Stress-induced Gene Expression in *Candida albicans*: Absence of a General Stress Response ▣

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We used transcriptional profiling to investigate the response of the fungal pathogen *Candida albicans* to temperature and osmotic and oxidative stresses under conditions that permitted >60% survival of the challenged cells. Each stress generated the transient induction of a specific set of genes including classic markers observed in the stress responses of other organisms. We noted that the classical hallmarks of the general stress response observed in *Saccharomyces cerevisiae* are absent from *C. albicans*; no *C. albicans* genes were significantly induced in a common response to the three stresses. This observation is supported by our inability to detect stress cross-protection in *C. albicans*. Similarly, in *C. albicans* there is essentially no induction of carbohydrate reserves like glycogen and trehalose in response to a mild stress, unlike the situation in *S. cerevisiae*. Thus *C. albicans* lacks the strong general stress response exhibited by *S. cerevisiae*.

INTRODUCTION

Unicellular organisms have developed different strategies, termed the adaptive response (Ruis and Schuller, 1995), to respond appropriately to environmental changes. Sudden and dramatic changes are defined as stresses and can directly affect the survival of the cells. In general, cells respond to stresses with transient changes in the expression of genes encoding products that serve to protect the cell against the encountered stress (Estruch, 2000).

The model unicellular eukaryotic yeast *Saccharomyces cerevisiae* has been extensively studied to determine the molecular basis for its responses to different stresses. Following a heat shock, yeast cells rapidly produce a large set of chaperones, the heat shock proteins (HSPs), which can help stabilize cellular proteins and block their thermal denaturation (Boy-Marcotte *et al.*, 1999). When challenged with a hyperosmotic stress, *S. cerevisiae* cells rapidly accumulate osmoprotective molecules like glycerol and produce more salt transporters to adapt their internal ionic strength to the new environment (Boy-Marcotte *et al.*, 1999; Proft and Serrano, 1999; Hohmann, 2002). Another stress response involves the production of detoxification enzymes when the cells are

faced with an increase in reactive oxygen species (Lee *et al.*, 1999).

S. cerevisiae is also able to induce a general stress response; in addition to the pattern of genes induced specifically by the stress, a general set of genes is induced that is common to all the stresses (Ruis and Schuller, 1995). This ability is linked to the function of the general stress response transcription factors Msn2p and Msn4p that recognize the stress responsive element (STRE; consensus "CCCCT") in the promoters of the stress response genes (Marchler *et al.*, 1993; Martinez-Pastor *et al.*, 1996; Schmitt and McEntee, 1996). This regulation is of great importance in the cross-protection to stress. It allows cells that have been challenged with a mild stress to acquire resistance to a stronger stress, even if the second stress is different from the first moderate one (Lewis *et al.*, 1995).

Candida albicans is a fungal pathogen that, when growing in the yeast form, is morphologically similar to *S. cerevisiae*. *C. albicans* has a commensal relationship with warm-blooded organisms and thus would be expected to live in a relatively stable environment in terms of temperature and osmotic conditions. In contrast, oxidative stress could be a frequent challenge for *C. albicans* cells as they are targeted by macrophage cells (Murphy, 1991). In this article, we present a global transcriptional analysis of *C. albicans* response to osmotic, thermal, and oxidative stress. We also investigated the presence of cross-protection to stresses and measured standard hallmarks of the general stress response like glycogen and trehalose accumulation in the stressed *C. albicans* cells.

MATERIALS AND METHODS

Growth Medium and Culture Conditions

Cultures of *C. albicans* strain SC5314 (Gillum *et al.*, 1984) were grown in 2% glucose, 2% bactopeptone, 1% yeast extract based medium (YPD).

For each stress, cultures were inoculated from a fresh colony and grown overnight in YPD at 30°C (or 23°C for the heat shock experiments). These were then diluted to an OD_{600} of 0.1 in 500 ml of fresh YPD and grown at the same initial temperature until an OD_{600} of 1. The culture was divided in two volumes of 250 ml; one sample was maintained as the control and the other subjected to the stress. Fifty-milliliter aliquots of the control and stress samples were removed at times 0, 10, 30, and 60 min after the initiation of the stress and centrifuged 2 min at 3500 rpm. The supernatants were then removed and the samples were quick-frozen and stored at −80°C.

For the heat shock experiments, the cells were transferred from 23 to 37°C in <1.5 min by immersing the sample in a water bath. To create the hyperosmotic shock, a prewarmed 6 M NaCl solution was added to the medium to generate a 0.3 M final concentration. Hydrogen peroxide (H_2O_2) was used for the oxidative stress. H_2O_2, 8 mM, at 30°C was added in the medium in order to obtain a 0.4 mM final concentration. For the latter two experiments, the same volume of water at 30°C was added to the control cultures.

In the cross-protection experiments, the mild stresses were generated as described above. Submersing the culture in a water bath at the required temperature created the strong thermal stress. To initiate the strong oxidative stress after the thermal shock (37°C for 30 min), the cells were allowed to cool to room temperature for 30 min before the oxidant solution was added to 1.6 mM H_2O_2 final concentration. After the stresses, the cultures were diluted to suitable concentrations and spread on YPD plates. The colonies were counted after 24 h. Each experiment was repeated four times.

Isolation of RNA and DNA Microarray Hybridization

We used slight variations of the methods and microarrays described (Nantel *et al.*, 2002). In all hybridizations, RNA from the stressed samples was labeled with Cy5, whereas the control RNA was labeled with Cy3. Detailed protocols can be obtained from the supplementary material (http://www.cbr.nrc.ca/genetics/stress/).

