RESEARCH ARTICLE

Overexpression of the *YAP1*, *PDE2*, and *STB3* genes enhances the tolerance of yeast to oxidative stress induced by 7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine

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Abstract

7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine (CTBT) is an antifungal agent that induces oxidative stress and enhances the activity of other antifungals with different modes of action. A genome-wide screening of *Saccharomyces cerevisiae* genomic library in the high-copy-number plasmid revealed three genes, *YAP1*, *PDE2*, and *STB3*, which increased the CTBT tolerance of the parental strain. The *YAP1* gene is known to activate many genes in response to oxidants. The *PDE2* and *STB3* genes encode the high-affinity cAMP phosphodiesterase and the transcription factor recognizing the ribosomal RNA processing element in promoter sequences, respectively. The protective effects of their overexpression against CTBT toxicity was observed in the absence of certain proteins involved in stress responses, cell wall integrity signaling, and chromatin remodeling. The enhanced CTBT tolerance of the *YAP1*, *PDE2,* and *STB3* transformants was a consequence of their high antioxidant enzyme activities at the beginning of CTBT treatment in comparison with that of the parental strain, for that they inactivated the CTBT-induced reactive oxygen species. These results point to the complex interplay among the oxidant sensing, cAMP-protein kinase A signaling, and transcription reprogramming of yeast cells, leading to their better adaptation to the stress imposed by CTBT.

Introduction

7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine (CTBT) is an antifungal agent that inhibits the growth of yeast (Cernicka *et al.*, 2007) and filamentous fungi (Culakova *et al.*, 2012). Beside its antifungal activity, CTBT enhances the efficiency of other antifungals with different targets in the cells, such as cycloheximide, azole antifungals, or 5-fluorocytosine (Cernicka *et al.*, 2007; Culakova *et al.*, 2012). Its growth-inhibitory activity observed in both wild-type and multidrug-resistant yeast strains is unaffected by deletion of the *PDR5* and *SNQ2* genes encoding the main multidrug resistance drug efflux pumps in *Saccharomyces cerevisiae* (Cernicka *et al.*, 2007).

At least 169 yeast deletion mutants have been identified as hypersensitive to CTBT. The deleted genes encode proteins involved mainly in the mitochondrial functions, DNA repair, transcription, chromatin remodeling, and the oxidative stress response. Transcriptome analysis of CTBT-treated yeast cells revealed a rapid induction of oxidant and stress defense genes that were dependent on the transcription factors encoded by the *YAP1, CIN5, MSN2*, and *MSN4* genes. At the same time, many yeast genes containing the polymerase A and C and ribosomal RNA processing element (RRPE) DNA-binding motifs in their promoters were repressed. These chemogenomic and transcriptome analysis results and the *in vivo* demonstration of CTBT-induced mitochondrial superoxide ($O_2^{\bullet -}$) production led to the conclusion that this compound causes oxidative stress, which enhances the activities of other drugs interfering with the metabolism of DNA, protein, and lipids, that is, the main targets of reactive oxygen species (ROS) in yeast cells (Batova *et al.*, 2010).

The antifungal activity of drugs can be suppressed by various defense mechanisms in treated cells. These mechanisms are mainly based on a decreased drug uptake, enhanced efflux of drugs from the cells by overproduced membrane transporters, the detoxification or sequestration of active compounds, and the overproduction, structural modification, or repair of the drug target (Kolaczkowska & Goffeau, 1999; Wolfger *et al.*, 2001; Sanglard & Odds, 2002; Prasad & Kapoor, 2005; Shapiro *et al.*, 2011). It was earlier demonstrated that the CTBT efflux mediated at least by transporters expressed under the control of the multidrug resistance transcription factors *PDR1* and *PDR3* does not contribute to the susceptibility of yeast to this compound (Cernicka *et al.*, 2007).

In the present study, we have used a genome-wide approach to reveal genes required for yeast cells to adapt and survive the cellular stress imposed by CTBT. We screened the yeast genomic library for multicopy suppressors of CTBT sensitivity and identified three protein-encoding genes that, when overexpressed, enhance the tolerance of yeast cells to CTBT.

Materials and methods

Strains and culturing conditions

The following haploid yeast strains were used: the *S. cerevisiae* BY4741 wild-type strain (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), its isogenic deletion mutants *yap1Δ, stb5Δ, msn2Δ, hog1Δ, mpk1Δ/slt2Δ, ras2Δ, rpd3Δ, sin3Δ, hos2Δ, set3Δ, hda1Δ, hda3Δ, sir2Δ, hst1Δ, hst3Δ*, and *atr1Δ* (EUROSCARF, http://web.uni-frankfurt.de/fb15/mikro/euroscarf), and the *msn2Δmsn4Δ* double mutant strain (*MATa ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100 msn2::HIS3 msn4::TRP1*; Schüller *et al.*, 2007). Cells were grown on glucose-rich (YPD) medium (2% glucose, 1% yeast extract, 2% bacto peptone), glycerol-rich (YPG) medium (as YPD but 2% glycerol instead of 2% glucose), or minimal (YNB) medium containing a 0.67% yeast nitrogen base without amino acids, 2% glucose and appropriate nutritional requirements. The media were solidified with 2% bactoagar. The *Escherichia coli* XL1-Blue strain was used as a host for transformation, plasmid amplification, and preparation. The bacterial strains were grown at 37 °C in Luria–Bertani medium (1% tryptone, 1% NaCl, 0.5% yeast extract, pH 7.4) supplemented with 100 μg mL^{-1} ampicillin for the selection of transformants.

Drug susceptibility assays

Drug susceptibility was determined in spot assays. Five-microliter aliquots of serially diluted suspensions of three independent clones grown in minimal medium were spotted onto minimal or rich media containing glucose or glycerol and supplemented with various concentrations of CTBT or other chemicals. Growth at 30 °C was scored after 5 or 7 days. The susceptibility to CTBT and other chemicals was also assessed using zone-inhibition assays. Approximately 10^7 cells were plated onto glucose or glycerol agar. The filter disks (diameter 6 mm), soaked with appropriate amounts of chemicals, were placed on the plates, which were incubated at 30 °C for 5 days before determination of the diameter of growth-inhibition zone.

Genomic library screening

The *S. cerevisiae* genomic library in a high-copy-number plasmid YEp13 (2 μm *LEU2* ori Amp^R Tet^R; Gbelska *et al.*, 1995) was used for the isolation of genes conferring CTBT resistance. Yeast transformation was carried out using the modified lithium acetate protocol (Nourani *et al.*, 1997) or by electroporation (Thompson *et al.*, 1998). Leucine-positive transformants were replica-plated onto YPD media containing 8 or 10 μg CTBT per mL. CTBT-resistant transformants were further analyzed by growth assays, using serial dilutions and spotting onto agar plates containing different concentrations of CTBT. Growth was scored after 7 days of incubation at 30 °C.

