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Microarray analyses of the metabolic responses of *Saccharomyces cerevisiae* to organic solvent dimethyl sulfoxide

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Abstract The toxic effects that organic solvents have on whole cells are important drawbacks in the application of these solvents in the production of fine chemicals by whole-cell stereoselective biotransformations. Although early studies found that organic solvents mainly destroyed the integrity of cell membranes by accumulating in the lipid bilayer of plasma membranes, the cellular metabolic responses to the presence of an organic solvent remain unclear. With the rapid development of genomics, it is possible to study cellular metabolism under perturbed conditions at the genome level. In this paper, the global gene expression profiles of *Saccharomyces cerevisiae* BY4743 grown in media with a high concentration of the organic solvent dimethyl sulfoxide (DMSO) were determined by microarray analysis of ~6,200 yeast open reading frames (ORFs). From cells grown in SD minimal medium containing 1.0% (v/v) DMSO, changes in transcript abundance greater than or equal to 2.5-fold were classified. Genomic analyses showed that 1,338 genes were significantly regulated by the presence of DMSO in yeast. Among them, only 400 genes were previously found to be responsive to general environmental stresses, such as temperature shock, amino acid starvation, nitrogen source depletion, and progression into stationary phase. The DMSO-responsive genes were involved in a variety of cellular functions, including carbohydrate, amino acid and lipid metabolism, cellular stress responses, and energy metabolism. Most of the genes in the lipid biosynthetic pathways were down-regulated by DMSO treatment, whereas genes involved in amino acid biosynthesis were mostly up-regulated. The results demonstrate that the application of microarray technology allows better interpretation of metabolic responses, and the information obtained will be useful for the construction of engineered yeast strains with better tolerance of organic solvents.

Keywords *Saccharomyces cerevisiae* · Gene expression profiling · Metabolic responses · Dimethyl sulfoxide

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Introduction

Organic solvents can dissolve lipophilic or water-insoluble compounds effectively, and their introduction into bioreaction systems permits the construction of homogeneous systems with these compounds [32]. Organic solvents have been widely used in the application of biotransformation with enzymes for several decades [8]. With ongoing research activities continuously revealing complicated chemical reactions that are effectively catalyzed by microorganisms, the potential for the application of organic solvents in biotransformation should not be understated. However, some biotransformations are cofactor-dependent (NADH or NADPH), and are often catalyzed by multicomponent enzyme systems, some of which are membrane

proteins. Because of the complexity of these biocatalytic systems, whole-cell in vivo cultures are usually favored over biotransformations using isolated enzymes [18].

On the other hand, organic solvents are generally known to have negative effects on the metabolism of microorganisms, and the toxic effects have been considered as serious drawbacks in the application of these solvents in environmental biotechnology and in the production of fine chemicals by whole-cell stereoselective biotransformation [18]. Although the detailed mechanism of their toxicity to microorganisms has not been studied extensively, it is thought that highly toxic organic solvents, such as toluene and hexane, destroy the integrity of cell membranes by accumulating in the lipid bilayer of plasma membranes [23, 33]. Recently, strains of *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, and *Escherichia coli* have been isolated with tolerance to toxic organic solvents [2, 3, 14, 26], and mechanisms of tolerance in these bacteria have been proposed [15]. Different mechanisms, such as the presence of an efflux system localized in the outer membrane, which actively decreases the amount of solvent in the cell [16, 29, 37], and alterations of the composition of the outer membrane [28, 36], were considered to contribute to organic-solvent tolerance in these strains. With the tolerance mechanism partially known, several successful attempts have been made to isolate the genes responsible for organic-solvent tolerance, and to construct high-tolerance bacterial strains [4, 25]. There are also several reports on eukaryotic microorganisms with organic-solvent tolerance [19, 23]. A *Saccharomyces cerevisiae* strain, KK-211, was isolated with extremely high tolerance to the organic solvent isooctane. Thirty isooctane-tolerance-associated genes were identified in the tolerant cells by mRNA differential display. The genes were found to be associated with numerous cellular functions, including cell stress, cell surface maintenance, the uptake of trehalose, and the production of glycogen [19, 23].

The rapid development of genomics in the past few years has provided access to genes and their regulatory elements. Microarray technology has been used to explore transcriptional profiles and genome differences for a variety of microorganisms, greatly facilitating our understanding of microbial metabolism [30, 31]. In this article, we examine the global effects of dimethyl sulfoxide (DMSO) on the metabolism of *S. cerevisiae* using gene expression profiling technology. Interpretation of the data results in a considerable amount of information that may help in understanding the metabolic responses of yeast to the organic solvent DMSO at the whole-genome level, and thus allow better exploration into possible mechanisms of DMSO tolerance. Considering that *S. cerevisiae* has been used in a number of different processes involving organic solvents within the pharmaceutical industry [34], the information reported here would be useful for the construction of engineered yeast strains with better organic-solvent tolerance.

Materials and methods

Strains and growth conditions

Saccharomyces cerevisiae wild-type strain BY4743 was purchased from the American Type Culture Center (ATCC, Manassas, Va.). Yeast was grown in YPD (complete) or SD (minimal) medium [17]. The cultures were started from a fresh single colony and grown in 1.0 ml YPD overnight at 30°C. The OD_{600} values of overnight cultures were normally around 2.0–3.0 after 16 h. After adjusting the OD_{600} to 1.0 with YPD media, each 2.0-ml culture was inoculated into three 250-ml flasks with 50 ml of SD medium. DMSO was added to the cultures at late log phase, when the OD_{600} reached 2.0 (~9–10 h). The cells were cultivated for another 2 h and then collected by centrifugation at 3000×g for 5 min at 4°C. Pellets were washed once with ice-cold water, and then lyophilized overnight at −20°C. The samples were stored at −80°C.