Data Analysis

Microarray data normalization and analysis was performed in GeneSpring software (Silicon Genetics, Redwood City, CA). Each gene's measured intensity was divided by its control channel value in each sample. When the control channel value was below 100.0, the data point was considered unacceptable. Intensity-dependent normalization was also applied, where the ratio was reduced to the residual of the Lowess fit of the intensity vs. ratio curve. To account for biological variability of individual genes and the absence of dye swapping, the logged ratios for each gene in each sample were divided by the average of the logs of the ratios from the control hybridizations ($t = 0$). Results presented consist of the average of three completely independent experiments. Identification of 972 genes with significant changes in transcript abundance in the 10- or 30-min time points compared with controls (false discovery rate < 10%) was done using the "Significance Analysis of Microarrays" algorithm (Tusher *et al.*, 2001). To ensure that our conclusions are not dependent on the mode of analysis, we achieved the same results when the expression data are normalized and analyzed as described (Nantel *et al.*, 2002). All of the gene lists and expression data can be downloaded from our web page at http://www.cbr.nrc.ca/genetics/stress/.

To compare gene expression data between *Candida* and *Saccharomyces,* we used the BlastP algorithm to compare the *Candida* protein sequences with the budding yeast proteome. In the presence of a significant homology (Blast E-value <10 exponet − 10), the *Candida* gene name was replaced with its strongest *Saccharomyces* homologue.

The search for consensus promoter sequences was completed using the data from the Saccharomyces Genome Database (Stanford: http://genome-www.stanford.edu/Saccharomyces/) and the CandidaDB World-Wide Web Server (Pasteur Institute: http://genolist.pasteur.fr/CandidaDB/).

Biochemical and Analytical Procedures

Determination of glycogen and trehalose were performed as described previously (Parrou and Francois, 1997).

RESULTS

Stresses and Technical Choices

We have applied global transcriptional profiling to examine the stress response pattern of *C. albicans*. Three commonly studied stresses (thermal, osmotic, and oxidative) have been investigated. We tested conditions similar to those previously used for stress analysis in *S. cerevisiae*. These conditions were chosen both because *S. cerevisiae* and *C. albicans* are physically similar cells that exhibit different natural life styles and because we wanted to be able to compare the *C. albicans* results with the extensive data sets that are available for the *S. cerevisiae* stress response. A rapid change from 23 to 37°C as a heat shock, addition of NaCl to a final concentration of 0.3 M as a hyperosmotic shock, and addition of hydrogen peroxide to a final concentration of 0.4 mM as a oxidative shock permitted >60% survival of the *C. albicans* cells, similar to the behavior of *S. cerevisiae* (Parrou *et al.*, 1997; Gasch *et al.*, 2000; Causton *et al.*, 2001). We chose to split the initial culture into a stressed and unstressed sample to ensure that the two samples differed only through the addition of the stress. We also examined the stressed sample over a time course and included the time point just before the addition of the stress to provide a further control.

Heat Shock Response

C. albicans cells respond to a rapid shift from 23 to 37°C with the transient induction of a variety of genes. The results obtained for the heat shock are presented on Figure 1A (left). For most genes this induction appears within 10 min of the initiation of the stress. Another group of genes is activated by 30 min. Some genes are continually induced from 10 to 30 min but most of the genes return to the basal level by 60 min.

To test the efficiency of the stress response, we have followed a panel of genes based on the behavior of their homologues in *S. cerevisiae*. In the latter organism, heat shock has been demonstrated to promote the formation of aggregated proteins that block the repressive activity of the transcriptional factor Hsp70p on the promoter of a family of HSPs (Shi *et al.*, 1998). Here, we followed the transcription of 6 HSPs (Figure 2A): *HSP12*, *HSP30*, *SSA4/HSP70*, *HSP78*, *HSP82*, and *HSP104*. There is no induction at all of *HSP30*; *HSP82* shows a weak induction in response to the heat shock, whereas *HSP12*, *HSP70*, *HSP78*, and *HSP104* show an induction equal or greater than twofold. These results demonstrate the ability of *C. albicans* to induce a classic response to heat shock by activating the production of the majority of an expected set of genes.
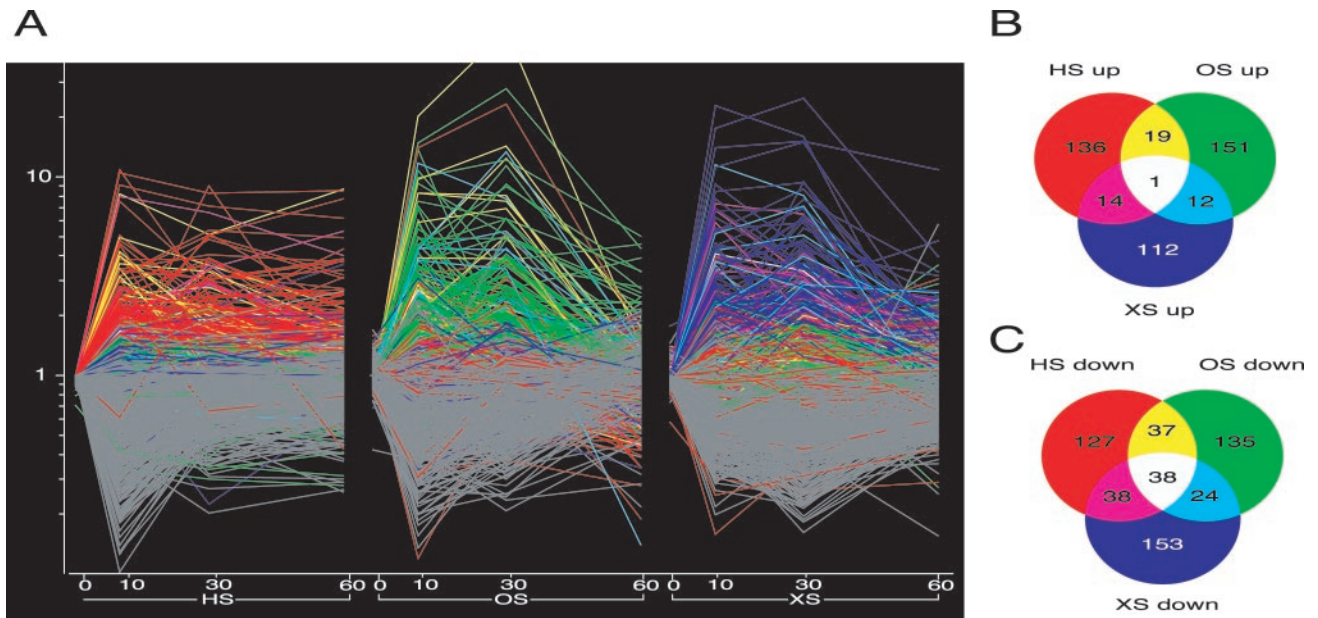
Figure 1. *Candida albicans* transcriptional response to stresses. (A) We selected 972 *C. albicans* genes that show a statistically significant variation following 10 or 30 min of thermal (HS), hyperosmotic (OS) and oxidative (XS) stresses (see MATERIALS AND METHODS for details). The (X) axis represents the time course and type of stress, the logarithmic (Y) axis corresponds to the mean normalized ratio of the change in transcript abundance. Each gene is represented by a single line that is colored according to the Venn Diagram in B. The specific heat inducible genes are red, the specific hyperosmotic induced genes are green and the specific oxidative induced genes are blue. Genes that can be induced by two or three stresses are colored accordingly to the Venn diagram representation (white, pink, yellow, and cyan). The Venn Diagram in C represents genes that are significantly reduced following a 10–30 min stress.