Recombinant DNA techniques and plasmids

Standard protocols were used to generate recombinant DNAs and for the restriction enzyme analysis and gel electrophoresis as described by Sambrook *et al.* (1989). Plasmid DNA from *E. coli* was prepared by an alkaline lysis method. Plasmid DNA from yeast cells was extracted according to Ward (1990). An empty vector pRS306K (*ARS1 KARS2 URA3* ori Amp^R) was used to introduce a plasmid-borne *URA3* gene into the host strain. Plasmid pRS426-ATR1 (2 μm *URA3* ori Amp^R) contained the *ATR1* gene under the control of the P_{GPD1} promoter (Kaya *et al.*, 2009). Plasmid pAdh1–Msn2–GFP contained the *MSN2* gene fused with GFP under the control of the P_{ADH1} promoter (Schüller *et al.*, 2007). Plasmid pRS425 (2 μm *LEU2* ori Amp^R) was used as an empty vector to introduce a plasmid-borne *LEU2* gene into the host strain and to subclone the *ERG6* gene on the HindIII-BamHI DNA fragment from the pED1 plasmid (Fig. 1). To obtain the YEp13-VTS1 and YEp13-STB3 plasmids, the SphI-SphI genomic fragments carrying the *PDE2* (2.8 kb) and *SEC7* (3.7 kb) DNA sequences were excised from the pED2 and pED3 plasmids, respectively.

DNA sequence analysis

The DNA sequences of the plasmid inserts were determined with an ABI Prism 3100 DNA sequencer

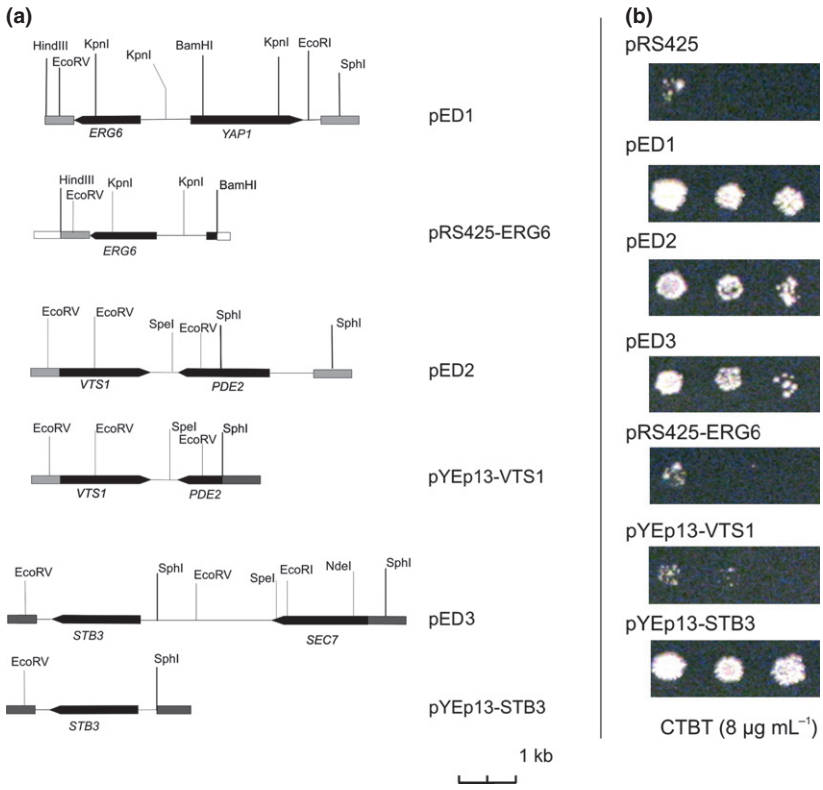

Fig. 1. CTBT tolerance of the yeast transformants bearing the *YAP1*, *PDE2*, and *STB3* genes on the high-copy-number plasmid. (a) Restriction maps of DNA inserts. (b) Growth of the BY4741 transformants on YPD medium containing CTBT.

(Applied Biosystems, Foster City, CA), using double-stranded plasmid DNA purified with Qiagen Plasmid kits (Qiagen, Hilden, Germany) and oligonucleotide primers flanking the BamHI cloning site in YEp13. Sequence data were analyzed using programs based on the BLAST algorithm by computing performed at the NCBI.

Measurements of ROS and specific enzyme activities and other analytical procedures

In all experiments, mid-log-phase (18 h) cultures of the parental strain BY4741 and its *YAP1*, *PDE2*, and *STB3* transformants were treated with a subinhibitory concentration (1 μg mL^{-1}) of CTBT for 1 h in shaken (150 r.p.m.) YNB liquid medium at 30 °C. The subinhibitory concentration resulted in not more than 25% of temporary growth inhibition (in 25-h cultures) in comparison with the untreated cultures. However, the cell number (optical density) of CTBT-treated cultures reached that of untreated 40-h cultures. Cell-free extracts were prepared using X-pressing (Dybecksgatan 10; Göteborg, Sweden). The protein content of the cell-free extract was measured by a modified Lowry method (Peterson, 1983). Specifically, to estimate the intracellular peroxide (H_2O_2) and $O_2^{\bullet-}$ levels, the indicators dihydrorhodamine 123 (DHR 123) and dihydroethidium (10 μM) were used, respectively. The extents of formation of rhodamine and ethidium were measured and quantified by BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA; Henderson & Chappell, 1993; Carter *et al.*, 1994). The excitation wavelength was 488 nm for both dyes, while the emission wavelengths were 530 using FL1 detector and 585 nm applying FL2 detector for rhodamine and ethidium, respectively. The specific activities of superoxide dismutases (Cu/Zn-SOD and Mn-SOD; Oberley & Spitz, 1984), glutathione reductase (GR; Pinto *et al.*, 1984), glutathione peroxidase (GPx; Chiu *et al.*, 1976), and catalase (CAT; Roggenkamp *et al.*, 1974), and the intracellular concentrations of glutathione (GSH) and oxidized GSH (glutathione disulfide, GSSG; Anderson, 1985) were determined using the well-established colorimetric assays.

Chemicals

CTBT was provided by G. Hajos (Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary). All other chemicals used in this study were of analytical grade and were bought from Sigma-Aldrich Ltd, with the exception of dihydroethidium, which was purchased from Fluka (Buchs, Switzerland).

Results

Isolation of CTBT defense genes

The yeast genomic library was screened for genes that, when overexpressed, confer resistance to CTBT. The *S. cerevisiae* BY4741 wild-type strain was transformed with the genomic library in the high-copy-number vector YEp13. The resulting transformants were selected for leucine prototrophy and CTBT resistance. From among about 20 000 transformants, 35 clones resistant to 8 μg mL^{-1} of CTBT were isolated. Restriction analysis of their plasmid DNA revealed three different restriction profiles. The plasmids with these profiles, named pED1, pED2, and pED3 (Fig. 1a), were analyzed in retransformation experiments and proved to confer CTBT resistance.

