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Gaining insight into the response logic of *Saccharomyces cerevisiae* to heat shock by combining expression profiles with metabolic pathways

Yanrui Ye, Yi Zhu, Li Pan, Lili Li, Xiaoning Wang, Ying Lin *

School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, PR China

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ABSTRACT

Extensive alteration of gene expression and metabolic remodeling enable the budding yeast *Saccharomyces cerevisiae* to ensure cellular homeostasis and adaptation to heat shock. The response logic of the cells to heat shock is still not entirely clear. In this study, we combined the expression profiles with metabolic pathways to investigate the logical relations between heat shock response metabolic pathways. The results showed that the heat-stressed *S. cerevisiae* cell accumulated trehalose and glycogen, which protect cellular proteins against denaturation, and modulate its phospholipid structure to sustain stability of the cell wall. The TCA cycle was enhanced, and the heat shock-induced turnover of amino acids and nucleotides served to meet the extra energy requirement due to heat-induced protein metabolism and modification. The enhanced respiration led to oxidative stress, and subsequently induced the aldehyde detoxification system. These results indicated that new insight into the response logic of *S. cerevisiae* to heat shock can be gained by integrating expression profiles and the logical relations between heat shock response metabolic pathways.

Yeast responds to potentially hazardous heat shock via the activation of a complicated response program to adapt and survive. This response includes rapid alterations in global transcription and metabolic remodeling [1].

Among the heat shock response genes, heat shock protein (HSP) genes have been known to have a vital role in coping with heat shock. Many HSPs function as molecular chaperones for protecting unfolded or unstable proteins from degradation or aggregation [2]. In *Saccharomyces cerevisiae*, this response is controlled by the heat shock transcription factor Hsf1p, which binds the heat shock response element (HSE) [3,4]. The heat shock response also involves the synthesis of metabolic enzymes and antioxidant defense proteins. The production of many of these proteins is induced under a variety of other environmental stress conditions, which is known as "environmental stress response" (ESR) [5]. The majority of the ESR genes are controlled by two highly related and partially redundant zinc-finger transcription factors called Msn2p and Msn4p [6]. In addition, many oxidative stress-responsive genes are involved in the heat shock response [5,7], suggesting that these genes may have important roles in coping with different types of stress.

In *S. cerevisiae*, the level of the disaccharide trehalose increases rapidly and dramatically after heat shock, along with the induction of HSPs [8]. Trehalose has been shown to stabilize the structures and enzymatic activities of proteins against thermal denaturation *in vitro*. Trehalose can prevent the aggregation of misfolded proteins [9], and serves as a positive regulator of the Hsf1p activity, suggesting that trehalose functions to protect proteins at the initial stages of the heat shock response before HSPs have been fully induced [10]. The polysaccharide glycogen, whose synthesis genes are regulated by Msn2p/Msn4p [11], has been found to accumulate after heat shock [12]. However, the role of glycogen in response to heat shock is poorly documented.

Although heat shock response metabolic pathways as well as HSPs have been studied extensively, the response logic between these metabolic pathways is unclear. In this study, we combined the expression profiles with the heat shock response metabolic pathways to investigate the response logic of yeast cells to heat shock.

Materials and methods

Strain and growth conditions. *S. cerevisiae* strain XQ1 was grown to $OD_{600} \approx 1$ in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) at 30 °C. Cells were collected by centrifugation, suspended in an equal volume of heated (42 °C) YPD medium, and incubated at 42 °C for growth. Samples were collected at 15 min and at 60 min, and total RNA was isolated. A sample of cells suspended in an equal volume of heated (30 °C) YPD medium was used as the unstressed reference.

RNA isolation and microarray hybridization. Total RNA was isolated by the acid/phenol method as described [13], and purified

* Corresponding author. Fax: +86 020 39380698.
E-mail address: feylin@scut.edu.cn (Y. Lin).

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with a NecleoSpin® Extract II kit (Machery-Nagel Corp., Germany) following the manufacturer's protocol. A yeast genome 70-mer oligonucleotide microarray was obtained from CapitalBio Corporation (Beijing, China). A 1-μg sample of total RNA was used to prepare fluorescent dye-labeled cDNA by the linear mRNA amplification procedure as described [14]. A DNA–DNA hybridization protocol was used to replace the RNA–DNA hybridizations in the present study to reduce cross-hybridization [15]. The labeled cDNAs were dissolved in 80 μl of hybridization solution (3× SSC, 0.2% (w/v) SDS, 5× Denhardt's solution, 25% (v/v) formamide) then denatured at 95 °C for 3 min before hybridization. A sample of the mixed hybridization buffer was placed onto a microarray slide and covered with a glass coverslip. The hybridizations were done with a BioMixer™ II (CapitalBio Corp., China). After hybridization, slides were washed with washing solution 1 (2× SSC, 0.2% SDS) and then with washing solution 2 (2× SSC) at 42 °C for 4 min. Self-hybridization of the control sample was used to evaluate the system noise.