Hyperosmotic Stress Response

The middle part of Figure 1A presents the results obtained for the hyperosmotic stress response. In terms of timing and intensity of induction, the results are similar to the heat shock experiments. We have investigated the behavior of a panel of genes similar to *S. cerevisiae* genes implicated in osmotic response. In this yeast, there is a complex but increasingly well-characterized pathway that directs the induction of two sets of genes that function to protect the cell from increases in the ionic strength in the medium (Estruch, 2000; Hohmann, 2002). One set of genes that includes *GPD1* (a glycerol-3-phosphate dehydrogenase; Norbeck *et al.*, 1996) and *GPP1* (a DL-glycerol-3-phosphatase; Albertyn *et al.*, 1994) is induced by the transcription factors Hot1p, Msn1p, Msn2p, and Msn4p (the latter two factors are also implicated in the general stress response of *S. cerevisiae*). The second set is regulated by Sko1p and includes the gene *ENA1* that encodes a plasma membrane Na$^+$ pump (Proft and Serrano, 1999). In *S. cerevisiae* there are also uninduced homologues of both the *GPD1* and *ENA1* genes, designated *GPD2* and *ENA2,* respectively (Garciadeblas *et al.*, 1993; Albertyn *et al.*, 1994; Ansell *et al.*, 1997; Proft and Serrano, 1999). Results from Figure 2B show that *ENA1* and *GPP1* in *C. albicans* have an increasing level of expression in response to the hyperosmotic shock. In addition, there is a more than twofold induction of both *GPD2* and *ENA2* after the shock (Figure 2B). This is consistent with an appropriate response of *C. albicans* to the hyperosmotic stress. However, *GPD1* induction never exceeds twofold. The annotation assignments for the *GPD* and *ENA* genes are based simply on the measure-

ment of sequence identities. However, based on the relative expression profiles during osmotic stress, both *ENA* genes of *C. albicans* behave like the *ENA1* gene of *S. cerevisiae*, whereas the *GPD1* and *GPD2* genes of *C. albicans* respond, respectively, like the *GPD2* and *GPD1* genes of *S. cerevisiae*.