DNA sequencing and computer-assisted analyses of the plasmid inserts revealed three different *S. cerevisiae* genomic regions. Plasmid pED1 with the 4.5-kb genomic insert carried the *YAP1* and *ERG6* genes. Plasmid pED2 harboring the 4.5-kb genomic insert carried the *VTS1* and *PDE2* genes. Plasmid pED3 with the 5.7-kb genomic insert carried the *STB3* gene and the N-terminal part of the *SEC7* gene. To identify the genes responsible for CTBT resistance, the *ERG6* gene was subcloned into the pRS425 vector. pED2 and pED3 were deleted for the *PDE2* and *SEC7* DNA sequences, respectively. Yeast transformants harboring the corresponding constructs were then analyzed for CTBT susceptibility. We found that only three genes, in the high-copy-number plasmid, *YAP1, PDE2*, and *STB3,* are responsible for CTBT resistance (Fig. 1b). The *YAP1* gene codes for the transcription factor that is a major regulator of the yeast defense to oxidative and xenobiotic stresses (Moye-Rowley, 2003; Rodrigues-Pousada *et al.*, 2010). The *PDE2* gene encodes the high-affinity cAMP phosphodiesterase, which plays a role in the protein kinase A (PKA) signaling pathway (Sass *et al.*, 1986). The *STB3* gene encodes the RRPE-binding protein involved in the glucose-induced transition from quiescence to growth. This transcription factor binds Sin3p, a member of the histone deacetylase complex involved in chromatin remodeling (Kasten & Stillman, 1997; Liko *et al.*, 2007).

Roles of the *YAP1*, *PDE2*, and *STB3* genes in CTBT tolerance

YAP1 is known to activate the expression of many genes involved in yeast multidrug resistance and oxidative stress protection. In a previous study, we identified 82 genes that were activated by Yap1p in CTBT-treated yeast cells and the *ATR1* gene displaying the highest response to CTBT treatment among the known major facilitator superfamily transporters involved in the control of multidrug resistance (Batova *et al.*, 2010). Therefore, we assessed the possible role of Atr1p, required for resistance to aminotriazole, nitroquinoline-*N*-oxide, and boron (Kanazawa *et al.*, 1988; Kaya *et al.*, 2009), in CTBT transport using the *atr1Δ* mutant strain and its transformants bearing the *ATR1* gene under the control of the *GPD1* promoter on a high-copy number plasmid. We observed that the *atr1Δ* mutant is hypersensitive to boric acid, whereas its pRS426-ATR1 transformants are resistant to this substrate of the Atr1p transporter, corroborating the published data (Kaya *et al.*, 2009). However, neither deletion of the *ATR1* gene nor its overexpression in yeast cells significantly altered their susceptibility to CTBT (Fig. 2). This indicates that CTBT is apparently not the substrate of Atr1p, and its overproduction only accompanies the activation of many genes involved in the oxidative stress response by Yap1p.

The role of the overexpressed *PDE2* gene in the control of CTBT tolerance in yeast was studied using the selected deletion mutants defective in the signal transduction pathways and transcription. Deletion mutants were

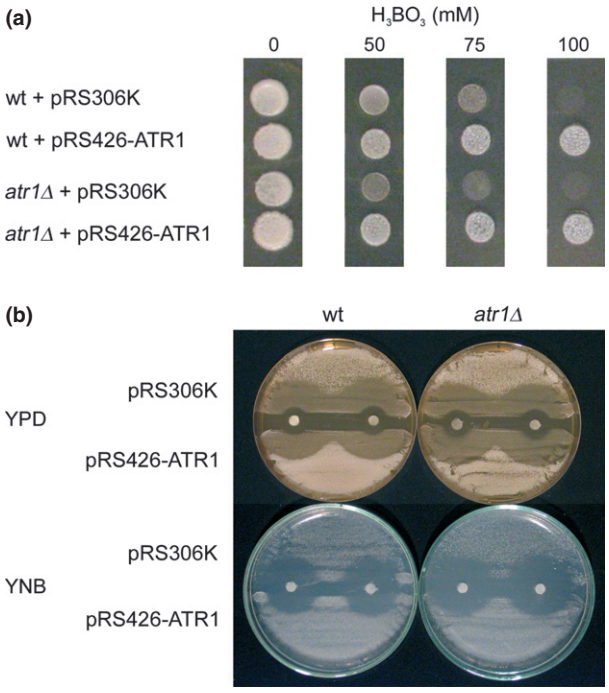

Fig. 2. Phenotype of the wild-type and *atr1Δ* mutant strains transformed with the plasmid-borne *ATR1* gene. (a) Growth of transformants on YPD plates in the presence of indicated concentrations of boric acid scored after 5 days of incubation at 30 °C. (b) Zone-inhibition assays of CTBT susceptibility of transformants on YPD and YNB medium. Amounts of CTBT per disk: 10 μg on YPD and 5 μg on YNB.

transformed with the *PDE2* gene borne on a high-copy-number plasmid pED2, and the CTBT susceptibility of the resulting transformants was assessed. As shown in Fig. 3, the overexpressed *PDE2* gene enhanced the CTBT tolerance in yeast transformants and allowed their growth at CTBT concentrations that already inhibited the proliferation of the host cells. The protective effect of the overexpressed *PDE2* against oxidative stress induced by CTBT was observed in the absence of the mitogen-activated protein kinases Hog1p and Mpk1p/Slt2p, which play roles in osmotic stress, oxidative stress, and cell wall integrity signaling (Paravicini *et al.*, 1992; Westfall *et al.*, 2004; Vilella *et al.*, 2005; Kim *et al.*, 2010). A similar effect was observed in mutants deleted in genes encoding transcription factors Yap1p, Stb5p, Msn2p, and Msn4p (Fig. 3), which have been shown to activate many genes involved in the oxidative and general stress responses (Martínez-Pastor *et al.*, 1996; Moye-Rowley, 2003; Larochelle *et al.*, 2006).

Our previous transcriptome analysis revealed that more than 120 genes activated by Msn2p and Msn4p transcription factors respond to CTBT treatment (Batova *et al.*, 2010). Because Msn2p and Msn4p have overlapping functions (Estruch & Carlson, 1993), we also used the pAdh1–Msn2–GFP plasmid to assess a role of the Msn2p transcription factor in the *PDE2* conferred CTBT tolerance. As also shown in Fig. 3, the *MSN2–GFP* fusion gene expressed from the strong constitutive P_{ADH1} promoter in the BY4741 host strain was not able to mimic the protective effect of the overexpressed pED2 borne *PDE2* gene. These results, together with the CTBT tolerance conferred by overexpressed *PDE2* to mutant lacking the Msn2p and Msn4p transcription factors, suggest that cAMP-activated PKA can employ diverse stress defense systems with overlapping function.

When the *STB3* gene on YEp13 was used instead of *PDE2,* a similar increase in the CTBT tolerance of the transformants was observed (Fig. 4). Moreover, deletion mutants bearing *STB3* on a high-copy-number plasmid displayed enhanced tolerances to CTBT in the absence of protein components of different histone deacetylase complexes, (Rpd3p, Sin3p), (Hos2p, Set3p), (Hda1p, Hda3p), and (Sir2p, Hst1p, Hst3p), or when the *RAS2* gene encoding the GTP-binding protein that stimulates the production of cAMP by adenylate cyclase was deleted (Fig. 4).

These results demonstrate the protective effect of overproduced Pde2p and Stb3p against CTBT toxicity, which is independent on the specific functions of the analyzed individual proteins involved in stress signaling, chromatin remodeling, and transcription. Apparently, the antioxidant defense of yeast cells in the presence of CTBT is the result of the cooperation of multiple signaling pathways and many genes involved in the control of the cell division cycle, energy metabolism, detoxification of oxidants, and repair of oxidative damaged DNA, proteins, and lipids.

