAGL0I05962g	*YJL146W*	*IDS2*	Protein involved in modulation of Ime2p activity during meiosis, appears to act indirectly to promote Ime2p-mediated late meiotic functions; found in growing cells and degraded during sporulation
CAGL0J02508g	*YOR009W*	*TIR4*	Cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; expressed under anaerobic conditions and required for anaerobic growth; transcription is also induced by cold shock
CAGL0J02530g	*YOR009W*	*TIR4*	Cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; expressed under anaerobic conditions and required for anaerobic growth; transcription is also induced by cold shock
CAGL0H08558g	*YPR049C*	*ATG11*	Adapter protein for pexophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway; directs receptor-bound cargo to the phagophore assembly site (PAS) for packaging into vesicles; required for recruiting other proteins to the (PAS)
CAGL0M05665g	*YNL015W*	*PBI2*	Cytosolic inhibitor of vacuolar proteinase B, required for efficient vacuole inheritance; with thioredoxin forms protein complex LMA1, which assists in priming SNARE molecules and promotes vacuole fusion
CAGL0I05060g	*YER088C*	*DOT6*	Protein involved in rRNA and ribosome biogenesis; binds polymerase A and C motif; subunit of the RPD3L histone deacetylase complex; similar to Tod6p; has chromatin specific SANT domain; involved in telomeric gene silencing and filamentation
CAGL0J03542g	*YCR068W*	*ATG15*	Lipase required for intravacuolar lysis of autophagic bodies and Cvt bodies; targeted to intravacuolar vesicles during autophagy via the multivesicular body (MVB) pathway
CAGL0G01320g	*YNL053W*	*MSG5*	Dual-specificity protein phosphatase; exists in 2 isoforms; required for maintenance of a low level of signaling through the cell integrity pathway, adaptive response to pheromone; regulates and is regulated by Slt2p; dephosphorylates Fus3p
CAGL0G05049g	*YDR063W*	*AIM7*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus; null mutant is viable and displays elevated frequency of mitochondrial genome loss
CAGL0F02101g	*YFL007W*	*BLM10*	Proteasome activator subunit; found in association with core particles, with and without the 19S regulatory

			particle; required for resistance to bleomycin, may be involved in protecting against oxidative damage; similar to mammalian PA200
CAGL0B00770g	*YCL038C*	*ATG22*	Vacuolar integral membrane protein required for efflux of amino acids during autophagic body breakdown in the vacuole; null mutation causes a gradual loss of viability during starvation
CAGL0B03615g			
CAGL0D01276g	*NA*		
CAGL0H05951g	*YPL107W*		
CAGL0L05236g	*YKL085W*	*MDH1*	Mitochondrial malate dehydrogenase, catalyzes interconversion of malate and oxaloacetate; involved in the tricarboxylic acid (TCA) cycle; phosphorylated
CAGL0L05742g	*YJL133W*	*MRS3*	Iron transporter that mediates Fe2+ transport across the inner mitochondrial membrane; mitochondrial carrier family member, similar to and functionally redundant with Mrs4p; active under low-iron conditions; may transport other cations
CAGL0J09130g	*YDL189W*	*RBS1*	Protein of unknown function, identified as a high copy suppressor of psk1 psk2 mutations that confer temperature-sensitivity for galactose utilization; proposed to bind single-stranded nucleic acids via its R3H domain
CAGL0K02629g	*YNL134C*		
CAGL0E06226g	*YKL157W*	*APE2*	Aminopeptidase yscII; may have a role in obtaining leucine from dipeptide substrates; sequence coordinates have changed since RT-PCR analysis showed that the adjacent ORF YKL158W comprises the 5' exon of APE2/YKL157W
CAGL0C04609g	*YJR099W*	*YUH1*	Ubiquitin C-terminal hydrolase that cleaves ubiquitin-protein fusions to generate monomeric ubiquitin; hydrolyzes the peptide bond at the C-terminus of ubiquitin; also the major processing enzyme for the ubiquitin-like protein Rub1p
CAGL0A02002g	*YFR017C*		
CAGL0K01639g	*YDL027C*		
CAGL0I01144g	*YHR105W*	*YPT35*	Endosomal protein of unknown function that contains a phox (PX) homology domain and binds to both phosphatidylinositol-3-phosphate (PtdIns(3)P) and proteins involved in ER-Golgi or vesicular transport
CAGL0G05357g	*YNL200C*		
CAGL0B02035g	*YDR129C*	*SAC6*	Fimbrin, actin-bundling protein; cooperates with Scp1p (calponin/transgelin) in the organization and maintenance of the actin cytoskeleton
CAGL0H05665g	*YPL091W*	*GLR1*	Cytosolic and mitochondrial glutathione oxidoreductase, converts oxidized glutathione to reduced glutathione; mitochondrial but not cytosolic form has a role in resistance to hyperoxia
CAGL0D03520g	*YKL195W*	*MIA40*	Essential protein of the mitochondrial intermembrane space (IMS); promotes retention of newly imported proteins; may do so by stabilizing client protein folding

			as part of a disulfide relay system or transferring metal to client proteins
CAGL0B00748g	*YCL039W*	*GID7*	Protein of unknown function, involved in proteasome-dependent catabolite inactivation of fructose-1,6-bisphosphatase; contains six WD40 repeats; computational analysis suggests that Gid7p and Moh1p have similar functions
CAGL0J09284g	*YDL129W*		
CAGL0L00539g	*YMR184W*	*ADD37*	Protein of unknown function involved in ER-associated protein degradation; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and is induced in response to the DNA-damaging agent MMS; YMR184W is not an essential gene
CAGL0J00539g	*YHR030C*	*SLT2*	Serine/threonine MAP kinase involved in regulating the maintenance of cell wall integrity and progression through the cell cycle; regulated by the PKC1-mediated signaling pathway
CAGL0L09251g	*NORBH*		
CAGL0G05962g	*YHR140W*		
CAGL0B03443g	*YLR253W*		
CAGL0D05082g	*YLL039C*	*UBI4*	Ubiquitin, becomes conjugated to proteins, marking them for selective degradation via the ubiquitin-26S proteasome system; essential for the cellular stress response; encoded as a polyubiquitin precursor comprised of 5 head-to-tail repeats
CAGL0M08360g	*YJL165C*	*HAL5*	Putative protein kinase; overexpression increases sodium and lithium tolerance, whereas gene disruption increases cation and low pH sensitivity and impairs potassium uptake, suggesting a role in regulation of Trk1p and/or Trk2p transporters
CAGL0L02607g	*YHR202W*		
CAGL0H10164g	*YBR057C*	*MUM2*	Cytoplasmic protein essential for meiotic DNA replication and sporulation; interacts with Orc2p, which is a component of the origin recognition complex
CAGL0L09207g	*YPR003C*		
CAGL0K10252g	*YOR124C*	*UBP2*	Ubiquitin-specific protease that removes ubiquitin from ubiquitinated proteins; interacts with Rsp5p and is required for MVB sorting of membrane proteins; can cleave polyubiquitin and has isopeptidase activity
CAGL0L08338g	*YIL138C*	*TPM2*	Minor isoform of tropomyosin, binds to and stabilizes actin cables and filaments, which direct polarized cell growth and the distribution of several organelles; appears to have distinct and also overlapping functions with Tpm1p
CAGL0M00880g	*YLR438W*	*CAR2*	L-ornithine transaminase (OTAse), catalyzes the second step of arginine degradation, expression is dually-regulated by allophanate induction and a specific arginine induction process; not nitrogen catabolite repression sensitive
CAGL0C02739g	*YAL008W*	*FUN14*	Mitochondrial protein of unknown function
CAGL0K02805g	*Y797*		

CAGL0J08547g	*YOR084W*	*LPX1*	Oleic acid-inducible, peroxisomal matrix localized lipase; transcriptionally activated by Yrm1p along with genes involved in multidrug resistance; peroxisomal import is dependent on the PTS1 receptor, Pex5p and on self-interaction
CAGL0A01870g	*YBL017C*	*PEP1*	Type I transmembrane sorting receptor for multiple vacuolar hydrolases; cycles between the late-Golgi and prevacuolar endosome-like compartments
CAGL0G02145g	*YOR069W*	*VPS5*	Nexin-1 homolog required for localizing membrane proteins from a prevacuolar/late endosomal compartment back to the late Golgi apparatus; structural component of the retromer membrane coat complex; forms a retromer subcomplex with Vps17p
CAGL0J08613g	*YOR088W*		
CAGL0G06182g	*YHR131C*		
CAGL0D01298g	*YPR074C*	*TKL1*	Transketolase, similar to Tkl2p; catalyzes conversion of xylulose-5-phosphate and ribose-5-phosphate to sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate in the pentose phosphate pathway; needed for synthesis of aromatic amino acids
CAGL0H02387g	*YMR261C*	*TPS3*	Regulatory subunit of trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate trehalose; expression is induced by stress conditions and repressed by the Ras-cAMP pathway
CAGL0I08305g	*YER024W*	*YAT2*	Carnitine acetyltransferase; has similarity to Yat1p, which is a carnitine acetyltransferase associated with the mitochondrial outer membrane
CAGL0M08184g	*YKL178C*	*STE3*	Receptor for a factor pheromone, transcribed in alpha cells and required for mating by alpha cells, couples to MAP kinase cascade to mediate pheromone response; ligand bound receptors are endocytosed and recycled to the plasma membrane; GPC
CAGL0D02134g	*YMR115W*	*MGR3*	Subunit of the mitochondrial (mt) i-AAA protease supercomplex, which degrades misfolded mitochondrial proteins; forms a subcomplex with Mgr1p that binds to substrates to facilitate proteolysis; required for growth of cells lacking mtDNA
CAGL0E01177g	*YDR155C*	*CPR1*	Cytoplasmic peptidyl-prolyl cis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds the drug cyclosporin A
CAGL0I00836g	*YMR140W*	*SIP5*	Protein of unknown function; interacts with both the Reg1p/Glc7p phosphatase and the Snf1p kinase
CAGL0E05654g	*YPL206C*	*PGC1*	Phosphatidyl Glycerol phospholipase C; regulates the phosphatidylglycerol (PG) content via a phospholipase C-type degradation mechanism; contains glycerophosphodiester phosphodiesterase motifs
CAGL0M00616g	*YJR126C*	*VPS70*	Protein of unknown function involved in vacuolar protein sorting
CAGL0H03971g	*YCR004C*	*YCP4*	Protein of unknown function, has sequence and structural similarity to flavodoxins; predicted to be palmitoylated; the authentic, non-tagged protein is detected in highly

			purified mitochondria in high-throughput studies
CAGL0M07007g	*YCR076C*		
CAGL0B02860g	*YLR356W*	*ATG33*	Putative protein of unknown function with similarity to SCM4; green fluorescent protein (GFP)-fusion protein localizes to mitochondria; YLR356W is not an essential gene
CAGL0B00858g	*YCL032W*	*STE50*	Protein involved in mating response, invasive/filamentous growth, and osmotolerance, acts as an adaptor that links G protein-associated Cdc42p-Ste20p complex to the effector Ste11p to modulate signal transduction
CAGL0M08492g	*YKL164C*	*PIR1*	O-glycosylated protein required for cell wall stability; attached to the cell wall via beta-1,3-glucan; mediates mitochondrial translocation of Apn1p; expression regulated by the cell integrity pathway and by Swi5p during the cell cycle
CAGL0F07953g	*YGR236C*	*SPG1*	Protein required for survival at high temperature during stationary phase; not required for growth on nonfermentable carbon sources; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0H04851g	*YML016C*	*PPZ1*	Serine/threonine protein phosphatase Z, isoform of Ppz2p; involved in regulation of potassium transport, which affects osmotic stability, cell cycle progression, and halotolerance
CAGL0L00473g	*YMR187C*		
CAGL0K00275g	*YKL213C*	*DOA1*	WD repeat protein required for ubiquitin-mediated protein degradation, forms complex with Cdc48p, plays a role in controlling cellular ubiquitin concentration; also promotes efficient NHEJ in postdiauxic/stationary phase
CAGL0D01270g			
CAGL0K01749g	*YDL019C*	*OSH2*	Member of an oxysterol-binding protein family with seven members in S. cerevisiae; family members have overlapping, redundant functions in sterol metabolism and collectively perform a function essential for viability
CAGL0C04499g	*YDL006W*	*PTC1*	Type 2C protein phosphatase (PP2C); inactivates the osmosensing MAPK cascade by dephosphorylating Hog1p; mutation delays mitochondrial inheritance; deletion reveals defects in precursor tRNA splicing, sporulation and cell separation
CAGL0I07139g	*YOR142W*	*LSC1*	Alpha subunit of succinyl-CoA ligase, which is a mitochondrial enzyme of the TCA cycle that catalyzes the nucleotide-dependent conversion of succinyl-CoA to succinate; phosphorylated
CAGL0H00418g	*YER175C*	*TMT1*	Trans-aconitate methyltransferase, cytosolic enzyme that catalyzes the methyl esterification of 3-isopropylmalate, an intermediate of the leucine biosynthetic pathway, and trans-aconitate, which inhibits the citric acid cycle
CAGL0G04477g	*NORBH*		
CAGL0I05852g	*YPL256C*	*CLN2*	G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition; late G1 specific expression depends on

			transcription factor complexes, MBF (Swi6p-Mbp1p) and SBF (Swi6p-Swi4p)
CAGL0L01265g	*YJR049C*	*UTR1*	ATP-NADH kinase; phosphorylates both NAD and NADH; active as a hexamer; enhances the activity of ferric reductase (Fre1p)
CAGL0J06182g	*YNL167C*	*SKO1*	Basic leucine zipper (bZIP) transcription factor of the ATF/CREB family, forms a complex with Tup1p and Ssn6p to both activate and repress transcription; cytosolic and nuclear protein involved in osmotic and oxidative stress responses
CAGL0D03982g	*YHR037W*	*PUT2*	Delta-1-pyrroline-5-carboxylate dehydrogenase, nuclear-encoded mitochondrial protein involved in utilization of proline as sole nitrogen source; deficiency of the human homolog causes HPII, an autosomal recessive inborn error of metabolism
CAGL0G06622g	*YNL101W*	*AVT4*	Vacuolar transporter, exports large neutral amino acids from the vacuole; member of a family of seven S. cerevisiae genes (AVT1-7) related to vesicular GABA-glycine transporters
CAGL0G05698g	*YDL215C*	*GDH2*	NAD(+)-dependent glutamate dehydrogenase, degrades glutamate to ammonia and alpha-ketoglutarate; expression sensitive to nitrogen catabolite repression and intracellular ammonia levels
CAGL0G03795g	*YLL024C*	*SSA2*	ATP binding protein involved in protein folding and vacuolar import of proteins; member of heat shock protein 70 (HSP70) family; associated with the chaperonin-containing T-complex; present in the cytoplasm, vacuolar membrane and cell wall
CAGL0H08063g	*YNL202W*	*SPS19*	Peroxisomal 2,4-dienoyl-CoA reductase, auxiliary enzyme of fatty acid beta-oxidation; homodimeric enzyme required for growth and sporulation on petroselineate medium; expression induced during late sporulation and in the presence of oleate
CAGL0I04048g	*YLR377C*	*FBP1*	Fructose-1,6-bisphosphatase, key regulatory enzyme in the gluconeogenesis pathway, required for glucose metabolism; undergoes either proteasome-mediated or autophagy-mediated degradation depending on growth conditions; interacts with Vid30p
CAGL0D00154g	*YPR192W*	*AQY1*	Spore-specific water channel that mediates the transport of water across cell membranes, developmentally controlled; may play a role in spore maturation, probably by allowing water outflow, may be involved in freeze tolerance
CAGL0G02673g	*YNL037C*	*IDH1*	Subunit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase, which catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the TCA cycle
CAGL0F01111g	*YOL032W*	*OPI10*	Protein with a possible role in phospholipid biosynthesis, based on inositol-excreting phenotype of the null mutant and its suppression by exogenous choline
CAGL0A04301g	*YBL056W*	*PTC3*	Type 2C protein phosphatase; dephosphorylates Hog1p (see also Ptc2p) to limit maximal kinase activity induced by osmotic stress; dephosphorylates T169 phosphorylated Cdc28p (see also Ptc2p); role in DNA checkpoint inactivation

CAGL0J04906g	*YJL049W*		
CAGL0F04345g	*YBR086C*	*IST2*	Plasma membrane protein that may be involved in osmotolerance, localizes to the mother cell in small-budded cells and to the bud in medium- and large-budded cells; mRNA is transported to the bud tip by an actomyosin-driven process
CAGL0E05962g	*YPL222W*	*FMP40*	Putative protein of unknown function; proposed to be involved in responding to environmental stresses; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0J06270g	*YDL176W*		
CAGL0G08338g	*YLR241W*		
CAGL0L11880g	*YER169W*	*RPH1*	JmjC domain-containing histone demethylase which can specifically demethylate H3K36 tri- and dimethyl modification states; transcriptional repressor of PHR1; Rph1p phosphorylation during DNA damage is under control of the MEC1-RAD53 pathway
CAGL0D03036g	*YJR001W*	*AVT1*	Vacuolar transporter, imports large neutral amino acids into the vacuole; member of a family of seven S. cerevisiae genes (AVT1-7) related to vesicular GABA-glycine transporters
CAGL0L03135g	*YKR031C*	*SPO14*	Phospholipase D, catalyzes the hydrolysis of phosphatidylcholine, producing choline and phosphatidic acid; involved in Sec14p-independent secretion; required for meiosis and spore formation; differently regulated in secretion and meiosis
CAGL0H00682g	*YMR196W*		
CAGL0J05874g	*YNL159C*	*ASI2*	Integral inner nuclear membrane protein that acts with Asi1p and Asi3p to ensure the fidelity of SPS-sensor signalling by maintaining the dormant repressed state of gene expression in the absence of inducing signals
CAGL0L09933g	*YOR042W*	*CUE5*	Protein containing a CUE domain that binds ubiquitin, which may facilitate intramolecular monoubiquitination; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm in a punctate pattern
CAGL0M09020g	*YJR095W*	*SFC1*	Mitochondrial succinate-fumarate transporter, transports succinate into and fumarate out of the mitochondrion; required for ethanol and acetate utilization
CAGL0M06633g	*YIL087C*	*AIM19*	Putative protein of unknown function; the authentic, non-tagged protein is detected in purified mitochondria in high-throughput studies; null mutant displays reduced respiratory growth
CAGL0K12034g	*YDR040C*	*ENA1*	P-type ATPase sodium pump, involved in Na+ and Li+ efflux to allow salt tolerance
CAGL0H09680g	*YER008C*	*SEC3*	Subunit of the exocyst complex (Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, Exo84p) which mediates targeting of post-Golgi vesicles to sites of active exocytosis; Sec3p specifically is a spatial landmark for secretion
CAGL0E01287g	*YDR148C*	*KGD2*	Dihydrolipoyl transsuccinylase, component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes the oxidative decarboxylation

			of alpha-ketoglutarate to succinyl-CoA in the TCA cycle; phosphorylated
CAGL0H08151g	*NORBH*		
CAGL0L07898g	*YCR019W*	*MAK32*	Protein necessary for structural stability of L-A double-stranded RNA-containing particles
CAGL0L05016g	*YKL072W*	*STB6*	Protein that binds Sin3p in a two-hybrid assay
CAGL0J08294g	*YNL275W*	*BOR1*	Boron efflux transporter of the plasma membrane; binds HCO3-, I-, Br-, NO3- and Cl-; has similarity to the characterized boron efflux transporter A. thaliana BOR1
CAGL0K07205g	*NORBH*		
CAGL0B04323g	*YBR026C*	*ETR1*	2-enoyl thioester reductase, member of the medium chain dehydrogenase/reductase family; localized to in mitochondria, where it has a probable role in fatty acid synthesis
CAGL0B03069g	*YLR354C*	*TAL1*	Transaldolase, enzyme in the non-oxidative pentose phosphate pathway; converts sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate to erythrose 4-phosphate and fructose 6-phosphate
CAGL0H02519g	*YMR253C*		
CAGL0F04851g	*YPR155C*	*NCA2*	Protein involved in regulation of mitochondrial expression of subunits 6 (Atp6p) and 8 (Atp8p) of the Fo-F1 ATP synthase; functions with Nca3p
CAGL0I02530g	*YHR176W*	*FMO1*	Flavin-containing monooxygenase, localized to the cytoplasmic face of the ER membrane; catalyzes oxidation of biological thiols to maintain the ER redox buffer ratio for correct folding of disulfide-bonded proteins
CAGL0L03982g	*YNL117W*	*MLS1*	Malate synthase, enzyme of the glyoxylate cycle, involved in utilization of non-fermentable carbon sources; expression is subject to carbon catabolite repression; localizes in peroxisomes during growth in oleic acid medium
CAGL0I06809g	*YER142C*	*MAG1*	3-methyl-adenine DNA glycosylase involved in protecting DNA against alkylating agents; initiates base excision repair by removing damaged bases to create abasic sites that are subsequently repaired
CAGL0E03498g	*NORBH*		
CAGL0G06842g	*YJL020C*	*BBC1*	Protein possibly involved in assembly of actin patches; interacts with an actin assembly factor Las17p and with the SH3 domains of Type I myosins Myo3p and Myo5p; localized predominantly to cortical actin patches
CAGL0M09229g	*YJR085C*		
CAGL0K00231g	*YKL215C*		
CAGL0L08932g	*YPL004C*	*LSP1*	Primary component of eisosomes, which are large immobile patch structures at the cell cortex associated with endocytosis, along with Pil1p and Sur7p; null mutants show activation of Pkc1p/Ypk1p stress resistance pathways
CAGL0G03245g	*YKR018C*		
CAGL0F00231g	*YJR077C*	*MIR1*	Mitochondrial phosphate carrier, imports inorganic

			phosphate into mitochondria; functionally redundant with Pic2p but more abundant than Pic2p under normal conditions; phosphorylated
CAGL0L10802g	*YCR045C*	*RRT12*	Putative protein of unknown function; non-essential gene identified in a screen for mutants with decreased levels of rDNA transcription
CAGL0I05148g	*YDL174C*	*DLD1*	D-lactate dehydrogenase, oxidizes D-lactate to pyruvate, transcription is heme-dependent, repressed by glucose, and derepressed in ethanol or lactate; located in the mitochondrial inner membrane
CAGL0H06633g	*YKR097W*	*PCK1*	Phosphoenolpyruvate carboxykinase, key enzyme in gluconeogenesis, catalyzes early reaction in carbohydrate biosynthesis, glucose represses transcription and accelerates mRNA degradation, regulated by Mcm1p and Cat8p, located in the cytosol
CAGL0G04081g	*YLR004C*	*THI73*	Putative plasma membrane permease proposed to be involved in carboxylic acid uptake and repressed by thiamine; substrate of Dbf2p/Mob1p kinase; transcription is altered if mitochondrial dysfunction occurs
CAGL0B03531g	*YCR015C*		
CAGL0F00649g	*YLR248W*	*RCK2*	Protein kinase involved in the response to oxidative and osmotic stress; identified as suppressor of S. pombe cell cycle checkpoint mutations
CAGL0I05214g	*YIL034C*	*CAP2*	Beta subunit of the capping protein (CP) heterodimer (Cap1p and Cap2p) which binds to the barbed ends of actin filaments preventing further polymerization; localized predominantly to cortical actin patches
CAGL0B02695g	*YDR255C*	*RMD5*	Conserved protein that has an E3-like ubiquitin ligase activity necessary for polyubiquitination and degradation of the gluconeogenic enzyme fructose-1,6-bisphosphatase; also required for sporulation; has a degenerate RING finger domain
CAGL0I03696g	*YDL146W*	*LDB17*	Protein involved in the regulation of endocytosis; transiently recruited to actin cortical patches in a SLA1-dependent manner after late coat component assembly; GFP-fusion protein localizes to the periphery, cytoplasm, bud, and bud neck
CAGL0C00671g	*YLR219W*	*MSC3*	Protein of unknown function, green fluorescent protein (GFP)-fusion protein localizes to the cell periphery; msc3 mutants are defective in directing meiotic recombination events to homologous chromatids; potential Cdc28p substrate
CAGL0E02651g	*YEL060C*	*PRB1*	Vacuolar proteinase B (yscB), a serine protease of the subtilisin family; involved in protein degradation in the vacuole and required for full protein degradation during sporulation
CAGL0M13343g	*YHR183W*	*GND1*	6-phosphogluconate dehydrogenase (decarboxylating), catalyzes an NADPH regenerating reaction in the pentose phosphate pathway; required for growth on D-glucono-delta-lactone and adaptation to oxidative stress
CAGL0K04235g	*YLR354C*	*TAL1*	Transaldolase, enzyme in the non-oxidative pentose phosphate pathway; converts sedoheptulose 7-phosphate

			and glyceraldehyde 3-phosphate to erythrose 4-phosphate and fructose 6-phosphate
CAGL0M12969g	*YIL077C*		
CAGL0K03575g	*YMR114C*		
CAGL0H08305g	*YDR051C*	*DET1*	Acid phosphatase involved in the non-vesicular transport of sterols in both directions between the endoplasmic reticulum and plasma membrane; deletion confers sensitivity to nickel
CAGL0I09009g	*YFR025C*	*HIS2*	Histidinolphosphatase, catalyzes the eighth step in histidine biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts; transcription is regulated by general amino acid control
CAGL0I07227g	*YOR136W*	*IDH2*	Subunit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase, which catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the TCA cycle; phosphorylated
CAGL0C03740g	*YEL007W*		
CAGL0K03839g	*YMR130W*		
CAGL0M07634g	*YMR016C*	*SOK2*	Nuclear protein that plays a regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway; negatively regulates pseudohyphal differentiation; homologous to several transcription factors
CAGL0L09086g	*YPR001W*	*CIT3*	Dual specificity mitochondrial citrate and methylcitrate synthase; catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate and that of propionyl-CoA and oxaloacetate to form 2-methylcitrate
CAGL0E05588g	*YOR346W*	*REV1*	Deoxycytidyl transferase, forms a complex with the subunits of DNA polymerase zeta, Rev3p and Rev7p; involved in repair of abasic sites in damaged DNA
CAGL0L04730g	*YGR112W*	*SHY1*	Mitochondrial inner membrane protein required for assembly of cytochrome c oxidase (complex IV); associates with complex IV assembly intermediates and complex III/complex IV supercomplexes; similar to human SURF1 involved in Leigh Syndrome
CAGL0I06424g	*YPR172W*		
CAGL0J00583g	*YHR028C*	*DAP2*	Dipeptidyl aminopeptidase, synthesized as a glycosylated precursor; localizes to the vacuolar membrane; similar to Ste13p
CAGL0C03696g	*YDR089W*		
CAGL0M02299g	*YPL150W*		
CAGL0M07205g	*YHR195W*	*NVJ1*	Nuclear envelope protein, anchored to the nuclear inner membrane, that interacts with the vacuolar membrane protein Vac8p to promote formation of nucleus-vacuole junctions during piecemeal microautophagy of the nucleus (PMN)
CAGL0H02893g	*YJL070C*		