Microarray data processing. Arrays were scanned using a confocal LuxScan™ (CapitalBio Corp., China) scanner and data were extracted with LuxScan™ 3.0 software (CapitalBio Corp., China). Normalization was done on the basis of a Lowess program [16]. For data extraction, spots with signal intensities below 800 units, after subtracting the background, were removed from both dye channels (Cy3 and Cy5). A "one class" method for the analysis of microarray software (SAM) was used to identify significantly differentially expressed genes [17]. Genes with false discovery rate (FDR) < 0.01, q-value <0.05, and expression fold change >2 were identified as significantly differentially expressed genes. Differentially expressed genes were clustered hierarchically by Gene Cluster 3.0 (Stanford University).

Gene ontology (GO) analysis of differentially expressed genes was done with GoTermFinder (http://go.princeton.edu/cgi-bin/GOTermFinder). Transcription factor (TF) activity was inferred by YEASTRACT [18] (http://www.yeastract.com/index.php). Pathway analysis was derived from SGD (http://www.yeastgenome.org/) and KEGG (http://www.genome.jp/kegg/).

Quantitative real time RT-PCR (qRT-PCR). The specificity of the primer pairs (Supplementary Table S1) was tested by amplification of genomic DNA before RT-PCR. The 18 S rRNA gene (*RDN18*) was used as an internal housekeeping control for expression. To avoid contamination with DNA, total RNA was digested with DNase I (Takara, Japan), and cDNA was synthesized with a Rever Tra Ace® qPCR RT Kit (Toyobo, Japan). Real time PCR, done with the Applied Biosystems 7500 real time PCR system using the SYBR® Green Realtime PCR Master Mix (Toyobo, Japan), used the following program: an initial denaturation step at 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s, 58 °C for 15 s, and a final step at 72 °C for 45 s.

Results and discussion

Acquisition of expression profiles and verification of microarray data

The heat shock experiments were done at 42 °C to invoke a typical and strong heat shock response. Biological replicates were collected for DNA microarray analysis to increase true positives. Self-hybridization of the control sample was done in independent triplicates, and the false-positive rate was reduced from 0.35% to 0.17%. A total of 1420 genes with a change of at least 2-fold, were identified as differentially expressed; 715 were up-regulated and 705 were down-regulated. As shown in Fig. 1, the clustered expression profiles of these genes from three independent experiments showed good reproducibility. To validate the microarray data, we analyzed the relative mRNA levels of several genes by qRT-PCR. The results showed that the microarray data are consistent with the qRT-PCR data (Fig. 3A).

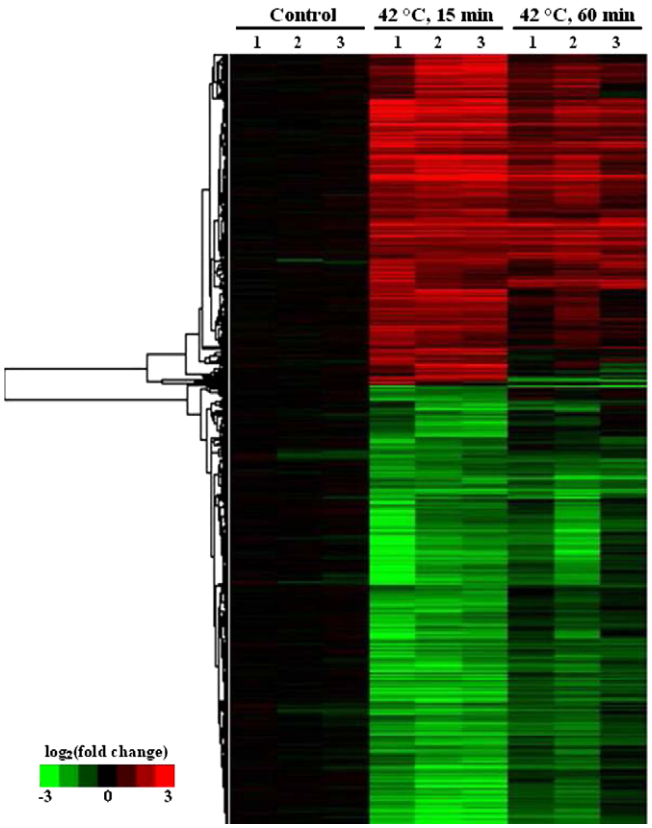

Fig. 1. Transcriptional profiles of genes with FDR <0.01, q-value <0.05, and expression fold change >2 are represented. Each horizontal strip represents a single gene. 1, 2 and 3 are biologically independent triplicates.