Antioxidant activity of yeast cells overexpressing *YAP1*, *PDE2*, and *STB3* genes

We found that CTBT (2 or 4 μg mL^{-1}) induces $O_2^{\bullet-}$ formation in cells grown in YPGal medium (measured via the oxidation of MitoSOX Red) and oxidative stress in yeast and filamentous fungi, resulting in cell death (Batova *et al.*, 2010; Culakova *et al.*, 2012). We therefore analyzed the $O_2^{\bullet-}$, H_2O_2, and GSH concentrations and specific activities of antioxidant enzymes such the SODs (which catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2) localized in the cytoplasm (Cu/Zn-SOD) or the mitochondria (Mn-SOD), CAT (which inactivates H_2O_2 to H_2O), GPx (which catalyzes the oxidation of GSH to GSSG with the concomitant consumption of H_2O_2), GR (which reduces GSSG to GSH at the expense of NADPH) in the parental strain BY4741 and its transformants bearing the *YAP1*, *PDE2*, and *STB3* genes on a high-copy-number plasmid. In all experiments, mid-log-phase (18-h) cultures multiplied in minimal (YNB) medium were used to obtain

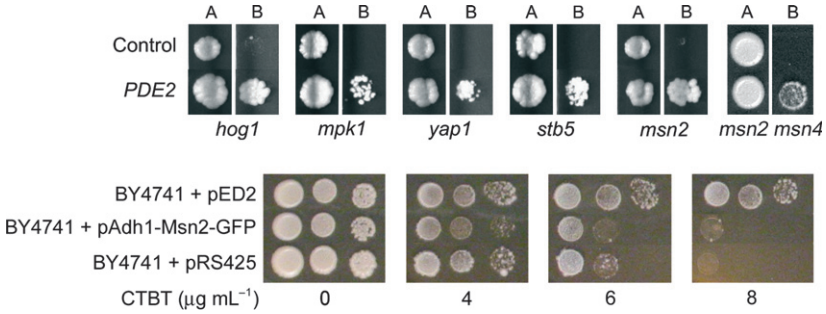

Fig. 3. CTBT tolerance of yeast deletion mutants induced by the overexpressed pED2 plasmid-borne *PDE2* gene. (a) Growth in the absence of CTBT. (b) Growth in the presence of CTBT.

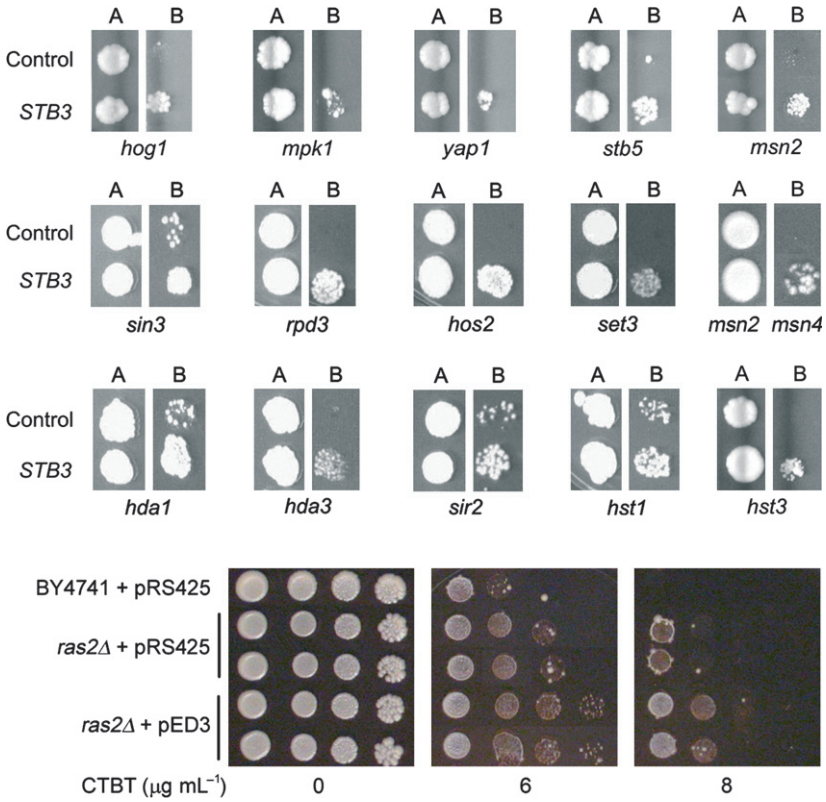

Fig. 4. CTBT tolerance of yeast deletion mutants induced by the overexpressed pED3 plasmid-borne *STB3* gene. (a) Growth in the absence of CTBT. (b) Growth in the presence of CTBT.

cells in the same physiological status and to eliminate the possible antioxidant effects of the complete (YPD) medium (Gazdag *et al.*, 2011). The results are presented in Fig. 5 and Table 1.

In comparison with the untreated cells, the CTBT-treated parental BY4741 cells exhibited (1) the same $O_2^{\bullet-}$, decreased H_2O_2 and significantly increased GSH and GSSG concentrations; and (2) the same CAT, GPx, and GR and significantly elevated specific activities of the SODs, especially Cu/Zn-SOD. Cellular redox homeostasis is normally maintained by high concentrations of GSH (up to 10 mM) in most living cells. Thus, GSH plays an important role in the response of yeast to oxidative stress (Halliwell & Gutteridge, 1991). These results suggested that CTBT causes a disturbed redox state in the cells and induces an adaptation process regulated at the level of the antioxidant system.

In comparison with the untreated parental BY4741 cells, the untreated *YAP1*, *PDE*2, and *STB3* transformants showed (1) the same (in *YAP1*) or a decreased (in the *PDE2* and *STB3* transformants) concentration of $O_2^{\bullet-}$; (2) a significantly decreased concentration of H_2O_2 in *STB3* and GSH in all transformants (an elevated level of GSSG in *STB3* and a decreased level in *YAP1* and decreased GSH/GSSG ratio in all transformants); and (3) the same CAT activity (in *YAP1*) or an increased level (in the *PDE2* and *STB3* transformants); a decreased GPx activity in all of them; and the same activity of GR (in *YAP1*) or a decreased activity (in the *PDE2* and *STB3* transformants). The overexpression of *YAP1*, *PDE2*, and *STB3* alone caused the disturbed redox homeostasis of the cells. *YAP1* induced the lowest level of disturbance.

In comparison with the untreated cells, the CTBT-treated transformants showed (1) a decreased concentration of $O_2^{\bullet-}$ in the *STB3* overexpressing cells; (2) a decreased concentration of H_2O_2 in the *YAP1* and *PDE2* transformants as a consequence of the significantly increased specific activity of GPx; (3) an increased GSH concentration and decreased Mn-SOD and CAT activities only in the *STB3* overexpressing cells; (4) no further increase in the activities of the SODs and of Cu/Zn-SOD after CTBT treatment, and (5) the other elements of the redox system were not influenced by CTBT treatment.

Significantly decreased GSH concentrations were detected in both untreated and CTBT-treated cells of the *YAP1*, *PDE2*, and *STB3* transformants, relative to the BY4741 host strain. These results are explained in part by decreased specific activity of GR in both untreated and CTBT-treated cells of the *PDE2* and *STB3* transformants.