Table 11.6: Genes down regulated uniquely by *C. glabrata* upon hyperosmotic stress treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0K00759g	*YGR211W*	*ZPR1*	Essential protein with two zinc fingers, present in the nucleus of growing cells but relocates to the cytoplasm in starved cells via a process mediated by Cpr1p; binds to translation elongation factor eEF-1 (Tef1p)
CAGL0L10758g	*YMR205C*	*PFK2*	Beta subunit of heterooctameric phosphofructokinase involved in glycolysis, indispensable for anaerobic growth, activated by fructose-2,6-bisphosphate and AMP, mutation inhibits glucose induction of cell cycle-related genes
CAGL0E05390g	*YOR335C*	*ALA1*	Cytoplasmic and mitochondrial alanyl-tRNA synthetase, required for protein synthesis; point mutation (cdc64-1 allele) causes cell cycle arrest at G1; lethality of null mutation is functionally complemented by human homolog
CAGL0G07084g	*YML021C*	*UNG1*	Uracil-DNA glycosylase, required for repair of uracil in DNA formed by spontaneous cytosine deamination, not required for strand-specific mismatch repair, cell-cycle regulated, expressed in late G1, localizes to mitochondria and nucleus
CAGL0C04983g	*YJR105W*	*ADO1*	Adenosine kinase, required for the utilization of S-adenosylmethionine (AdoMet); may be involved in recycling adenosine produced through the methyl cycle
CAGL0G08250g	*YLR244C*	*MAP1*	Methionine aminopeptidase, catalyzes the cotranslational removal of N-terminal methionine from nascent polypeptides; function is partially redundant with that of Map2p
CAGL0K12804g	*YLR074C*	*BUD20*	Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern
CAGL0J04928g	*YJL050W*	*MTR4*	ATP-dependent 3'-5' RNA helicase, involved in nuclear RNA processing and degredation both as a component of the TRAMP complex and in TRAMP independent processes; member of the Dead-box family of helicases
CAGL0L03245g	*YKR038C*	*KAE1*	Highly conserved putative glycoprotease proposed to be involved in transcription as a component of the EKC protein complex with Bud32p, Cgi121p, Pcc1p, and Gon7p; also identified as a component of the KEOPS protein complex
CAGL0K00825g	*YGR208W*	*SER2*	Phosphoserine phosphatase of the phosphoglycerate pathway, involved in serine and glycine biosynthesis, expression is regulated by the available nitrogen source
CAGL0L12760g	*YPL048W*	*CAM1*	Nuclear protein required for transcription of MXR1; binds the MXR1 promoter in the presence of other nuclear factors; binds calcium and phospholipids; has similarity to translational cofactor EF-1 gamma
CAGL0K01793g	*YDL018C*	*ERP3*	Protein with similarity to Emp24p and Erv25p, member of the p24 family involved in ER to Golgi transport

CAGL0J09614g	*YDL208W*	*NHP2*	Nuclear protein related to mammalian high mobility group (HMG) proteins, essential for function of H/ACA-type snoRNPs, which are involved in 18S rRNA processing
CAGL0H08800g	*YPL225W*		
CAGL0H07887g	*YGL234W*	*ADE5,7*	Bifunctional enzyme of the 'de novo' purine nucleotide biosynthetic pathway, contains aminoimidazole ribotide synthetase and glycinamide ribotide synthetase activities
CAGL0H07161g	*YDR317W*	*HIM1*	Protein of unknown function involved in DNA repair
CAGL0A03971g	*YLR200W*	*YKE2*	Subunit of the heterohexameric Gim/prefoldin protein complex involved in the folding of alpha-tubulin, beta-tubulin, and actin
CAGL0L10868g	*YOR271C*	*FSF1*	Putative protein, predicted to be an alpha-isopropylmalate carrier; belongs to the sideroblastic-associated protein family; non-tagged protein is detected in purified mitochondria; likely to play a role in iron homeostasis
CAGL0A00979g	*YLR325C*	*RPL38*	Protein component of the large (60S) ribosomal subunit, has similarity to rat L38 ribosomal protein
CAGL0M08448g	*YKL165C*	*MCD4*	Protein involved in glycosylphosphatidylinositol (GPI) anchor synthesis; multimembrane-spanning protein that localizes to the endoplasmic reticulum; highly conserved among eukaryotes
CAGL0L08756g	*YPL012W*	*RRP12*	Protein required for export of the ribosomal subunits; associates with the RNA components of the pre-ribosomes; contains HEAT-repeats
CAGL0C01661g	*YBR246W*	*RRT2*	Putative protein of unknown function; non-essential gene identified in a screen for mutants with increased levels of rDNA transcription; null mutants display a weak carboxypeptidase Y missorting/secretion phenotype
CAGL0J07458g	*YNL232W*	*CSL4*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; predicted to contain an S1 RNA binding domain; has similarity to human hCsl4p (EXOSC1)
CAGL0K06061g	*YOR167C*	*RPS28A*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps28Bp and has similarity to rat S28 ribosomal protein
CAGL0L08624g	*YFL007W*	*BLM10*	Proteasome activator subunit; found in association with core particles, with and without the 19S regulatory particle; required for resistance to bleomycin, may be involved in protecting against oxidative damage; similar to mammalian PA200
CAGL0M12881g	*NORBH*		
CAGL0L10714g	*YMR202W*	*ERG2*	C-8 sterol isomerase, catalyzes the isomerization of the delta-8 double bond to the delta-7 position at an intermediate step in ergosterol biosynthesis
CAGL0J00649g	*YHR025W*	*THR1*	Homoserine kinase, conserved protein required for threonine biosynthesis; expression is regulated by the GCN4-mediated general amino acid control pathway
CAGL0M09911g	*YLR405W*	*DUS4*	Dihydrouridine synthase, member of a widespread family of conserved proteins including Smm1p, Dus1p, and Dus3p

CAGL0K12848g	*YFL045C*	*SEC53*	Phosphomannomutase, involved in synthesis of GDP-mannose and dolichol-phosphate-mannose; required for folding and glycosylation of secretory proteins in the ER lumen
CAGL0L10560g	*YHR064C*	*SSZ1*	Hsp70 protein that interacts with Zuo1p (a DnaJ homolog) to form a ribosome-associated complex that binds the ribosome via the Zuo1p subunit; also involved in pleiotropic drug resistance via sequential activation of PDR1 and PDR5; binds ATP
CAGL0L07348g	*YDL102W*	*POL3*	Catalytic subunit of DNA polymerase delta; required for chromosomal DNA replication during mitosis and meiosis, intragenic recombination, repair of double strand DNA breaks, and DNA replication during nucleotide excision repair (NER)
CAGL0H07821g	*YGL238W*	*CSE1*	Nuclear envelope protein that mediates the nuclear export of importin alpha (Srp1p), homolog of metazoan CAS protein, required for accurate chromosome segregation
CAGL0H10384g	*YDL111C*	*RRP42*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hRrp42p (EXOSC7)
CAGL0F01705g	*YLR060W*	*FRS1*	Beta subunit of cytoplasmic phenylalanyl-tRNA synthetase, forms a tetramer with Frs2p to generate active enzyme; able to hydrolyze mis-aminoacylated tRNA-Phe, which could contribute to translational quality control
CAGL0B02717g	*YLR153C*	*ACS2*	Acetyl-coA synthetase isoform which, along with Acs1p, is the nuclear source of acetyl-coA for histone acetylation; mutants affect global transcription; required for growth on glucose; expressed under anaerobic conditions
CAGL0I01232g	*YHR110W*	*ERP5*	Protein with similarity to Emp24p and Erv25p, member of the p24 family involved in ER to Golgi transport
CAGL0L02893g	*YOR205CP*		
CAGL0K09570g	*YNL130C*	*CPT1*	Cholinephosphotransferase, required for phosphatidylcholine biosynthesis and for inositol-dependent regulation of EPT1 transcription
CAGL0H02453g	*YMR258C*		
CAGL0K06281g	*YDR454C*	*GUK1*	Guanylate kinase, converts GMP to GDP; required for growth and mannose outer chain elongation of cell wall N-linked glycoproteins
CAGL0J06424g	*YGL201C*	*MCM6*	Protein involved in DNA replication; component of the Mcm2-7 hexameric complex that binds chromatin as a part of the pre-replicative complex
CAGL0L12364g	*YPL028W*	*ERG10*	Acetyl-CoA C-acetyltransferase (acetoacetyl-CoA thiolase), cytosolic enzyme that transfers an acetyl group from one acetyl-CoA molecule to another, forming acetoacetyl-CoA; involved in the first step in mevalonate biosynthesis
CAGL0G08734g	*YGL147C*	*RPL9A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl9Bp and has similarity to E. coli L6 and rat L9 ribosomal proteins
CAGL0B04433g	*YBR021W*	*FUR4*	Uracil permease, localized to the plasma membrane; expression is tightly regulated by uracil levels and

			environmental cues
CAGL0B04961g	*YLR172C*	*DPH5*	Methyltransferase required for synthesis of diphthamide, which is a modified histidine residue of translation elongation factor 2 (Eft1p or Eft2p); not essential for viability; GFP-Dph5p fusion protein localizes to the cytoplasm
CAGL0G02783g	*YIL104C*	*SHQ1*	Chaperone protein required for the assembly of box H/ACA snoRNPs and thus for pre-rRNA processing, forms a complex with Naf1p and interacts with H/ACA snoRNP components Nhp2p and Cbf5p; homology with known Hsp90p cochaperones
CAGL0A01540g	*YGL030W*	*RPL30*	Protein component of the large (60S) ribosomal subunit, has similarity to rat L30 ribosomal protein; involved in pre-rRNA processing in the nucleolus; autoregulates splicing of its transcript
CAGL0G06666g	*YNL102W*	*POL1*	Catalytic subunit of the DNA polymerase I alpha-primase complex, required for the initiation of DNA replication during mitotic DNA synthesis and premeiotic DNA synthesis
CAGL0K00671g	*YJL191W*	*RPS14B*	Ribosomal protein 59 of the small subunit, required for ribosome assembly and 20S pre-rRNA processing; mutations confer cryptopleurine resistance; nearly identical to Rps14Ap and similar to E. coli S11 and rat S14 ribosomal proteins
CAGL0H08712g	*YBR200W*	*BEM1*	Protein containing SH3-domains, involved in establishing cell polarity and morphogenesis; functions as a scaffold protein for complexes that include Cdc24p, Ste5p, Ste20p, and Rsr1p
CAGL0M10681g	*YNR024W*	*MPP6*	Nuclear exosome-associated RNA binding protein; involved in surveillance of pre-rRNAs and pre-mRNAs, and the degradation of cryptic non-coding RNAs (ncRNA); copurifies with ribosomes
CAGL0K01551g	*YDL031W*	*DBP10*	Putative ATP-dependent RNA helicase of the DEAD-box protein family, constituent of 66S pre-ribosomal particles; essential protein involved in ribosome biogenesis
CAGL0L08008g	*NORBH*		
CAGL0M10527g	*YBL004W*	*UTP20*	Component of the small-subunit (SSU) processome, which is involved in the biogenesis of the 18S rRNA
CAGL0M03619g	*YNL292W*	*PUS4*	Pseudouridine synthase, catalyzes only the formation of pseudouridine-55 (Psi55), a highly conserved tRNA modification, in mitochondrial and cytoplasmic tRNAs; PUS4 overexpression leads to translational derepression of GCN4 (Gcd- phenotype)
CAGL0D04576g	*YPR118W*	*MRI1*	5'-methylthioribose-1-phosphate isomerase; catalyzes the isomerization of 5-methylthioribose-1-phosphate to 5-methylthioribulose-1-phosphate in the methionine salvage pathway
CAGL0E00363g	*NA*		
CAGL0F06523g	*YKL078W*	*DHR2*	Predominantly nucleolar DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis
CAGL0F06809g	*YIR026C*	*YVH1*	Protein phosphatase involved in vegetative growth at low temperatures, sporulation, and glycogen accumulation;

			mutants are defective in 60S ribosome assembly; member of the dual-specificity family of protein phosphatases
CAGL0I02354g	*YHR169W*	*DBP8*	ATPase, putative RNA helicase of the DEAD-box family; component of 90S preribosome complex involved in production of 18S rRNA and assembly of 40S small ribosomal subunit; ATPase activity stimulated by association with Esp2p
CAGL0A00627g	*YAR002CA*		
CAGL0D03718g	*YPR041W*	*TIF5*	Translation initiation factor eIF-5; N-terminal domain functions as a GTPase-activating protein to mediate hydrolysis of ribosome-bound GTP; C-terminal domain is the core of ribosomal preinitiation complex formation
CAGL0M00506g	*YJR132W*	*NMD5*	Karyopherin, a carrier protein involved in nuclear import of proteins; importin beta homolog
CAGL0H07425g	*YBR155W*	*CNS1*	TPR-containing co-chaperone; binds both Hsp82p (Hsp90) and Ssa1p (Hsp70) and stimulates the ATPase activity of SSA1, ts mutants reduce Hsp82p function while over expression suppresses the phenotypes of an HSP82 ts allele and a cpr7 deletion
CAGL0M00638g	*YLR449W*	*FPR4*	Peptidyl-prolyl cis-trans isomerase (PPIase) (proline isomerase) localized to the nucleus; catalyzes isomerization of proline residues in histones H3 and H4, which affects lysine methylation of those histones
CAGL0M04323g	*YLR131C*	*ACE2*	Transcription factor that activates expression of early G1-specific genes, localizes to daughter cell nuclei after cytokinesis and delays G1 progression in daughters, localization is regulated by phosphorylation; potential Cdc28p substrate
CAGL0G07106g	*YML022W*	*APT1*	Adenine phosphoribosyltransferase, catalyzes the formation of AMP from adenine and 5-phosphoribosylpyrophosphate; involved in the salvage pathway of purine nucleotide biosynthesis
CAGL0E05566g	*YOR344C*	*TYE7*	Serine-rich protein that contains a basic-helix-loop-helix (bHLH) DNA binding motif; binds E-boxes of glycolytic genes and contributes to their activation; may function as a transcriptional activator in Ty1-mediated gene expression
CAGL0J05786g	*YALI0E27720G*		
CAGL0M04961g	*YMR235C*	*RNA1*	GTPase activating protein (GAP) for Gsp1p, involved in nuclear transport
CAGL0J07920g	*YNL256W*	*FOL1*	Multifunctional enzyme of the folic acid biosynthesis pathway, has dihydropteroate synthetase, dihydro-6-hydroxymethylpterin pyrophosphokinase, and dihydroneopterin aldolase activities
CAGL0G04983g	*YLR363WA*		
CAGL0B01232g	*YLR186W*	*EMG1*	Member of the alpha/beta knot fold methyltransferase superfamily; required for maturation of 18S rRNA and for 40S ribosome production; interacts with RNA and with S-adenosylmethionine; associates with spindle/microtubules; forms homodimers
CAGL0L08602g	*YHR201C*	*PPX1*	Exopolyphosphatase, hydrolyzes inorganic polyphosphate (poly P) into Pi residues; located in the cytosol, plasma

			membrane, and mitochondrial matrix
CAGL0L05566g	*YJL125C*	*GCD14*	Subunit of tRNA (1-methyladenosine) methyltransferase, with Gcd10p, required for the modification of the adenine at position 58 in tRNAs, especially tRNAi-Met; first identified as a negative regulator of GCN4 expression
CAGL0E05698g	*YPL208W*	*RKM1*	SET-domain lysine-N-methyltransferase, catalyzes the formation of dimethyllysine residues on the large ribsomal subunit protein L23a (RPL23A and RPL23B)
CAGL0M13959g	*YMR312W*	*ELP6*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; required for Elongator structural integrity
CAGL0H02673g	*YKR060W*	*UTP30*	Subunit of U3-containing 90S preribosome complex involved in production of 18S rRNA and assembly of small ribosomal subunit
CAGL0J03780g	*YOR239W*	*ABP140*	Nonessential protein that binds actin filaments and localizes to actin patches and cables, has similarity to S-adenosylmethionine (AdoMet)-dependent methyltransferases
CAGL0B04345g	*YBR025C*	*OLA1*	P-loop ATPase with similarity to human OLA1 and bacterial YchF; identified as specifically interacting with the proteasome; protein levels are induced by hydrogen peroxide
CAGL0J01023g	*YJL035C*	*TAD2*	Subunit of tRNA-specific adenosine-34 deaminase, forms a heterodimer with Tad3p that converts adenosine to inosine at the wobble position of several tRNAs
CAGL0I01254g	*YHR111W*	*UBA4*	Protein that activates Urm1p before its conjugation to proteins (urmylation); one target is the thioredoxin peroxidase Ahp1p, suggesting a role of urmylation in the oxidative stress response
CAGL0H07271g	*YDR315C*	*IPK1*	Inositol 1,3,4,5,6-pentakisphosphate 2-kinase, nuclear protein required for synthesis of 1,2,3,4,5,6-hexakisphosphate (phytate), which is integral to cell function; has 2 motifs conserved in other fungi; ipk1 gle1 double mutant is inviable
CAGL0G09317g	*YGR195W*	*SKI6*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hRrp41p (EXOSC4)
CAGL0H09438g	*YER016W*	*BIM1*	Microtubule-binding protein that together with Kar9p makes up the cortical microtubule capture site and delays the exit from mitosis when the spindle is oriented abnormally
CAGL0E04334g	*YHR007C*	*ERG11*	Lanosterol 14-alpha-demethylase, catalyzes the C-14 demethylation of lanosterol to form 4,4"-dimethyl cholesta-8,14,24-triene-3-beta-ol in the ergosterol biosynthesis pathway; member of the cytochrome P450 family
CAGL0M01056g	*YDR339C*	*FCF1*	Putative PINc domain nuclease required for early cleavages of 35S pre-rRNA and maturation of 18S rRNA; component of the SSU (small subunit) processome involved in 40S ribosomal subunit biogenesis; copurifies with Faf1p
CAGL0C01419g	*YPL184C*	*MRN1*	RNA-binding protein proposed to be involved in

			translational regulation; binds specific categories of mRNAs, including those that contain upstream open reading frames (uORFs) and internal ribosome entry sites (IRES)
CAGL0M10912g	*YNR032C-A*	*HUB1*	Ubiquitin-like protein modifier, may function in modification of Sph1p and Hbt1p, functionally complemented by the human or S. pombe ortholog; mechanism of Hub1p adduct formation not yet clear
CAGL0K08382g	*YJL136C*	*RPS21B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps21Ap and has similarity to rat S21 ribosomal protein
CAGL0J07898g	*YNL255C*	*GIS2*	Protein with seven cysteine-rich CCHC zinc-finger motifs, similar to human CNBP, proposed to be involved in the RAS/cAMP signaling pathway
CAGL0L04350g	*YOR159C*	*SME1*	Core Sm protein Sm E; part of heteroheptameric complex (with Smb1p, Smd1p, Smd2p, Smd3p, Smx3p, and Smx2p) that is part of the spliceosomal U1, U2, U4, and U5 snRNPs; homolog of human Sm E
CAGL0F02761g	*YDR408C*	*ADE8*	Phosphoribosyl-glycinamide transformylase, catalyzes a step in the 'de novo' purine nucleotide biosynthetic pathway
CAGL0D06160g	*YGL043W*	*DST1*	General transcription elongation factor TFIIS, enables RNA polymerase II to read through blocks to elongation by stimulating cleavage of nascent transcripts stalled at transcription arrest sites
CAGL0H06193g	*YAL019W*	*FUN30*	Protein whose overexpression affects chromosome stability, potential Cdc28p substrate; homolog of Snf2p; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0B00880g	*YCL031C*	*RRP7*	Essential protein involved in rRNA processing and ribosome biogenesis
CAGL0K07612g	*YKL130C*	*SHE2*	RNA-binding protein that binds specific mRNAs and interacts with She3p; part of the mRNA localization machinery that restricts accumulation of certain proteins to the bud
CAGL0E03223g	*YGR158C*	*MTR3*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hMtr3p (EXOSC6)
CAGL0H02255g	*YMR266W*	*RSN1*	Membrane protein of unknown function; overexpression suppresses NaCl sensitivity of sro7 mutant cells by restoring sodium pump (Ena1p) localization to the plasma membrane
CAGL0G01276g	*YNL050C*		
CAGL0A04125g	*YLR192C*	*HCR1*	Dual function protein involved in translation initiation as a substoichiometric component (eIF3j) of translation initiation factor 3 (eIF3) and required for processing of 20S pre-rRNA; binds to eIF3 subunits Rpg1p and Prt1p and 18S rRNA
CAGL0H00935g	*YPL239W*	*YAR1*	Cytoplasmic ankyrin-repeat containing protein of unknown function, proposed to link the processes of 40S ribosomal subunit biogenesis and adaptation to osmotic and oxidative stress; expression repressed by heat shock

CAGL0M00176g	*YJR148W*	*BAT2*	Cytosolic branched-chain amino acid aminotransferase, homolog of murine ECA39; highly expressed during stationary phase and repressed during logarithmic phase
CAGL0D03454g	*YGR173W*	*RBG2*	Protein with similarity to mammalian developmentally regulated GTP-binding protein
CAGL0M01210g	*YHR085W*	*IPI1*	Essential component of the Rix1 complex (with Rix1p and Ipi3p) that is required for processing of ITS2 sequences from 35S pre-rRNA; Rix1 complex associates with Mdn1p in pre-60S ribosomal particles
CAGL0C00759g	*YLR223C*	*IFH1*	Essential protein with a highly acidic N-terminal domain; IFH1 exhibits genetic interactions with FHL1, overexpression interferes with silencing at telomeres and HM loci; potential Cdc28p substrate
CAGL0H09218g	*YGL224C*	*SDT1*	Pyrimidine nucleotidase; overexpression suppresses the 6-AU sensitivity of transcription elongation factor S-II, as well as resistance to other pyrimidine derivatives
CAGL0G03113g	*YGR095C*	*RRP46*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hRrp46p (EXOSC5)
CAGL0D03300g	*YLR022C*	*SDO1*	Essential protein involved in 60S ribosome maturation; ortholog of the human protein (SBDS) responsible for autosomal recessive Shwachman-Bodian-Diamond Syndrome; highly conserved across archae and eukaryotes
CAGL0K06215g	*YDR449C*	*UTP6*	Nucleolar protein, component of the small subunit (SSU) processome containing the U3 snoRNA that is involved in processing of pre-18S rRNA
CAGL0J09944g	*YNL065W*	*AQR1*	Plasma membrane multidrug transporter of the major facilitator superfamily, confers resistance to short-chain monocarboxylic acids and quinidine; involved in the excretion of excess amino acids
CAGL0G10109g	*YPR190C*	*RPC82*	RNA polymerase III subunit C82
CAGL0G09801g	*YPR175W*	*DPB2*	Second largest subunit of DNA polymerase II (DNA polymerase epsilon), required for normal yeast chromosomal replication; expression peaks at the G1/S phase boundary; potential Cdc28p substrate
CAGL0I04290g	*YGL169W*	*SUA5*	Single-stranded telomeric DNA-binding protein, required for normal telomere length; null mutant lacks N6-threonylcarbamoyl adenosine (t6A) modification in the anticodon loop of ANN-decoding tRNA; member of conserved YrdC/Sua5 family
CAGL0M06369g	*YPL086C*	*ELP3*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; exhibits histone acetyltransferase activity that is directed to histones H3 and H4; disruption confers resistance to K. lactis zymotoxin
CAGL0H07205g	*YHR069C*	*RRP4*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; predicted to contain RNA binding domains; has similarity to human hRrp4p (EXOSC2)
CAGL0D00616g	*YLR406C*	*RPL31B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl31Ap and has similarity to rat L31 ribosomal protein; associates with the karyopherin

			Sxm1p; loss of both Rpl31p and Rpl39p confers lethality
CAGL0A03212g	*YDR384C*	*ATO3*	Plasma membrane protein, regulation pattern suggests a possible role in export of ammonia from the cell; phosphorylated in mitochondria; member of the TC 9.B.33 YaaH family of putative transporters
CAGL0J07722g	*YNL246W*	*VPS75*	NAP family histone chaperone; binds to histones and Rtt109p, stimulating histone acetyltransferase activity; possesses nucleosome assembly activity in vitro; proposed role in vacuolar protein sorting and in double-strand break repair
CAGL0K06033g	*NA*		
CAGL0K04499g	*YGR061C*	*ADE6*	Formylglycinamidine-ribonucleotide (FGAM)-synthetase, catalyzes a step in the 'de novo' purine nucleotide biosynthetic pathway
CAGL0M11154g	*YDR060W*	*MAK21*	Constituent of 66S pre-ribosomal particles, required for large (60S) ribosomal subunit biogenesis; involved in nuclear export of pre-ribosomes; required for maintenance of dsRNA virus; homolog of human CAATT-binding protein
CAGL0E02717g	*YOR006C*	*TSR3*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to both the cytoplasm and the nucleus
CAGL0G05874g	*YHR143W-A*	*RPC10*	RNA polymerase subunit, found in RNA polymerase complexes I, II, and III
CAGL0H05863g	*YPL101W*	*ELP4*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; required for Elongator structural integrity
CAGL0K06567g	*YHR010W*	*RPL27A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl27Bp and has similarity to rat L27 ribosomal protein
CAGL0M13915g	*YMR310C*		
CAGL0G07535g	*YOR294W*	*RRS1*	Essential protein that binds ribosomal protein L11 and is required for nuclear export of the 60S pre-ribosomal subunit during ribosome biogenesis; mouse homolog shows altered expression in Huntington's disease model mice
CAGL0F00627g	*YCL037C*	*SRO9*	Cytoplasmic RNA-binding protein that associates with translating ribosomes; involved in heme regulation of Hap1p as a component of the HMC complex, also involved in the organization of actin filaments; contains a La motif
CAGL0I06490g	*YPR169W*	*JIP5*	Essential protein of unknown function; interacts with proteins involved in RNA processing, ribosome biogenesis, ubiquitination and demethylation; tagged protein localizes to nucleus and nucleolus; similar to WDR55, a human WD repeat protein
CAGL0H09064g	*YHR128W*	*FUR1*	Uracil phosphoribosyltransferase, synthesizes UMP from uracil; involved in the pyrimidine salvage pathway
CAGL0C04301g	*YDR002W*	*YRB1*	Ran GTPase binding protein; involved in nuclear protein import and RNA export, ubiquitin-mediated protein degradation during the cell cycle; shuttles between the nucleus and cytoplasm; is essential; homolog of human