Oxidative Stress Response

The results obtained for the oxidative shock are presented on Figure 1A (right). As for the heat and hyperosmotic shocks, the profile of expression is that of a stress as the genes are rapidly and transiently induced. We have also chosen a set of *C. albicans* homologues of the *S. cerevisiae* genes responsive to oxidative stress (Figure 2C). These *S. cerevisiae* genes are implicated in the detoxification of the cell, and the expression of most of the antioxidant genes is induced by the transcription factors Yap1p and Skn7p (Lee *et al.*, 1999). We chose *C. albicans CTA1* as the homolog of *CTT1* (the cytoplasmic catalase T); there appears to be only one catalase in *C. albicans*, and the profile of expression of *CaCTA1* matches *ScCTT1* and not that of the noninducible *ScCTA1* (unpublished data). We found an induction greater than fourfold for *TTR1* (a glutathione reductase), *TRX1* (a thioredoxin), *CTA1*, and *TRR1* (a thioredoxin reductase) in response to the oxidative stress (Figure 2C). On the other hand, *SOD2* (a superoxide dismutase) does not appear inducible. Significantly, there is a sixfold early activation of *CAP1*, the *C. albicans* homologue of the *S. cerevisiae YAP1* (Alarco and Raymond, 1999). Recent work in *S. cerevisiae* also demonstrates an induction of *YAP1* expression in re-
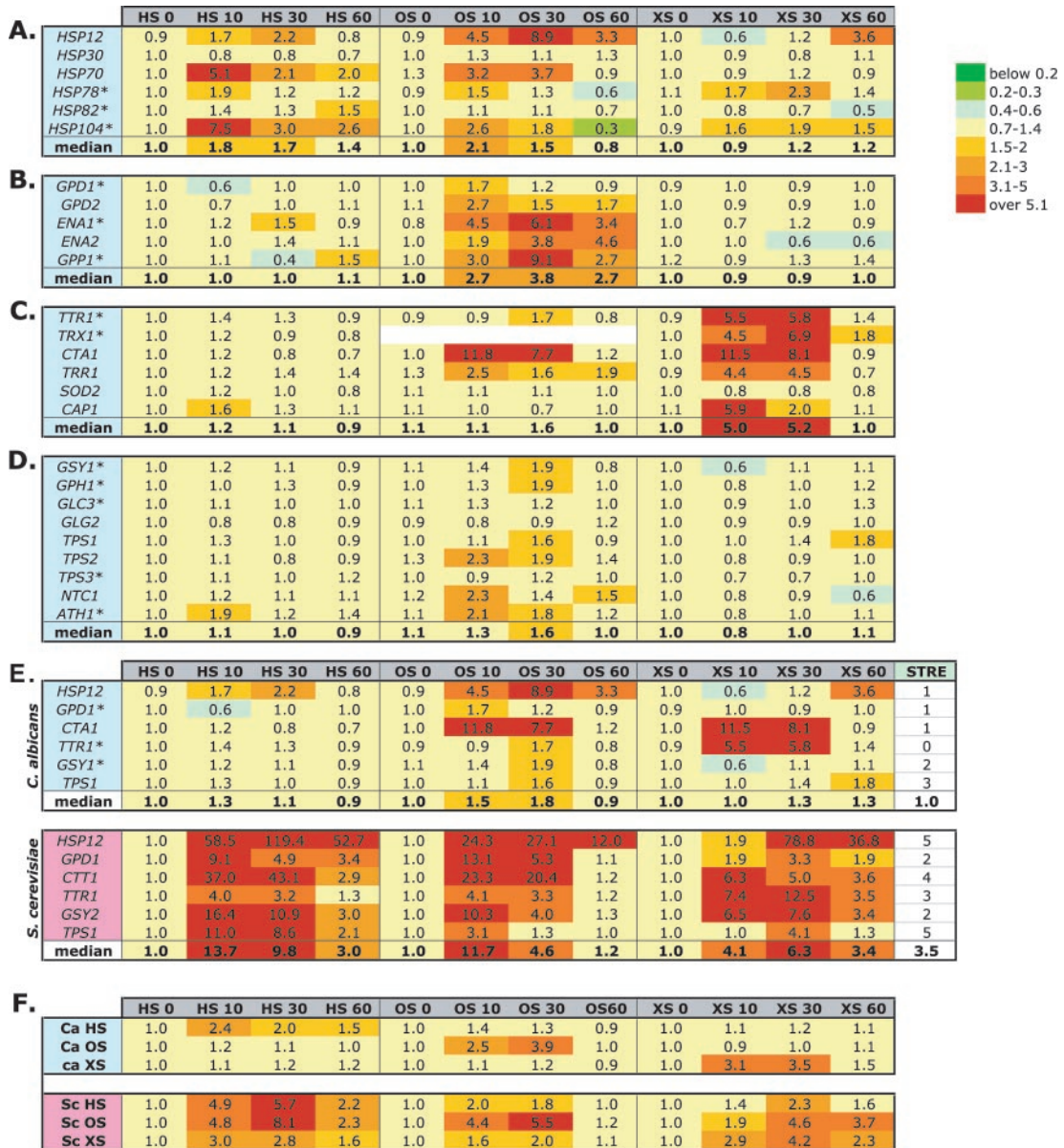
A.

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60
HSP12	0.9	1.7	2.2	0.8	0.9	4.5	8.9	3.3	1.0	0.6	1.2	3.6
HSP30	1.0	0.8	0.8	0.7	1.0	1.3	1.1	1.3	1.0	0.9	0.8	1.1
HSP70	1.0	5.1	2.1	2.0	1.3	3.2	3.7	0.9	1.0	0.9	1.2	0.9
HSP78*	1.0	1.9	1.2	1.2	0.9	1.5	1.3	0.6	1.1	1.7	2.3	1.4
HSP82*	1.0	1.4	1.3	1.5	1.0	1.1	1.1	0.7	1.0	0.8	0.7	0.5
HSP104*	1.0	7.5	3.0	2.6	1.0	2.6	1.8	0.3	0.9	1.6	1.9	1.5
median	1.0	1.8	1.7	1.4	1.0	2.1	1.5	0.8	1.0	0.9	1.2	1.2

Legend:
- below 0.2
- 0.2–0.3
- 0.4–0.6
- 0.7–1.4
- 1.5–2
- 2.1–3
- 3.1–5
- over 5.1

B.

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60
GPD1*	1.0	0.6	1.0	1.0	1.0	1.7	1.2	0.9	1.0	1.0	0.9	1.0
GPD2	1.0	0.7	1.0	1.1	1.1	2.7	1.5	1.7	1.0	0.9	0.9	1.0
ENA1*	1.0	1.2	1.5	0.9	0.8	4.5	6.1	3.4	1.0	0.7	1.2	0.9
ENA2	1.0	1.0	1.4	1.1	1.0	1.9	3.8	4.6	1.0	1.0	0.6	0.6
GPP1*	1.0	1.1	0.4	1.5	1.0	3.0	9.1	2.7	1.2	0.9	1.3	1.4
median	1.0	1.0	1.0	1.1	1.0	2.7	3.8	2.7	1.0	0.9	0.9	1.0

C.