Gene ontology and transcription factor analysis

Gene ontology (GO) annotations for the differentially expressed genes reveal several significant trends in the gene expression program (Table 1 and Supplementary Table S2). To further investigate transcriptional regulation, these genes were grouped on the basis of documented transcription factors, ordered by the percentage of genes that are regulated by the TFs (Table 2). The GO enrichment of "unfolded protein binding" (p = 1.23E−5) and "chaperone regulator activity" (p = 2.46E−4), and the enrichment of genes regulated by Hsf1p, Msn2p, and Msn4p, are consistent with the fact that HSPs, many of which function as molecular chaperones, are induced by heat shock [2], and that Msn2p/Msn4p regulate the expression of ESR genes in response to various types of stress [6]. It is noteworthy that the most strongly enriched GO annotation in terms of "molecular function" is oxidoreductase activity. In addition, 42.2% of Yap1p-regulated genes and 34% of Met4p-regulated genes, which regulate the expression of antioxidant enzyme genes in response to oxidative stress, were induced, indicating that extensive oxidative stress is involved in heat shock, which is consistent with the results of previous studies [5,7]. Heat shock affects transcription of ribosomal protein genes as well as the stability of their mRNAs. A significant number of the down-regulated genes, which are closely related to the TFs Sfp1p, Rap1p, Fhl1p, and Ifh1p, were involved in ribosome biogenesis (p = 3.20E−71). Sfp1p is a regulator of ribosomal protein (RP) genes, whereas its binding to promoters is stress-sensitive [19]. Rap1p, Fhl1p, and Ifh1p have been reported to regulate the RP genes. It has been shown that binding of Rap1 to the promoter of RP genes excludes nucleosomes, and recruits Fhl1p and Ifh1p to drive transcription [20]. Binding or interactions of these TFs may be disrupted in response

Table 1
GO terms enrichment of differentially expressed genes.

GO term	Corrected *p*-value	Gene
Induced (715 genes)		
Oxidoreductase activity	1.27E−15	82
Catalytic activity	9.27E−6	303
Unfolded protein binding	1.23E−5	25
Peroxidase activity	1.55E−4	11
Oxidoreductase activity, acting on peroxide as acceptor	1.55E−4	11
Aldo–keto reductase activity	2.75E−4	8
Antioxidant activity	4.72E−4	12
Repressed (705 genes)		
Structural constituent of ribosome	3.20E−71	135
RNA methyltransferase activity	5.34E−9	18
RNA polymerase activity	2.04E−5	17
S-Adenosylmethionine-dependent methyltransferase activity	2.15E−5	23
ATP-dependent helicase activity	3.79E−5	20

to heat shock, leading to the subsequent repression of RP gene expression. This regulation is extremely important for the economy of the yeast cell, because it is affected largely at the level of transcription, where expression of RP genes accounts for about 50% of the total RNA polymerase II transcription [21].

Exploration of response logics between heat shock-responsive metabolic pathways

The global view of changes in gene expression provides a rough picture of the way in which the cells adapt to heat shock. To understand the response logic of the yeast cells to heat shock, metabolic pathways obtained from the KEGG and SGD databases were integrated into a pathway map that can be divided into five modules according to the expression profiles (Fig. 2). The pathway modules 1–5 are the thermoprotectant module, the aldehyde detoxification module, the phospholipid synthesis module, the TCA cycle module, and the module for amino acid and nucleotide metabolism, respectively.

In module 1, the genes of trehalose and glycogen metabolism are up-regulated (Figs. 2 and 3). The trehalose synthase genes *TPS1* and *TPS2*, and the trehalase genes *ATH1* and *NTH1* are regulated by Msn2p/Msn4p rather than by Hsf1p. The co-induction of trehalose synthase and trehalase (Fig. 3A) supports the suggestions that trehalose is a positive regulator of the heat-induced activity of Hsf1p and functions to protect proteins at the initial stages of heat shock response before HSPs have been fully induced [10], and that degradation of trehalose is critical for recovery from heat shock because high levels of trehalose can interfere with normal protein activity by stabilizing proteins in nonnative conformations and inhibiting the refolding of these denatured proteins by HSPs [22].