			RanBP1
CAGL0G02167g	*YKR079C*	*TRZ1*	tRNA 3'-end processing endonuclease tRNase Z; also localized to mitochondria and interacts genetically with Rex2 exonuclease; homolog of the human candidate prostate cancer susceptibility gene ELAC2
CAGL0L04928g	*YMR047C*	*NUP116*	Subunit of the nuclear pore complex (NPC) that is localized to both sides of the pore; contains a repetitive GLFG motif that interacts with mRNA export factor Mex67p and with karyopherin Kap95p; homologous to Nup100p
CAGL0B00792g	*YCL037C*	*SRO9*	Cytoplasmic RNA-binding protein that associates with translating ribosomes; involved in heme regulation of Hap1p as a component of the HMC complex, also involved in the organization of actin filaments; contains a La motif
CAGL0K02497g	*YPR016C*	*TIF6*	Constituent of 66S pre-ribosomal particles, has similarity to human translation initiation factor 6 (eIF6); may be involved in the biogenesis and or stability of 60S ribosomal subunits
CAGL0I03366g	*YDL166C*	*FAP7*	Essential NTPase required for small ribosome subunit synthesis, mediates processing of the 20S pre-rRNA at site D in the cytoplasm but associates only transiently with 43S preribosomes via Rps14p, may be the endonuclease for site D
CAGL0M13871g	*YMR308C*	*PSE1*	Karyopherin/importin that interacts with the nuclear pore complex; acts as the nuclear import receptor for specific proteins, including Pdr1p, Yap1p, Ste12p, and Aft1p
CAGL0C03630g	*YNR043W*	*MVD1*	Mevalonate pyrophosphate decarboxylase, essential enzyme involved in the biosynthesis of isoprenoids and sterols, including ergosterol; acts as a homodimer
CAGL0F00913g	*YAL017W*	*PSK1*	One of two (see also PSK2) PAS domain containing S/T protein kinases; coordinately regulates protein synthesis and carbohydrate metabolism and storage in response to a unknown metabolite that reflects nutritional status
CAGL0L08068g	*YCR028C-A*	*RIM1*	Single-stranded DNA-binding protein essential for mitochondrial genome maintenance; involved in mitochondrial DNA replication
CAGL0C03003g	*YCR051W*		
CAGL0L07700g	*YCR014C*	*POL4*	DNA polymerase IV, undergoes pair-wise interactions with Dnl4p-Lif1p and Rad27p to mediate repair of DNA double-strand breaks by non-homologous end joining (NHEJ); homologous to mammalian DNA polymerase beta
CAGL0L08206g	*YCR035C*	*RRP43*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hRrp43p (OIP2, EXOSC8)
CAGL0D00220g	*YAL059W*	*ECM1*	Protein of unknown function, localized in the nucleoplasm and the nucleolus, genetically interacts with MTR2 in 60S ribosomal protein subunit export
CAGL0K09020g	*YOR253W*	*NAT5*	Subunit of the N-terminal acetyltransferase NatA (Nat1p, Ard1p, Nat5p); N-terminally acetylates many proteins, which influences multiple processes such as the cell cycle,

			heat-shock resistance, mating, sporulation, and telomeric silencing
CAGL0I05654g	*YNR012W*	*URK1*	Uridine/cytidine kinase, component of the pyrimidine ribonucleotide salvage pathway that converts uridine into UMP and cytidine into CMP; involved in the pyrimidine deoxyribonucleotide salvage pathway, converting deoxycytidine into dCMP
CAGL0E03289g	*YGR162W*	*TIF4631*	Translation initiation factor eIF4G, subunit of the mRNA cap-binding protein complex (eIF4F) that also contains eIF4E (Cdc33p); associates with the poly(A)-binding protein Pab1p, also interacts with eIF4A (Tif1p); homologous to Tif4632p
CAGL0G02629g	*YIL096C*		
CAGL0K02475g	*YPR017C*	*DSS4*	Guanine nucleotide dissociation stimulator for Sec4p, functions in the post-Golgi secretory pathway; binds zinc, found both on membranes and in the cytosol
CAGL0I04752g	*YBR029C*	*CDS1*	Phosphatidate cytidylyltransferase (CDP-diglyceride synthetase); an enzyme that catalyzes that conversion of CTP + phosphate into diphosphate + CDP-diaclglyerol, a critical step in the synthesis of all major yeast phospholipids
CAGL0M05885g	*YKR063C*	*LAS1*	Essential nuclear protein possibly involved in bud formation and morphogenesis; mutants require the SSD1-v allele for viability
CAGL0K03795g	*YMR128W*	*ECM16*	Essential DEAH-box ATP-dependent RNA helicase specific to the U3 snoRNP, predominantly nucleolar in distribution, required for 18S rRNA synthesis
CAGL0H05093g	*YLR384C*	*IKI3*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; maintains structural integrity of Elongator; homolog of human IKAP, mutations in which cause familial dysautonomia (FD)
CAGL0K05313g	*YPR033C*	*HTS1*	Cytoplasmic and mitochondrial histidine tRNA synthetase; encoded by a single nuclear gene that specifies two messages; efficient mitochondrial localization requires both a presequence and an amino-terminal sequence
CAGL0K01045g	*YGR083C*	*GCD2*	Delta subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a negative regulator of GCN4 expression
CAGL0E05500g	*YOR341W*	*RPA190*	RNA polymerase I subunit; largest subunit of RNA polymerase I
CAGL0G07975g	*YNR038W*	*DBP6*	Essential protein involved in ribosome biogenesis; putative ATP-dependent RNA helicase of the DEAD-box protein family
CAGL0K10472g	*YFR009W*	*GCN20*	Positive regulator of the Gcn2p kinase activity, forms a complex with Gcn1p; proposed to stimulate Gcn2p activation by an uncharged tRNA
CAGL0C03784g	*YIL008W*	*URM1*	Ubiquitin-like protein with weak sequence similarity to ubiquitin; depends on the E1-like activating enzyme Uba4p; molecular function of the Urm1p pathway is unknown, but it is required for normal growth,

			particularly at high temperature
CAGL0H10406g	*YDL112W*	*TRM3*	2'-O-ribose methyltransferase, catalyzes the ribose methylation of the guanosine nucleotide at position 18 of tRNAs
CAGL0F06853g	*YKL021C*	*MAK11*	Protein involved in an early, nucleolar step of 60S ribosomal subunit biogenesis; essential for cell growth and replication of killer M1 dsRNA virus; contains four beta-transducin repeats
CAGL0L12408g	*YPL030W*	*TRM44*	tRNA(Ser) Um(44) 2'-O-methyltransferase; involved in maintaining levels of the tRNA-Ser species tS(CGA) and tS(UGA); conserved among metazoans and fungi but there does not appear to be a homolog in plants; TRM44 is a non-essential gene
CAGL0K06787g	*YBR218C*	*PYC2*	Pyruvate carboxylase isoform, cytoplasmic enzyme that converts pyruvate to oxaloacetate; highly similar to isoform Pyc1p but differentially regulated; mutations in the human homolog are associated with lactic acidosis
CAGL0I09240g	*YBR261C*	*TAE1*	Putative S-adenosylmethionine-dependent methyltransferase of the seven beta-strand family; has a role in protein synthesis; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; YBR261C is not an essential gene
CAGL0L10120g	*YOR048C*	*RAT1*	Nuclear 5' to 3' single-stranded RNA exonuclease, involved in RNA metabolism, including rRNA and snRNA processing as well as mRNA transcription termination
CAGL0B00220g	*YLR186W*	*EMG1*	Member of the alpha/beta knot fold methyltransferase superfamily; required for maturation of 18S rRNA and for 40S ribosome production; interacts with RNA and with S-adenosylmethionine; associates with spindle/microtubules; forms homodimers
CAGL0A04257g	*YER088C*	*DOT6*	Protein involved in rRNA and ribosome biogenesis; binds polymerase A and C motif; subunit of the RPD3L histone deacetylase complex; similar to Tod6p; has chromatin specific SANT domain; involved in telomeric gene silencing and filamentation
CAGL0C03531g	*YNR046W*	*TRM112*	Subunit of tRNA methyltransferase (MTase) complexes in combination with Trm9p and Trm11p; subunit of complex with Mtq2p that methylates Sup45p (eRF1) in the ternary complex eRF1-eRF3-GTP; deletion confers resistance to zymocin
CAGL0L10780g	*YNR016C*	*ACC1*	Acetyl-CoA carboxylase, biotin containing enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA; required for de novo biosynthesis of long-chain fatty acids
CAGL0K11506g	*YKL113C*	*RAD27*	5' to 3' exonuclease, 5' flap endonuclease, required for Okazaki fragment processing and maturation as well as for long-patch base-excision repair; member of the S. pombe RAD2/FEN1 family
CAGL0G09955g	*YPR183W*	*DPM1*	Dolichol phosphate mannose (Dol-P-Man) synthase of the ER membrane, catalyzes the formation of Dol-P-Man from Dol-P and GDP-Man; required for glycosyl phosphatidylinositol membrane anchoring, O mannosylation, and protein glycosylation

CAGL0K08426g	*YGL200C*	*EMP24*	Integral membrane component of endoplasmic reticulum-derived COPII-coated vesicles, which function in ER to Golgi transport
CAGL0H09592g	*YER011W*	*TIR1*	Cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; expression is downregulated at acidic pH and induced by cold shock and anaerobiosis; abundance is increased in cells cultured without shaking
CAGL0I05764g	*YNR009W*	*NRM1*	Transcriptional co-repressor of MBF (MCB binding factor)-regulated gene expression; Nrm1p associates stably with promoters via MBF to repress transcription upon exit from G1 phase
CAGL0H01589g			
CAGL0I00770g	*YMR144W*		
CAGL0L03828g	*YNL111C*	*CYB5*	Cytochrome b5, involved in the sterol and lipid biosynthesis pathways; acts as an electron donor to support sterol C5-6 desaturation
CAGL0B02607g	*YDR232W*	*HEM1*	5-aminolevulinate synthase, catalyzes the first step in the heme biosynthetic pathway; an N-terminal signal sequence is required for localization to the mitochondrial matrix; expression is regulated by Hap2p-Hap3p
CAGL0J00275g	*YDR280W*	*RRP45*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase PH and to human hRrp45p (PM/SCL-75, EXOSC9)
CAGL0E00759g	*YDR173C*	*ARG82*	Inositol polyphosphate multikinase (IPMK), sequentially phosphorylates Ins(1,4,5)P3 to form Ins(1,3,4,5,6)P5; also has diphosphoinositol polyphosphate synthase activity; regulates arginine-, phosphate-, and nitrogen-responsive genes
CAGL0K06919g	*YBR227C*	*MCX1*	Mitochondrial matrix protein; putative ATP-binding chaperone with non-proteolytic function; similar to bacterial ClpX proteins
CAGL0E02255g	*YOL109W*	*ZEO1*	Peripheral membrane protein of the plasma membrane that interacts with Mid2p; regulates the cell integrity pathway mediated by Pkc1p and Slt2p; the authentic protein is detected in a phosphorylated state in highly purified mitochondria
CAGL0H02409g	*YMR260C*	*TIF11*	Translation initiation factor eIF1A, essential protein that forms a complex with Sui1p (eIF1) and the 40S ribosomal subunit and scans for the start codon; C-terminus associates with Fun12p (eIF5B); N terminus interacts with eIF2 and eIF3
CAGL0D05060g	*YGR123C*	*PPT1*	Protein serine/threonine phosphatase with similarity to human phosphatase PP5; present in both the nucleus and cytoplasm; expressed during logarithmic growth; computational analyses suggest roles in phosphate metabolism and rRNA processing
CAGL0F03641g	*YML018C*		
CAGL0M07876g	*YJR024C*	*MDE1*	5'-methylthioribulose-1-phosphate dehydratase; acts in the methionine salvage pathway; potential Smt3p sumoylation substrate; expression downregulated by caspofungin and deletion mutant is caspofungin resistant

CAGL0K09042g	*YOR252W*	*TMA16*	Protein of unknown function that associates with ribosomes
CAGL0A02112g	*YHR019C*	*DED81*	Cytosolic asparaginyl-tRNA synthetase, required for protein synthesis, catalyzes the specific attachment of asparagine to its cognate tRNA
CAGL0L08114g	*NA*		
CAGL0J11748g	*YMR006C*	*PLB2*	Phospholipase B (lysophospholipase) involved in phospholipid metabolism; displays transacylase activity in vitro; overproduction confers resistance to lysophosphatidylcholine
CAGL0F09207g	*YHR208W*	*BAT1*	Mitochondrial branched-chain amino acid aminotransferase, homolog of murine ECA39; highly expressed during logarithmic phase and repressed during stationary phase
CAGL0K07524g	*YKL128C*	*PMU1*	Putative phosphomutase, contains a region homologous to the active site of phosphomutases; overexpression suppresses the histidine auxotrophy of an ade3 ade16 ade17 triple mutant and the temperature sensitivity of a tps2 mutant
CAGL0I05302g	*YIL026C*	*IRR1*	Subunit of the cohesin complex, which is required for sister chromatid cohesion during mitosis and meiosis and interacts with centromeres and chromosome arms, essential for viability
CAGL0C01925g	*YGL246C*	*RAI1*	Nuclear protein that binds to and stabilizes the exoribonuclease Rat1p, required for pre-rRNA processing
CAGL0D06622g	*YLL021W*	*SPA2*	Component of the polarisome, which functions in actin cytoskeletal organization during polarized growth; acts as a scaffold for Mkk1p and Mpk1p cell wall integrity signaling components; potential Cdc28p substrate
CAGL0K10362g	*YOR130C*	*ORT1*	Ornithine transporter of the mitochondrial inner membrane, exports ornithine from mitochondria as part of arginine biosynthesis; human ortholog is associated with hyperammonaemia-hyperornithinaemia-homocitrullinuria (HHH) syndrome
CAGL0G01628g	*YNL035C*		
CAGL0M08118g	*YJL177W*	*RPL17B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl17Ap and has similarity to E. coli L22 and rat L17 ribosomal proteins
CAGL0J02046g	*NORBH*		
CAGL0L11660g	*YOR116C*	*RPO31*	RNA polymerase III subunit C160, part of core enzyme; similar to bacterial beta-prime subunit
CAGL0J04510g	*YLR418C*	*CDC73*	Component of the Paf1p complex that binds to and modulates the activity of RNA polymerases I and II; required for expression of certain genes, modification of some histones, and telomere maintenance
CAGL0E00781g	*YDR172W*	*SUP35*	Translation termination factor eRF3; altered protein conformation creates the [PSI(+)] prion, a dominant cytoplasmically inherited protein aggregate that alters translational fidelity and creates a nonsense suppressor phenotype
CAGL0D00880g	*YDL060W*	*TSR1*	Protein required for processing of 20S pre-rRNA in the

			cytoplasm, associates with pre-40S ribosomal particles
CAGL0D04488g	*YPR116W*	*RRG8*	Putative protein of unknown function, required for mitochondrial genome maintenance; null mutation results in a decrease in plasma membrane electron transport
CAGL0L05676g	*YJL130C*	*URA2*	Bifunctional carbamoylphosphate synthetase (CPSase)-aspartate transcarbamylase (ATCase), catalyzes the first two enzymatic steps in the de novo biosynthesis of pyrimidines; both activities are subject to feedback inhibition by UTP
CAGL0H07249g	*YHR068W*	*DYS1*	Deoxyhypusine synthase, catalyzes formation of deoxyhypusine, the first step in hypusine biosynthesis; triggers posttranslational hypusination of translation elongation factor eIF-5A and regulates its intracellular levels; tetrameric
CAGL0F04323g	*YBL035C*	*POL12*	B subunit of DNA polymerase alpha-primase complex, required for initiation of DNA replication during mitotic and premeiotic DNA synthesis; also functions in telomere capping and length regulation
CAGL0F01683g	*YLR062C*	*BUD28*	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 98% of ORF overlaps the verified gene RPL22A; diploid mutant displays a weak budding pattern phenotype in a systematic assay
CAGL0J01507g	*YMR076C*	*PDS5*	Protein required for establishment and maintenance of sister chromatid condensation and cohesion, colocalizes with cohesin on chromosomes, may function as a protein-protein interaction scaffold; also required during meiosis
CAGL0J10362g	*YGL101W*		
CAGL0L04092g	*YNL123W*	*NMA111*	Serine protease and general molecular chaperone; involved in response to heat stress and promotion of apoptosis; may contribute to lipid homeostasis; sequence similarity to the mammalian Omi/HtrA2 family of serine proteases
CAGL0J00209g	*YGR024C*	*THG1*	tRNAHis guanylyltransferase, adds a guanosine residue to the 5' end of tRNAHis after transcription and RNase P cleavage; couples nuclear division and migration to cell budding and cytokinesis; essential enzyme conserved among eukaryotes
CAGL0K12012g	*YDR037W*	*KRS1*	Lysyl-tRNA synthetase
CAGL0L11176g	*YML060W*	*OGG1*	Mitochondrial glycosylase/lyase that specifically excises 7,8-dihydro-8-oxoguanine residues located opposite cytosine or thymine residues in DNA, repairs oxidative damage to mitochondrial DNA, contributes to UVA resistance
CAGL0C03289g	*YLL048C*	*YBT1*	Transporter of the ATP-binding cassette (ABC) family involved in bile acid transport; similar to mammalian bile transporters
CAGL0H00847g	*YPL244C*	*HUT1*	Protein with a role in UDP-galactose transport to the Golgi lumen, has similarity to human UDP-galactose transporter UGTrel1, exhibits a genetic interaction with S. cerevisiae ERO1
CAGL0F01727g	*YLR059C*	*REX2*	3'-5' RNA exonuclease; involved in 3'-end processing of U4 and U5 snRNAs, 5S and 5.8S rRNAs, and RNase P

			and RNase MRP RNA; localized to mitochondria and null suppresses escape of mtDNA to nucleus in yme1 yme2 mutants; RNase D exonuclease
CAGL0I03234g	*YEL026W*	*SNU13*	RNA binding protein, part of U3 snoRNP involved in rRNA processing, part of U4/U6-U5 tri-snRNP involved in mRNA splicing, similar to human 15.5K protein
CAGL0J02816g	*YER049W*	*TPA1*	Protein of unknown function; interacts with Sup45p (eRF1), Sup35p (eRF3) and Pab1p; has a role in translation termination efficiency, mRNA poly(A) tail length and mRNA stability
CAGL0C01287g	*YIL114C*	*POR2*	Putative mitochondrial porin (voltage-dependent anion channel), related to Por1p but not required for mitochondrial membrane permeability or mitochondrial osmotic stability
CAGL0H10604g	*YNL016W*	*PUB1*	Poly (A)+ RNA-binding protein, abundant mRNP-component protein that binds mRNA and is required for stability of many mRNAs; component of glucose deprivation induced stress granules, involved in P-body-dependent granule assembly
CAGL0H07931g	*YGL232W*	*TAN1*	Putative tRNA acetyltransferase, RNA-binding protein required for the formation of the modified nucleoside N(4)-acetylcytidine in serine and leucine tRNAs but not required for the same modification in 18S rRNA
CAGL0M02453g	*YPL144W*	*POC4*	Component of a heterodimeric Poc4p-Irc25p chaperone involved in assembly of alpha subunits into the 20S proteasome; may regulate formation of proteasome isoforms with alternative subunits under different conditions
CAGL0J03476g	*YCR072C*	*RSA4*	WD-repeat protein involved in ribosome biogenesis; may interact with ribosomes; required for maturation and efficient intra-nuclear transport or pre-60S ribosomal subunits, localizes to the nucleolus
CAGL0E01155g	*YDR156W*	*RPA14*	RNA polymerase I subunit A14
CAGL0B03091g	*YLR353W*	*BUD8*	Protein involved in bud-site selection; diploid mutants display a unipolar budding pattern instead of the wild-type bipolar pattern, and bud at the proximal pole
CAGL0H10252g	*YBR061C*	*TRM7*	2'-O-ribose methyltransferase, methylates the 2'-O-ribose of nucleotides at positions 32 and 34 of the tRNA anticodon loop
CAGL0G07529g	*YJL179W*	*PFD1*	Subunit of heterohexameric prefoldin, which binds cytosolic chaperonin and transfers target proteins to it; involved in the biogenesis of actin and of alpha- and gamma-tubulin
CAGL0J04532g	*YLR419W*		
CAGL0G00748g	*YAR008W*	*SEN34*	Subunit of the tRNA splicing endonuclease, which is composed of Sen2p, Sen15p, Sen34p, and Sen54p; Sen34p contains the active site for tRNA 3' splice site cleavage and has similarity to Sen2p and to Archaeal tRNA splicing endonuclease
CAGL0I03080g	*YEL021W*	*URA3*	Orotidine-5'-phosphate (OMP) decarboxylase, catalyzes the sixth enzymatic step in the de novo biosynthesis of pyrimidines, converting OMP into uridine monophosphate (UMP); converts 5-FOA into 5-fluorouracil, a toxic

			compound
CAGL0L01243g	*YEL042W*	*GDA1*	Guanosine diphosphatase located in the Golgi, involved in the transport of GDP-mannose into the Golgi lumen by converting GDP to GMP after mannose is transferred its substrate
CAGL0G02761g	*YIL103W*	*DPH1*	Protein required, along with Dph2p, Kti11p, Jjj3p, and Dph5p, for synthesis of diphthamide, which is a modified histidine residue of translation elongation factor 2 (Eft1p or Eft2p); may act in a complex with Dph2p and Kti11p
CAGL0K07700g	*YFL023W*	*BUD27*	Protein involved in bud-site selection, nutrient signaling, and gene expression controlled by TOR kinase; diploid mutants show a random budding pattern rather than the wild-type bipolar pattern; plays a role in regulating Ty1 transposition
CAGL0C02475g	*YER030W*	*CHZ1*	Histone chaperone for Htz1p/H2A-H2B dimer; required for the stabilization of the Chz1p-Htz1-H2B complex; has overlapping function with Nap1p; null mutant displays weak sensitivity to MMS and benomyl; contains a highly conserved CHZ motif
CAGL0H04411g	*YOL021C*	*DIS3*	Exosome core complex catalytic subunit; possesses both endonuclease and 3'-5' exonuclease activity; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; has similarity to E. coli RNase R and to human DIS3
CAGL0L03003g	*YKR026C*	*GCN3*	Alpha subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a positive regulator of GCN4 expression
CAGL0K09724g	*YNL135C*	*FPR1*	Peptidyl-prolyl cis-trans isomerase (PPIase), binds to the drugs FK506 and rapamycin; also binds to the nonhistone chromatin binding protein Hmo1p and may regulate its assembly or function
CAGL0K09416g	*YGL211W*	*NCS6*	Protein required for thiolation of the uridine at the wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation and in invasive and pseudohyphal growth; inhibits replication of Brome mosaic virus in S. cerevisiae
CAGL0I05940g	*YJL145W*	*SFH5*	Non-classical phosphatidylinositol transfer protein (PITP); exhibits PI- but not PC-transfer activity; localizes to the peripheral endoplasmic reticulum, cytosol and microsomes; similar to Sec14p
CAGL0I01958g	*YHR154W*	*RTT107*	Protein implicated in Mms22-dependent DNA repair during S phase, DNA damage induces phosphorylation by Mec1p at one or more SQ/TQ motifs; interacts with Mms22p and Slx4p; has four BRCT domains; has a role in regulation of Ty1 transposition
CAGL0H01441g	*YDR300C*	*PRO1*	Gamma-glutamyl kinase, catalyzes the first step in proline biosynthesis
CAGL0C01749g	*YBR242W*		
CAGL0D00550g	*YKL181W*	*PRS1*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes
CAGL0G03729g	*YOR372C*	*NDD1*	Transcriptional activator essential for nuclear division;

			localized to the nucleus; essential component of the mechanism that activates the expression of a set of late-S-phase-specific genes
CAGL0I07997g	*YOL076W*	*MDM20*	Non-catalytic subunit of the NatB N-terminal acetyltransferase, which catalyzes N-acetylation of proteins with specific N-terminal sequences; involved in mitochondrial inheritance and actin assembly
CAGL0M11110g	*YDR087C*	*RRP1*	Essential evolutionarily conserved nucleolar protein necessary for biogenesis of 60S ribosomal subunits and processing of pre-rRNAs to mature rRNAs, associated with several distinct 66S pre-ribosomal particles
CAGL0D03740g	*YHR062C*	*RPP1*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends
CAGL0B03927g	*YJR014W*	*TMA22*	Protein of unknown function; associates with ribosomes and has a putative RNA binding domain; interacts with Tma20p; similar to human GRAP and human DRP1, which interacts with human Tma20p homolog MCT-1
CAGL0I09196g	*YBR259W*		
CAGL0L04356g	*YOR160W*	*MTR10*	Nuclear import receptor, mediates the nuclear localization of proteins involved in mRNA-nucleus export; promotes dissociation of mRNAs from the nucleus-cytoplasm mRNA shuttling protein Npl3p
CAGL0J06006g	*YNL153C*	*GIM3*	Subunit of the heterohexameric cochaperone prefoldin complex which binds specifically to cytosolic chaperonin and transfers target proteins to it
CAGL0E04994g	*YNL067W*	*RPL9B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl9Ap and has similarity to E. coli L6 and rat L9 ribosomal proteins
CAGL0K07656g	*YFL022C*	*FRS2*	Alpha subunit of cytoplasmic phenylalanyl-tRNA synthetase, forms a tetramer with Frs1p to form active enzyme; evolutionarily distant from mitochondrial phenylalanyl-tRNA synthetase based on protein sequence, but substrate binding is similar
CAGL0M11880g	*YCR044C*	*PER1*	Protein of the endoplasmic reticulum, required for GPI-phospholipase A2 activity that remodels the GPI anchor as a prerequisite for association of GPI-anchored proteins with lipid rafts; functionally complemented by human ortholog PERLD1
CAGL0G06226g	*YAL024C*	*LTE1*	Putative GDP/GTP exchange factor required for mitotic exit at low temperatures; acts as a guanine nucleotide exchange factor (GEF) for Tem1p, which is a key regulator of mitotic exit; physically associates with Ras2p-GTP
CAGL0I09438g	*YBR271W*		
CAGL0E02315g	*YOL012C*	*HTZ1*	Histone variant H2AZ, exchanged for histone H2A in nucleosomes by the SWR1 complex; involved in transcriptional regulation through prevention of the spread of silent heterochromatin
CAGL0A01914g	*YHR099W*	*TRA1*	Subunit of SAGA and NuA4 histone acetyltransferase complexes; interacts with acidic activators (e.g., Gal4p) which leads to transcription activation; similar to human TRRAP, which is a cofactor for c-Myc mediated

			oncogenic transformation
CAGL0J10208g	*YNL072W*	*RNH201*	Ribonuclease H2 catalytic subunit, removes RNA primers during Okazaki fragment synthesis; homolog of RNAse HI (the S. cerevisiae homolog of mammalian RNAse HII is RNH1); related to human AGS4 that causes Aicardi-Goutieres syndrome
CAGL0C01221g	*YDR211W*	*GCD6*	Catalytic epsilon subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a negative regulator of GCN4 expression
CAGL0M13717g	*YMR300C*	*ADE4*	Phosphoribosylpyrophosphate amidotransferase (PRPPAT; amidophosphoribosyltransferase), catalyzes first step of the 'de novo' purine nucleotide biosynthetic pathway
CAGL0F02079g	*YFL008W*	*SMC1*	Subunit of the multiprotein cohesin complex, essential protein involved in chromosome segregation and in double-strand DNA break repair; SMC chromosomal ATPase family member, binds DNA with a preference for DNA with secondary structure
CAGL0K10780g	*YML056C*	*IMD4*	Inosine monophosphate dehydrogenase, catalyzes the first step of GMP biosynthesis, member of a four-gene family in S. cerevisiae, constitutively expressed
CAGL0B04851g	*YCL014W*	*BUD3*	Protein involved in bud-site selection and required for axial budding pattern; localizes with septins to bud neck in mitosis and may constitute an axial landmark for next round of budding
CAGL0J06374g	*YDL130W*	*RPP1B*	Ribosomal protein P1 beta, component of the ribosomal stalk, which is involved in interaction of translational elongation factors with ribosome; accumulation is regulated by phosphorylation and interaction with the P2 stalk component
CAGL0L07370g	*YDL103C*	*QRI1*	UDP-N-acetylglucosamine pyrophosphorylase, catalyzes the formation of UDP-N-acetylglucosamine (UDP-GlcNAc), which is important in cell wall biosynthesis, protein N-glycosylation, and GPI anchor biosynthesis
CAGL0C01947g	*YGL245W*	*GUS1*	Glutamyl-tRNA synthetase (GluRS), forms a complex with methionyl-tRNA synthetase (Mes1p) and Arc1p; complex formation increases the catalytic efficiency of both tRNA synthetases and ensures their correct localization to the cytoplasm
CAGL0G02535g	*YKR095W-A*	*PCC1*	Proposed transcription factor involved in the expression of genes regulated by alpha-factor and galactose; component of the EKC/KEOPS protein complex with Kae1p, Gon7p, Bud32p, and Cgi121p; related to human cancer-testis antigens
CAGL0D04114g	*YHR042W*	*NCP1*	NADP-cytochrome P450 reductase; involved in ergosterol biosynthesis; associated and coordinately regulated with Erg11p
CAGL0L11770g	*YER164W*	*CHD1*	Nucleosome remodeling factor that functions in regulation of transcription elongation; contains a chromo domain, a helicase domain and a DNA-binding domain; component of both the SAGA and SLIK complexes
CAGL0G06336g	*YAL029C*	*MYO4*	One of two type V myosin motors (along with MYO2)