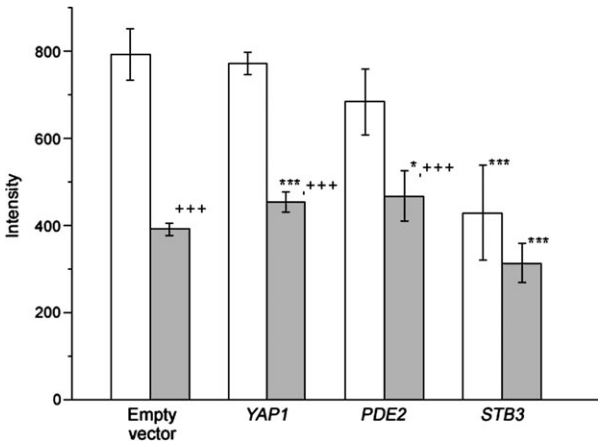

Fig. 5. Intracellular H_2O_2 content detected as oxidation of DHR 123 in the *Saccharomyces cerevisiae* BY4741 parental strain and its transformants containing an empty vector pRS425 and the *YAP1*, *PDE2*, and *STB3* genes on YEp13 plasmid without or after treatment with a subinhibitory concentration of CTBT (1 μg mL^{-1}) for 1 h in mid-log-phase cultures grown in YNB medium at 30 °C. Untreated cultures (empty columns), CTBT-treated cultures (gray columns). The average fluorescence intensity of rhodamine of 10 000 cells from 5×10^6 cells mL^{-1} culture was monitored flow cytometrically at 21 °C. Data are presented as the mean ± SD of the results of four independent experiments. Significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Values were calculated via the Student's *t*-test. $^{+++}$ Significant differences between untreated and CTBT-treated samples. */*** Significant differences between the CTBT-treated BY4741 parental strain and its CTBT-treated transformants overexpressing the *YAP1*, *PDE2*, and *STB3* genes.

CTBT treatment induced 48% and 19% increases only of GSH in the parental and *STB3* overexpressing cells, respectively. The strain-dependent changes in the specific activities of the antioxidant enzymes, the concentration of GSSG, and the ratio GSH/GSSG in the *YAP1*, *PDE2*, and *STB3* overexpressing cells proved the disturbed redox state of the transformants.

Discussion

The survival of yeast cells under stress conditions depends on their ability to respond appropriately to alterations in the environment. Cells have to sense and transmit stress signals and to activate stress defense mechanisms by reprogramming their gene expressions. In this study, we have shown that the three overexpressed genes, *YAP1*, *PDE2*, and *STB3*, can enhance the tolerance of yeast cells to CTBT, which induces $O_2^{\bullet-}$ production and oxidative stress. *YAP1* and *STB3* encode transcription factors. *PDE2* controls the intracellular cAMP level, which modulates the Gpr1-cAMP-PKA and Ras-cAMP-PKA signaling pathways (Xue *et al.*, 1998; Thevelein & de Winde, 1999). This relatively small number of identified genes enhancing the CTBT tolerance contrasts with the 169 genes whose deletions led to the increased sensitivity of yeast cells to this compound (Batova *et al.*, 2010). Apparently, the overexpression of each identified gene does not necessarily have an opposite effect to that of its deletion on the susceptibility of yeast cells to CTBT.

The *YAP1* gene has been identified in genome-wide screenings both for CTBT sensitivity, using the collection of deletion mutants (Batova *et al.*, 2010), and for CTBT resistance, using the DNA library of genes in the high-copy-number plasmid. Plasmids carrying the *YAP1* gene were recovered most frequently. Besides the DNA insert of pED1, some of them also contained the complete *GIS4* gene adjacent to *YAP1*. The *YAP1* gene encodes the main transcriptional activator involved in the oxidative stress response activating the expression of many genes in response to drugs and oxidants such as H_2O_2, organic peroxides, $O_2^{\bullet-}$-generating paraquat, menadione, and also diamide (Moye-Rowley *et al.*, 1989; Lee *et al.*, 1999; Herrero *et al.*, 2008; Salin *et al.*, 2008). Yap1p activity is limited by its binding protein Ybp1p and by its availability in the nucleus (Gulshan *et al.*, 2011). As we have shown previously, Yap1p responds quickly to CTBT treatment and accumulates in the nucleus. Transcriptome analysis of the CTBT-treated cells revealed at least 82 genes containing the Yap1p DNA-binding element in their promoter (Batova *et al.*, 2010). Among the genes with the highest response to CTBT, those encoding peroxiredoxins, thioredoxins, thioredoxin reductase, cytochrome *c* peroxidase, glutathione S-transferase, and SODs were identified. They are all known players in the antioxidant defense of yeast cells (Herrero *et al.*, 2008). Yap1p also activates genes that encode the drug efflux pumps involved in multidrug resistance. One of its targets, the *ATR1* gene, which displays a four times higher expression in CTBT-treated cells as compared with the untreated control (Batova *et al.*, 2010), was found not to be involved in the CTBT efflux (Fig. 2).

The *PDE2* gene codes for a high-affinity cAMP phosphodiesterase that negatively regulates the cAMP-dependent protein kinase in the Ras2-cAMP-PKA and Gpr1-cAMP-PKA signaling pathways (Xue *et al.*, 1998; Thevelein & de Winde, 1999). The overexpression of *PDE2* has been found to confer yeast resistance to H_2O_2 (Charizanis *et al.*, 1999; Park *et al.*, 2005) and cisplatin, a potent DNA-damaging agent (Burger *et al.*, 2000). It also decreases the intracellular concentration of cAMP (Park *et al.*, 2005), which leads to the inhibition of cAMP-dependent PKA activity. The cAMP signaling pathway represses the function of the Skn7p transcription factor (Charizanis *et al.*, 1999), which is linked to oxidative stress tolerance (Krems *et al.*, 1996; Fassler & West, 2011). The enhanced PKA activity leads to the production

Table 1. Specific GSH, GSSG, and superoxide concentrations and specific SODs, CAT, GPx, and GR enzyme activities of the *Saccharomyces cerevisiae* BY4741 parental strain containing an empty vector pRS425 and the *YAP1*, *PDE2*, and *STB3* genes on YEp13 plasmid after 1 h treatment (signed with –t) with subinhibitory concentration of CTBT (1 μg mL^{-1}). Mid-log phase cells were grown in YNB medium at 30 °C