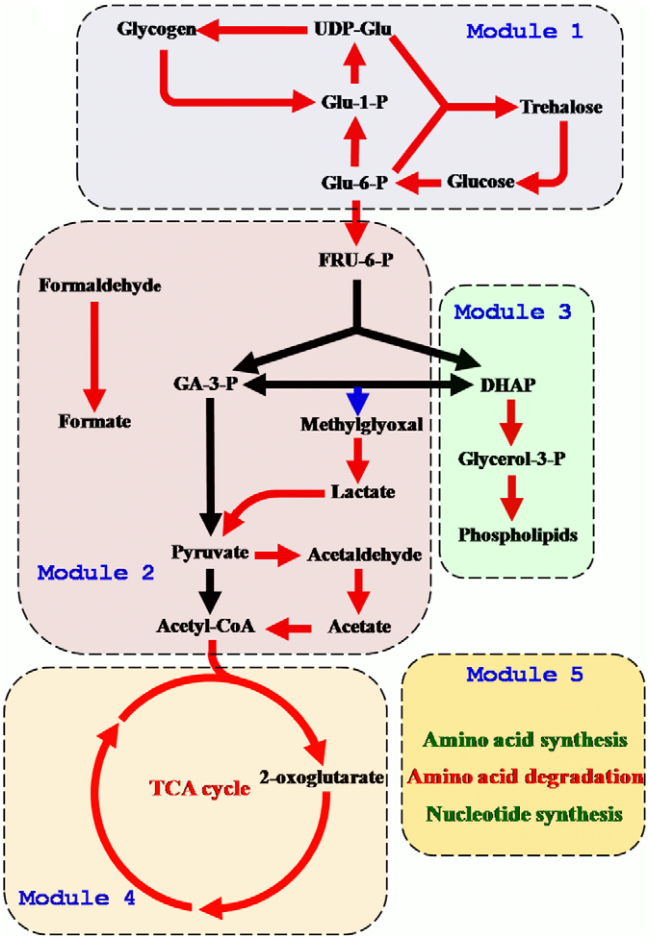

Fig. 2. Metabolic remodeling inferred from the differentially expressed genes. Only key metabolites are indicated. Directions of arrows indicate the direction of the flow of metabolites inferred from the expression profiles. Red arrows represent pathways in which genes are induced while black arrows represent pathways in which genes are unchanged. The blue arrow represents the nonenzymatic reaction of methylglyoxal formation. Pathways in module 5 with red lettering represent pathways in which genes are induced, whereas green lettering represents pathways in which genes are repressed. Modules 1–5 are the thermoprotectant synthesis module, the aldehyde detoxification module, the phospholipids synthesis module, the TCA cycle module, and the module for amino acid and nucleotides metabolism, respectively. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

Table 2
Transcription factor enrichment. Only TFs that regulate >20% genes in the induced or repressed list are shown.

Transcription factor	Gene (%)	TF description
Induced (715 genes)		
Yap1p	42.2	Regulates transcriptional response to oxidative stress
Met4p	34.0	Regulates sulfur amino acid metabolism and oxidative stress defense
Sok2p	31.6	Has a regulatory role in the cAMP-dependent protein kinase (PKA) signal transduction pathway
Hsf1p	31.0	Activates multiple genes in response to stress
Msn2p	29.2	Related to Msn4p; regulates the general stress response
Aft1p	27.3	Involved in iron utilization and homeostasis
Msn4p	25.3	Related to Msn2p; regulates the general stress response
Repressed (705 genes)		
Sfp1p	52.2	Controls the expression of many ribosome biogenesis genes
Met4p	34.2	Regulates sulfur amino acid metabolism and oxidative stress defense
Rap1p	33.2	Activates genes encoding ribosomal proteins and glycolytic enzymes
Fhl1p	26.1	Involved in the expression of ribosomal protein (RP) genes
Rpn4p	23.0	Activates expression of proteasome genes