			involved in actin-based transport of cargos; required for mRNA transport, including ASH1 mRNA, and facilitating the growth and movement of ER tubules into the growing bud along with She3p
CAGL0K09284g	*YCR057C*	*PWP2*	Conserved 90S pre-ribosomal component essential for proper endonucleolytic cleavage of the 35 S rRNA precursor at A0, A1, and A2 sites; contains eight WD-repeats; PWP2 deletion leads to defects in cell cycle and bud morphogenesis
CAGL0H09658g	*YER009W*	*NTF2*	Nuclear envelope protein, interacts with GDP-bound Gsp1p and with proteins of the nuclear pore to transport Gsp1p into the nucleus where it is an essential player in nucleocytoplasmic transport
CAGL0M13563g	*YMR292W*	*GOT1*	Evolutionarily conserved non-essential protein present in early Golgi cisternae that may be involved in ER-Golgi transport at a step after vesicle tethering to Golgi membranes, exhibits membrane topology similar to that of Sft2p
CAGL0J02266g	*YER007CA*		
CAGL0D05214g	*YFR032C-A*	*RPL29*	Protein component of the large (60S) ribosomal subunit, has similarity to rat L29 ribosomal protein; not essential for translation, but required for proper joining of the large and small ribosomal subunits and for normal translation rate
CAGL0K07854g	*YPR082C*	*DIB1*	17-kDa component of the U4/U6aU5 tri-snRNP, plays an essential role in pre-mRNA splicing, orthologue of hDIM1, the human U5-specific 15-kDa protein
CAGL0M09801g	*YLR285W*	*NNT1*	Putative nicotinamide N-methyltransferase, has a role in rDNA silencing and in lifespan determination
CAGL0I09350g	*YBR267W*	*REI1*	Cytoplasmic pre-60S factor; required for the correct recycling of shuttling factors Alb1, Arx1 and Tif6 at the end of the ribosomal large subunit biogenesis; involved in bud growth in the mitotic signaling network
CAGL0K09966g	*YOR361C*	*PRT1*	eIF3b subunit of the core complex of translation initiation factor 3 (eIF3), essential for translation; part of a subcomplex (Prt1p-Rpg1p-Nip1p) that stimulates binding of mRNA and tRNA(i)Met to ribosomes
CAGL0H00957g	*YPL237W*	*SUI3*	Beta subunit of the translation initiation factor eIF2, involved in the identification of the start codon; proposed to be involved in mRNA binding
CAGL0F06831g	*YIR033W*	*MGA2*	ER membrane protein involved in regulation of OLE1 transcription, acts with homolog Spt23p; inactive ER form dimerizes and one subunit is then activated by ubiquitin/proteasome-dependent processing followed by nuclear targeting
CAGL0L08184g	*YCR034W*	*FEN1*	Fatty acid elongase, involved in sphingolipid biosynthesis; acts on fatty acids of up to 24 carbons in length; mutations have regulatory effects on 1,3-beta-glucan synthase, vacuolar ATPase, and the secretory pathway
CAGL0B01463g	*YOL142W*	*RRP40*	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm; predicted to contain both S1 and KH RNA

			binding domains; has similarity to human hRrp40p (EXOSC3)
CAGL0E05874g	*YPL217C*	*BMS1*	GTPase required for synthesis of 40S ribosomal subunits and for processing the 35S pre-rRNA at sites A0, A1, and A2; interacts with Rcl1p, which stimulates its GTPase and U3 snoRNA binding activities; has similarity to Tsr1p
CAGL0J10802g	*YHR072W-A*	*NOP10*	Constituent of small nucleolar ribonucleoprotein particles containing H/ACA-type snoRNAs, which are required for pseudouridylation and processing of pre-18S rRNA
CAGL0H08415g	*YDR045C*	*RPC11*	RNA polymerase III subunit C11; mediates pol III RNA cleavage activity and is important for termination of transcription; homologous to TFIIS
CAGL0L02799g	*YOR210W*	*RPB10*	RNA polymerase subunit ABC10-beta, common to RNA polymerases I, II, and III
CAGL0M04873g	*YMR230W*	*RPS10B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps10Ap and has similarity to rat ribosomal protein S10
CAGL0L05082g	*YKL077W*		
CAGL0L09669g	*YKL014C*	*URB1*	Nucleolar protein required for the normal accumulation of 25S and 5.8S rRNAs, associated with the 27SA2 pre-ribosomal particle; proposed to be involved in the biogenesis of the 60S ribosomal subunit
CAGL0H07183g	*YHR070W*	*TRM5*	tRNA(m(1)G37)methyltransferase, methylates a tRNA base adjacent to the anticodon that has a role in prevention of frameshifting; highly conserved across Archaea, Bacteria, and Eukarya
CAGL0L10978g	*YOR276W*	*CAF20*	Phosphoprotein of the mRNA cap-binding complex involved in translational control, repressor of cap-dependent translation initiation, competes with eIF4G for binding to eIF4E
CAGL0K09152g	*YOR246C*		
CAGL0I09064g	*YFR028C*	*CDC14*	Protein phosphatase required for mitotic exit; located in the nucleolus until liberated by the FEAR and Mitotic Exit Network in anaphase, enabling it to act on key substrates to effect a decrease in CDK/B-cyclin activity and mitotic exit
CAGL0D01562g	*YPR062W*	*FCY1*	Cytosine deaminase, zinc metalloenzyme that catalyzes the hydrolytic deamination of cytosine to uracil; of biomedical interest because it also catalyzes the deamination of 5-fluorocytosine (5FC) to form anticancer drug 5-fluorouracil (5FU)
CAGL0A00495g	*YGL008C*	*PMA1*	Plasma membrane H+-ATPase, pumps protons out of the cell; major regulator of cytoplasmic pH and plasma membrane potential; part of the P2 subgroup of cation-transporting ATPases
CAGL0J04598g	*YLR420W*	*URA4*	Dihydroorotase, catalyzes the third enzymatic step in the de novo biosynthesis of pyrimidines, converting carbamoyl-L-aspartate into dihydroorotate
CAGL0L03846g	*YNL112W*	*DBP2*	Essential ATP-dependent RNA helicase of the DEAD-box protein family, involved in nonsense-mediated mRNA decay and rRNA processing
CAGL0L04532g	*YOR168W*	*GLN4*	Glutamine tRNA synthetase, monomeric class I tRNA

			synthetase that catalyzes the specific glutaminylation of tRNA(Glu); N-terminal domain proposed to be involved in enzyme-tRNA interactions
CAGL0B01122g	*YLR180W*	*SAM1*	S-adenosylmethionine synthetase, catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine; one of two differentially regulated isozymes (Sam1p and Sam2p)
CAGL0M09086g	*YJR092W*	*BUD4*	Protein involved in bud-site selection and required for axial budding pattern; localizes with septins to bud neck in mitosis and may constitute an axial landmark for next round of budding; potential Cdc28p substrate
CAGL0I07535g	*YOL098C*		
CAGL0H02783g	*YJL076W*	*NET1*	Core subunit of the RENT complex, which is a complex involved in nucleolar silencing and telophase exit; stimulates transcription by RNA polymerase I and regulates nucleolar structure
CAGL0D06336g	*YGL050W*	*TYW3*	tRNA methyltransferase required for synthesis of wybutosine, a modified guanosine found at the 3'-position adjacent to the anticodon of phenylalanine tRNA which supports reading frame maintenance by stabilizing codon-anticodon interactions
CAGL0L08118g	*YCR031C*	*RPS14A*	Ribosomal protein 59 of the small subunit, required for ribosome assembly and 20S pre-rRNA processing; mutations confer cryptopleurine resistance; nearly identical to Rps14Bp and similar to E. coli S11 and rat S14 ribosomal proteins
CAGL0H09372g	*YGR185C*	*TYS1*	Cytoplasmic tyrosyl-tRNA synthetase, required for cytoplasmic protein synthesis; interacts with positions 34 and 35 of the tRNATyr anticodon; mutations in human ortholog YARS are associated with Charcot-Marie-Tooth (CMT) neuropathies
CAGL0M12639g	*YIL064W*	*SEE1*	Protein with a role in intracellular transport; has sequence similarity to S-adenosylmethionine-dependent methyltransferases of the seven beta-strand family
CAGL0J00341g	*YHR047C*	*AAP1*	Arginine/alanine aminopeptidase, overproduction stimulates glycogen accumulation
CAGL0L13156g	*YLR073C*	*RFU1*	Protein that inhibits Doa4p deubiquitinating activity; contributes to ubiquitin homeostasis by regulating the conversion of free ubiquitin chains to ubiquitin monomers by Doa4p; GFP-fusion protein localizes to endosomes
CAGL0L07678g	*YPL266W*	*DIM1*	Essential 18S rRNA dimethylase (dimethyladenosine transferase), responsible for conserved m6(2)Am6(2)A dimethylation in 3'-terminal loop of 18S rRNA, part of 90S and 40S pre-particles in nucleolus, involved in pre-ribosomal RNA processing
CAGL0L01551g	*YML052W*	*SUR7*	Plasma membrane protein that localizes to furrow-like invaginations (MCC patches); component of eisosomes; associated with endocytosis, along with Pil1p and Lsp1p; sporulation and plasma membrane sphingolipid content are altered in mutants
CAGL0J03454g	*YNL021W*	*HDA1*	Putative catalytic subunit of a class II histone deacetylase complex that also contains Hda2p and Hda3p; Hda1p interacts with the Hda2p-Hda3p subcomplex to form an

			active tetramer; deletion increases histone H2B, H3 and H4 acetylation
CAGL0J10406g	*YGL105W*	*ARC1*	Protein that binds tRNA and methionyl- and glutamyl-tRNA synthetases (Mes1p and Gus1p), delivering tRNA to them, stimulating catalysis, and ensuring their localization to the cytoplasm; also binds quadruplex nucleic acids
CAGL0B03795g	*YJR007W*	*SUI2*	Alpha subunit of the translation initiation factor eIF2, involved in the identification of the start codon; phosphorylation of Ser51 is required for regulation of translation by inhibiting the exchange of GDP for GTP
CAGL0C03369g	*YNR053C*	*NOG2*	Putative GTPase that associates with pre-60S ribosomal subunits in the nucleolus and is required for their nuclear export and maturation
CAGL0K06809g	*YBR220C*		
CAGL0J02222g	*YER002W*	*NOP16*	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis
CAGL0J08250g	*YNL273W*	*TOF1*	Subunit of a replication-pausing checkpoint complex (Tof1p-Mrc1p-Csm3p) that acts at the stalled replication fork to promote sister chromatid cohesion after DNA damage, facilitating gap repair of damaged DNA; interacts with the MCM helicase
CAGL0G03003g	*YGR090W*	*UTP22*	Possible U3 snoRNP protein involved in maturation of pre-18S rRNA, based on computational analysis of large-scale protein-protein interaction data
CAGL0A03674g	*YBR141C*		
CAGL0K08294g	*YKR054C*	*DYN1*	Cytoplasmic heavy chain dynein, microtubule motor protein, required for anaphase spindle elongation; involved in spindle assembly, chromosome movement, and spindle orientation during cell division, targeted to microtubule tips by Pac1p
CAGL0M11638g	*YLR107W*	*REX3*	RNA exonuclease; required for maturation of the RNA component of RNase MRP; functions redundantly with Rnh70p and Rex2p in processing of U5 snRNA and RNase P RNA; member of RNase D family of exonucleases
CAGL0F04939g	*YLR274W*	*MCM5*	Component of the hexameric MCM complex, which is important for priming origins of DNA replication in G1 and becomes an active ATP-dependent helicase that promotes DNA melting and elongation when activated by Cdc7p-Dbf4p in S-phase
CAGL0I09328g	*YBR265W*	*TSC10*	3-ketosphinganine reductase, catalyzes the second step in phytosphingosine synthesis, essential for growth in the absence of exogenous dihydrosphingosine or phytosphingosine, member of short chain dehydrogenase/reductase protein family
CAGL0I07667g	*YOL093W*	*TRM10*	tRNA methyltransferase, methylates the N-1 position of guanosine in tRNAs
CAGL0G01782g	*YPR104C*	*FHL1*	Transcriptional activator with similarity to DNA-binding domain of Drosophila forkhead but unable to bind DNA in vitro; required for rRNA processing; isolated as a suppressor of splicing factor prp4

CAGL0D04642g	*YPR120C*	*CLB5*	B-type cyclin involved in DNA replication during S phase; activates Cdc28p to promote initiation of DNA synthesis; functions in formation of mitotic spindles along with Clb3p and Clb4p; most abundant during late G1 phase
CAGL0M07898g	*YLR045C*	*STU2*	Microtubule-associated protein (MAP) of the XMAP215/Dis1 family; regulates microtubule dynamics during spindle orientation and metaphase chromosome alignment; interacts with spindle pole body component Spc72p
CAGL0L04136g	*YNL126W*	*SPC98*	Component of the microtubule-nucleating Tub4p (gamma-tubulin) complex; interacts with Spc110p at the spindle pole body (SPB) inner plaque and with Spc72p at the SPB outer plaque
CAGL0J02948g	*YER060W*	*FCY21*	Putative purine-cytosine permease, very similar to Fcy2p but cannot substitute for its function
CAGL0M00484g	*YJR133W*	*XPT1*	Xanthine-guanine phosphoribosyl transferase, required for xanthine utilization and for optimal utilization of guanine
CAGL0E01353g	*YLR130C*	*ZRT2*	Low-affinity zinc transporter of the plasma membrane; transcription is induced under low-zinc conditions by the Zap1p transcription factor
CAGL0F03861g	*YMR208W*	*ERG12*	Mevalonate kinase, acts in the biosynthesis of isoprenoids and sterols, including ergosterol, from mevalonate
CAGL0L10846g	*YCR047C*	*BUD23*	Methyltransferase, methylates residue G1575 of 18S rRNA; required for rRNA processing and nuclear export of 40S ribosomal subunits independently of methylation activity; diploid mutant displays random budding pattern
CAGL0J04576g	*YKL191W*	*DPH2*	Protein required, along with Dph1p, Kti11p, Jjj3p, and Dph5p, for synthesis of diphthamide, which is a modified histidine residue of translation elongation factor 2 (Eft1p or Eft2p); may act in a complex with Dph1p and Kti11p
CAGL0H09614g	*YER011W*	*TIR1*	Cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; expression is downregulated at acidic pH and induced by cold shock and anaerobiosis; abundance is increased in cells cultured without shaking
CAGL0B02145g	*YHR149C*	*SKG6*	Integral membrane protein that localizes primarily to growing sites such as the bud tip or the cell periphery; potential Cdc28p substrate; Skg6p interacts with Zds1p and Zds2p
CAGL0B03707g	*YJR006W*	*POL31*	DNA polymerase III (delta) subunit, essential for cell viability; involved in DNA replication and DNA repair
CAGL0H07051g	*YDR321W*	*ASP1*	Cytosolic L-asparaginase, involved in asparagine catabolism
CAGL0D04180g	*YIL091C*	*UTP25*	Nucleolar protein of unknown function; proposed to function as an RNA helicase based on structure prediction and remote homology searches; essential for viability
CAGL0M11616g	*YLR106C*	*MDN1*	Huge dynein-related AAA-type ATPase (midasin), forms extended pre-60S particle with the Rix1 complex (Rix1p-Ipi1p-Ipi3p), may mediate ATP-dependent remodeling of 60S subunits and subsequent export from nucleoplasm to cytoplasm
CAGL0B02321g	*YML115C*	*VAN1*	Component of the mannan polymerase I, which contains

			Van1p and Mnn9p and is involved in the first steps of mannan synthesis; mutants are vanadate-resistant
CAGL0G05379g	*YNL199C*	*GCR2*	Transcriptional activator of genes involved in glycolysis; interacts and functions with the DNA-binding protein Gcr1p
CAGL0L11528g	*YHR101C*	*BIG1*	Integral membrane protein of the endoplasmic reticulum, required for normal content of cell wall beta-1,6-glucan
CAGL0L12782g	*NA*		
CAGL0D03190g	*YLR017W*	*MEU1*	Methylthioadenosine phosphorylase (MTAP), catalyzes the initial step in the methionine salvage pathway; affects polyamine biosynthesis through regulation of ornithine decarboxylase (Spe1p) activity; regulates ADH2 gene expression
CAGL0J09086g	*YDL191W*	*RPL35A*	Protein component of the large (60S) ribosomal subunit, identical to Rpl35Bp and has similarity to rat L35 ribosomal protein
CAGL0L11506g	*YML075C*	*HMG1*	One of two isozymes of HMG-CoA reductase that catalyzes the conversion of HMG-CoA to mevalonate, which is a rate-limiting step in sterol biosynthesis; localizes to the nuclear envelope; overproduction induces the formation of karmellae
CAGL0J01485g	*YHL013C*	*OTU2*	Protein of unknown function that may interact with ribosomes, based on co-purification experiments; member of the ovarian tumor-like (OTU) superfamily of predicted cysteine proteases; shows cytoplasmic localization
CAGL0E02101g	*YOL115W*	*PAP2*	Non-canonical poly(A) polymerase, involved in nuclear RNA degradation as a component of the TRAMP complex; catalyzes polyadenylation of hypomodified tRNAs, and snoRNA and rRNA precursors; overlapping but non-redundant functions with Trf5p
CAGL0J06666g	*YML108W*		
CAGL0G03267g	*YBL069W*	*AST1*	Peripheral membrane protein that interacts with the plasma membrane ATPase Pma1p and has a role in its targeting to the plasma membrane, possibly by influencing its incorporation into lipid rafts
CAGL0A04213g	*YBL051C*	*PIN4*	Protein involved in G2/M phase progression and response to DNA damage, interacts with Rad53p; contains an RNA recognition motif, a nuclear localization signal, and several SQ/TQ cluster domains; hyperphosphorylated in response to DNA damage
CAGL0A02090g	*YHR020W*		
CAGL0J06600g	*YML106W*	*URA5*	Major orotate phosphoribosyltransferase (OPRTase) isozyme that catalyzes the fifth enzymatic step in de novo biosynthesis of pyrimidines, converting orotate into orotidine-5'-phosphate; minor OPRTase encoded by URA10
CAGL0M05973g	*YKR048C*	*NAP1*	Protein that interacts with mitotic cyclin Clb2p; required for the regulation of microtubule dynamics during mitosis; controls bud morphogenesis; involved in the transport of H2A and H2B histones to the nucleus; phosphorylated by CK2
CAGL0E00517g	*YCR087CA*		

CAGL0C01199g	*YDR213W*	*UPC2*	Sterol regulatory element binding protein, induces transcription of sterol biosynthetic genes and of DAN/TIR gene products; Ecm22p homolog; relocates from intracellular membranes to perinuclear foci on sterol depletion
CAGL0E02343g	*YOL010W*	*RCL1*	Subunit of U3-containing 90S preribosome processome complex involved in 18S rRNA biogenesis and small ribosomal subunit assembly; stimulates Bms1p GTPase and U3 binding activity; similar to RNA cyclase-like proteins but no activity detected
CAGL0C02585g	*YLR397C*	*AFG2*	ATPase of the CDC48/PAS1/SEC18 (AAA) family, forms a hexameric complex; may be involved in degradation of aberrant mRNAs
CAGL0L03025g	*YKR025W*	*RPC37*	RNA polymerase III subunit C37
CAGL0E01067g	*YDR161W*		
CAGL0H04081g	*YML126C*	*ERG13*	3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, catalyzes the formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA; involved in the second step in mevalonate biosynthesis
CAGL0G05742g	*YDL213C*	*NOP6*	Putative RNA-binding protein implicated in ribosome biogenesis; contains an RNA recognition motif (RRM) and has similarity to hydrophilins; NOP6 may be a fungal-specific gene as no homologs have been yet identified in higher eukaryotes
CAGL0K05049g	*YNL221C*	*POP1*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends; binds to the RPR1 RNA subunit in RNase P
CAGL0M03773g	*YNL300W*	*TOS6*	Glycosylphosphatidylinositol-dependent cell wall protein, expression is periodic and decreases in respone to ergosterol perturbation or upon entry into stationary phase; depletion increases resistance to lactic acid
CAGL0D01606g	*YPR058W*	*YMC1*	Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis; member of the mitochondrial carrier (MCF) family; has similarity with Ymc2p
CAGL0M02871g	*YJL186W*	*MNN5*	Alpha-1,2-mannosyltransferase, responsible for addition of the second alpha-1,2-linked mannose of the branches on the mannan backbone of oligosaccharides, localizes to an early Golgi compartment
CAGL0J07436g	*YNL231C*	*PDR16*	Phosphatidylinositol transfer protein (PITP) controlled by the multiple drug resistance regulator Pdr1p, localizes to lipid particles and microsomes, controls levels of various lipids, may regulate lipid synthesis, homologous to Pdr17p
CAGL0F00187g	*YMR319C*	*FET4*	Low-affinity Fe(II) transporter of the plasma membrane
CAGL0E05434g	*YOR337W*	*TEA1*	Ty1 enhancer activator required for full levels of Ty enhancer-mediated transcription; C6 zinc cluster DNA-binding protein
CAGL0H08019g	*YNL207W*	*RIO2*	Essential serine kinase involved in the processing of the 20S pre-rRNA into mature 18S rRNA; has similarity to Rio1p

CAGL0K00957g	*YGR200C*	*ELP2*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; target of Kluyveromyces lactis zymocin
CAGL0L12804g	*YPL050C*	*MNN9*	Subunit of Golgi mannosyltransferase complex also containing Anp1p, Mnn10p, Mnn11p, and Hoc1p that mediates elongation of the polysaccharide mannan backbone; forms a separate complex with Van1p that is also involved in backbone elongation
CAGL0M02783g	*YPL127C*	*HHO1*	Histone H1, a linker histone required for nucleosome packaging at restricted sites; suppresses DNA repair involving homologous recombination; not required for telomeric silencing, basal transcriptional repression, or efficient sporulation
CAGL0I05588g	*YNR015W*	*SMM1*	Dihydrouridine synthase, member of a family of dihydrouridine synthases including Dus1p, Smm1p, Dus3p, and Dus4p; modifies uridine residues at position 20 of cytoplasmic tRNAs
CAGL0L08096g	*YJL186W*	*MNN5*	Alpha-1,2-mannosyltransferase, responsible for addition of the second alpha-1,2-linked mannose of the branches on the mannan backbone of oligosaccharides, localizes to an early Golgi compartment
CAGL0H04807g	*YML014W*	*TRM9*	tRNA methyltransferase, catalyzes esterification of modified uridine nucleotides in tRNA(Arg3) and tRNA(Glu), likely as part of a complex with Trm112p; deletion confers resistance to zymocin
CAGL0E06644g	*NA*		
CAGL0A04323g	*YBL057C*	*PTH2*	One of two (see also PTH1) mitochondrially-localized peptidyl-tRNA hydrolases; negatively regulates the ubiquitin-proteasome pathway via interactions with ubiquitin-like ubiquitin-associated proteins; dispensable for cell growth
CAGL0B01397g	*YOL144W*	*NOP8*	Nucleolar protein required for 60S ribosomal subunit biogenesis
CAGL0K02937g	*YKL027W*		
CAGL0D04884g	*YPR137W*	*RRP9*	Protein involved in pre-rRNA processing, associated with U3 snRNP; component of small ribosomal subunit (SSU) processosome; ortholog of the human U3-55k protein
CAGL0C05181g	*YOL061W*	*PRS5*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes
CAGL0L05170g	*YKL082C*	*RRP14*	Essential protein, constituent of 66S pre-ribosomal particles; interacts with proteins involved in ribosomal biogenesis and cell polarity; member of the SURF-6 family
CAGL0B00330g	*YCL061C*	*MRC1*	S-phase checkpoint protein required for DNA replication; interacts with and stabilizes Pol2p at stalled replication forks during stress, where it forms a pausing complex with Tof1p and is phosphorylated by Mec1p; protects uncapped telomeres
CAGL0F00319g	*YJR074W*	*MOG1*	Conserved nuclear protein that interacts with GTP-Gsp1p, which is a Ran homolog of the Ras GTPase family, and stimulates nucleotide release, involved in nuclear protein

			import, nucleotide release is inhibited by Yrb1p
CAGL0A04169g	*YLR190W*	*MMR1*	Phosphorylated protein of the mitochondrial outer membrane, localizes only to mitochondria of the bud; interacts with Myo2p to mediate mitochondrial distribution to buds; mRNA is targeted to the bud via the transport system involving She2p
CAGL0M05797g	*YGL063W*	*PUS2*	Mitochondrial tRNA:pseudouridine synthase; acts at positions 27 and 28, but not at position 72; efficiently and rapidly targeted to mitochondria, specifically dedicated to mitochondrial tRNA modification
CAGL0G08085g	*YDR093W*	*DNF2*	Aminophospholipid translocase (flippase) that localizes primarily to the plasma membrane; contributes to endocytosis, protein transport and cell polarity; type 4 P-type ATPase
CAGL0I04444g	*YAR015W*	*ADE1*	N-succinyl-5-aminoimidazole-4-carboxamide ribotide (SAICAR) synthetase, required for 'de novo' purine nucleotide biosynthesis; red pigment accumulates in mutant cells deprived of adenine
CAGL0L09581g	*YBR252W*	*DUT1*	dUTPase, catalyzes hydrolysis of dUTP to dUMP and PPi, thereby preventing incorporation of uracil into DNA during replication; critical for the maintenance of genetic stability
CAGL0M03091g	*YJL087C*	*TRL1*	tRNA ligase, required for tRNA splicing; composed of three essential domains containing the phosphodiesterase, polynucleotide kinase, and ligase activities required for ligation; localized at the inner membrane of the nuclear envelope
CAGL0M06589g	*YDR020C*	*DAS2*	Putative protein of unknown function; non-essential gene identified in a screen for mutants with increased levels of rDNA transcription; weak similarity with uridine kinases and with phosphoribokinases
CAGL0H08734g	*YPR043W*	*RPL43A*	Protein component of the large (60S) ribosomal subunit, identical to Rpl43Bp and has similarity to rat L37a ribosomal protein; null mutation confers a dominant lethal phenotype
CAGL0I07645g	*YOL094C*	*RFC4*	Subunit of heteropentameric Replication factor C (RF-C), which is a DNA binding protein and ATPase that acts as a clamp loader of the proliferating cell nuclear antigen (PCNA) processivity factor for DNA polymerases delta and epsilon
CAGL0K02387g	*YGL171W*	*ROK1*	ATP-dependent RNA helicase of the DEAD box family; required for 18S rRNA synthesis
CAGL0A00385g	*YGL016W*	*KAP122*	Karyopherin beta, responsible for import of the Toa1p-Toa2p complex into the nucleus; binds to nucleoporins Nup1p and Nup2p; may play a role in regulation of pleiotropic drug resistance
CAGL0F00429g	*YJR069C*	*HAM1*	Conserved protein with deoxyribonucleoside triphosphate pyrophosphohydrolase activity, mediates exclusion of noncanonical purines from deoxyribonucleoside triphosphate pools; mutant is sensitive to the base analog 6-N-hydroxylaminopurine
CAGL0I05500g	*YER099C*	*PRS2*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide,

			histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes
CAGL0M05775g	*YPL211W*	*NIP7*	Nucleolar protein required for 60S ribosome subunit biogenesis, constituent of 66S pre-ribosomal particles; physically interacts with Nop8p and the exosome subunit Rrp43p
CAGL0D02860g	*YLL033W*	*IRC19*	Putative protein of unknown function; YLL033W is not an essential gene but mutant is defective in spore formation; null mutant displays increased levels of spontaneous Rad52p foci

Table 11.7: GO terms associated with genes commonly up regulated by *S. cerevisiae* and *C. glabrata*.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Cellular response to oxidative stress	$5.38\ e^{-8}$	*UGA2 YDL124W YPR1 GRX2 HSP12 GRE3 YJR096W MCR1 GAD1 GCY1*	10	67
Response to stress	$5.89\ e^{-8}$	*SSA3 TPS1 NTH1 TPS2 HSP42 HSP78 HSP31 HSP12 GRE3 XBP1 MNN4 HSP104 DDR48 ATH1*	14	152
Trehalose biosynthetic process	$1.74\ e^{-6}$	*TPS1 TPS2 UGP1 PGM2*	4	7

Table 11.8: GO terms associated with genes commonly down regulated by *S. cerevisiae* and *C. glabrata*.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Ribosome biogenesis	$1.00\ e^{-14}$	*RIO1 PNO1 NOC2 PUS7 YTM1 RRP36 NOP58 NOP4 NOG1 NAN1 NOP53 RRP15 NOC4*	89	170
Translation	$7.14\ e^{-10}$	*MAK16 FUN12 RBG1 ILS1 RPG1 GRS1 SUP45 RPL21A RPS11A RLI1 RPL42A SSB2 CDC33*	33	318

Table 11.9: GO terms associated with genes uniquely up regulated by *S. cerevisiae*.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Protein kinase activity	6.77 e^{-5}	*CDC28 CDC7 VHS1 SPS1 PKP2 FMP48 IKS1 PTK2 PAN3 TDA1 NPR1 PKH2 CKA2*	13	126
Cellular response to oxidative stress	0.000662	*PRX1 GRX7 TRR1 MTL1 DOT5 MSN2 NCE103 OXR1*	8	67
NADPH regeneration	0.000777	*YMR315W ALD4 ALD6*	3	7

Table 11.10: GO terms associated with genes uniquely down regulated by *S. cerevisiae*

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Structural constituent of ribosome	1.00 e^{-14}	*MAK16 RPL4A RPS9B RPP1A RPS16B RPS13 RPP2B RPL12A RPS2*	28	218
Translation	1.00 e^{-14}	*EFB1 MAK16 RPL4A RPS9B RPP1A RPS16B RPS9A RPS6A CDC60*	31	318

Table 11.11: GO terms associated with genes uniquely up regulated by *C. glabrata*.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Proline biosynthetic process	0.001178	*PRO1 PUT2 PRO2*	3	5
Protein dephosphorylation	8.41 e^{-5}	*PTC3 PTC6 PTC1 GIP2 PTP3 HAL5 PPZ1 MIH1 MSG5*	9	38
Peroxisome	0.000402	*PEX22 LDH1 PEX5 CTA1 PEX14 PEX18 TES1 PEX13 MLS1*	11	66

Table 11.12: GO terms associated with genes uniquely down regulated by *C. glabrata*.