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60
TTR1*	1.0	1.4	1.3	0.9	0.9	0.9	1.7	0.8	0.9	5.5	5.8	1.4
TRX1*	1.0	1.2	0.9	0.8					1.0	4.5	6.9	1.8
CTA1	1.0	1.2	0.8	0.7	1.0	11.8	7.7	1.2	1.0	11.5	8.1	0.9
TRR1	1.0	1.2	1.4	1.4	1.3	2.5	1.6	1.9	0.9	4.4	4.5	0.7
SOD2	1.0	1.2	1.0	0.8	1.1	1.1	1.1	1.0	1.0	0.8	0.8	0.8
CAP1	1.0	1.6	1.3	1.1	1.1	1.0	0.7	1.0	1.1	5.9	2.0	1.1
median	1.0	1.2	1.1	0.9	1.1	1.1	1.6	1.0	1.0	5.0	5.2	1.0

D.

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60
GSY1*	1.0	1.2	1.1	0.9	1.1	1.4	1.9	0.8	1.0	0.6	1.1	1.1
GPH1*	1.0	1.0	1.3	0.9	1.0	1.3	1.9	1.0	1.0	0.8	1.0	1.2
GLC3*	1.0	1.1	1.0	1.0	1.1	1.3	1.2	1.0	1.0	0.9	1.0	1.3
GLG2	1.0	0.8	0.8	0.9	0.9	0.8	0.9	1.2	1.0	0.9	0.9	1.0
TPS1	1.0	1.3	1.0	0.9	1.0	1.1	1.6	0.9	1.0	1.0	1.4	1.8
TPS2	1.0	1.1	0.8	0.9	1.3	2.3	1.9	1.4	1.0	0.8	0.9	1.0
TPS3*	1.0	1.1	1.0	1.2	1.0	0.9	1.2	1.0	1.0	0.7	0.7	1.0
NTC1	1.0	1.2	1.1	1.1	1.2	2.3	1.4	1.5	1.0	0.8	0.9	0.6
ATH1*	1.0	1.9	1.2	1.4	1.1	2.1	1.8	1.2	1.0	0.8	1.0	1.1
median	1.0	1.1	1.0	0.9	1.1	1.3	1.6	1.0	1.0	0.8	1.0	1.1

E.

C. albicans

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60	STRE
HSP12	0.9	1.7	2.2	0.8	0.9	4.5	8.9	3.3	1.0	0.6	1.2	3.6	1
GPD1*	1.0	0.6	1.0	1.0	1.0	1.7	1.2	0.9	0.9	1.0	0.9	1.0	1
CTA1	1.0	1.2	0.8	0.7	1.0	11.8	7.7	1.2	1.0	11.5	8.1	0.9	1
TTR1*	1.0	1.4	1.3	0.9	0.9	0.9	1.7	0.8	0.9	5.5	5.8	1.4	0
GSY1*	1.0	1.2	1.1	0.9	1.1	1.4	1.9	0.8	1.0	0.6	1.1	1.1	2
TPS1	1.0	1.3	1.0	0.9	1.0	1.1	1.6	0.9	1.0	1.0	1.4	1.8	3
median	1.0	1.3	1.1	0.9	1.0	1.5	1.8	0.9	1.0	1.0	1.3	1.3	1.0

S. cerevisiae

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS 60	XS 0	XS 10	XS 30	XS 60	STRE
HSP12	1.0	58.5	119.4	52.7	1.0	24.3	27.1	12.0	1.0	1.9	78.8	36.8	5
GPD1	1.0	9.1	4.9	3.4	1.0	13.1	5.3	1.1	1.0	1.9	3.3	1.9	2
CTT1	1.0	37.0	43.1	2.9	1.0	23.3	20.4	1.2	1.0	6.3	5.0	3.6	4
TTR1	1.0	4.0	3.2	1.3	1.0	4.1	3.3	1.2	1.0	7.4	12.5	3.5	3
GSY2	1.0	16.4	10.9	3.0	1.0	10.3	4.0	1.3	1.0	6.5	7.6	3.4	2
TPS1	1.0	11.0	8.6	2.1	1.0	3.1	1.3	1.0	1.0	1.0	4.1	1.3	5
median	1.0	13.7	9.8	3.0	1.0	11.7	4.6	1.2	1.0	4.1	6.3	3.4	3.5

F.

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS60	XS 0	XS 10	XS 30	XS 60
Ca HS	1.0	2.4	2.0	1.5	1.0	1.4	1.3	0.9	1.0	1.1	1.2	1.1
Ca OS	1.0	1.2	1.1	1.0	1.0	2.5	3.9	1.0	1.0	0.9	1.0	1.1
ca XS	1.0	1.1	1.2	1.2	1.0	1.1	1.2	0.9	1.0	3.1	3.5	1.5

	HS 0	HS 10	HS 30	HS 60	OS 0	OS 10	OS 30	OS60	XS 0	XS 10	XS 30	XS 60
Sc HS	1.0	4.9	5.7	2.2	1.0	2.0	1.8	1.0	1.0	1.4	2.3	1.6
Sc OS	1.0	4.8	8.1	2.3	1.0	4.4	5.5	1.2	1.0	1.9	4.6	3.7
Sc XS	1.0	3.0	2.8	1.6	1.0	1.6	2.0	1.1	1.0	2.9	4.2	2.3