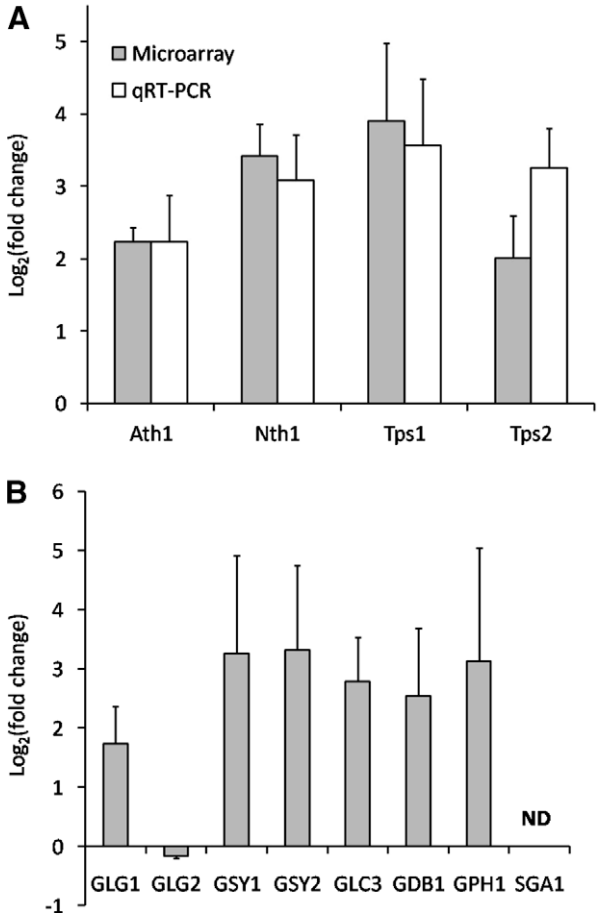

Fig. 3. Expression of genes involved in trehalose and glycogen metabolism. (A) Genes in trehalose metabolism. Gray bar, microarray data; white bar, qRT-PCR data. (B) Microarray results of genes in glycogen metabolism. ND, not detectable in the microarray. The results are reported as mean ± SE (n = 3).

Glycogen, known as a storage carbohydrate, has been found to accumulate in response to a wide variety of environmental stress, including heat shock [12]; however, its role in response to heat shock is poorly documented. The expression of glycogen metabolism genes was up-regulated, except the *GLG2* isozyme of *GLG1* (Fig. 3B). This is consistent with previous observations that isozymes involved in carbohydrate metabolism are expressed differentially in response to stress [5]. The similarity between glycogen and trehalose metabolism suggests that they may have somewhat similar roles in response to heat shock. Therefore, module 1 was considered to be involved in thermal protection and the regulation of Hsf1p activity.

Aldehydes are toxic metabolites that react as weak mutagens with macromolecules and subsequently disrupt cellular functions. Methylglyoxal (MG) is a typical 2-oxoaldehyde derived from glycolysis. The detoxification systems of MG and formaldehyde are similar, in that they both involve a glutathione-dependent enzymatic system [23,24]. Most of the genes involved in detoxification systems of aldehydes are up-regulated in response to heat shock (module 2 in Figs. 2, 4A). The enhancement of formaldehyde and MG detoxification supports the previous suggestion that GSH can serve as a cofactor for the detoxification of various cytotoxic compounds and has an important role in maintaining cell viability at higher temperatures [25]. However, unlike previous reports [23,25], expression of *GSH1* and *GSH2*, which are involved in GSH synthesis, was not up-regulated in this study. This inconsistency can be explained by the regeneration of GSH in the formaldehyde and MG detoxification systems. In addition, aldo–keto reductases (AKRs) catalyze a range of aldehyde and ketone substrates [26], including MG [27,28]. It has been shown that AKRs can catalyze MG very efficiently and are induced strongly by GSH depletion [29]. Among the 10 AKR genes, 8 were up-regulated (Table 1 and Fig. 4B). Furthermore, it has been reported that disruption of at least three AKR genes results in a heat shock phenotype [26]. These results suggest that AKRs may partially overlap in their aldehyde detoxification activity, and aldehyde detoxification is critical for cells to survive heat shock. Thus, they were proposed to be involved in the aldehyde detoxification module in response to heat shock.