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
RNA binding	1.62 e^{-10}	*PIN4 SRO9 RRP43 DBP10 RRP42 TRM3 NHP2 NOP6 RRP45 PRO1 RRP12 MRN1*	51	337
Sterol biosynthetic process	3.12 e^{-6}	*UPC2 ERG11 NCP1 HMG1 ERG13 ERG2 ERG12*	10	29
Ergosterol biosynthetic process	0.000243	*ERG11 NCP1 HMG1 ERG13 ERG2 ERG12 ERG10*	7	23

Figure 11.3: Protein sequence comparisons of the glycerol-3-dehydrogenases in *C. glabrata*. Protein sequences were obtained from Génolevures **[25]** and sequence comparisons conducted using ClustalW **[113]**. Colours denote amino acid properties: RED - Small and hydrophobic, BLUE – acidic, MAGENTA – basic, GREEN – Hydroxyl, sulfhydryl or amine. Symbols denote: * (asterisk) - identical amino acid, **:** (colon) – conserved substitution (same group), **.** (period) – semi-conserved substitution (group with similar properties).

Figure 11.4: Syntenic context of *GPD1* and *GPD2*. Using YGOB **[29]**, the syntenic context of the glycerol-3-dehydrogenases, GPD1 and GPD2 in *S. cerevisiae* were compared to their homologues in *C. glabrata*.

```
CAGL0J04202g      MSDAGRKNFSDKLNEGLTPDSQKSTWDKGKEFVTDETDKLAGKFQGEENKGVAQGMHDSA 60
YFL014W           MSDAGRKGFGEKASEALKPDSQKSYAEQGKEYITDKADKVAGKVQPEDNKGVFQGVHDSA 60
                  *******.*.:* .*.*.******  ::***::**::**:***.* *:**** **:****

CAGL0J04202g      QKGADEAN--AESYADTAREYMDAAKSKLNDAVEYVSKSVHGGEK---- 103
YFL014W           EKGKDNAEGQGESLADQARDYMGAAKSKLNDAVEYVSGRVHGEEDPTKK 109
                  :** *:*:  .** ** **:**.**************  *** *.
```

Figure 11.5: Sequence comparisons of *C. glabrata* CAGL0J04202g and *S. cerevisiae* Hsp12. Protein sequences were obtained from Génolevures **[25]** and sequence comparisons conducted using ClustalW **[113]**. Colours denote amino acid properties: RED - Small and hydrophobic, BLUE – acidic, MAGENTA – basic, GREEN – Hydroxyl, sulfhydryl or amine. Symbols denote: * (asterisk) - identical amino acid, **:** (colon) – conserved substitution (same group), **.** (period) – semi-conserved substitution (group with similar properties).

```
CAGL0H02563g.aa   MKVSNVVLGAIAIA-AVNAQNASNHSSSSKNAANALP-ANNAYTAGVAGAAVAGALAFLI 58
YMR251W-A         MKLSQVVVSAVAFTGLVSAANSSN-SSSSKNAAQPIAGLNNGKVAGAAGVALAGALAFLI 59
                  **:*:**:.*:*::  *.* *:** ********:.:.  **. .**.**.*:********
```

Figure 11.6: Sequence comparisons of *C. glabrata* CAGL0H02563g and *S. cerevisiae* Hor7. Protein sequences were obtained from Génolevures **[25]** and sequence comparisons conducted using ClustalW **[113]**. Colours denote amino acid properties: RED - Small and hydrophobic, MAGENTA – basic, GREEN – Hydroxyl, sulfhydryl or amine. Symbols denote: * (asterisk) - identical amino acid, **:** (colon) – conserved substitution (same group), **.** (period) – semi-conserved substitution (group with similar properties).

```
CAGL0H02563g.aa   MKVSNVVLGAIAIAAVNAQNASNHSSSSKNAAN--------ALPANNAYTAGVAGAAVAG 52
YDR524C-B         MQFKTIVAAFATVAAVQAANVSTNGSNRTNGSNTTSTKISTGAAASNALGAGVFGAAVAA 60
                  *:...:* .  ::***:* *.*.:.*. .*.:*        . .*.**  *** *****.

CAGL0H02563g.aa   ALAFLI 58
YDR524C-B         GVAFLF 66
                  .:***:
```

Figure 11.7: Sequence comparisons of *C. glabrata* CAGL0H02563g and *S. cerevisiae* YDR524C-B. Protein sequences were obtained from Génolevures **[25]** and sequence comparisons conducted using ClustalW **[113]**. Colours denote amino acid properties: RED - Small and hydrophobic, MAGENTA – basic, GREEN – Hydroxyl, sulfhydryl or amine. Symbols denote: * (asterisk) - identical amino acid, **:** (colon) – conserved substitution (same group), **.** (period) – semi-conserved substitution (group with similar properties).

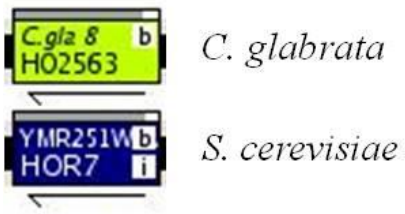

Figure 11.8: Syntenic context of *HOR7* and *CAGL0H02563g.* Using YGOB **[29]**, the syntenic context of the *HOR7* in *S. cerevisiae* was compared to *CAGL0H02563g* in *C. glabrata*, confirming its homology.

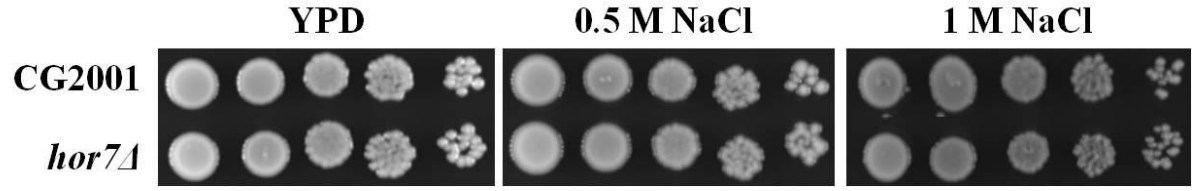

Figure 11.9: Phenotypic screening of wild type *C. glabrata* and *hor7* to hyperosmotic stress. Each strain was serial diluted and spotted on to media containing the indicated stress. Pictures were taken after 2 days. Representative of two technical and two biological replicates.

Table 11.13: Non-homologous and functionally unknown genes up regulated upon hyperosmotic stress treatment by *C. glabrata*.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Description**
CAGL0J11550g	*YNL195C*	Protein of unknown function with similarity to globins
CAGL0H03289g	*YGL082W*	Putative protein of unknown function
CAGL0L10582g	*YMR196W*	Putative protein of unknown function
CAGL0M09339g	*YBL107C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0J01331g	*YMR090W*	Protein of unknown function with similarity to succinate dehydrogenase cytochrome b subunit
CAGL0A01694g	*YGL036W*	Putative protein of unknown function, conserved in fungi
CAGL0C04543g	*YJR096W*	Putative protein of unknown function
CAGL0I08151g	*YALI0E33297G*	
CAGL0G07062g	*YML020W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0C04785g	*YJR115W*	Putative protein of unknown function
CAGL0H02563g		
CAGL0I02046g	*YPR127W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0K04763g		
CAGL0K04719g	*YNL208W*	Cysteinyl-tRNA synthetase
CAGL0G07645g		
CAGL0L06248g	*YBR085C-A*	
CAGL0J05324g	*YJL068C*	Putative protein of unknown function

CAGL0D05104g		
CAGL0K04939g	*YNL217W*	Cysteinyl-tRNA synthetase
CAGL0M12474g	*YIL055C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0J11616g		
CAGL0C03674g	*YNR040W*	Putative zinc-cluster protein of unknown function
CAGL0F06919g	*YIR035C*	Putative protein of unknown function
CAGL0C01771g	*YBR241C*	Putative protein of unknown function
CAGL0G03773g	*YLL023C*	Putative protein of unknown function with similarity to Pip2p, an oleate-specific transcriptional activator of peroxisome proliferation
CAGL0M02915g		
CAGL0J05390g		
CAGL0H02541g	*YMR252C*	Putative protein of unknown function
CAGL0I05610g	*YNR014W*	Protein that interacts specifically in vivo with phospholipid translocase (flippase) Dnf3p
CAGL0J05962g	*YNL155W*	Cell wall protein of unknown function
CAGL0H08261g	*YOR019W*	Putative protein of unknown function
CAGL0D01254g		
CAGL0D03938g	*YHR035W*	Protein of unknown function that may interact with ribosomes, based on co-purification experiments
CAGL0K04631g	*YGR067C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0J07084g	*YPL113C*	Putative protein kinase of unknown cellular role
CAGL0K12958g	*YML131W*	Putative protein of unknown function with similarity to human PEX5Rp (peroxin protein 5 related protein)
CAGL0I01276g	*YHR112C*	Protein of unknown function required for establishment of sister chromatid cohesion
CAGL0J08481g	*YDR506C*	Protein of unknown function that localizes to the nuclear side of the spindle pole body and along short spindles
CAGL0K09218g	*YCR061W*	Putative protein of unknown function
CAGL0A00341g	*YGL010W*	Putative protein of unknown function
CAGL0A01892g		
CAGL0A03410g		
CAGL0B01727g	*YDR109C*	Putative protein of unknown function
CAGL0M11000g	*YNR034W-A*	
CAGL0J01397g	*YMR087W*	Putative protein of unknown function
CAGL0K09702g	*YNL134C*	Putative protein of unknown function, contains DHHC domain, also predicted to have thiol-disulfide oxidoreductase active site
CAGL0G06886g	*YJL017W*	Minor succinate dehydrogenase isozyme
CAGL0E02981g	*YGR149W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0G05544g		
CAGL0C00451g	*YBR137W*	Putative protein of unknown function

CAGL0K08228g	*YKR051W*	Protein of unconfirmed function
CAGL0D00990g	*YDL057W*	Putative protein of unknown function
CAGL0M03839g	*YNL305C*	Putative protein of unknown function
CAGL0M08206g	*YJL171C*	Putative protein of unknown function
CAGL0F02717g		
CAGL0L03938g	*YNL115C*	Putative protein of unknown function with similarity to dehydrogenases from other model organisms
CAGL0F04191g	*YBL029CA*	
CAGL0I07887g		
CAGL0I02794g	*YOR114W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0K04037g		
CAGL0I04180g		
CAGL0E04548g	*YOR020W-A*	
CAGL0I10582g	*YGR127W*	Putative protein of unknown function
CAGL0G06446g		
CAGL0M05467g	*YBR204C*	Putative ion transporter, similar to mammalian electroneutral Na(+)-(K+)-C1- cotransporter family
CAGL0H00781g	*YPL247C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0G06006g	*YHR138C*	Putative protein of unknown function
CAGL0B01595g		
CAGL0H01837g		
CAGL0M09647g	*YMR155W*	Putative protein of unknown function
CAGL0H07337g		
CAGL0H03311g		
CAGL0K03663g		
CAGL0B03615g		
CAGL0D01276g		
CAGL0H05951g	*YPL107W*	Putative protein of unknown function
CAGL0K02629g	*YNL134C*	Putative protein of unknown function, contains DHHC domain, also predicted to have thiol-disulfide oxidoreductase active site
CAGL0A02002g	*YFR017C*	Putative mitochondrial transport protein
CAGL0K01639g	*YDL027C*	Putative protein of unknown function
CAGL0G05357g	*YNL200C*	Protein of unknown function with similarity to globins
CAGL0J09284g	*YDL129W*	Putative protein of unknown function
CAGL0L09251g		
CAGL0G05962g	*YHR140W*	Putative protein of unknown function
CAGL0B03443g	*YLR253W*	Putative protein of unknown function
CAGL0J04004g	*YOR228C*	Protein of unknown function required for establishment of sister chromatid cohesion
CAGL0L02607g	*YHR202W*	Putative protein of unknown function

CAGL0L09207g	*YPR003C*	Putative transporter, member of the mitochondrial carrier family
CAGL0K02805g	*Y797*	
CAGL0J08613g	*YOR088W*	Zinc-regulated transcription factor, protein phosphatase involved in vegetative growth at low temperatures, sporulation, and glycogen accumulation
CAGL0G06182g	*YHR131C*	Putative protein of unknown function
CAGL0M08426g	*YJL163C*	Putative protein of unknown function, predicted to encode a triose phosphate transporter subfamily member based on phylogenetic analysis
CAGL0M07007g	*YCR076C*	Putative protein of unknown function
CAGL0M12793g	*YER079W*	Putative protein of unknown function
CAGL0L00473g	*YMR187C*	Putative protein of unknown function
CAGL0D01270g		
CAGL0G04477g		
CAGL0J04906g	*YJL049W*	Putative protein of unknown function with similarity to AMP deaminases
CAGL0J06270g	*YDL176W*	Putative transporter, member of the sugar porter family
CAGL0G08338g	*YLR241W*	Putative protein of unknown function
CAGL0H00682g	*YMR196W*	Putative protein of unknown function
CAGL0J09394g	*YDL124W*	Putative protein of unknown function
CAGL0H08151g		
CAGL0K07205g		
CAGL0H02519g	*YMR253C*	Putative protein of unknown function
CAGL0H10120g	*YBR056W*	Putative metalloprotease
CAGL0E03498g		
CAGL0M09229g	*YJR085C*	Putative protein of unknown function
CAGL0K00231g	*YKL215C*	Putative protein of unknown function
CAGL0G03245g	*YKR018C*	Putative protein of unknown function
CAGL0B03531g	*YCR015C*	Putative protein of unknown function
CAGL0M04763g	*YOR289W*	Protein of unknown function
CAGL0L05720g	*YJL132W*	Putative protein of unknown function
CAGL0M12969g	*YIL077C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0K03575g	*YMR114C*	Protein of unknown function
CAGL0K03839g	*YMR130W*	Predicted transporter of the mitochondrial inner membrane
CAGL0I06424g	*YPR172W*	Putative protein of unknown function with similarity to telomere-encoded helicases
CAGL0C03696g	*YDR089W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0M02299g	*YPL150W*	Putative protein of unknown function, predicted to be palmitoylated
CAGL0H02893g	*YJL070C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0B03817g	*YJR008W*	Retrotransposon TYA Gag and TYB Pol genes

Table 11.14: Non-homologous and functionally unknown genes down regulated upon hyperosmotic stress treatment by *C. glabrata*.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Description**
CAGL0F05115g	*YLR413W*	Putative helicase with limited sequence similarity to human Rb protein
CAGL0H08800g	*YPL225W*	Putative protein of unknown function
CAGL0M12881g		
CAGL0L02893g	*YOR205CP*	
CAGL0H02453g	*YMR258C*	Protein with NADP(H) oxidoreductase activity
CAGL0F04103g	*YBL028C*	3'-->5' exonuclease and endonuclease with a possible role in apoptosis
CAGL0L08008g		
CAGL0E00363g		
CAGL0A00627g	*YAR002CA*	
CAGL0J05786g	*YALI0E27720G*	
CAGL0G04983g	*YLR363WA*	
CAGL0D00836g	*YDL063C*	Putative lipase
CAGL0G01276g	*YNL050C*	TyB Gag-Pol protein
CAGL0A02189g	*YDR341C*	Putative protein of unknown function
CAGL0K06033g		
CAGL0M13915g	*YMR310C*	Putative protein of unknown function with similarity to phosphoserine phosphatases
CAGL0C03003g	*YCR051W*	Putative protein of unknown function
CAGL0E01573g	*YNL313C*	Putative protein of unknown function, deletion confers reduced fitness in saline
CAGL0L10164g	*YOR051C*	Protein of unknown function required for cell viability
CAGL0G02629g	*YIL096C*	Putative protein of unknown function
CAGL0H01589g		
CAGL0I00770g	*YMR144W*	Predicted transporter of the mitochondrial inner membrane
CAGL0F03641g	*YML018C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0L08114g		
CAGL0M04719g	*YOR287C*	Protein of unknown function
CAGL0G01628g	*YNL035C*	Retrotransposon TYA Gag gene co-transcribed with TYB Pol
CAGL0J02046g		
CAGL0I08547g	*YER156C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0J10362g	*YGL101W*	Putative protein of unknown function
CAGL0L07832g	*YCR016W*	Protein of unknown function
CAGL0J04532g	*YLR419W*	Putative protein of unknown function with similarity to hexokinases
CAGL0M06941g	*YNL022C*	Putative protein of unknown function with strong similarity to alanyl-tRNA synthases from Eubacteria

CAGL0J07744g	*YNL247W*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0C01749g	*YBR242W*	Putative protein of unknown function
CAGL0I09196g	*YBR259W*	Putative protein of unknown function
CAGL0I09438g	*YBR271W*	Putative protein of unknown function
CAGL0J02266g	*YER007CA*	
CAGL0L05082g	*YKL077W*	Putative protein of unknown function
CAGL0K09152g	*YOR246C*	Phosphatase with some similarity to GPM1/YKL152C, a phosphoglycerate mutase
CAGL0I07535g	*YOL098C*	Retrotransposon TYA Gag and TYB Pol genes
CAGL0A03674g	*YBR141C*	Serine hydrolase
CAGL0H02431g	*YMR259C*	Protein with NADP(H) oxidoreductase activity
CAGL0L12782g		
CAGL0J06666g	*YML108W*	Putative protein of unknown function with similarity to helicases
CAGL0A02090g	*YHR020W*	Putative protein of unknown function
CAGL0E00517g	*YCR087CA*	
CAGL0E01067g	*YDR161W*	Putative protein of unknown function
CAGL0E06644g		
CAGL0K02937g	*YKL027W*	Methionine-R-sulfoxide reductase, reduces the R enantiomer of free Met-SO

Descriptions taken from SGD **[45].**

12 Appendix III

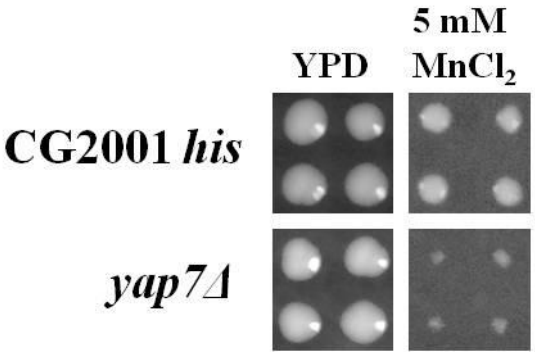

Figure 12.1: Sensitivity of Wild Type and *yap7* mutants to $MnCl_2$. Each strain was spotted four times in a square on to media containing the indicated stress. Pictures were taken after 2 days (YPD) and 4 days ($MnCl_2$). Representative of two technical and two biological replicates.

Table 12.1: Genes up regulated by oxidative stress and macrophage engulfment

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0B01507g	*YOL140W*	*ARG8*	Acetylornithine aminotransferase, catalyzes the fourth step in the biosynthesis of the arginine precursor ornithine
CAGL0I09724g	*YOR306C*	*MCH5*	Plasma membrane riboflavin transporter; facilitates the uptake of vitamin B2; required for FAD-dependent processes; sequence similarity to mammalian monocarboxylate permeases, however mutants are not deficient in monocarboxylate transport
CAGL0L04664g	*YOR176W*	*HEM15*	Ferrochelatase, a mitochondrial inner membrane protein, catalyzes the insertion of ferrous iron into protoporphyrin IX, the eighth and final step in the heme biosynthetic pathway
CAGL0L11902g	*YER170W*	*ADK2*	Mitochondrial adenylate kinase, catalyzes the reversible synthesis of GTP and AMP from GDP and ADP; may serve as a back-up for synthesizing GTP or ADP depending on metabolic conditions; 3' sequence of ADK2 varies with strain background
CAGL0H10142g	*YDR035W*	*ARO3*	3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, catalyzes the first step in aromatic amino acid biosynthesis and is feedback-inhibited by phenylalanine or high concentration of tyrosine or tryptophan
CAGL0F07359g	*YGL117W*		
CAGL0M05533g	*YBR208C*	*DUR1,2*	Urea amidolyase, contains both urea carboxylase and allophanate hydrolase activities, degrades urea to CO2 and NH3; expression sensitive to nitrogen catabolite repression and induced by allophanate, an intermediate in allantoin degradation
CAGL0M12551g	*YIL057C*	*RGI2*	Putative protein of unknown function; expression induced under carbon limitation and repressed under high glucose
CAGL0G02563g	*YKR098C*	*UBP11*	Ubiquitin-specific protease that cleaves ubiquitin from ubiquitinated proteins
CAGL0G05269g	*YDR070C*	*FMP16*	Putative protein of unknown function; proposed to be involved in responding to conditions of stress; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0C04785g	*YJR115W*		
CAGL0F05071g	*YLR284C*	*ECI1*	Peroxisomal delta3,delta2-enoyl-CoA isomerase, hexameric protein that converts 3-hexenoyl-CoA to trans-2-hexenoyl-CoA, essential for the beta-oxidation of unsaturated fatty acids, oleate-induced
CAGL0K10868g	*YDR256C*	*CTA1*	Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1p) during fatty acid beta-oxidation
CAGL0F06875g	*YIR034C*	*LYS1*	Saccharopine dehydrogenase (NAD+, L-lysine-forming), catalyzes the conversion of saccharopine to L-lysine, which is the final step in the lysine biosynthesis pathway
CAGL0F04521g	*YBL043W*	*ECM13*	Non-essential protein of unknown function; induced by

			treatment with 8-methoxypsoralen and UVA irradiation
CAGL0E01815g	*YLR120C*	*YPS1*	Aspartic protease, attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
CAGL0G06732g	*YNL104C*	*LEU4*	Alpha-isopropylmalate synthase (2-isopropylmalate synthase); the main isozyme responsible for the first step in the leucine biosynthesis pathway
CAGL0D05280g	*YFR030W*	*MET10*	Subunit alpha of assimilatory sulfite reductase, which converts sulfite into sulfide
CAGL0I09108g	*YNL125C*	*ESBP6*	Protein with similarity to monocarboxylate permeases, appears not to be involved in transport of monocarboxylates such as lactate, pyruvate or acetate across the plasma membrane
CAGL0M14047g	*YMR318C*	*ADH6*	NADPH-dependent medium chain alcohol dehydrogenase with broad substrate specificity; member of the cinnamyl family of alcohol dehydrogenases; may be involved in fusel alcohol synthesis or in aldehyde tolerance
CAGL0K12958g	*YML131W*		
CAGL0J06402g	*YDL131W*	*LYS21*	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p
CAGL0A03740g	*YGL205W*	*POX1*	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway; localized to the peroxisomal matrix
CAGL0C03443g	*YNR050C*	*LYS9*	Saccharopine dehydrogenase (NADP+, L-glutamate-forming); catalyzes the formation of saccharopine from alpha-aminoadipate 6-semialdehyde, the seventh step in lysine biosynthesis pathway; exhibits genetic and physical interactions with TRM112
CAGL0L05434g	*YKR042W*	*UTH1*	Mitochondrial outer membrane and cell wall localized SUN family member required for mitochondrial autophagy; involved in the oxidative stress response, life span during starvation, mitochondrial biogenesis, and cell death
CAGL0F03399g	*YMR272C*	*SCS7*	Sphingolipid alpha-hydroxylase, functions in the alpha-hydroxylation of sphingolipid-associated very long chain fatty acids, has both cytochrome b5-like and hydroxylase/desaturase domains, not essential for growth
CAGL0J00561g	*YHR029C*	*YHI9*	Protein of unknown function; null mutant is defective in unfolded protein response; possibly involved in a membrane regulation metabolic pathway; member of the PhzF superfamily, though most likely not involved in phenazine production
CAGL0H08844g	*YMR173W*	*DDR48*	DNA damage-responsive protein, expression is increased in response to heat-shock stress or treatments that produce DNA lesions; contains multiple repeats of the amino acid sequence NNNDSYGS
CAGL0M04675g	*YOR285W*	*RDL1*	Protein of unknown function, localized to the mitochondrial outer membrane
CAGL0E04356g	*YHR008C*	*SOD2*	Mitochondrial superoxide dismutase, protects cells against oxygen toxicity; phosphorylated
CAGL0L09273g	*YPR006C*	*ICL2*	2-methylisocitrate lyase of the mitochondrial matrix, functions in the methylcitrate cycle to catalyze the conversion of 2-