Figure 2. Normalized data for a selection of *C. albicans* genes and comparison of *C. albicans* and *S. cerevisiae* response to stress through the behavior of the homologous genes. (A) to (D) show expression of *C. albicans* homologues of *S. cerevisiae* genes induced by stresses. (A) A selection of heat inducible genes. Data in (B) correspond to a panel of hyperosmotic responsive genes. (C) The selection of oxidative stress activated genes; (D) the genes implicated in carbohydrate reserve metabolism. (E) A selection of general stress response genes from *S. cerevisiae* (pink) and the behavior of their *C. albicans* homologues (blue), during the time course for the different stresses. The STRE column indicates the number of STRE identified in the promoter of the genes. The median of the levels of expression of the presented genes was calculated and the results are presented for *C. albicans* (blue) and the homologous genes of *S. cerevisiae* (pink) for each stress. The star after a gene name reflects an association with a *S. cerevisiae* gene only by homology. For (F), the sets of *C. albicans* genes induced at least two times by a stress were screened in order to identify the genes possessing an homologue in *S. cerevisiae* that is induced by the same stress. The median of the levels of expression of these genes during the selection stress but also during the two others, was calculated and the results are presented for *C. albicans* (blue) and the homologous genes of *S. cerevisiae* (pink) for each stress. HS, thermal stress; OS, hyperosmotic stress; XS, oxidative stress. Each value is colored based on its level according to the legend.

sponse to oxidative stress (Gasch *et al.*, 2000). The strong response of *CAP1* may be due in part to autoinduction; there is a perfect consensus site for *CAP1/YAP1* binding in the *CAP1* promoter (TGACTAA in position −380 relative to the +1 start of translation). Overall the results demonstrate the ability of *C. albicans* to respond transcriptionally to an oxidative stress.

Is There a General Stress Response in C. albicans?

Because all the stresses generated responses that were similar in intensity and duration, we were able to compare them to find those genes induced by all the stresses and thus identify potential components of a general stress response in *C. albicans*. On Figure 1A the expression profiles of the genes have been colored based on the Venn diagram in Figure 1B. This analysis shows that the *C. albicans* induced stress responses are quite specific. Although 139–183 genes are induced by each stress, statistical analysis shows little overlap between the list of induced genes. We have also investigated genes that are consistently repressed after 10–30 min of treatment by the different stresses. As shown in Figure 1C, there is considerably more overlap between the lists of stress-repressed genes compared with the stress-induced genes (Figure 1B). This is not surprising because all the stresses have the common feature of reducing growth rates. The nature of the repressed common genes reflects a reduction in growth and many are implicated in RNA production or maturation. Analysis of the expression profiles by hierarchical clustering has also failed to reveal a common set of induced genes.

From the increasing amount of global transcriptional analysis, we have been able to compare the data for the transcriptional response to stress in *S. cerevisiae* (Gasch *et al.*, 2000) with the current data from *C. albicans*. The comprehensive study of the response of *S. cerevisiae* to a wide variety of stresses is accessible at http://genome-www.stanford.edu/yeast_stress/. Similar stresses (thermal, oxidative, and osmotic) were applied in each study, although some experimental details were different. Figure 2E presents the induction pattern of a set of genes that are classically studied as markers of the general stress response in *S. cerevisiae*, together with the behavior of their *C. albicans* homologues. Because the data processing strategies are different from those applied in the current study, the differences of induction intensities may not reflect the relative physiological levels of gene expression. It is remarkable that although the selected *S. cerevisiae* genes are significantly induced by all the stresses, none of the *Candida* homologues are induced more than two times by all three of the analyzed stresses in the 10–30-min period following the stress. Because the commonly responsive genes are inducible in *S. cerevisiae* through the general stress response mechanism and the STRE motifs of their promoters, we checked for the presence of such elements in the promoters of the homologous genes in *C. albicans* (Figure 2E). Most of the candidate general-stress-response genes from *C. albicans* possess potential STRE elements in their promoters. Thus the absence of the general response is not simply due to the absence of the potential *cis*-acting regulatory elements. However, the numbers of consensus sites are low relative to the situation in *S. cerevisiae* and their functionality remains to be demonstrated. Overall there is no convincing evidence for a general stress response in *C. albicans*. This point is illustrated by Figure 2F that presents an overview of the behavior of homologous genes from *C. albicans* and *S. cerevisiae* to stress: the data correspond to the median of the *C. albicans* genes induced by a stress and possessing a homologue in *S. cerevisiae* responding to this stress as well. This highlights the fact that the genes induced by a given stress are typically not inducible by other stresses in *C. albicans*, in contrast with the situation in *S. cerevisiae*.
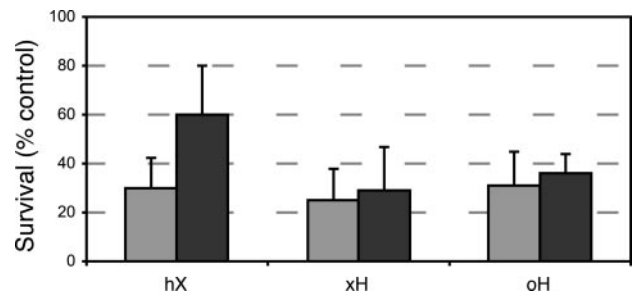

Figure 3. Cross-protection phenomenon in *C. albicans*. The data represent the survival level of cells after exposure to a mild stress followed by a different strong stress: a mild heat stress followed by a strong oxidative stress -hX-, a mild oxidative stress followed by a strong heat shock -xH- and a mild hyperosmotic stress followed by a strong heat shock -oH- (see MATERIALS AND METHODS section for details). The control (gray bars) corresponds to cells unexposed to the mild stress and the data presents the level of survival compared with the nonstressed cells. The assay (dark bars) corresponds to cells exposed to the mild stress and the data presents the level of survival compared with the sample that has not been submitted to stresses. The results are the median of four independent assays.