Heat shock causes modulation of the lipid structures and hence *de novo* synthesis of phospholipids was involved (module 3 in Fig. 2). Phosphatidylcholine (PC) comprises 50% of total cellular phospholipids, and has a major role in maintaining cellular integrity in response to stress. It has been shown that the growth of yeast cells that are defective in PC synthesis is inhibited at elevated temperature [30]. Cardiolipin (CL) is an anionic phospholipid localized predominantly in the mitochondrial inner membrane. The *crd1* mutant, which is defective in CL synthesis, exhibits severe vacuolar defects and sensitivity to heat shock [31]. The induction of *OPI3* (2.78 ± 1.50) and *CRD1* (2.14 ± 0.22) implied an increase in PC and CL content, supporting the importance of these phospholipids in the sustentation under heat shock. In addition, the expression of some genes involved in sphingolipid production, such as *LCB5* (2.49 ± 0.46), *YDC1* (2.88 ± 0.68), *DPL1* (2.70 ± 0.21), and *YPC1* (7.52 ± 2.71), was up-regulated. The products of these genes catalyze the synthesis of phytosphingosine, dihydrosphingosine and their phosphates, which have been implicated as secondary messengers in signaling pathways that regulate amino acid and nucleotide metabolism [32–34] (see below).

The induction of genes involved in protein metabolism and modification (Supplementary Table S2) suggests that energy is required. It has been known that increased temperature enhances the consumption of oxygen in respiration, and heat shock may enhance the generation of reactive oxygen species (ROS) in mitochondria caused by an enhanced respiration rate [6,25]. Our results further support this possibility by showing that expression of most of the genes involved was induced (module 4 in Fig. 2 and Table 1). A considerable proportion of the genes in the TCA cycle (p = 0.02) are up-regulated, indicating that respiration, which produces ATP, was enhanced in response to the increased energy requirement. ROS generation (p = 1.55E−4) was involved in the enhancement of respiration, indicating that the enhanced respiration is probably attributed to the oxidative stress experienced in heat shock.

Expression of ribosomal protein (RP) genes, which accounts for a high proportion of the overall protein synthetic capacity, is down-regulated (Table 1). Amino acid and nucleotide synthesis was repressed, and amino acid degradation was induced (module 5 in Fig. 2 and Supplementary Fig. S1). These results suggest that the decreased synthesis of normal proteins saves energy.

In summary, the response logic of *S. cerevisiae* cells to heat shock can be inferred by combining gene expression profiles with metabolic pathways. As shown in Fig. 2, yeast cells confronted with heat stress accumulate trehalose and glycogen, which protect cellular proteins (module 1), and alter phospholipid structure, which sustains cell wall stability (module 3). The TCA cycle is enhanced (module 4), and the synthesis of amino acids and nucleotides is repressed (module 5), which together meet the increased energy requirement due to heat-induced protein metabolism and modification. The enhanced respiration leads to oxidative stress, which subsequently induces the aldehyde detoxification system (module 2). In conclusion, we have gained new insight into the response logic of heat shock response by showing the integrated actions of

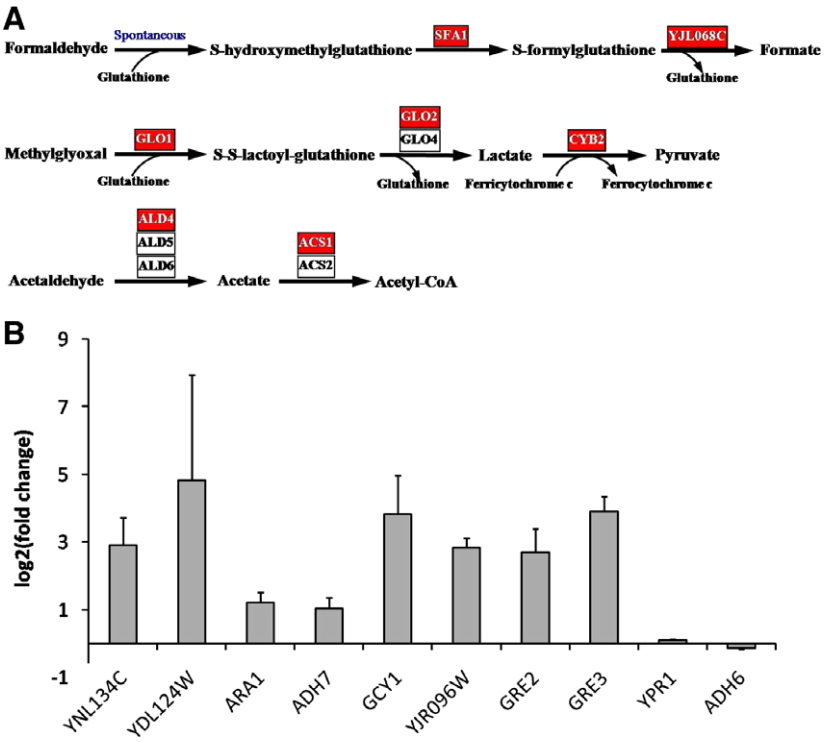

Fig. 4. Expression of genes involved in aldehyde detoxification. (A) Detoxification pathways of formaldehyde, methylglyoxal and acetaldehyde. Genes in red boxes are induced, whereas genes in white boxes are not induced. (B) Expression of aldo–keto reductase genes. Results are reported as mean ± SE ($n = 3$). (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