			methylisocitrate to succinate and pyruvate; ICL2 transcription is repressed by glucose and induced by ethanol
CAGL0C05115g	*YOL058W*	*ARG1*	Arginosuccinate synthetase, catalyzes the formation of L-argininosuccinate from citrulline and L-aspartate in the arginine biosynthesis pathway; potential Cdc28p substrate
CAGL0I08987g	*YHR018C*	*ARG4*	Argininosuccinate lyase, catalyzes the final step in the arginine biosynthesis pathway
CAGL0F03267g	*YDR326C*	*YSP2*	Protein involved in programmed cell death; mutant shows resistance to cell death induced by amiodarone or intracellular acidification
CAGL0H02585g	*YMR250W*	*GAD1*	Glutamate decarboxylase, converts glutamate into gamma-aminobutyric acid (GABA) during glutamate catabolism; involved in response to oxidative stress
CAGL0F02101g	*YFL007W*	*BLM10*	Proteasome activator subunit; found in association with core particles, with and without the 19S regulatory particle; required for resistance to bleomycin, may be involved in protecting against oxidative damage; similar to mammalian PA200
CAGL0M03971g	*YNL311C*		
CAGL0G04741g	*YNL104C*	*LEU4*	Alpha-isopropylmalate synthase (2-isopropylmalate synthase); the main isozyme responsible for the first step in the leucine biosynthesis pathway
CAGL0K02629g	*YNL134C*		
CAGL0A02002g	*YFR017C*		
CAGL0L09251g	*NORBH*		
CAGL0J04004g	*YOR228C*		
CAGL0L00759g	*YER055C*	*HIS1*	ATP phosphoribosyltransferase, a hexameric enzyme, catalyzes the first step in histidine biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts; transcription is regulated by general amino acid control
CAGL0G02739g	*YIL101C*	*XBP1*	Transcriptional repressor that binds to promoter sequences of the cyclin genes, CYS3, and SMF2; expression is induced by stress or starvation during mitosis, and late in meiosis; member of the Swi4p/Mbp1p family; potential Cdc28p substrate
CAGL0I08305g	*YER024W*	*YAT2*	Carnitine acetyltransferase; has similarity to Yat1p, which is a carnitine acetyltransferase associated with the mitochondrial outer membrane
CAGL0D02134g	*YMR115W*	*MGR3*	Subunit of the mitochondrial (mt) i-AAA protease supercomplex, which degrades misfolded mitochondrial proteins; forms a subcomplex with Mgr1p that binds to substrates to facilitate proteolysis; required for growth of cells lacking mtDNA
CAGL0H02101g	*YHR087W*	*RTC3*	Protein of unknown function involved in RNA metabolism; has structural similarity to SBDS, the human protein mutated in Shwachman-Diamond Syndrome (the yeast SBDS ortholog = SDO1); null mutation suppresses cdc13-1 temperature sensitivity
CAGL0K07788g	*YBR115C*	*LYS2*	Alpha aminoadipate reductase, catalyzes the reduction of

			alpha-aminoadipate to alpha-aminoadipate 6-semialdehyde, which is the fifth step in biosynthesis of lysine; activation requires posttranslational phosphopantetheinylation by Lys5p
CAGL0E03762g	*YHL027W*	*RIM101*	Transcriptional repressor involved in response to pH and in cell wall construction; required for alkaline pH-stimulated haploid invasive growth and sporulation; activated by proteolytic processing; similar to A. nidulans PacC
CAGL0J09240g	*YDL131W*	*LYS21*	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p
CAGL0K12254g	*YBR105C*	*VID24*	Peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulates fructose-1,6-bisphosphatase (FBPase) targeting to the vacuole; promotes proteasome-dependent catabolite degradation of FBPase
CAGL0K11616g	*YKL120W*	*OAC1*	Mitochondrial inner membrane transporter, transports oxaloacetate, sulfate, thiosulfate, and isopropylmalate; member of the mitochondrial carrier family
CAGL0C01243g	*YIL116W*	*HIS5*	Histidinol-phosphate aminotransferase, catalyzes the seventh step in histidine biosynthesis; responsive to general control of amino acid biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts
CAGL0C01595g	*YBR248C*	*HIS7*	Imidazole glycerol phosphate synthase (glutamine amidotransferase:cyclase), catalyzes the fifth and sixth steps of histidine biosynthesis and also produces 5-aminoimidazole-4-carboxamide ribotide (AICAR), a purine precursor
CAGL0L00649g	*YAL054C*	*ACS1*	Acetyl-coA synthetase isoform which, along with Acs2p, is the nuclear source of acetyl-coA for histone acetlyation; expressed during growth on nonfermentable carbon sources and under aerobic conditions
CAGL0E05962g	*YPL222W*	*FMP40*	Putative protein of unknown function; proposed to be involved in responding to environmental stresses; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0F07029g	*YGL125W*	*MET13*	Major isozyme of methylenetetrahydrofolate reductase, catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in the methionine biosynthesis pathway
CAGL0H06633g	*YKR097W*	*PCK1*	Phosphoenolpyruvate carboxykinase, key enzyme in gluconeogenesis, catalyzes early reaction in carbohydrate biosynthesis, glucose represses transcription and accelerates mRNA degradation, regulated by Mcm1p and Cat8p, located in the cytosol
CAGL0M04763g	*YOR289W*		
CAGL0K03465g	*YMR108W*	*ILV2*	Acetolactate synthase, catalyses the first common step in isoleucine and valine biosynthesis and is the target of several classes of inhibitors, localizes to the mitochondria; expression of the gene is under general amino acid control
CAGL0M07634g	*YMR016C*	*SOK2*	Nuclear protein that plays a regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway; negatively regulates pseudohyphal differentiation;

			homologous to several transcription factors
CAGL0A00363g	*YGL009C*	*LEU1*	Isopropylmalate isomerase, catalyzes the second step in the leucine biosynthesis pathway
CAGL0L06138g	*YGL186C*	*TPN1*	Plasma membrane pyridoxine (vitamin B6) transporter; member of the purine-cytosine permease subfamily within the major facilitator superfamily; proton symporter with similarity to Fcy21p, Fcy2p, and Fcy22p
CAGL0J10846g	*YHR071W*	*PCL5*	Cyclin, interacts with and phosphorylated by Pho85p cyclin-dependent kinase (Cdk), induced by Gcn4p at level of transcription, specifically required for Gcn4p degradation, may be sensor of cellular protein biosynthetic capacity

Descriptions taken from SGD **[45]**.

Table 12.2: Genes down regulated by oxidative stress and macrophage engulfment

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0J09614g	*YDL208W*	*NHP2*	Nuclear protein related to mammalian high mobility group (HMG) proteins, essential for function of H/ACA-type snoRNPs, which are involved in 18S rRNA processing
CAGL0G04499g	*YJL105W*	*SET4*	Protein of unknown function, contains a SET domain
CAGL0M12881g	*NORBH*		
CAGL0L10714g	*YMR202W*	*ERG2*	C-8 sterol isomerase, catalyzes the isomerization of the delta-8 double bond to the delta-7 position at an intermediate step in ergosterol biosynthesis
CAGL0B04433g	*YBR021W*	*FUR4*	Uracil permease, localized to the plasma membrane; expression is tightly regulated by uracil levels and environmental cues
CAGL0J00869g	*YKL144C*	*RPC25*	RNA polymerase III subunit C25, required for transcription initiation; forms a heterodimer with Rpc17p; paralog of Rpb7p
CAGL0G03443g	*YER110C*	*KAP123*	Karyopherin beta, mediates nuclear import of ribosomal proteins prior to assembly into ribosomes and import of histones H3 and H4; localizes to the nuclear pore, nucleus, and cytoplasm; exhibits genetic interactions with RAI1
CAGL0F06809g	*YIR026C*	*YVH1*	Protein phosphatase involved in vegetative growth at low temperatures, sporulation, and glycogen accumulation; mutants are defective in 60S ribosome assembly; member of the dual-specificity family of protein phosphatases
CAGL0G07106g	*YML022W*	*APT1*	Adenine phosphoribosyltransferase, catalyzes the formation of AMP from adenine and 5-phosphoribosylpyrophosphate; involved in the salvage pathway of purine nucleotide biosynthesis
CAGL0D05940g	*YGR175C*	*ERG1*	Squalene epoxidase, catalyzes the epoxidation of squalene to 2,3-oxidosqualene; plays an essential role in the ergosterol-biosynthesis pathway and is the specific target of the antifungal drug terbinafine
CAGL0C02343g	*YER036C*	*ARB1*	ATPase of the ATP-binding cassette (ABC) family involved in 40S and 60S ribosome biogenesis, has similarity to Gcn20p; shuttles from nucleus to cytoplasm, physically interacts with Tif6p, Lsg1p
CAGL0B02475g	*YML123C*	*PHO84*	High-affinity inorganic phosphate (Pi) transporter and low-affinity manganese transporter; regulated by Pho4p and Spt7p; mutation confers resistance to arsenate; exit from the ER during maturation requires Pho86p
CAGL0G00154g	*YGR285C*	*ZUO1*	Cytosolic ribosome-associated chaperone that acts, together with Ssz1p and the Ssb proteins, as a chaperone for nascent polypeptide chains; contains a DnaJ domain and functions as a J-protein partner for Ssb1p and Ssb2p
CAGL0E04334g	*YHR007C*	*ERG11*	Lanosterol 14-alpha-demethylase, catalyzes the C-14 demethylation of lanosterol to form 4,4"-dimethyl cholesta-8,14,24-triene-3-beta-ol in the ergosterol biosynthesis pathway; member of the cytochrome P450 family
CAGL0J00385g	*YHR049W*	*FSH1*	Putative serine hydrolase that localizes to both the nucleus and

			cytoplasm; sequence is similar to S. cerevisiae Fsh2p and Fsh3p and the human candidate tumor suppressor OVCA2
CAGL0F02563g	*YDR399W*	*HPT1*	Dimeric hypoxanthine-guanine phosphoribosyltransferase, catalyzes the formation of both inosine monophosphate and guanosine monophosphate; mutations in the human homolog HPRT1 can cause Lesch-Nyhan syndrome and Kelley-Seegmiller syndrome
CAGL0E05522g	*YOR342C*		
CAGL0K01859g	*YDL014W*	*NOP1*	Nucleolar protein, component of the small subunit processome complex, which is required for processing of pre-18S rRNA; has similarity to mammalian fibrillarin
CAGL0L11132g	*YML058W*	*SML1*	Ribonucleotide reductase inhibitor involved in regulating dNTP production; regulated by Mec1p and Rad53p during DNA damage and S phase
CAGL0L03872g	*YNL113W*	*RPC19*	RNA polymerase subunit, common to RNA polymerases I and III
CAGL0H09064g	*YHR128W*	*FUR1*	Uracil phosphoribosyltransferase, synthesizes UMP from uracil; involved in the pyrimidine salvage pathway
CAGL0B00792g	*YCL037C*	*SRO9*	Cytoplasmic RNA-binding protein that associates with translating ribosomes; involved in heme regulation of Hap1p as a component of the HMC complex, also involved in the organization of actin filaments; contains a La motif
CAGL0C03630g	*YNR043W*	*MVD1*	Mevalonate pyrophosphate decarboxylase, essential enzyme involved in the biosynthesis of isoprenoids and sterols, including ergosterol; acts as a homodimer
CAGL0J01848g	*YPR010C*	*RPA135*	RNA polymerase I subunit A135
CAGL0B02794g	*YLR359W*	*ADE13*	Adenylosuccinate lyase, catalyzes two steps in the 'de novo' purine nucleotide biosynthetic pathway; expression is repressed by adenine and activated by Bas1p and Pho2p; mutations in human ortholog ADSL cause adenylosuccinase deficiency
CAGL0J04070g	*YOR224C*	*RPB8*	RNA polymerase subunit ABC14.5, common to RNA polymerases I, II, and III
CAGL0M11066g	*YGR109C*	*CLB6*	B-type cyclin involved in DNA replication during S phase; activates Cdc28p to promote initiation of DNA synthesis; functions in formation of mitotic spindles along with Clb3p and Clb4p; most abundant during late G1
CAGL0B00220g	*YLR186W*	*EMG1*	Member of the alpha/beta knot fold methyltransferase superfamily; required for maturation of 18S rRNA and for 40S ribosome production; interacts with RNA and with S-adenosylmethionine; associates with spindle/microtubules; forms homodimers
CAGL0L03828g	*YNL111C*	*CYB5*	Cytochrome b5, involved in the sterol and lipid biosynthesis pathways; acts as an electron donor to support sterol C5-6 desaturation
CAGL0E02255g	*YOL109W*	*ZEO1*	Peripheral membrane protein of the plasma membrane that interacts with Mid2p; regulates the cell integrity pathway mediated by Pkc1p and Slt2p; the authentic protein is detected in a phosphorylated state in highly purified mitochondria
CAGL0B04125g	*YPR110C*	*RPC40*	RNA polymerase subunit, common to RNA polymerase I and

			III
CAGL0A04037g	*YLR196W*	*PWP1*	Protein with WD-40 repeats involved in rRNA processing; associates with trans-acting ribosome biogenesis factors; similar to beta-transducin superfamily
CAGL0F03927g	*YMR217W*	*GUA1*	GMP synthase, an enzyme that catalyzes the second step in the biosynthesis of GMP from inosine 5'-phosphate (IMP); transcription is not subject to regulation by guanine but is negatively regulated by nutrient starvation
CAGL0K00781g	*YGR210C*		
CAGL0I03234g	*YEL026W*	*SNU13*	RNA binding protein, part of U3 snoRNP involved in rRNA processing, part of U4/U6-U5 tri-snRNP involved in mRNA splicing, similar to human 15.5K protein
CAGL0J03476g	*YCR072C*	*RSA4*	WD-repeat protein involved in ribosome biogenesis; may interact with ribosomes; required for maturation and efficient intra-nuclear transport or pre-60S ribosomal subunits, localizes to the nucleolus
CAGL0I03080g	*YEL021W*	*URA3*	Orotidine-5'-phosphate (OMP) decarboxylase, catalyzes the sixth enzymatic step in the de novo biosynthesis of pyrimidines, converting OMP into uridine monophosphate (UMP); converts 5-FOA into 5-fluorouracil, a toxic compound
CAGL0L03003g	*YKR026C*	*GCN3*	Alpha subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a positive regulator of GCN4 expression
CAGL0K10780g	*YML056C*	*IMD4*	Inosine monophosphate dehydrogenase, catalyzes the first step of GMP biosynthesis, member of a four-gene family in S. cerevisiae, constitutively expressed
CAGL0E00979g	*YDR165W*	*TRM82*	Subunit of a tRNA methyltransferase complex composed of Trm8p and Trm82p that catalyzes 7-methylguanosine modification of tRNA
CAGL0H00440g	*YJR124C*		
CAGL0M06457g	*YBR187W*	*GDT1*	Putative protein of unknown function; expression is reduced in a gcr1 null mutant; GFP-fusion protein localizes to the vacuole; expression pattern and physical interactions suggest a possible role in ribosome biogenesis
CAGL0B01012g	*YCL025C*	*AGP1*	Low-affinity amino acid permease with broad substrate range, involved in uptake of asparagine, glutamine, and other amino acids; expression is regulated by the SPS plasma membrane amino acid sensor system (Ssy1p-Ptr3p-Ssy5p)
CAGL0M05599g	*YBR162C*	*TOS1*	Covalently-bound cell wall protein of unknown function; identified as a cell cycle regulated SBF target gene; deletion mutants are highly resistant to treatment with beta-1,3-glucanase; has sequence similarity to YJL171C
CAGL0L13156g	*YLR073C*	*RFU1*	Protein that inhibits Doa4p deubiquitinating activity; contributes to ubiquitin homeostasis by regulating the conversion of free ubiquitin chains to ubiquitin monomers by Doa4p; GFP-fusion protein localizes to endosomes
CAGL0H05137g	*YPL061W*	*ALD6*	Cytosolic aldehyde dehydrogenase, activated by Mg2+ and utilizes NADP+ as the preferred coenzyme; required for conversion of acetaldehyde to acetate; constitutively expressed;

			locates to the mitochondrial outer surface upon oxidative stress
CAGL0J02948g	*YER060W*	*FCY21*	Putative purine-cytosine permease, very similar to Fcy2p but cannot substitute for its function
CAGL0G02409g	*YKR092C*	*SRP40*	Nucleolar, serine-rich protein with a role in preribosome assembly or transport; may function as a chaperone of small nucleolar ribonucleoprotein particles (snoRNPs); immunologically and structurally to rat Nopp140
CAGL0I07799g	*YBR154C*	*RPB5*	RNA polymerase subunit ABC27, common to RNA polymerases I, II, and III; contacts DNA and affects transactivation
CAGL0K12034g	*YDR040C*	*ENA1*	P-type ATPase sodium pump, involved in Na+ and Li+ efflux to allow salt tolerance
CAGL0G02189g	*YKR080W*	*MTD1*	NAD-dependent 5,10-methylenetetrahydrafolate dehydrogenase, plays a catalytic role in oxidation of cytoplasmic one-carbon units; expression is regulated by Bas1p and Bas2p, repressed by adenine, and may be induced by inositol and choline
CAGL0H04081g	*YML126C*	*ERG13*	3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, catalyzes the formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA; involved in the second step in mevalonate biosynthesis
CAGL0K02541g	*YHL011C*	*PRS3*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes
CAGL0M12122g	*YAL036C*	*RBG1*	Member of the DRG family of GTP-binding proteins; associates with translating ribosomes; interacts with Tma46p, Ygr250cp, Gir2p and Yap1p via two-hybrid
CAGL0L09581g	*YBR252W*	*DUT1*	dUTPase, catalyzes hydrolysis of dUTP to dUMP and PPi, thereby preventing incorporation of uracil into DNA during replication; critical for the maintenance of genetic stability
CAGL0C02211g	*YEL040W*	*UTR2*	Chitin transglycosylase that functions in the transfer of chitin to beta(1-6) and beta(1-3) glucans in the cell wall; similar to and functionally redundant with Crh1; glycosylphosphatidylinositol (GPI)-anchored protein localized to bud neck
CAGL0I05500g	*YER099C*	*PRS2*	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes

Descriptions taken from SGD **[45]**.

Table 12.3: Genes dependent on *YAP1* in *S. cerevisiae* and *C. glabrata*.

C. glabrata Systematic Name	*S. cerevisiae* Systematic Name	*S. cerevisiae* Standard Name	*S. cerevisiae* Description
CAGL0J01331g	*YMR090W*		
CAGL0M11682g	*YLR108C*		
CAGL0J07612g	*YNL241C*	*ZWF1*	Glucose-6-phosphate dehydrogenase (G6PD), catalyzes the first step of the pentose phosphate pathway; involved in adapting to oxidatve stress; homolog of the human G6PD which is deficient in patients with hemolytic anemia
CAGL0I01408g	*YJR048W*	*CYC1*	Cytochrome c, isoform 1; electron carrier of the mitochondrial intermembrane space that transfers electrons from ubiquinone-cytochrome c oxidoreductase to cytochrome c oxidase during cellular respiration
CAGL0G09042g	*YPL202C*	*AFT2*	Iron-regulated transcriptional activator; activates genes involved in intracellular iron use and required for iron homeostasis and resistance to oxidative stress; similar to Aft1p
CAGL0K05687g	*YHR179W*	*OYE2*	Widely conserved NADPH oxidoreductase containing flavin mononucleotide (FMN), homologous to Oye3p with slight differences in ligand binding and catalytic properties; may be involved in sterol metabolism
CAGL0K04719g	*YNL208W*		
CAGL0H00484g	*YJR122W*	*IBA57*	Mitochondrial matrix protein involved in the incorporation of iron-sulfur clusters into mitochondrial aconitase-type proteins; activates the radical-SAM family members Bio2p and Lip5p; interacts with Ccr4p in the two-hybrid system
CAGL0L01859g	*YKL040C*	*NFU1*	Protein involved in iron metabolism in mitochondria; similar to NifU, which is a protein required for the maturation of the Fe/S clusters of nitrogenase in nitrogen-fixing bacteria
CAGL0K10868g	*YDR256C*	*CTA1*	Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1p) during fatty acid beta-oxidation
CAGL0F00825g	*YOL049W*	*GSH2*	Glutathione synthetase, catalyzes the ATP-dependent synthesis of glutathione (GSH) from gamma-glutamylcysteine and glycine; induced by oxidative stress and heat shock
CAGL0K12958g	*YML131W*		
CAGL0J06402g	*YDL131W*	*LYS21*	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p
CAGL0E05280g	*YOL151W*	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase); stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway
CAGL0K10890g	*YHR179W*	*OYE2*	Widely conserved NADPH oxidoreductase containing flavin mononucleotide (FMN), homologous to Oye3p with slight differences in ligand binding and catalytic properties; may be involved in sterol metabolism

CAGL0K05813g	*YDR513W*	*GRX2*	Cytoplasmic glutaredoxin, thioltransferase, glutathione-dependent disulfide oxidoreductase involved in maintaining redox state of target proteins, also exhibits glutathione peroxidase activity, expression induced in response to stress
CAGL0K09702g	*YNL134C*		
CAGL0K00803g	*YGR209C*	*TRX2*	Cytoplasmic thioredoxin isoenzyme of the thioredoxin system which protects cells against oxidative and reductive stress, forms LMA1 complex with Pbi2p, acts as a cofactor for Tsa1p, required for ER-Golgi transport and vacuole inheritance
CAGL0G02101g	*YKR076W*	*ECM4*	Omega class glutathione transferase; not essential; similar to Ygr154cp; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
CAGL0E04356g	*YHR008C*	*SOD2*	Mitochondrial superoxide dismutase, protects cells against oxygen toxicity; phosphorylated
CAGL0F06017g	*YMR038C*	*CCS1*	Copper chaperone for superoxide dismutase Sod1p, involved in oxidative stress protection; Met-X-Cys-X2-Cys motif within the N-terminal portion is involved in insertion of copper into Sod1p under conditions of copper deprivation
CAGL0K02629g	*YNL134C*		
CAGL0J04048g	*YOR226C*	*ISU2*	Conserved protein of the mitochondrial matrix, required for synthesis of mitochondrial and cytosolic iron-sulfur proteins, performs a scaffolding function in mitochondria during Fe/S cluster assembly; isu1 isu2 double mutant is inviable
CAGL0L01111g	*YDL168W*	*SFA1*	Bifunctional enzyme containing both alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase activities, functions in formaldehyde detoxification and formation of long chain and complex alcohols, regulated by Hog1p-Sko1p
CAGL0H05665g	*YPL091W*	*GLR1*	Cytosolic and mitochondrial glutathione oxidoreductase, converts oxidized glutathione to reduced glutathione; mitochondrial but not cytosolic form has a role in resistance to hyperoxia
CAGL0K08536g	*YKL103C*	*LAP4*	Vacuolar aminopeptidase yscI; zinc metalloproteinase that belongs to the peptidase family M18; often used as a marker protein in studies of autophagy and cytosol to vacuole targeting (CVT) pathway
CAGL0A02530g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress
CAGL0E05170g	*YOL151W*	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase); stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway
CAGL0J09240g	*YDL131W*	*LYS21*	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p
CAGL0I01166g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress

CAGL0J09394g	*YDL124W*		
CAGL0J07986g	*YNL260C*		
CAGL0C01705g	*YBR244W*	*GPX2*	Phospholipid hydroperoxide glutathione peroxidase induced by glucose starvation that protects cells from phospholipid hydroperoxides and nonphospholipid peroxides during oxidative stress

Descriptions taken from SGD **[45]**.

Table 12.4: Gene dependent on *YAP1* in response to oxidative stress and benomyl treatment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0F07293g	*YGL114W*		
CAGL0L07656g	*YML004C*	*GLO1*	Monomeric glyoxalase I, catalyzes the detoxification of methylglyoxal (a by-product of glycolysis) via condensation with glutathione to produce S-D-lactoylglutathione; expression regulated by methylglyoxal levels and osmotic stress
CAGL0M11682g	*YLR108C*		
CAGL0C04785g	*YJR115W*		
CAGL0J07612g	*YNL241C*	*ZWF1*	Glucose-6-phosphate dehydrogenase (G6PD), catalyzes the first step of the pentose phosphate pathway; involved in adapting to oxidatve stress; homolog of the human G6PD which is deficient in patients with hemolytic anemia
CAGL0F04279g	*YBL033C*	*RIB1*	GTP cyclohydrolase II; catalyzes the first step of the riboflavin biosynthesis pathway
CAGL0I04884g	*YDL007W*	*RPT2*	One of six ATPases of the 19S regulatory particle of the 26S proteasome involved in the degradation of ubiquitinated substrates; required for normal peptide hydrolysis by the core 20S particle
CAGL0L00803g	*YER054C*	*GIP2*	Putative regulatory subunit of the protein phosphatase Glc7p, involved in glycogen metabolism; contains a conserved motif (GVNK motif) that is also found in Gac1p, Pig1p, and Pig2p
CAGL0B01419g	*YOL143C*	*RIB4*	Lumazine synthase (6,7-dimethyl-8-ribityllumazine synthase, also known as DMRL synthase); catalyzes synthesis of immediate precursor to riboflavin
CAGL0J09262g	*YDL130WA*		
CAGL0J03212g	*YER073W*	*ALD5*	Mitochondrial aldehyde dehydrogenase, involved in regulation or biosynthesis of electron transport chain components and acetate formation; activated by K+; utilizes NADP+ as the preferred coenzyme; constitutively expressed
CAGL0H05973g	*YPL108W*		
CAGL0F00825g	*YOL049W*	*GSH2*	Glutathione synthetase, catalyzes the ATP-dependent synthesis of glutathione (GSH) from gamma-glutamylcysteine and glycine; induced by oxidative stress and heat shock
CAGL0F04521g	*YBL043W*	*ECM13*	Non-essential protein of unknown function; induced by treatment with 8-methoxypsoralen and UVA irradiation
CAGL0M14047g	*YMR318C*	*ADH6*	NADPH-dependent medium chain alcohol dehydrogenase with

			broad substrate specificity; member of the cinnamyl family of alcohol dehydrogenases; may be involved in fusel alcohol synthesis or in aldehyde tolerance
CAGL0K12958g	*YML131W*		
CAGL0G09977g	*YPR184W*	*GDB1*	Glycogen debranching enzyme containing glucanotranferase and alpha-1,6-amyloglucosidase activities, required for glycogen degradation; phosphorylated in mitochondria
CAGL0K08800g	*YMR009W*	*ADI1*	Acireductone dioxygenease involved in the methionine salvage pathway; ortholog of human MTCBP-1; transcribed with YMR010W and regulated post-transcriptionally by RNase III (Rnt1p) cleavage; ADI1 mRNA is induced in heat shock conditions
CAGL0E05280g	*YOL151W*	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase); stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway
CAGL0K10890g	*YHR179W*	*OYE2*	Widely conserved NADPH oxidoreductase containing flavin mononucleotide (FMN), homologous to Oye3p with slight differences in ligand binding and catalytic properties; may be involved in sterol metabolism
CAGL0J00561g	*YHR029C*	*YHI9*	Protein of unknown function; null mutant is defective in unfolded protein response; possibly involved in a membrane regulation metabolic pathway; member of the PhzF superfamily, though most likely not involved in phenazine production
CAGL0K09702g	*YNL134C*		
CAGL0L06402g	*YDR135C*	*YCF1*	Vacuolar glutathione S-conjugate transporter of the ATP-binding cassette family, has a role in detoxifying metals such as cadmium, mercury, and arsenite; also transports unconjugated bilirubin; similar to human cystic fibrosis protein CFTR
CAGL0G05830g	*YHR146W*	*CRP1*	Protein that binds to cruciform DNA structures
CAGL0L01177g	*YEL047C*		
CAGL0K05973g	*YLR259C*	*HSP60*	Tetradecameric mitochondrial chaperonin required for ATP-dependent folding of precursor polypeptides and complex assembly; prevents aggregation and mediates protein refolding after heat shock; role in mtDNA transmission; phosphorylated
CAGL0M04675g	*YOR285W*	*RDL1*	Protein of unknown function, localized to the mitochondrial outer membrane
CAGL0J05852g	*YIR036*		
CAGL0G01672g	*YPR108W*	*RPN7*	Essential, non-ATPase regulatory subunit of the 26S proteasome, similar to another S. cerevisiae regulatory subunit, Rpn5p, as well as to mammalian proteasome subunits
CAGL0G02101g	*YKR076W*	*ECM4*	Omega class glutathione transferase; not essential; similar to Ygr154cp; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
CAGL0F06017g	*YMR038C*	*CCS1*	Copper chaperone for superoxide dismutase Sod1p, involved in oxidative stress protection; Met-X-Cys-X2-Cys motif within the N-terminal portion is involved in insertion of copper into Sod1p under conditions of copper deprivation
CAGL0J04048g	*YOR226C*	*ISU2*	Conserved protein of the mitochondrial matrix, required for

			synthesis of mitochondrial and cytosolic iron-sulfur proteins, performs a scaffolding function in mitochondria during Fe/S cluster assembly; isu1 isu2 double mutant is inviable
CAGL0J00451g	*YGR192C*	*TDH3*	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3 bis-phosphoglycerate; detected in the cytoplasm and cell-wall
CAGL0L01111g	*YDL168W*	*SFA1*	Bifunctional enzyme containing both alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase activities, functions in formaldehyde detoxification and formation of long chain and complex alcohols, regulated by Hog1p-Sko1p
CAGL0H05665g	*YPL091W*	*GLR1*	Cytosolic and mitochondrial glutathione oxidoreductase, converts oxidized glutathione to reduced glutathione; mitochondrial but not cytosolic form has a role in resistance to hyperoxia
CAGL0A02530g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress
CAGL0G03927g	*YLL028W*	*TPO1*	Polyamine transporter that recognizes spermine, putrescine, and spermidine; catalyzes uptake of polyamines at alkaline pH and excretion at acidic pH; phosphorylation enhances activity and sorting to the plasma membrane
CAGL0F01815g	*YALI0E33297G*		
CAGL0H05137g	*YPL061W*	*ALD6*	Cytosolic aldehyde dehydrogenase, activated by Mg2+ and utilizes NADP+ as the preferred coenzyme; required for conversion of acetaldehyde to acetate; constitutively expressed; locates to the mitochondrial outer surface upon oxidative stress
CAGL0I01166g	*YDR353W*	*TRR1*	Cytoplasmic thioredoxin reductase, key regulatory enzyme that determines the redox state of the thioredoxin system, which acts as a disulfide reductase system and protects cells against both oxidative and reductive stress
CAGL0M09229g	*YJR085C*		
CAGL0I09009g	*YFR025C*	*HIS2*	Histidinolphosphatase, catalyzes the eighth step in histidine biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts; transcription is regulated by general amino acid control
CAGL0C01705g	*YBR244W*	*GPX2*	Phospholipid hydroperoxide glutathione peroxidase induced by glucose starvation that protects cells from phospholipid hydroperoxides and nonphospholipid peroxides during oxidative stress

Descriptions taken from SGD **[45]**.