Cross-protection to Stress in C. albicans

In *C. albicans*, as for *S. cerevisiae*, a mild stress is able to protect the yeast against a subsequent stronger stress that would otherwise kill the cells. Thus pretreatment with a mild heat shock ensures the resistance of the *C. albicans* cells to a strong heat shock (Arguelles, 1997), and a mild oxidative stress protects against a strong increase of ROS (Jamieson *et al.*, 1996). However, one of the most important properties of the general stress response in *S. cerevisiae* is the ability to create an induced cross-protection. In this situation the two stresses are of different natures so that, for example, a mild heat shock will protect the cells from a lethal hyperosmotic shock (Lewis *et al.*, 1995). We have investigated the existence of such a cross-protection phenomenon in *C. albicans*. Figure 3 presents three cross-protection experiments. The results show a twofold increase of resistance in a mild heat stress followed by a strong oxidative stress and no improvement in survival in the case of the mild oxidative stress or hyperosmotic stress followed by a strong heat shock. Thus, the acquired resistance is weak to nonexistent in *C. albicans* (maximum twofold) compared with the more than 100-fold increase of *S. cerevisiae* survival (Wieser *et al.*, 1991; Lewis *et al.*, 1995).

Response of the Reserve Sugars to a Stress

Another physiological consequence of the general stress response in *S. cerevisiae* is the accumulation of glycogen and trehalose after a stress. Parrou *et al.* (1997) have described that the stresses trigger the production of glycogen up to three times the basal level in response to heat shock and have detected an accumulation in response to the hyperosmotic and oxidative stresses. Similarly, *S. cerevisiae* weakly induces the production of trehalose after these same stresses. This low accumulation is due to a turn-over phenomenon with induction of genes implicated in both trehalose production and degradation (Parrou *et al.*, 1997). We have tested the production of storage sugars in *C. albicans* in response to
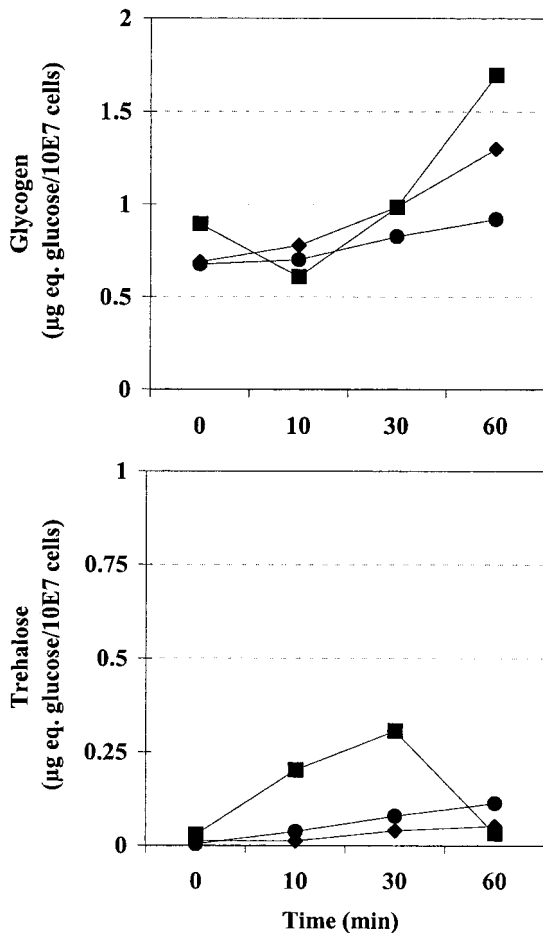
Figure 4. Effects of stresses on the carbohydrate reserve levels of *C. albicans*. Glycogen (A) and trehalose (B) are measured at different times after the stress. Thermal stress (●) corresponds to a temperature shift from 23–37°C, hyperosmotic stress (■) is triggered by challenging the culture with 0.3 M NaCl final, and oxidative stress (◆) is due to the addition of H_2O_2 to a final concentration of 0.4 mM.

stress. As shown in Figure 4, there is essentially no accumulation of the reserve sugars in response to either thermal or oxidative stresses. This lack of accumulation cannot be explained through a turnover mechanism, as there is no induction of the genes implied in trehalose or glycogen metabolism. Hyperosmotic stress only slightly increased glycogen and trehalose production. These results agree with the microarray data because several genes involved in reserve sugar metabolism are induced nearly twofold by the hyperosmotic stress (Figure 2D). Taken as a whole, the accumulation of the reserve sugars is not dramatically changed by the application of the stresses, probably because of the weaker response of the genes.

DISCUSSION

C. albicans exhibits the classical transient transcriptional induction of specific genes in response to environmental

stresses. Thermal, osmotic, and oxidative stresses each induce specific sets of >100 genes. Many of these induced genes encode proteins that serve to protect the cell. However, in *C. albicans* each stress response is unique: there are essentially no genes that are induced in common by all the stresses. This situation is in marked contrast to that of *S. cerevisiae*, which exhibits a coordinated induction of a set of genes in response to several stresses.