metabolic remodeling throughout the cell. Further studies of the interactions between transcription and metabolic remodeling are needed to shed more light on the mechanism of heat shock response and adaptation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.05.071.

References

[1] S. Lindquist, Heat-shock proteins and stress tolerance in microorganisms, Curr. Opin. Genet. Dev. 2 (1992) 748–755.
[2] J.P. Hendrick, F.U. Hartl, Molecular chaperone functions of heat-shock proteins, Annu. Rev. Biochem. 62 (1993) 349–384.
[3] J.S. Hahn, Z. Hu, D.J. Thiele, V.R. Iyer, Genome-wide analysis of the biology of stress responses through heat shock transcription factor, Mol. Cell. Biol. 24 (2004) 5249–5256.
[4] P.K. Sorger, H.R.B. Pelham, Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation, Cell 54 (1988) 855–864.
[5] A.P. Gasch, P.T. Spellman, C.M. Kao, O. Carmel-Harel, M.B. Eisen, G. Storz, D. Botstein, P.O. Brown, Genomic expression programs in the response of yeast cells to environmental changes, Mol. Biol. Cell 11 (2000) 4241–4257.
[6] G. Marchler, C. Schuller, G. Adam, H. Ruis, A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions, EMBO J. 12 (1993) 1997–2003.
[7] J.F. Davidson, B. Whyte, P.H. Bissinger, R.H. Schiestl, Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 93 (1996) 5116–5121.
[8] T. Hottiger, T. Boller, A. Wiemken, Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in *Saccharomyces cerevisiae* cells subjected to temperature shifts, FEBS Lett. 220 (1987) 113–115.
[9] T. Hottiger, C. De Virgilio, M.N. Hall, T. Boller, A. Wiemken, The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins in vitro, Eur. J. Biochem. 219 (1994) 187–193.
[10] L.K. Conlin, H.C.M. Nelson, The Natural osmolyte trehalose is a positive regulator of the heat-induced activity of yeast heat shock transcription factor, Mol. Cell. Biol. 27 (2007) 1505–1515.
[11] A. Smith, M.P. Ward, S. Garrett, Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation, EMBO J. 17 (1998) 3556–3564.
[12] J. Francois, J.L. Parrou, Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*, FEMS Microbiol. Rev. 25 (2001) 125–145.
[13] K. Kohrer, H. Domdey, Preparation of high molecular weight RNA, Methods Enzymol. 194 (1991) 398–405.
[14] T.A. Patterson, E.K. Lobenhofer, S.B. Fulmer-Smentek, P.J. Collins, T.-M. Chu, W. Bao, H. Fang, E.S. Kawasaki, J. Hager, I.R. Tikhonova, S.J. Walker, L. Zhang, P. Hurban, F. de Longueville, J.C. Fuscoe, W. Tong, L. Shi, R.D. Wolfinger, Performance comparison of one-color and two-color platforms within the Microarray Quality Control (MAQC) project, Nat. Biotech. 24 (2006) 1140–1150.
[15] A.C. Eklund, L.R. Turner, P. Chen, R.V. Jensen, G. deFeo, A.R. Kopf-Sill, Z. Szallasi, Replacing cRNA targets with cDNA reduces microarray cross-hybridization, Nat. Biotechnol. 24 (2006) 1071–1073.
[16] Y.H. Yang, S. Dudoit, P. Luu, D.M. Lin, V. Peng, J. Ngai, T.P. Speed, Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation, Nucleic Acids Res. 30 (2002) e15.
[17] V.G. Tusher, R. Tibshirani, G. Chu, Significance analysis of microarrays applied to the ionizing radiation response, Proc. Natl. Acad. Sci. USA 98 (2001) 5116–5121.
[18] M.C. Teixeira, P. Monteiro, P. Jain, S. Tenreiro, A.R. Fernandes, N.P. Mira, M. Alenquer, A.T. Freitas, A.L. Oliveira, I. Sa-Correia, The YEASTRACT database: a tool for the analysis of transcription regulatory associations in *Saccharomyces cerevisiae*, Nucleic Acids Res. 34 (2006) D446–D451.
[19] R.M. Marion, A. Regev, E. Segal, Y. Barash, D. Koller, N. Friedman, E.K. O'Shea, Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression, Proc. Natl. Acad. Sci. USA 101 (2004) 14315–14322.
[20] Y. Zhao, K.B. McIntosh, D. Rudra, S. Schawalder, D. Shore, J.R. Warner, Fine-structure analysis of ribosomal protein gene transcription, Mol. Cell. Biol. 26 (2006) 4853–4862.
[21] J.R. Warner, The economics of ribosome biosynthesis in yeast, Trends Biochem. Sci. 24 (1999) 437–440.
[22] S. Wera, E. De Schrijver, I. Geyskens, S. Nwaka, J.M. Thevelein, Opposite roles of trehalase activity in heat-shock recovery and heat-shock survival in *Saccharomyces cerevisiae*, Biochem. J. 343 (1999) 621–626.
[23] M.R. Fernandez, J.A. Biosca, A. Norin, H. Jornvall, X. Pares, Class III alcohol dehydrogenase from *Saccharomyces cerevisiae*: structural and enzymatic features