	Concentration/Activity			
Parameter	Empty vector	*YAP1*	*PDE2*	*STB3*
$O_2^{\bullet-}$	231 ± 4.5	221 ± 12	169 ± 14***	135 ± 2***
$O_2^{\bullet-}$ –t	224 ± 7.5	196 ± 23	152 ± 5.6	118 ± 3.2^{+++}
GSH†	5.69 ± 0.2	2.73 ± 0.36***	1.62 ± 0.21***	1.69 ± 0.21***
GSH† –t	8.56 ± 0.58$^{\#\#\#}$	2.59 ± 0.41	1.50 ± 0.26	2.28 ± 0.13^{+++}
GSSG†	0.057 ± 0.004	0.039 ± 0.077***	0.05 ± 0.006	0.072 ± 0.007**
GSSG† –t	0.077 ± 0.006$^{\#\#\#}$	0.039 ± 0.004	0.05 ± 0.005	0.080 ± 0.005
GSH/GSSG	99.1 ± 4.1	71.1 ± 5.2***	32.6 ± 2.9***	23.7 ± 4.45***
GSH/GSSG –t	111.6 ± 10.4	62.1 ± 4.3^{+}	29.5 ± 5.8	28.4 ± 2.87
SODs‡	4.76 ± 1.05	12.72 ± 0.71***	10.24 ± 2.43***	10.13 ± 0.81***
SODs‡ –t	11.67 ± 3.7$^{\#\#\#}$	11.45 ± 1.84	10.37 ± 0.71	11.09 ± 1.03
Mn-SOD‡	1.22 ± 0.08	0.81 ± 0.17***	0.87 ± 0.24*	0.58 ± 0.1***
Mn-SOD‡ –t	1.33 ± 0.11	1.05 ± 0.18	0.78 ± 0.077	0.34 ± 0.13^{+}
Cu/Zn-SOD‡	3.54 ± 1.13	11.91 ± 0.79***	9.37 ± 2.57***	9.55 ± 0.81***
Cu/Zn-SOD‡ –t	10.35 ± 3.83$^{\#\#\#}$	10.4 ± 1.74	9.59 ± 0.71	10.72 ± 1.01
CAT§	0.10 ± 0.04	0.097 ± 0.026	0.16 ± 0.03*	0.33 ± 0.022***
CAT§ –t	0.084 ± 0.015	0.088 ± 0.023	0.13 ± 0.05	0.24 ± 0.04^{++}
GPx¶	4.45 ± 0.53	2.31 ± 0.46***	1.56 ± 0.38***	2.91 ± 0.28***
GPx¶ –t	4.40 ± 0.27	3.47 ± 0.28^{++}	2.64 ± 0.37^{++}	3.14 ± 0.51
GR¶	47.80 ± 2.25	46.86 ± 3.04	41.78 ± 1.71**	44.3 ± 1.3*
GR¶ –t	47.85 ± 2.65	52.03 ± 3.87	41.58 ± 3.33	45.1 ± 2.9

Values were expressed as means ± SD, calculated from the data of four independent experiments.

†Specific concentrations are given in μmol (mg dry biomass)$^{-1}$.

‡Specific activities are given in unit (min mg protein)$^{-1}$.

§Specific activities are given in μmol (min mg protein)$^{-1}$.

¶Specific activities are given in nmol (min mg protein)$^{-1}$.

Significance: $^{\#,*,+}P < 0.05$; $^{\#\#,**,++}P < 0.01$; $^{\#\#\#,***,+++}P < 0.001$. Values were calculated via the Student's *t*-test.

$^{\#}$//$^{\#\#\#}$Significant differences between untreated cells of BY4741 and its CTBT-treated cells.

*//***Significant differences between untreated cells of BY4741 and untreated cells of transformants.

$^{+}$//$^{+++}$Significant differences between untreated and CTBT-treated cells of transformants.

of mitochondria that are prone to the production of ROS (Leadsham & Gourlay, 2010). The cAMP-PKA signaling also inhibits the nuclear localization of Msn2p and Msn4p (Görner *et al.*, 1998; Jacquet *et al.*, 2003), the two homologous transcription factors that mediate a transient cellular response to multiple stresses and to changes in the nutritional environment (Gasch *et al.*, 2000). More than a hundred genes with Msn2p/Msn4p DNA-binding stress response elements in their promoter regions have been identified as overexpressed in CTBT-treated yeast cells (Batova *et al.*, 2010). The proteins encoded by members of the Msn2p/Msn4p regulon belong in several functional classes and include enzymes involved in carbohydrate metabolism, antioxidant proteins and proteins involved in protein degradation. However, the *PDE2* overexpression was sufficient to confer the CTBT tolerance in yeast mutant lacking the Msn2p/Msn4p or Yap1p transcription factors (Fig. 3). This observation corroborates the hydrogen peroxide resistance conferred by overexpressed *PDE2* in the *msn2Δmsn4Δ* double mutant (Park *et al.*, 2005) and suggests that PKA can employ diverse stress defense systems with overlapping function (Park *et al.*, 2005; Avrahami-Moyal *et al.*, 2012).

Stb3p is the transcription factor that recognizes the RRPE sequence in the promoters of its target genes. It has been isolated in a screen looking for Sin3p-binding partners (Kasten & Stillman, 1997). Sin3p is a component of the two distinct histone deacetylase complexes Rpd3L and Rpd3S (Rundlett *et al.*, 1996). While histone deacetylation is generally associated with the gene repression (Struhl, 1998), its role in transcriptional activation has been appreciated only recently (Bernstein *et al.*, 2000). Histone deacetylases have been found to be required for the activation of osmotic stress genes (Proft & Struhl, 2002), DNA damage-induced genes (Sharma *et al.*, 2007), as well as the *PDR5* gene encoding the main multidrug resistance efflux pump (Borecka-Melkusova *et al.*, 2008). Stb3p can act both as an activator (Liko *et al.*, 2007) and as a repressor (Liko *et al.*, 2010) of gene expression. The *STB3* overexpression reduced the expression of genes for

ribosomal proteins and diminished the rate of amino acid incorporation in Hos2p-dependent manner (Liko *et al.*, 2010). Recent transcriptome analysis revealed that at least 150 genes harboring the RRPE sequences in their promoters are responsive to CTBT treatment of yeast cells. Among them, almost a hundred genes, involved mainly in ribosome biogenesis and protein synthesis, were downregulated (Batova *et al.*, 2010), and 40 of them were overlapping with those identified in *STB3* overexpressed cells (Liko *et al.*, 2010). Such downregulation of gene expression is saving energy needed for synthesis of the translation machinery and represents a general response to stress conditions encountered by yeast cells. They can respond to ROS exposure by delaying progression through the cell division cycle that may enable them to repair any macromolecular damage without passing it on to their daughter cells. In fact, several Stb3p target genes, including *GPX1*, *UBX6*, *YAP7*, and those also activated by Yap1p, such as YML131W, *SOD2*, *HSP31*, *GTT2*, *OYE3*, *BCY1*, and *AAD14*, were upregulated in CTBT-treated cells (Batova *et al.*, 2010) and could account for the beneficial effect of *STB3* overexpression. This effect was not abolished by the absence of specific components of signaling pathways (Hog1p, Mpk1p, Ras2p) and transcription factors (Yap1p, Stb5p, Msn2p/Msn4p) involved in yeast cell stress responses. It was also observed in the absence of studied individual subunits of histone deacetylase complexes (Fig. 4) probably because of their partially overlapping (Sharma *et al.*, 2007) and dual role in transcriptional regulation (Dovey *et al.*, 2010). Further studies are needed to disclose the exact role of Stb3p in the control of oxidative stress response in yeast cells.

The protective effect of the overexpressed *YAP1*, *PDE2*, and *STB3* genes against CTBT toxicity was accompanied by changes in the overall regulation of the antioxidant system of the yeast cells. It should be emphasized in advance that the overexpression of the *YAP1, PDE2*, and *STB3* genes caused a disturbed internal redox state of the untreated cells, which resulted in decreased concentrations of $O_2^{\bullet-}$, H_2O_2, and GSH in the transformants. This phenomenon was accompanied by the upregulation of CAT and the downregulation of GR in the *PDE2* and *STB3* transformants, and by the upregulation of Cu/Zn-SOD in all transformants (Fig. 5, Table 1). A single gene mutation-induced alteration in the redox state of yeast cells has already been reported (Gazdag *et al.*, 2003, 2011), but these are the first results indicating the consequences of overexpressed *YAP1, PDE2*, and *STB3* genes in redox processes and their regulation.