It is evident that comparisons of global cellular responses among different systems have many potential complications. There are no standard conditions for stress induction and for transcription profile analysis, so details of the activating process, measurement and analysis of the response will vary. In this set of experiments we chose conditions that were broadly compatible with those of Gasch *et al.* (2000), but the major consideration was to obtain comparable intensities among the transcriptional responses within our experiments in order to easily compare our *C. albicans* results. The comparison with the *S. cerevisiae* results is less straightforward; even with a restructuring of the *S. cerevisiae* data to fit the *C. albicans* analysis strategy, applied quantitative comparisons have to be handled with care. For example, it is risky to consider the greater variation of *S. cerevisiae* to the same thermal stress (Figure 2E) as defining a direct physiological difference between *S. cerevisiae* and *C. albicans*. However, even with variation in the quantification and the absence of general stress response in *C. albicans*, it is clear that the pattern of response to each stress is relatively similar between *S. cerevisiae* and *C. albicans*, especially when it comes to protective genes such as ROS scavengers. In addition, the comparison is facilitated by the fact that 80% of the *S. cerevisiae* stress-inducible genes possess an orthologue in *C. albicans*. This work illustrates the power and limits of DNA microarray analysis to compare the response of different species to similar conditions.

There are also many unique distinctions between the responses of the two fungi to similar stresses. For example, *HSP30* is well induced by heat shock in *S. cerevisiae* (12-fold in the results of Gasch *et al.* [2000]) but not at all in *C. albicans* (Figure 2A). In *C. albicans* *HSP12* and *CTA1* are strongly activated by hyperosmotic shock but poorly by thermal stress relative to the behavior of the homologous genes in *S. cerevisiae* (Figure 2E; Schuller *et al.*, 1994; Varela *et al.*, 1995). There are possible explanations for these differences between the two yeasts: *HSP12* has so far not been demonstrated to be a chaperone protein (Zara *et al.*, 2002) and *HSP30* is induced by heat shock in *S. cerevisiae* through a HSE- and Msn2/4p-independent mechanism that remains to be unraveled (Seymour and Piper, 1999); moreover, *CTT1* and *HSP12* are inducible by osmotic stress in a *msn2 msn4* double mutant (Rep *et al.*, 1999). Overall, one should keep in mind that these results are derived from global microarray analyses and must be supported by more specific experiments. However, the cross-protection experiments (Figure 3) and the analysis of reserve sugar production (Figure 4) strongly support the reliability of the microarray results. One should note for this last point that recent reports (Arguelles, 1997; Van Dijck *et al.*, 2002) have demonstrated the accumulation of trehalose in response to a strong heat shock in *C. albicans*. Nevertheless, it appears that *C. albicans* is much less efficient in accumulating reserve sugars after a moderate stress than is *S. cerevisiae*. Because this phenome-

non depends on the STRE and the general stress response (Parrou *et al.*, 1999; Zahringer *et al.*, 2000), the evidence, both physiological as well as transcriptional, strongly supports the lack of a general stress response in *C. albicans*.

In *S. cerevisiae*, the specific transcription factors Msn2p and Msn4p are implicated in the general stress response (Martinez-Pastor *et al.*, 1996; Schmitt and McEntee, 1996). Although STREs are present in the promoters of many *C. albicans* stress responsive genes, their number of copies per promoter is reduced compared with the equivalent *S. cerevisiae* genes (Figure 2E and unpublished data). The absence of general stress response, together with the relative lack of STREs in stress-responsive genes brings into question the possible role for the transcription factors Msn2p and Msn4p. Candidate homologues of the Msn2p and Msn4p transcription factors exist in *C. albicans* (Straffon and Brown, personal communication); their function is currently under study. However, even in *S. cerevisiae*, the Msn2/Msn4-STRE regulatory circuit is not the only way to induce genes in response to different stresses.

The *Candida* lineage appears to have initiated more than 150 million years ago (Pesole *et al.*, 1995). As a consequence, there has been extensive opportunity for divergence from other fungi such as *S. cerevisiae*. This divergence is emphasized by the presence of ~2000 genes in *C. albicans* that have no homologues in *S. cerevisiae*. The differences in environmental constraints for *C. albicans* could have allowed a different evolution of signal transduction pathways (Kadosh and Johnson, 2001). The current work shows that this is true for the regulatory circuit involved in the general response to stress. Knowledge of the unique wiring of the regulatory pathways in *C. albicans* will be important in the development of specific drugs against this pathogenic yeast. It will also be necessary to categorize the numerous currently uncharacterized genes of this pathogenic yeast involved in the stress processes. It is noteworthy that 49% of the orthologous genes similarly induced in *C. albicans* and *S. cerevisiae* are of unknown function, vs. 16% implicated in stress and 15% implicated in metabolic processes.

This study highlights the transcriptional behavior *C. albicans* genes in response to stresses and this profiling allows answers to some outstanding questions. For example, *C. albicans* decode the standard CUG codon as serine (Pesole *et al.*, 1995; Santos *et al.*, 1997). Recent experiments (Santos *et al.*, 1999) have modified *S. cerevisiae* to allow expression of a ser-CUG codon; this modification triggered the general stress response process. The authors speculated that the advantage of a constitutive general stress response could be the reason for the use of a nonstandard code in *C. albicans*. However, our results do not support this speculation, because there is no general stress response in *C. albicans*, and so, the selective "advantage" of the ser-CUG codon is questionable. Recently it has also been suggested "the general stress response exactly reflects the needs of the cell under any environmental conditions" (Hohmann, 2002). This need is not true for *C. albicans* and this discrepancy emphasizes the point that generalizations from even such successful model systems as *S. cerevisiae* may not be possible. Ultimately it is likely that experimental analyses in many systems will be necessary to understand the general properties of cellular response to stress.

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