Table 12.5: YAP1 dependent genes with YRE-A sites.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0K06677g	*YBR213W*	*MET8*	Bifunctional dehydrogenase and ferrochelatase, involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis
CAGL0B01419g	*YOL143C*	*RIB4*	Lumazine synthase (6,7-dimethyl-8-ribityllumazine synthase, also known as DMRL synthase); catalyzes synthesis of immediate precursor to riboflavin
CAGL0K12958g	*YML131W*		
CAGL0H09064g	*YHR128W*	*FUR1*	Uracil phosphoribosyltransferase, synthesizes UMP from uracil; involved in the pyrimidine salvage pathway
CAGL0K09702g	*YNL134C*		
CAGL0F08085g	*YGR243W*	*FMP43*	Putative protein of unknown function; expression regulated by osmotic and alkaline stresses; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0K02585g	*YHL009C*	*YAP3*	Basic leucine zipper (bZIP) transcription factor
CAGL0K02629g	*YNL134C*		
CAGL0L01111g	*YDL168W*	*SFA1*	Bifunctional enzyme containing both alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase activities, functions in formaldehyde detoxification and formation of long chain and complex alcohols, regulated by Hog1p-Sko1p
CAGL0I06787g	*YER143W*	*DDI1*	DNA damage-inducible v-SNARE binding protein, contains a ubiquitin-associated (UBA) domain, may act as a negative regulator of constitutive exocytosis, may play a role in S-phase checkpoint control
CAGL0I01782g	*YGR218W*	*CRM1*	Major karyopherin, involved in export of proteins, RNAs, and ribosomal subunits from the nucleus; exportin
CAGL0F03861g	*YMR208W*	*ERG12*	Mevalonate kinase, acts in the biosynthesis of isoprenoids and sterols, including ergosterol, from mevalonate
CAGL0I06809g	*YER142C*	*MAG1*	3-methyl-adenine DNA glycosylase involved in protecting DNA against alkylating agents; initiates base excision repair by removing damaged bases to create abasic sites that are subsequently repaired
CAGL0L04268g	*YOR155C*	*ISN1*	Inosine 5'-monophosphate (IMP)-specific 5'-nucleotidase, catalyzes the breakdown of IMP to inosine, does not show similarity to known 5'-nucleotidases from other organisms

Descriptions taken from SGD **[45]**.

Table 12.6: YAP1 dependent genes with YRE-O sites.

C. glabrata Systematic Name	*S. cerevisiae* Systematic Name	*S. cerevisiae* Standard Name	*S. cerevisiae* Description
CAGL0L05258g	*YKL086W*	*SRX1*	Sulfiredoxin, contributes to oxidative stress resistance by reducing cysteine-sulfinic acid groups in the peroxiredoxins Tsa1p and Ahp1p that are formed upon exposure to oxidants; conserved in higher eukaryotes
CAGL0G08041g	*YDR091C*	*RLI1*	Essential iron-sulfur protein required for ribosome biogenesis and translation initiation; facilitates binding of a multifactor complex (MFC) of translation initiation factors to the small ribosomal subunit; predicted ABC family ATPase
CAGL0M08448g	*YKL165C*	*MCD4*	Protein involved in glycosylphosphatidylinositol (GPI) anchor synthesis; multimembrane-spanning protein that localizes to the endoplasmic reticulum; highly conserved among eukaryotes
CAGL0D00352g	*YKL188C*	*PXA2*	Subunit of a heterodimeric peroxisomal ATP-binding cassette transporter complex (Pxa1p-Pxa2p), required for import of long-chain fatty acids into peroxisomes; similarity to human adrenoleukodystrophy transporter and ALD-related proteins
CAGL0F07293g	*YGL114W*		
CAGL0H03773g	*YNL002C*	*RLP7*	Nucleolar protein with similarity to large ribosomal subunit L7 proteins; constituent of 66S pre-ribosomal particles; plays an essential role in processing of precursors to the large ribosomal subunit RNAs
CAGL0G04499g	*YJL105W*	*SET4*	Protein of unknown function, contains a SET domain
CAGL0J01331g	*YMR090W*		
CAGL0B01771g	*YDR110W*	*FOB1*	Nucleolar protein that binds the rDNA replication fork barrier (RFB) site; required for replication fork blocking, recombinational hotspot activity, condensin recruitment to RFB and rDNA repeat segregation; related to retroviral integrases
CAGL0G05269g	*YDR070C*	*FMP16*	Putative protein of unknown function; proposed to be involved in responding to conditions of stress; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0C04785g	*YJR115W*		
CAGL0F04279g	*YBL033C*	*RIB1*	GTP cyclohydrolase II; catalyzes the first step of the riboflavin biosynthesis pathway
CAGL0G09042g	*YPL202C*	*AFT2*	Iron-regulated transcriptional activator; activates genes involved in intracellular iron use and required for iron homeostasis and resistance to oxidative stress; similar to Aft1p
CAGL0K04719g	*YNL208W*		
CAGL0H00484g	*YJR122W*	*IBA57*	Mitochondrial matrix protein involved in the incorporation of iron-sulfur clusters into mitochondrial aconitase-type proteins; activates the radical-SAM family members Bio2p and Lip5p; interacts with Ccr4p in the two-hybrid system
CAGL0H03091g	*YGL091C*	*NBP35*	Essential iron-sulfur cluster binding protein localized in the cytoplasm; forms a complex with Cfd1p that is involved in iron-sulfur protein assembly in the cytosol; similar to P-loop

			NTPases
CAGL0L01485g	*YML048W*	*GSF2*	ER localized integral membrane protein that may promote secretion of certain hexose transporters, including Gal2p; involved in glucose-dependent repression
CAGL0L01925g	*YKL035W*	*UGP1*	UDP-glucose pyrophosphorylase (UGPase), catalyses the reversible formation of UDP-Glc from glucose 1-phosphate and UTP, involved in a wide variety of metabolic pathways, expression modulated by Pho85p through Pho4p
CAGL0K01397g	*YDL045C*	*FAD1*	Flavin adenine dinucleotide (FAD) synthetase, performs the second step in synthesis of FAD from riboflavin
CAGL0J02794g	*YER048W-A*	*ISD11*	Protein required for mitochondrial iron-sulfur cluster biosynthesis
CAGL0F04521g	*YBL043W*	*ECM13*	Non-essential protein of unknown function; induced by treatment with 8-methoxypsoralen and UVA irradiation
CAGL0M06369g	*YPL086C*	*ELP3*	Subunit of Elongator complex, which is required for modification of wobble nucleosides in tRNA; exhibits histone acetyltransferase activity that is directed to histones H3 and H4; disruption confers resistance to K. lactis zymotoxin
CAGL0K08800g	*YMR009W*	*ADI1*	Acireductone dioxygenease involved in the methionine salvage pathway; ortholog of human MTCBP-1; transcribed with YMR010W and regulated post-transcriptionally by RNase III (Rnt1p) cleavage; ADI1 mRNA is induced in heat shock conditions
CAGL0B00792g	*YCL037C*	*SRO9*	Cytoplasmic RNA-binding protein that associates with translating ribosomes; involved in heme regulation of Hap1p as a component of the HMC complex, also involved in the organization of actin filaments; contains a La motif
CAGL0D00220g	*YAL059W*	*ECM1*	Protein of unknown function, localized in the nucleoplasm and the nucleolus, genetically interacts with MTR2 in 60S ribosomal protein subunit export
CAGL0B01727g	*YDR109C*		
CAGL0L12122g	*YER180C*	*ISC10*	Protein required for sporulation, transcript is induced 7.5 hours after induction of meiosis, expected to play significant role in the formation of reproductive cells
CAGL0M07744g	*NORBH*		
CAGL0L06402g	*YDR135C*	*YCF1*	Vacuolar glutathione S-conjugate transporter of the ATP-binding cassette family, has a role in detoxifying metals such as cadmium, mercury, and arsenite; also transports unconjugated bilirubin; similar to human cystic fibrosis protein CFTR
CAGL0H02959g	*YGL096W*	*TOS8*	Homeodomain-containing protein and putative transcription factor found associated with chromatin; target of SBF transcription factor; induced during meiosis and under cell-damaging conditions; similar to Cup9p transcription factor
CAGL0G08844g	*YIL130W*	*ASG1*	Zinc cluster protein proposed to function as a transcriptional regulator involved in the stress response; null mutants have a respiratory deficiency, calcofluor white sensitivity and slightly increased cycloheximide resistance
CAGL0L13046g	*YHR122W*		
CAGL0L00671g	*YER056C*	*FCY2*	Purine-cytosine permease, mediates purine (adenine, guanine,

			and hypoxanthine) and cytosine accumulation
CAGL0H02739g	*YLR163C*	*MAS1*	Smaller subunit of the mitochondrial processing protease (MPP), essential processing enzyme that cleaves the N-terminal targeting sequences from mitochondrially imported proteins
CAGL0J02112g	*YIL003W*	*CFD1*	Highly conserved, iron-sulfur cluster binding protein localized in the cytoplasm; forms a complex with Nbp35p that is involved in iron-sulfur protein assembly in the cytosol
CAGL0K04279g	*YGR049W*	*SCM4*	Potential regulatory effector of CDC4 function, suppresses a temperature-sensitive allele of CDC4, tripartite protein structure in which a charged region separates two uncharged domains, not essential for mitosis or meiosis
CAGL0H06919g	*YML130C*	*ERO1*	Thiol oxidase required for oxidative protein folding in the endoplasmic reticulum
CAGL0B01078g	*YLR177W*		
CAGL0H01375g	*YDR297W*	*SUR2*	Sphinganine C4-hydroxylase, catalyses the conversion of sphinganine to phytosphingosine in sphingolipid biosyntheis
CAGL0M06171g	*YBR173C*	*UMP1*	Short-lived chaperone required for correct maturation of the 20S proteasome; may inhibit premature dimerization of proteasome half-mers; degraded by proteasome upon completion of its assembly
CAGL0K08536g	*YKL103C*	*LAP4*	Vacuolar aminopeptidase yscI; zinc metalloproteinase that belongs to the peptidase family M18; often used as a marker protein in studies of autophagy and cytosol to vacuole targeting (CVT) pathway
CAGL0F08305g	*YGR257C*	*MTM1*	Mitochondrial protein of the mitochondrial carrier family, involved in activating mitochondrial Sod2p probably by facilitating insertion of an essential manganese cofactor
CAGL0M13519g	*YMR290C*	*HAS1*	ATP-dependent RNA helicase; localizes to both the nuclear periphery and nucleolus; highly enriched in nuclear pore complex fractions; constituent of 66S pre-ribosomal particles
CAGL0J08547g	*YOR084W*	*LPX1*	Oleic acid-inducible, peroxisomal matrix localized lipase; transcriptionally activated by Yrm1p along with genes involved in multidrug resistance; peroxisomal import is dependent on the PTS1 receptor, Pex5p and on self-interaction
CAGL0E05654g	*YPL206C*	*PGC1*	Phosphatidyl Glycerol phospholipase C; regulates the phosphatidylglycerol (PG) content via a phospholipase C-type degradation mechanism; contains glycerophosphodiester phosphodiesterase motifs
CAGL0J00429g	*YHR051W*	*COX6*	Subunit VI of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; expression is regulated by oxygen levels
CAGL0M06105g	*YBR170C*	*NPL4*	Endoplasmic reticulum and nuclear membrane protein, forms a complex with Cdc48p and Ufd1p that recognizes ubiquitinated proteins in the endoplasmic reticulum and delivers them to the proteasome for degradation
CAGL0J09240g	*YDL131W*	*LYS21*	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p

CAGL0F01925g	*YLR051C*	*FCF2*	Essential nucleolar protein involved in the early steps of 35S rRNA processing; interacts with Faf1p; member of a transcriptionally co-regulated set of genes called the RRB regulon
CAGL0C05467g	*YDL233W*		
CAGL0L00649g	*YAL054C*	*ACS1*	Acetyl-coA synthetase isoform which, along with Acs2p, is the nuclear source of acetyl-coA for histone acetlyation; expressed during growth on nonfermentable carbon sources and under aerobic conditions
CAGL0M09020g	*YJR095W*	*SFC1*	Mitochondrial succinate-fumarate transporter, transports succinate into and fumarate out of the mitochondrion; required for ethanol and acetate utilization
CAGL0J09394g	*YDL124W*		
CAGL0I07799g	*YBR154C*	*RPB5*	RNA polymerase subunit ABC27, common to RNA polymerases I, II, and III; contacts DNA and affects transactivation
CAGL0J01485g	*YHL013C*	*OTU2*	Protein of unknown function that may interact with ribosomes, based on co-purification experiments; member of the ovarian tumor-like (OTU) superfamily of predicted cysteine proteases; shows cytoplasmic localization
CAGL0E05676g	*YPL207W*	*TYW1*	Protein required for the synthesis of wybutosine, a modified guanosine found at the 3'-position adjacent to the anticodon of phenylalanine tRNA which supports reading frame maintenance by stabilizing codon-anticodon interactions
CAGL0B02904g	*NORBH*		
CAGL0H02937g	*YJL069C*	*UTP18*	Possible U3 snoRNP protein involved in maturation of pre-18S rRNA, based on computational analysis of large-scale protein-protein interaction data
CAGL0H06633g	*YKR097W*	*PCK1*	Phosphoenolpyruvate carboxykinase, key enzyme in gluconeogenesis, catalyzes early reaction in carbohydrate biosynthesis, glucose represses transcription and accelerates mRNA degradation, regulated by Mcm1p and Cat8p, located in the cytosol
CAGL0F00649g	*YLR248W*	*RCK2*	Protein kinase involved in the response to oxidative and osmotic stress; identified as suppressor of S. pombe cell cycle checkpoint mutations

Descriptions taken from SGD **[45].**

Table 12.7: YAP1 dependent genes with both YRE-O and YRE-A sites.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0I05060g	*YER088C*	*DOT6*	Protein involved in rRNA and ribosome biogenesis; binds polymerase A and C motif; subunit of the RPD3L histone deacetylase complex; similar to Tod6p; has chromatin specific SANT domain; involved in telomeric gene silencing and filamentation

Descriptions taken from SGD **[45].**

13 Appendix IV

Table 13.1: GO terms associated with genes up regulated by *C. glabrata* solely by oxidative stress compared to combinatorial and single hyperosmotic stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Cellular amino acid biosynthetic process	1.00 e^{-14}	*LYS2 HIS7 ILV6 HIS4 LYS21 ARO3 TRP4 HOM3 HIS1 TRP2 MET6 LEU1 TRP5 ASN2 BAT1 HIS5 MET28 TRP3 MET17 ILV5 ADI1 ILV2 LEU4 LYS9 HIS3*	25	98
Proteasome complex	6.82 e^{-7}	*PRE7 UMP1 RPN3 RPN11 RPN12 PRE5 PRE6 RPT5 RPN7*	9	46

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 13.2: GO terms associated with genes up regulated by *C. glabrata* in response to oxidative stress and combinatorial stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Oxidative stress response	1.61 e^{-8}	*GPX2 TRX3 TRR1 GRX4 SOD2 SRX1 AHP1 TSA1 GRE2*	9	55
Homeostasis of metal ions (Na, K, Ca etc.)	0.001213	*OCT1 AHP1 SSQ1 CCS1 ISU1 ISA2*	6	98

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 13.3: GO terms associated with genes up regulated uniquely by *C. glabrata* under combinatorial stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Protein phosphorylation	$9.58\ e^{-8}$	*FUS3 CHK1 KIN1 CMK1 STE20 YCK1 IKS1 YAK1 ELM1 YPK1 NNK1 KNS1 RIM11 FPK1 PKH2 HRK1 SKS1 TPK2 ISR1*	24	133
Peroxisome organization	$1.19\ e^{-5}$	*OAF1 PEX32 ADR1 PEX3 PEX28 PEX2 PEX30 PEX6 PEX15*	9	29
Sequence-specific DNA binding	0.000158	*OAF1 MATALPHA1 TEC1 RPN4 STP4 STB3 ADR1 PDR1 MGA1 YAP3 CST6 GSM1 PHD1 HAP1 YAP1 GAT2 YRM1 AFT2 USV1*	21	165
Sporulation resulting in formation of a cellular spore	0.008145	*OAF1 SEF1 UBX7 SPS22 FMP45 SPS1 SPO11 SSP1 GSM1 TGL4 CDA2 SPO1*	12	103
Autophagy	0.002249	*CIS1 MON1 ATG7 ATG23 ATG4 ATG2 ATG21 ATG13*	8	45

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 13.4: GO terms associated with genes down regulated uniquely by *C. glabrata* under combinatorial stress treatment (15 minutes).

GO Term	p-Value	Example Genes	Number of Genes Regulated	Number of Genes in GO Term
Translation	$1.00\ e^{-14}$	*EFB1 RPL32 RPS9B RPS29B RPP1A RPS16B DTD1 SES1 RPS13 GIR2 RPS6A RPL5 CDC60 RPL7B*	57	318
Cell cycle	0.000114	*MCM2 RIF1 MCD1 CDC13 PSF1 WBP1 CIN8 SMC2 SWE1 CDC6 CDC45 CLB4 CDC5 CLN1 CDC31 CLB2*	31	316

GO term enrichment analysis (p-value <0.01) was performed using gene ontology inferred from homology with *S. cerevisiae*, using FunSpec **[145]**.

Table 13.5: Genes uniquely up regulated by combinatorial stress and macrophage engulfment.

C. glabrata **Systematic Name**	***S. cerevisiae*** **Systematic Name**	***S. cerevisiae*** **Standard Name**	***S. cerevisiae*** **Description**
CAGL0E01837g	*YDR144C*	*MKC7*	GPI-anchored aspartyl protease (yapsin) involved in protein processing; shares functions with Yap3p and Kex2p
CAGL0L02167g	*YKR009C*	*FOX2*	Multifunctional enzyme of the peroxisomal fatty acid beta-oxidation pathway; has 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities
CAGL0I01980g	*YNL257C*	*SIP3*	Protein that activates transcription through interaction with DNA-bound Snf1p, C-terminal region has a putative leucine zipper motif; potential Cdc28p substrate
CAGL0M03465g	*YCR010C*	*ADY2*	Acetate transporter required for normal sporulation; phosphorylated in mitochondria
CAGL0M07293g	*YPL058C*	*PDR12*	Plasma membrane ATP-binding cassette (ABC) transporter, weak-acid-inducible multidrug transporter required for weak organic acid resistance; induced by sorbate and benzoate and regulated by War1p; mutants exhibit sorbate hypersensitivity
CAGL0H06787g	*YIL160C*	*POT1*	3-ketoacyl-CoA thiolase with broad chain length specificity, cleaves 3-ketoacyl-CoA into acyl-CoA and acetyl-CoA during beta-oxidation of fatty acids
CAGL0A03740g	*YGL205W*	*POX1*	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway; localized to the peroxisomal matrix
CAGL0L03267g	*YKR039W*	*GAP1*	General amino acid permease; localization to the plasma membrane is regulated by nitrogen source
CAGL0H09460g	*YER015W*	*FAA2*	Long chain fatty acyl-CoA synthetase; accepts a wider range of acyl chain lengths than Faa1p, preferring C9:0-C13:0; involved in the activation of endogenous pools of fatty acids
CAGL0L09108g	*YPR002W*	*PDH1*	Mitochondrial protein that participates in respiration, induced by diauxic shift; homologous to E. coli PrpD, may take part in the conversion of 2-methylcitrate to 2-methylisocitrate
CAGL0M12947g	*YIL077C*		
CAGL0M02387g	*YPL147W*	*PXA1*	Subunit of a heterodimeric peroxisomal ATP-binding cassette transporter complex (Pxa1p-Pxa2p), required for import of long-chain fatty acids into peroxisomes; similarity to human adrenoleukodystrophy transporter and ALD-related proteins
CAGL0J03058g	*YER065C*	*ICL1*	Isocitrate lyase, catalyzes the formation of succinate and glyoxylate from isocitrate, a key reaction of the glyoxylate cycle; expression of ICL1 is induced by growth on ethanol and repressed by growth on glucose
CAGL0G05720g	*YNL183C*	*NPR1*	Protein kinase that stabilizes several plasma membrane amino acid transporters by antagonizing their ubiquitin-mediated degradation
CAGL0J04884g	*YBR273C*	*UBX7*	UBX (ubiquitin regulatory X) domain-containing protein that interacts with Cdc48p
CAGL0K10714g	*YOR018W*	*ROD1*	Membrane protein that binds the ubiquitin ligase Rsp5p via its 2 PY motifs; overexpression confers resistance to the GST substrate o-dinitrobenzene,zinc, and calcium; proposed to

			regulate the endocytosis of plasma membrane proteins
CAGL0D06424g	*YLR304C*	*ACO1*	Aconitase, required for the tricarboxylic acid (TCA) cycle and also independently required for mitochondrial genome maintenance; phosphorylated; component of the mitochondrial nucleoid; mutation leads to glutamate auxotrophy
CAGL0K12254g	*YBR105C*	*VID24*	Peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulates fructose-1,6-bisphosphatase (FBPase) targeting to the vacuole; promotes proteasome-dependent catabolite degradation of FBPase
CAGL0E04884g	*YDR216W*	*ADR1*	Carbon source-responsive zinc-finger transcription factor, required for transcription of the glucose-repressed gene ADH2, of peroxisomal protein genes, and of genes required for ethanol, glycerol, and fatty acid utilization
CAGL0L00649g	*YAL054C*	*ACS1*	Acetyl-coA synthetase isoform which, along with Acs2p, is the nuclear source of acetyl-coA for histone acetlyation; expressed during growth on nonfermentable carbon sources and under aerobic conditions
CAGL0D06688g	*YOR374W*	*ALD4*	Mitochondrial aldehyde dehydrogenase, required for growth on ethanol and conversion of acetaldehyde to acetate; phosphorylated; activity is K+ dependent; utilizes NADP+ or NAD+ equally as coenzymes; expression is glucose repressed
CAGL0L06072g	*YER130C*	*COM2*	Protein of unknown function
CAGL0F04631g	*YBL049W*	*MOH1*	Protein of unknown function, has homology to kinase Snf7p; not required for growth on nonfermentable carbon sources; essential for viability in stationary phase
CAGL0H07469g	*YFR019W*	*FAB1*	1-phosphatidylinositol-3-phosphate 5-kinase; vacuolar membrane kinase that generates phosphatidylinositol (3,5)P2, which is involved in vacuolar sorting and homeostasis
CAGL0C04587g	*YJR098C*		
CAGL0L00583g	*YPL230W*	*USV1*	Putative transcription factor containing a C2H2 zinc finger; mutation affects transcriptional regulation of genes involved in growth on non-fermentable carbon sources, response to salt stress and cell wall biosynthesis
CAGL0L06094g	*YGL184C*	*STR3*	Cystathionine beta-lyase, converts cystathionine into homocysteine

Descriptions taken from SGD **[45].**

Table 13.6: Genes uniquely down regulated by combinatorial stress and macrophage engulfment.