Short-term (20 min) treatment with higher CTBT concentrations (2–4 μg mL^{-1}) induced elevated $O_2^{\bullet-}$ production in the parental BY4741 cells (Batova *et al.*, 2010). A longer-term treatment (60 min) with a lower CTBT concentration (1 μg mL^{-1}) decreased the level of $O_2^{\bullet-}$ in cells overexpressing the *PDE2* and *STB3* genes, and also the concentration of H_2O_2 in the parental strain and its transformants via upregulation of the specific activity of Cu/Zn-SOD in all examined strains. The activity of CAT was higher in the *PDE2* and *STB3* transformants, as was that of GPx in the transformants overexpressing the *YAP1*, *PDE2*, and *STB3* genes as compared with the untreated parental cells. The phenotypic resistance of the *YAP1*, *PDE2*, and *STB3* transformants was a consequence of the upregulation of some of their antioxidant enzymes in comparison with the parental BY4741 strain. At the beginning of redox-active CTBT treatment, they could promptly inactivate the CTBT-induced ROS. However, the GSH concentration was decreased in all transformants, and the CTBT treatment elevated its level only in the parental cells and the *STB3* transformant. These results suggested that GSH is merely the first line, while the antioxidant enzymes are the determining factor maintaining redox homeostasis.

In conclusion, the genome-wide screening allowed us to identify the *YAP1*, *PDE2*, and *STB3* genes that, when overexpressed, enhance the CTBT tolerance of yeast cells. The protective effects of these genes are apparently connected with the adaptation of the yeast genome expression to the presence of CTBT in its chemical environment, leading to activation of the genes involved in the oxidative and general stress responses, resulting in a higher antioxidant capacity of yeast cells.

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References

Anderson ME (1985) Determination of glutathione and glutathione disulphide in biological samples. *Methods Enzymol* **113**: 548–555.

Avrahami-Moyal L, Braun S & Engelberg D (2012) Overexpression of *PDE2* or *SSD1-V* in *Saccharomyces cerevisiae* W303-1A strain renders it ethanol-tolerant. *FEMS Yeast Res* **12**: 447–455.

Batova M, Klobucnikova V, Oblasova Z, Gregan J, Zahradnik P, Hapala I, Subik J & Schüller C (2010) Chemogenomic and transcriptome analysis identifies mode of action of the chemosensitizing agent CTBT (7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine). *BMC Genomics* **11**: 153.

Bernstein BE, Tong JK & Schreiber SL (2000) Genome-wide studies of histone deacetylase function in yeast. *Proc Natl Acad Sci USA* **97**: 13708–13713.

Borecka-Melkusova S, Kozovska Z, Hikkel I, Dzugasova V & Subik J (2008) *RPD3* and *ROM2* are required for multidrug resistance in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **8**: 414–424.

Burger H, Capello A, Schenk PW, Stoter G, Brouwer J & Nooter K (2000) A genome-wide screening in *Saccharomyces cerevisiae* for genes that confer resistance to the anticancer agent cisplatin. *Biochem Biophys Res Commun* **269**: 767–774.

Carter WO, Narayanan PK & Robinson JP (1994) Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. *J Leukoc Biol* **55**: 253–258.

Cernicka J, Kozovska Z, Hnatova M, Valachovic M, Hapala I, Riedl Z, Hajos G & Subik J (2007) Chemosensitization of drug-resistant and drug-sensitive yeast cells to antifungals. *Int J Antimicrob Agents* **29**: 170–178.

Charizanis C, Juhnke H, Krems B & Entian KD (1999) The oxidative stress response mediated via Pos9/Skn7 is negatively regulated by the Ras/PKA pathway in *Saccharomyces cerevisiae*. *Mol Gen Genet* **261**: 740–752.

Chiu DTY, Stults FH & Tappel AL (1976) Purification and properties of rat lung soluble glutathione peroxidase. *Biochim Biophys Acta* **445**: 558–566.

Culakova H, Dzugasova V, Gbelska Y & Subik J (2012) CTBT (7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine) producing ROS affects growth and viability of filamentous fungi. *FEMS Microbiol Lett* **328**: 138–143.

Dovey OM, Foster CT & Cowley SM (2010) Emphasizing the positive. A role for histone deacetylases in transcriptional activation. *Cell Cycle* **9**: 2700–2701.

Estruch F & Carlson M (1993) Two homologous zinc finger genes identified by multicopy suppression in a SNF1 protein kinase mutant of *Saccharomyces cerevisiae*. *Mol Cell Biol* **13**: 3872–3881.

Fassler JS & West AH (2011) Fungal Skn7 stress responses and their relationship to virulence. *Eukaryot Cell* **10**: 156–167.

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D & Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* **11**: 4241–4257.

Gazdag Z, Pócsi I, Belágyi J, Emri T, Blaskó Á, Takács K & Pesti M (2003) Chromate tolerance caused by reduced hydroxyl radical production and decreased glutathione reductase activity in *Schizosaccharomyces pombe*. *J Basic Microbiol* **43**: 96–103.

Gazdag Z, Fujs S, Koszegi B, Kálmán N, Papp G, Emri T, Belágyi J, Pócsi I, Raspor P & Pesti M (2011) The abc1-/coq8- respiratory-deficient mutant of *Schizosaccharomyces pombe* suffers from glutathione underproduction and hyperaccumulates Cd^{2+}. *Folia Microbiol* **56**: 353–359.

Gbelska Y, Mockovciakova D, Timko J & Subik J (1995) The properties of the multicopy suppressor of the *ogd1* mutation in yeast. *J Basic Microbiol* **35**: 229–232.

Görner W, Durchschlag E, Martinez-Pastor MT, Estruch F, Ammerer G, Hamilton B, Ruis H & Schüller C (1998) Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev* **12**: 586–597.

Gulshan K, Lee SS & Moye-Rowley WS (2011) Differential oxidant tolerance determined by the key transcription factor Yap1 is controlled by levels of the Yap1-binding protein, Ybp1. *J Biol Chem* **286**: 34071–34081.

Halliwell B & Gutteridge JMC (1991) *Free Radicals in Biology and Medicine*, 2nd edn. Oxford University Press, New York, NY.

Henderson LM & Chappell JB (1993) Dihydrorhodamine 123: a fluorescent probe for superoxide generation? *Eur J Biochem* **217**: 973–980.

Herrero E, Ros J, Bellí G & Cabiscol E (2008) Redox control and oxidative stress in yeast cells. *Biochim Biophys Acta* **1780**: 1217–1235.

Jacquet M, Renault G, Lallet S, De Mey J & Goldbeter A (2003) Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in *Saccharomyces cerevisiae*. *J Cell Biol* **161**: 497–505.

Kanazawa S, Driscoll M & Struhl K (1988) *ATR1*, a *Saccharomyces cerevisiae* gene encoding a transmembrane protein required for aminotriazole resistance. *Mol Cell Biol* **8**: 664–673.