differ toward the human/mammalian forms in a manner consistent with functional needs in formaldehyde detoxication, FEBS Lett. 370 (1995) 23–26.
[24] K. Maeta, S. Izawa, Y. Inoue, Methylglyoxal, a metabolite derived from glycolysis, functions as a signal initiator of the high osmolarity glycerol-mitogen-activated protein kinase cascade and calcineurin/Crz1-mediated pathway in *Saccharomyces cerevisiae*, J. Biol. Chem. 280 (2005) 253–260.
[25] K. Sugiyama, A. Kawamura, S. Izawa, Y. Inoue, Role of glutathione in heat-shock-induced cell death of *Saccharomyces cerevisiae*, Biochem. J. 352 (2000) 71–78.
[26] Q. Chang, T.A. Griest, T.M. Harter, J.M. Petrash, Functional studies of aldo–keto reductases in *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1773 (2007) 321–329.
[27] J. Aguilera, J. Prieto, The *Saccharomyces cerevisiae* aldose reductase is implied in the metabolism of methylglyoxal in response to stress conditions, Curr. Genet. 39 (2001) 273–283.
[28] C.N. Chen, L. Porubleva, G. Shearer, M. Svrakic, L.G. Holden, J.L. Dover, M. Johnston, P.R. Chitnis, D.H. Kohl, Associating protein activities with their genes: rapid identification of a gene encoding a methylglyoxal reductase in the yeast *Saccharomyces cerevisiae*, Yeast 20 (2003) 545–554.
[29] C.H. Choi, S.J. Park, S.Y. Jeong, H.S. Yim, S.O. Kang, Methylglyoxal accumulation by glutathione depletion leads to cell cycle arrest in *Dictyostelium*, Mol. Microbiol. 70 (2008) 1293–1304.
[30] A.G. Howe, V. Zaremberg, C.R. McMaster, Cessation of growth to prevent cell death due to inhibition of phosphatidylcholine synthesis is impaired at 37 °C in *Saccharomyces cerevisiae*, J. Biol. Chem. 277 (2002) 44100–44107.
[31] S. Chen, M. Tarsio, P.M. Kane, M.L. Greenberg, Cardiolipin mediates cross talk between the mitochondria and the vacuole, Chem. Phys. Lipids 154 (2008) S20.
[32] G.M. Jenkins, A. Richards, T. Wahl, C. Mao, L. Obeid, Y. Hannun, Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*, J. Biol. Chem. 272 (1997) 32566–32572.
[33] S.R. Ferguson-Yankey, M.S. Skrzypek, R.L. Lester, R.C. Dickson, Mutant analysis reveals complex regulation of sphingolipid long chain base phosphates and long chain bases during heat stress in yeast, Yeast 19 (2002) 573–586.
[34] L.A. Cowart, Y. Okamoto, F.R. Pinto, J.L. Gandy, J.S. Almeida, Y.A. Hannun, Roles for sphingolipid biosynthesis in mediation of specific programs of the heat stress response determined through gene expression profiling, J. Biol. Chem. 278 (2003) 30328–30338.