C. glabrata Systematic Name	*S. cerevisiae* Systematic Name	*S. cerevisiae* Standard Name	*S. cerevisiae* Description
CAGL0J03366g	*YER083C*	*GET2*	Subunit of the GET complex; involved in insertion of proteins into the ER membrane; required for the retrieval of HDEL proteins from the Golgi to the ER in an ERD2 dependent fashion and for meiotic nuclear division
CAGL0F08547g	*YAL003W*	*EFB1*	Translation elongation factor 1 beta; stimulates nucleotide exchange to regenerate EF-1 alpha-GTP for the next elongation cycle; part of the EF-1 complex, which facilitates binding of aminoacyl-tRNA to the ribosomal A site
CAGL0J11858g	*YEL050C*	*RML2*	Mitochondrial ribosomal protein of the large subunit, has similarity to E. coli L2 ribosomal protein; fat21 mutant allele causes inability to utilize oleate and may interfere with activity of the Adr1p transcription factor
CAGL0H08283g	*YOR020C*	*HSP10*	Mitochondrial matrix co-chaperonin that inhibits the ATPase activity of Hsp60p, a mitochondrial chaperonin; involved in protein folding and sorting in the mitochondria; 10 kD heat shock protein with similarity to E. coli groES
CAGL0J08415g	*YDR502C*	*SAM2*	S-adenosylmethionine synthetase, catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine; one of two differentially regulated isozymes (Sam1p and Sam2p)
CAGL0L02365g	*YEL002C*	*WBP1*	Beta subunit of the oligosaccharyl transferase (OST) glycoprotein complex; required for N-linked glycosylation of proteins in the endoplasmic reticulum
CAGL0H05511g	*YPL081W*	*RPS9A*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins
CAGL0B04697g	*YCL001W*	*RER1*	Protein involved in retention of membrane proteins, including Sec12p, in the ER; localized to Golgi; functions as a retrieval receptor in returning membrane proteins to the ER
CAGL0L03311g	*YKR043C*		
CAGL0J07678g	*YNL244C*	*SUI1*	Translation initiation factor eIF1; component of a complex involved in recognition of the initiator codon; modulates translation accuracy at the initiation phase
CAGL0H03641g	*YNL010W*		
CAGL0A03168g	*YDR382W*	*RPP2B*	Ribosomal protein P2 beta, a component of the ribosomal stalk, which is involved in the interaction between translational elongation factors and the ribosome; regulates the accumulation of P1 (Rpp1Ap and Rpp1Bp) in the cytoplasm
CAGL0E00869g	*YLR150W*	*STM1*	Protein required for optimal translation under nutrient stress; perturbs association of Yef3p with ribosomes; involved in TOR signaling; binds G4 quadruplex and purine motif triplex nucleic acid; helps maintain telomere structure
CAGL0M06083g	*YPL106C*	*SSE1*	ATPase that is a component of the heat shock protein Hsp90 chaperone complex; binds unfolded proteins; member of the heat shock protein 70 (HSP70) family; localized to the cytoplasm
CAGL0J02992g	*YER063W*	*THO1*	Conserved nuclear RNA-binding protein; specifically binds to

			transcribed chromatin in a THO- and RNA-dependent manner, genetically interacts with shuttling hnRNP NAB2; overproduction suppresses transcriptional defect caused by hpr1 mutation
CAGL0G02475g	*YKR094C*	*RPL40B*	Fusion protein, identical to Rpl40Ap, that is cleaved to yield ubiquitin and a ribosomal protein of the large (60S) ribosomal subunit with similarity to rat L40; ubiquitin may facilitate assembly of the ribosomal protein into ribosomes
CAGL0J02354g	*YIL018W*	*RPL2B*	Protein component of the large (60S) ribosomal subunit, identical to Rpl2Ap and has similarity to E. coli L2 and rat L8 ribosomal proteins; expression is upregulated at low temperatures
CAGL0E03938g	*YLL045C*	*RPL8B*	Ribosomal protein L4 of the large (60S) ribosomal subunit, nearly identical to Rpl8Ap and has similarity to rat L7a ribosomal protein; mutation results in decreased amounts of free 60S subunits
CAGL0G08668g	*YNL066W*	*SUN4*	Cell wall protein related to glucanases, possibly involved in cell wall septation; member of the SUN family
CAGL0A04521g	*YER102W*	*RPS8B*	Protein component of the small (40S) ribosomal subunit; identical to Rps8Ap and has similarity to rat S8 ribosomal protein
CAGL0M06501g	*YBR189W*	*RPS9B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Ap and has similarity to E. coli S4 and rat S9 ribosomal proteins
CAGL0K11418g	*YDR226W*	*ADK1*	Adenylate kinase, required for purine metabolism; localized to the cytoplasm and the mitochondria; lacks cleavable signal sequence
CAGL0M13849g	*YMR307W*	*GAS1*	Beta-1,3-glucanosyltransferase, required for cell wall assembly and also has a role in transcriptional silencing; localizes to the cell surface via a glycosylphosphatidylinositol (GPI) anchor; also found at the nuclear periphery
CAGL0H04521g	*YBL092W*	*RPL32*	Protein component of the large (60S) ribosomal subunit, has similarity to rat L32 ribosomal protein; overexpression disrupts telomeric silencing
CAGL0D03146g	*YJL004C*	*SYS1*	Integral membrane protein of the Golgi required for targeting of the Arf-like GTPase Arl3p to the Golgi; multicopy suppressor of ypt6 null mutation
CAGL0M10219g	*YKL008C*	*LAC1*	Ceramide synthase component, involved in synthesis of ceramide from C26(acyl)-coenzyme A and dihydrosphingosine or phytosphingosine, functionally equivalent to Lag1p
CAGL0L01353g	*YEL034W*	*HYP2*	Translation elongation factor eIF-5A, previously thought to function in translation initiation; similar to and functionally redundant with Anb1p; structural homolog of bacterial EF-P; undergoes an essential hypusination modification
CAGL0K06149g	*YDR447C*	*RPS17B*	Ribosomal protein 51 (rp51) of the small (40s) subunit; nearly identical to Rps17Ap and has similarity to rat S17 ribosomal protein
CAGL0M12408g	*YIL052C*	*RPL34B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl34Ap and has similarity to rat L34 ribosomal protein
CAGL0M07095g	*YHR190W*	*ERG9*	Farnesyl-diphosphate farnesyl transferase (squalene synthase), joins two farnesyl pyrophosphate moieties to form squalene in the sterol biosynthesis pathway

CAGL0J10164g	*YNL069C*	*RPL16B*	N-terminally acetylated protein component of the large (60S) ribosomal subunit, binds to 5.8 S rRNA; has similarity to Rpl16Ap, E. coli L13 and rat L13a ribosomal proteins; transcriptionally regulated by Rap1p
CAGL0M11946g	*YAL042W*	*ERV46*	Protein localized to COPII-coated vesicles, forms a complex with Erv41p; involved in the membrane fusion stage of transport
CAGL0G01452g	*YNL044W*	*YIP3*	Protein localized to COPII vesicles, proposed to be involved in ER to Golgi transport; interacts with members of the Rab GTPase family and Yip1p; also interacts with Rtn1p
CAGL0I02992g	*YNL281W*	*HCH1*	Heat shock protein regulator that binds to Hsp90p and may stimulate ATPase activity; originally identified as a high-copy number suppressor of a HSP90 loss-of-function mutation; GFP-fusion protein localizes to the cytoplasm and nucleus
CAGL0J08349g	*YNL278W*	*CAF120*	Part of the evolutionarily-conserved CCR4-NOT transcriptional regulatory complex involved in controlling mRNA initiation, elongation, and degradation
CAGL0M02849g	*YLR048W*	*RPS0B*	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps0Ap; required for maturation of 18S rRNA along with Rps0Ap; deletion of either RPS0 gene reduces growth rate, deletion of both genes is lethal
CAGL0M13805g	*YGR279C*	*SCW4*	Cell wall protein with similarity to glucanases; scw4 scw10 double mutants exhibit defects in mating
CAGL0F02937g	*YEL054C*	*RPL12A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl12Bp; rpl12a rpl12b double mutant exhibits slow growth and slow translation; has similarity to E. coli L11 and rat L12 ribosomal proteins
CAGL0H04741g	*YML012W*	*ERV25*	Protein that forms a heterotrimeric complex with Erp1, Erp2p, and Emp24, member of the p24 family involved in endoplasmic reticulum to Golgi transport
CAGL0G09669g	*YLR291C*	*GCD7*	Beta subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a negative regulator of GCN4 expression
CAGL0L12540g	*YPL037C*	*EGD1*	Subunit beta1 of the nascent polypeptide-associated complex (NAC) involved in protein targeting, associated with cytoplasmic ribosomes; enhances DNA binding of the Gal4p activator; homolog of human BTF3b
CAGL0J03234g	*YIL069C*	*RPS24B*	Protein component of the small (40S) ribosomal subunit; identical to Rps24Ap and has similarity to rat S24 ribosomal protein
CAGL0G01826g	*YPR102C*	*RPL11A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl11Bp; involved in ribosomal assembly; depletion causes degradation of proteins and RNA of the 60S subunit; has similarity to E. coli L5 and rat L11
CAGL0H09196g	*YGL225W*	*VRG4*	Golgi GDP-mannose transporter; regulates Golgi function and glycosylation in Golgi
CAGL0I00484g	*YLR300W*	*EXG1*	Major exo-1,3-beta-glucanase of the cell wall, involved in cell wall beta-glucan assembly; exists as three differentially glycosylated isoenzymes
CAGL0K12650g	*YFL037W*	*TUB2*	Beta-tubulin; associates with alpha-tubulin (Tub1p and Tub3p) to form tubulin dimer, which polymerizes to form microtubules

CAGL0L11462g	*YLR448W*	*RPL6B*	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl6Ap and to rat L6 ribosomal protein; binds to 5.8S rRNA
CAGL0A02794g	*YDR367W*	*KEI1*	Component of inositol phosphorylceramide (IPC) synthase; forms a complex with Aur1p and regulates its activity; required for IPC synthase complex localization to the Golgi; post-translationally processed by Kex2p; KEI1 is an essential gene
CAGL0I04532g	*YBR015C*	*MNN2*	Alpha-1,2-mannosyltransferase, responsible for addition of the first alpha-1,2-linked mannose to form the branches on the mannan backbone of oligosaccharides, localizes to an early Golgi compartment
CAGL0L06886g	*YMR142C*	*RPL13B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Ap; not essential for viability; has similarity to rat L13 ribosomal protein
CAGL0A03278g	*YBR084CA*		
CAGL0K05467g	*YOR074C*	*CDC21*	Thymidylate synthase, required for de novo biosynthesis of pyrimidine deoxyribonucleotides; expression is induced at G1/S
CAGL0J07238g	*YOR369C*	*RPS12*	Protein component of the small (40S) ribosomal subunit; has similarity to rat ribosomal protein S12
CAGL0E00737g	*YDR174W*	*HMO1*	Chromatin associated high mobility group (HMG) family member involved in genome maintenance; rDNA-binding component of the Pol I transcription system; associates with a 5'-3' DNA helicase and Fpr1p, a prolyl isomerase
CAGL0F07073g	*YGL123W*	*RPS2*	Protein component of the small (40S) subunit, essential for control of translational accuracy; phosphorylation by C-terminal domain kinase I (CTDK-I) enhances translational accuracy; similar to E. coli S5 and rat S2 ribosomal proteins
CAGL0J00165g	*YLR333C*	*RPS25B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps25Ap and has similarity to rat S25 ribosomal protein
CAGL0M12991g	*YIL078W*	*THS1*	Threonyl-tRNA synthetase, essential cytoplasmic protein
CAGL0D04290g	*YMR079W*	*SEC14*	Phosphatidylinositol/phosphatidylcholine transfer protein; involved in regulating PtdIns, PtdCho, and ceramide metabolism, products of which regulate intracellular transport and UPR; functionally homologous to mammalian PITPs
CAGL0H00462g	*YJR123W*	*RPS5*	Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins
CAGL0A01562g	*YGR148C*	*RPL24B*	Ribosomal protein L30 of the large (60S) ribosomal subunit, nearly identical to Rpl24Ap and has similarity to rat L24 ribosomal protein; not essential for translation but may be required for normal translation rate
CAGL0M07161g	*YHR193C*	*EGD2*	Alpha subunit of the heteromeric nascent polypeptide-associated complex (NAC) involved in protein sorting and translocation, associated with cytoplasmic ribosomes
CAGL0J11462g	*YNL190W*		
CAGL0M08514g	*YJL158C*	*CIS3*	Mannose-containing glycoprotein constituent of the cell wall; member of the PIR (proteins with internal repeats) family

CAGL0K05027g	*YNL220W*	*ADE12*	Adenylosuccinate synthase, catalyzes the first step in synthesis of adenosine monophosphate from inosine 5'monophosphate during purine nucleotide biosynthesis; exhibits binding to single-stranded autonomously replicating (ARS) core sequence
CAGL0M06303g	*YPL090C*	*RPS6A*	Protein component of the small (40S) ribosomal subunit; identical to Rps6Bp and has similarity to rat S6 ribosomal protein
CAGL0H10076g	*YBR054W*	*YRO2*	Putative protein of unknown function; the authentic, non-tagged protein is detected in a phosphorylated state in highly purified mitochondria in high-throughput studies; transcriptionally regulated by Haa1p
CAGL0K09130g	*YOR247W*	*SRL1*	Mannoprotein that exhibits a tight association with the cell wall, required for cell wall stability in the absence of GPI-anchored mannoproteins; has a high serine-threonine content; expression is induced in cell wall mutants
CAGL0E01221g	*YDR152W*	*GIR2*	Highly-acidic cytoplasmic RWD domain-containing protein of unknown function; interacts with Rbg1p and Gcn1p; associates with translating ribosomes; putative intrinsically unstructured protein
CAGL0M02695g	*YPL131W*	*RPL5*	Protein component of the large (60S) ribosomal subunit with similarity to E. coli L18 and rat L5 ribosomal proteins; binds 5S rRNA and is required for 60S subunit assembly
CAGL0H05643g	*YPL090C*	*RPS6A*	Protein component of the small (40S) ribosomal subunit; identical to Rps6Bp and has similarity to rat S6 ribosomal protein
CAGL0D05742g	*YJL002C*	*OST1*	Alpha subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes asparagine-linked glycosylation of newly synthesized proteins
CAGL0J03652g	*YPL160W*	*CDC60*	Cytosolic leucyl tRNA synthetase, ligates leucine to the appropriate tRNA
CAGL0M00814g	*YLR441C*	*RPS1A*	Ribosomal protein 10 (rp10) of the small (40S) subunit; nearly identical to Rps1Bp and has similarity to rat S3a ribosomal protein
CAGL0B04587g	*YNL108C*		
CAGL0F09031g	*YJR145C*	*RPS4A*	Protein component of the small (40S) ribosomal subunit; mutation affects 20S pre-rRNA processing; identical to Rps4Bp and has similarity to rat S4 ribosomal protein
CAGL0G05071g	*YDR062W*	*LCB2*	Component of serine palmitoyltransferase, responsible along with Lcb1p for the first committed step in sphingolipid synthesis, which is the condensation of serine with palmitoyl-CoA to form 3-ketosphinganine
CAGL0I06160g	*YJL158C*	*CIS3*	Mannose-containing glycoprotein constituent of the cell wall; member of the PIR (proteins with internal repeats) family
CAGL0G00990g	*YLR340W*	*RPP0*	Conserved ribosomal protein P0 similar to rat P0, human P0, and E. coli L10e; shown to be phosphorylated on serine 302
CAGL0G07227g	*YML026C*	*RPS18B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps18Ap and has similarity to E. coli S13 and rat S18 ribosomal proteins
CAGL0I00814g	*YDL081C*	*RPP1A*	Ribosomal stalk protein P1 alpha, involved in the interaction between translational elongation factors and the ribosome; accumulation of P1 in the cytoplasm is regulated by

			phosphorylation and interaction with the P2 stalk component
CAGL0M12727g	*YIL070C*	*MAM33*	Acidic protein of the mitochondrial matrix involved in oxidative phosphorylation; related to the human complement receptor gC1q-R
CAGL0F01045g	*YOL040C*	*RPS15*	Protein component of the small (40S) ribosomal subunit; has similarity to E. coli S19 and rat S15 ribosomal proteins
CAGL0J08734g	*YAL023C*	*PMT2*	Protein O-mannosyltransferase, transfers mannose residues from dolichyl phosphate-D-mannose to protein serine/threonine residues; acts in a complex with Pmt1p, can instead interact with Pmt5p in some conditions; target for new antifungals
CAGL0K04543g	*YGR063C*	*SPT4*	Protein involved in the regulating Pol I and Pol II transcription, pre-mRNA processing, kinetochore function, and gene silencing; forms a complex with Spt5p
CAGL0D01408g	*YPR069C*	*SPE3*	Spermidine synthase, involved in biosynthesis of spermidine and also in biosynthesis of pantothenic acid; spermidine is required for growth of wild-type cells
CAGL0I04818g	*YBR038W*	*CHS2*	Chitin synthase II; catalyzes transfer of N-acetylglucosamine (GlcNAc) to chitin upon activation of zymogenic form; required for chitin synthesis in the primary septum during cytokinesis; localization regulated by Cdk1p during mitosis
CAGL0K07414g	*YMR242C*	*RPL20A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl20Bp and has similarity to rat L18a ribosomal protein
CAGL0G07018g	*YML018C*		
CAGL0M03575g	*YNL290W*	*RFC3*	Subunit of heteropentameric Replication factor C (RF-C), which is a DNA binding protein and ATPase that acts as a clamp loader of the proliferating cell nuclear antigen (PCNA) processivity factor for DNA polymerases delta and epsilon
CAGL0A00209g	*YGL022W*	*STT3*	Subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes asparagine-linked glycosylation of newly synthesized proteins; forms a subcomplex with Ost3p and Ost4p and is directly involved in catalysis
CAGL0L05148g	*YKL081W*	*TEF4*	Translation elongation factor EF-1 gamma
CAGL0G00682g	*YAR002CA*		
CAGL0J11000g	*YPL199C*		
CAGL0B00550g	*YCL050C*	*APA1*	Diadenosine 5',5''-P1,P4-tetraphosphate phosphorylase I (AP4A phosphorylase), involved in catabolism of bis(5'-nucleosidyl) tetraphosphates; has similarity to Apa2p
CAGL0G09130g	*YPL198W*	*RPL7B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl7Ap and has similarity to E. coli L30 and rat L7 ribosomal proteins; contains a conserved C-terminal Nucleic acid Binding Domain (NDB2)

Descriptions taken from SGD **[45].**

Table 13.7: Non-homologous and functionally unknown genes uniquely regulated by combinatorial stress.

C. glabrata Systematic Name	*S. cerevisiae* Systematic Name	*S. cerevisiae* Standard Name	*S. cerevisiae* Description
CAGL0K07271g	*YML006C*	*GIS4*	CAAX box containing protein of unknown function, proposed to be involved in the RAS/cAMP signaling pathway
CAGL0B02629g	*YDR251W*	*PAM1*	Essential protein of unknown function; exhibits variable expression during colony morphogenesis; overexpression permits survival without protein phosphatase 2A, inhibits growth, and induces a filamentous phenotype
CAGL0E04620g	*YBR078W*	*ECM33*	GPI-anchored protein of unknown function, has a possible role in apical bud growth; GPI-anchoring on the plasma membrane crucial to function; phosphorylated in mitochondria; similar to Sps2p and Pst1p
CAGL0J05302g	*YJL066C*	*MPM1*	Mitochondrial membrane protein of unknown function, contains no hydrophobic stretches
CAGL0D06600g	*YLL019C*	*KNS1*	Nonessential putative protein kinase of unknown cellular role; member of the LAMMER family of protein kinases, which are serine/threonine kinases also capable of phosphorylating tyrosine residues
CAGL0C00407g	*YLR094C*	*GIS3*	Protein of unknown function
CAGL0L06072g	*YER130C*	*COM2*	Protein of unknown function
CAGL0F08745g	*YLR327C*	*TMA10*	Protein of unknown function that associates with ribosomes
CAGL0H10362g	*YDL110C*	*TMA17*	Protein of unknown function that associates with ribosomes; heterozygous deletion demonstrated increases in chromosome instability in a rad9 deletion background; protein abundance is decreased upon intracellular iron depletion
CAGL0M05995g	*YKR046C*	*PET10*	Protein of unknown function that co-purifies with lipid particles; expression pattern suggests a role in respiratory growth; computational analysis of large-scale protein-protein interaction data suggests a role in ATP/ADP exchange
CAGL0G02541g	*YKR096W*	*ESL2*	Protein of unknown function that may interact with ribosomes, based on co-purification experiments; green fluorescent protein (GFP)-fusion protein localizes to the nucleus and cytoplasm; predicted to contain a PINc domain
CAGL0L08074g	*YCR030C*	*SYP1*	Protein of unknown function that may regulate assembly and disassembly of the septin ring; colocalizes and interacts with septin subunits; potential role in actin cytoskeletal organization
CAGL0K03883g	*YMR132C*	*JLP2*	Protein of unknown function, contains sequence that closely resembles a J domain (typified by the E. coli DnaJ protein)
CAGL0F04631g	*YBL049W*	*MOH1*	Protein of unknown function, has homology to kinase Snf7p; not required for growth on nonfermentable

			carbon sources; essential for viability in stationary phase
CAGL0K03949g	*YMR135C*	*GID8*	Protein of unknown function, involved in proteasome-dependent catabolite inactivation of fructose-1,6-bisphosphatase; contains LisH and CTLH domains, like Vid30p; dosage-dependent regulator of START
CAGL0F06545g	*YMR063W*	*RIM9*	Protein of unknown function, involved in the proteolytic activation of Rim101p in response to alkaline pH; has similarity to A. nidulans PalI; putative membrane protein
CAGL0B00616g	*YCL048W*	*SPS22*	Protein of unknown function, redundant with Sps2p for the organization of the beta-glucan layer of the spore wall
CAGL0G02651g	*YIL097W*	*FYV10*	Protein of unknown function, required for survival upon exposure to K1 killer toxin; involved in proteasome-dependent catabolite inactivation of FBPase; contains CTLH domain; plays role in anti-apoptosis
CAGL0C02409g	*YER033C*	*ZRG8*	Protein of unknown function; authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; GFP-fusion protein is localized to the cytoplasm; transcription induced under conditions of zinc deficiency
CAGL0B00814g	*YCL034W*	*LSB5*	Protein of unknown function; binds Las17p, which is a homolog of human Wiskott-Aldrich Syndrome protein involved in actin patch assembly and actin polymerization; may mediate disassembly of the Pan1 complex from the endocytic coat
CAGL0L08448g	*YPR149W*	*NCE102*	Protein of unknown function; contains transmembrane domains; involved in secretion of proteins that lack classical secretory signal sequences; component of the detergent-insoluble glycolipid-enriched complexes (DIGs)
CAGL0K02563g	*YHL010C*	*ETP1*	Putative protein of unknown function that is required for growth on ethanol; contains a zinc finger region and has homology to human BRAP2, which is a cytoplasmic protein that binds nuclear localization sequences
CAGL0H03201g	*YGL085W*	*LCL3*	Putative protein of unknown function, has homology to Staphylococcus aureus nuclease; green fluorescent protein (GFP)-fusion protein localizes to mitochondria and is induced in response to the DNA-damaging agent MMS
CAGL0C05533g	*YDL237W*	*AIM6*	Putative protein of unknown function, required for respiratory growth; YDL237W is not an essential gene
CAGL0F08085g	*YGR243W*	*FMP43*	Putative protein of unknown function; expression regulated by osmotic and alkaline stresses; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies
CAGL0L09999g	*YOR044W*	*IRC23*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion localizes to the ER; null mutant displays increased levels of spontaneous

			Rad52p foci
CAGL0I07821g	*YOL087C*	*DUF1*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; deletion mutant is sensitive to various chemicals including phenanthroline, sanguinarine, and nordihydroguaiaretic acid
CAGL0L01067g	*YDL173W*	*PAR32*	Putative protein of unknown function; hyperphosphorylated upon rapamycin treatment in a Tap42p-dependent manner; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; PAR32 is not an essential gene
CAGL0I09966g	*YOL053W*	*AIM39*	Putative protein of unknown function; null mutant displays elevated frequency of mitochondrial genome loss
CAGL0D02728g	*YHR199C*	*AIM46*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; null mutant displays elevated frequency of mitochondrial genome loss
CAGL0K08844g	*YHL021C*	*AIM17*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; null mutant displays reduced frequency of mitochondrial genome loss
CAGL0L03674g	*YJL103C*	*GSM1*	Putative zinc cluster protein of unknown function; proposed to be involved in the regulation of energy metabolism, based on patterns of expression and sequence analysis
CAGL0K10428g	*YFR017C*	-	-
CAGL0I10252g		-	-
CAGL0K08030g	*YPR091C*	-	-
CAGL0K03377g	*YMR102C*	-	-
CAGL0L01045g		-	-
CAGL0K08470g		-	-
CAGL0I10626g	*YGR125W*	-	-
CAGL0A01089g	*YPL272C*	-	-
CAGL0C04213g	*YBR005WP*	-	-
CAGL0K07183g		-	-
CAGL0M03685g	*YNL295W*	-	-
CAGL0J09262g	*YDL130WA*	-	-
CAGL0I02596g		-	-
CAGL0K11638g	*YMR102C*	-	-
CAGL0M08316g	*YKL171W*	-	-
CAGL0E06380g	*YKL151C*	-	-
CAGL0G07601g	*YBR287W*	-	-
CAGL0C04939g	*YJR107W*	-	-

CAGL0D05456g	*NORBH*	-	-
CAGL0M09493g	*YMR160W*	-	-
CAGL0F01881g		-	-
CAGL0F08767g		-	-
CAGL0B02926g		-	-
CAGL0L03355g	*YKR045C*	-	-
CAGL0J01595g	*YPR015C*	-	-
CAGL0H06413g	*YLR390WA*	-	-
CAGL0K10824g	*YLR149C*	-	-
CAGL0L06974g	*YDL086W*	-	-
CAGL0D06666g		-	-
CAGL0M12947g	*YIL077C*	-	-
CAGL0K11110g	*YDR239C*	-	-
CAGL0L10318g	*YOR059C*	-	-
CAGL0H10032g		-	-
CAGL0J01419g	*YMR086W*	-	-
CAGL0A04081g	*YLR194C*	-	-
CAGL0D00660g	*YDL073W*	-	-
CAGL0L02519g	*YOR378W*	-	-
CAGL0M12078g		-	-
CAGL0G02057g	*YOR062C*	-	-
CAGL0L03762g	*YOR097C*	-	-
CAGL0H05203g	*YPL066W*	-	-
CAGL0F07975g	*YGR237C*	-	-
CAGL0M03421g		-	-
CAGL0I08019g	*YOL075C*	-	-
CAGL0J00187g	*YGR026W*	-	-
CAGL0G01122g		-	-
CAGL0B01078g	*YLR177W*	-	-
CAGL0K12584g	*YFL034W*	-	-
CAGL0L07634g	*YML002W*	-	-
CAGL0I05984g	*YJL147C*	-	-
CAGL0K08206g	*YGL140C*	-	-
CAGL0L06578g	*YGR226C*	-	-
CAGL0H00979g	*YPL236C*	-	-
CAGL0E04510g	*YOR022C*	-	-
CAGL0K12716g	*YFL040W*	-	-
CAGL0I10516g	*YGR130C*	-	-
CAGL0F02519g	*YJL206C*	-	-

CAGL0K11297g	*YDR248C*	-	-
CAGL0M08734g	*YDR262W*	-	-
CAGL0F08855g	*YGR015C*	-	-
CAGL0K12980g		-	-
CAGL0G04169g	*YDR306C*	-	-
CAGL0K08118g	*YPR097W*	-	-
CAGL0K07073g	*YBR235W*	-	-
CAGL0L10340g	*YOR060C*	-	-
CAGL0F08877g	*YGR016W*	-	-
CAGL0D02266g	*YOR352W*	-	-
CAGL0M01078g	*YDR338C*	-	-
CAGL0A02299g		-	-
CAGL0L07524g		-	-
CAGL0E06072g	*YPL229W*	-	-
CAGL0K00341g	*NORBH*	-	-
CAGL0C04587g	*YJR098C*	-	-
CAGL0L12914g	*YMR031C*	-	-
CAGL0J07018g	*YPL109C*	-	-
CAGL0H04279g		-	-
CAGL0M10956g	*YCR073WA*	-	-

Descriptions taken from SGD **[45].**