Kasten MM & Stillman DJ (1997) Identification of the *Saccharomyces cerevisiae* genes *STB1-STB5* encoding Sin3p binding proteins. *Mol Gen Genet* **256**: 376–386.

Kaya A, Karakaya HC, Fomenko DE, Gladyshev VN & Koc A (2009) Identification of a novel system for boron transport: Atr1 is a main boron exporter in yeast. *Mol Cell Biol* **29**: 3665–3674.

Kim KY, Truman AW, Caesar S, Schlenstedt G & Levin DE (2010) Yeast Mpk1 cell wall integrity mitogen-activated protein kinase regulates nucleocytoplasmic shuttling of the Swi6 transcriptional regulator. *Mol Biol Cell* **21**: 1609–1619.

Kolaczkowska A & Goffeau A (1999) Regulation of pleiotropic drug resistance in yeast. *Drug Resist Updat* **2**: 403–414.

Krems B, Charizanis C & Entian KD (1996) The response regulator-like protein Pos9/Skn7 of *Saccharomyces cerevisiae* is involved in oxidative stress resistance. *Curr Genet* **29**: 327–334.

Larochelle M, Drouin S, Robert F & Turcotte B (2006) Oxidative stress-activated zinc cluster protein Stb5 has dual activator/repressor functions required for pentose phosphate pathway regulation and NADPH production. *Mol Cell Biol* **26**: 6690–6701.

Leadsham JE & Gourlay CW (2010) cAMP/PKA signaling balances respiratory activity with mitochondria dependent apoptosis via transcriptional regulation. *BMC Cell Biol* **11**: 92.

Lee J, Godon C, Lagniel G, Spector D, Garin J, Labarre J & Toledano MB (1999) Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J Biol Chem* **274**: 16040–16046.

Liko D, Slattery MG & Heideman W (2007) Stb3 binds to ribosomal RNA processing element motifs that control

transcriptional responses to growth in *Saccharomyces cerevisiae*. *J Biol Chem* **282**: 26623–26628.

Liko D, Conway MK, Grunwald DS & Heideman W (2010) Stb3 plays a role in the glucose-induced transition from quiescence to growth in *Saccharomyces cerevisiae*. *Genetics* **185**: 797–810.

Martínez-Pastor MT, Marchler G, Schüller C, Marchler-Bauer A, Ruis H & Estruch F (1996) The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *EMBO J* **15**: 2227–2235.

Moye-Rowley WS (2003) Regulation of the transcriptional response to oxidative stress in fungi: similarities and differences. *Eukaryot Cell* **2**: 381–389.

Moye-Rowley WS, Harshman KD & Parker CS (1989) Yeast *YAP1* encodes a novel form of the jun family of transcriptional activator proteins. *Genes Dev* **3**: 283–292.

Nourani A, Papajova D, Delahodde A, Jacq C & Subik J (1997) Clustered amino acid substitutions in the yeast transcription regulator Pdr3p increase pleiotropic drug resistance and identify a new central regulatory domain. *Mol Gen Genet* **256**: 397–405.

Oberley LW & Spitz DR (1984) Assay of superoxide dismutase activity in tumor tissue. *Methods Enzymol* **105**: 457–464.

Paravicini G, Cooper M, Friedli L, Smith DJ, Carpentier JL, Klig LS & Payton MA (1992) The osmotic integrity of the yeast cell requires a functional *PKC1* gene product. *Mol Cell Biol* **12**: 4896–4905.

Park JI, Grant CM & Dawes IW (2005) The high-affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae* is the major determinant of cAMP levels in stationary phase: involvement of different branches of the Ras-cyclic AMP pathway in stress responses. *Biochem Biophys Res Commun* **327**: 311–319.

Peterson GL (1983) Determination of total protein. *Methods Enzymol* **91**: 86–105.

Pinto MC, Mata AM & Lopez-Barea I (1984) Reversible inactivation of *Saccharomyces cerevisiae* glutathione reductase under reducing conditions. *Arch Biochem Biophys* **228**: 1–12.

Prasad R & Kapoor K (2005) Multidrug resistance in yeast *Candida*. *Int Rev Cytol* **242**: 215–248.

Proft M & Struhl K (2002) Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. *Mol Cell* **9**: 1307–1317.

Rodrigues-Pousada C, Menezes RA & Pimentel C (2010) The Yap family and its role in stress response. *Yeast* **27**: 245–258.

Roggenkamp R, Sahm H & Wagner F (1974) Microbial assimilation of methanol induction and function of catalase in *Candida boidinii*. *FEBS Lett* **41**: 283–286.

Rundlett SE, Carmen AA, Kobayashi R, Bavykin S, Turner BM & Grunstein M (1996) HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc Natl Acad Sci USA* **93**: 14503–14508.

Salin H, Fardeau V, Piccini E, Lelandais G, Tanty V, Lemoine S, Jacq C & Devaux F (2008) Structure and properties of transcriptional networks driving selenite stress response in yeasts. *BMC Genomics* **9**: 333.

Sambrook J, Fritsch EF & Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanglard D & Odds FC (2002) Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* **2**: 73–85.

Sass P, Field J, Nikawa J, Toda T & Wigler M (1986) Cloning and characterization of the high-affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **83**: 9303–9307.

Schüller C, Mamnun YM, Wolfger H, Rockwell N, Thorner J & Kuchler K (2007) Membrane-active compounds activate the transcription factors Pdr1 and Pdr3 connecting pleiotropic drug resistance and membrane lipid homeostasis in *Saccharomyces cerevisiae*. *Mol Biol Cell* **18**: 4932–4944.

Shapiro RS, Robbins N & Cowen LE (2011) Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol Mol Biol Rev* **75**: 213–267.

Sharma VM, Tomar RS, Dempsey AE & Reese JC (2007) Histone deacetylases *RPD3* and *HOS2* regulate the transcriptional activation of DNA damage-induced genes. *Mol Cell Biol* **27**: 3199–3210.

Struhl K (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* **12**: 599–606.

Thevelein JM & de Winde JH (1999) Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* **33**: 904–918.

Thompson JR, Register E, Curotto J, Kurtz M & Kelly R (1998) An improved protocol for the preparation of yeast cells for transformation by electroporation. *Yeast* **14**: 565–571.

Vilella F, Herrero E, Torres J & de la Torre-Ruiz MA (2005) Pkc1 and the upstream elements of the cell integrity pathway in *Saccharomyces cerevisiae*, Rom2 and Mtl1, are required for cellular responses to oxidative stress. *J Biol Chem* **280**: 9149–9159.

Ward AC (1990) Single step purification of shuttle vectors from yeast for high frequency back-transformation into *E. coli*. *Nucleic Acid Res* **18**: 5319.

Westfall PJ, Ballon DR & Thorner J (2004) When the stress of your environment makes you go HOG wild. *Science* **306**: 1511–1512.

Wolfger H, Mamnun YM & Kuchler K (2001) Fungal ABC proteins: pleiotropic drug resistance, stress response and cellular detoxification. *Res Microbiol* **152**: 375–389.

Xue Y, Batlle M & Hirsch JP (1998) *GPR1* encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway. *EMBO J* **17**: 1996–2007.