RNA extraction and microarray preparation

Approximately 18 ± 1 mg of lyophilized yeast cells in a 1.5-ml microcentrifuge tube was rehydrated in 75 μl RNA Later (Ambion, Austin, Tex.) and incubated for 30 min. Subsequently, 875 μl Trizol Reagent (GibcoBRL, Rockville, Md.) was added to each tube. The tubes were vortexed for 15 sand allowed rest for 45 s. This was repeated for a total of 5 min. Chloroform (240ôμl, HPLC grade, RNAase-free) was added to each tube. The tubes were vortexed for 30 s, incubated for 10 min at room temperature (RT), and centrifuged at 12,500×g in a refrigerated Eppendorf centrifuge at 4°C for 5 min. The aqueous phase (570 μl) was removed and placed in a RNAase-free 2-ml tube. Nuclease-free water (430 μl, Ambion), and 1.0 ml 100% isopropanol were added to each tube. The tubes were mixed thoroughly by inversion, incubated for 10 min at RT, and centrifuged for 20 min as before. Pellets were washed with 400 μl 70% ethanol and centrifuged for 10 min. The pellet was then dissolved in 100 μl nuclease-free water. RNA quality was determined using the Bioanalyzer 2100 and the RNA 6000 assay (Agilent Technologies, Palo Alto, Calif.) according to the manufacturer's instructions. RNA concentrations were determined spectrophotometrically by measuring the absorption at 260 nm in an Ultrospec 2000 (Pharmacia Biotech, Piscataway, N.J.). Microarrays containing ~6,200 *Sacchoromyces cerevisiae* genes, essentially covering the entire genome, were generated by Agilent Technologies using oligonucleotides 60 bases in length synthesized in situ by an ink-jet printing method [13].

Microarray hybridization

Each RNA sample was labeled with either Cy3 or Cy5 using (Agilent Technologies' Fluorescent Linear Amplification Kit essentially according to the supplier's instructions . Labeled cRNAs were evaluated using the RNA 6000 assay on the Agilent Bioanalyzer 2100. Labeled cRNA concentrations were determined spectrophotometrically by measuring the absorption at 260 nm. Probe solutions containing 125 ng of labeled cRNA for each mutant and its paired control were prepared using Agilent Technologies in situ Hybridization Reagent Kit. Each pair of samples to be hybridized was independently labeled and hybridized utilizing fluor reversal for a total of two hybridizations per sample pair. The microarrays were scanned simultaneously in the Cy3 and Cy5 channels with a 48-slide, Dual Laser DNA Microarray Scanner (Agilent Technologies) at 10-μm resolution using default settings.

Microarray data processing and analysis

Image Analysis Software (Version A.4.0.45, Agilent Technologies) was used for image analysis. Each feature was determined from the array's associated pattern file and a detection algorithm. Intensity values for each feature were determined after subtracting background derived from an average of negative control features. Features with unusual pixel intensity statistics (high non-uniformity, saturation in either channel) were excluded from downstream analyses. Data was loaded into the Rosetta Resolver database (Rosetta Inpharmatics, Kirkland, Wash.) for storage and analysis. Data were evaluated after combining results from fluor reversal replicate hybridizations. The annotation of yeast ORFs was updated from the Proteome Bioknowledge Library on February 22, 2002 (Incyte Genomics, Palo Alto, Calif.).

Results

Global gene expression monitoring of *S. cerevisiae* grown in media containing DMSO

The availability of the complete yeast genome sequence facilitates metabolic studies in this model organism. We have implemented microarray technology to investigate the metabolic effects of the organic solvent DMSO on yeast at the genome level. For microarray analyses, the level of gene expression from essentially every gene in the *S. cerevisiae* genome was examined simultaneously using DNA microarray technology (Aligent Technologies). Over 6,200 unique ORFs were represented on a single array. Total RNA was isolated from both DMSO-treated and untreated yeast samples. The fluorescent signals were detected by scanning. Normalization and ratio determination were carried out with Rosetta Resolver software. The sample yeast cells for gene-expression profiling were collected from late log phase. Baseline experiments (untreated) were done in 20 replicates; the data obtained were compared by pair-wise analyses and then averaged. The results showed less than 4% of genes with transcript abundance changes and only 0.73% of the genes showed changes greater than 1.5-fold among the control samples (data not shown). These analyses demonstrate that the experimental protocols and detection methods used were very reliable and reproducible [12].

Although the growth kinetics of *S. cerevisiae* were not affected by 1% DMSO in the media tested (Fig. 1A), the pair-wise comparison showed that yeast have significant global metabolic responses to the presence of DMSO. The gene-expression profiles of yeast grown in medium with or without DMSO are shown in Fig. 1. Using a cutoff value of a 2.5-fold change in transcript abundance and a p value <0.005, 1,338 genes were identified as responsive to the presence of DMSO, including the majority of genes from carbohydrate, amino acid, and lipid metabolism pathways. Of these, 658 genes were down-regulated (2.5-fold decreased expression) and 680 genes were up-regulated (2.5-fold increased expression) (Fig. 1B); 891 genes are functionally known or have homologies previously found in other organisms,

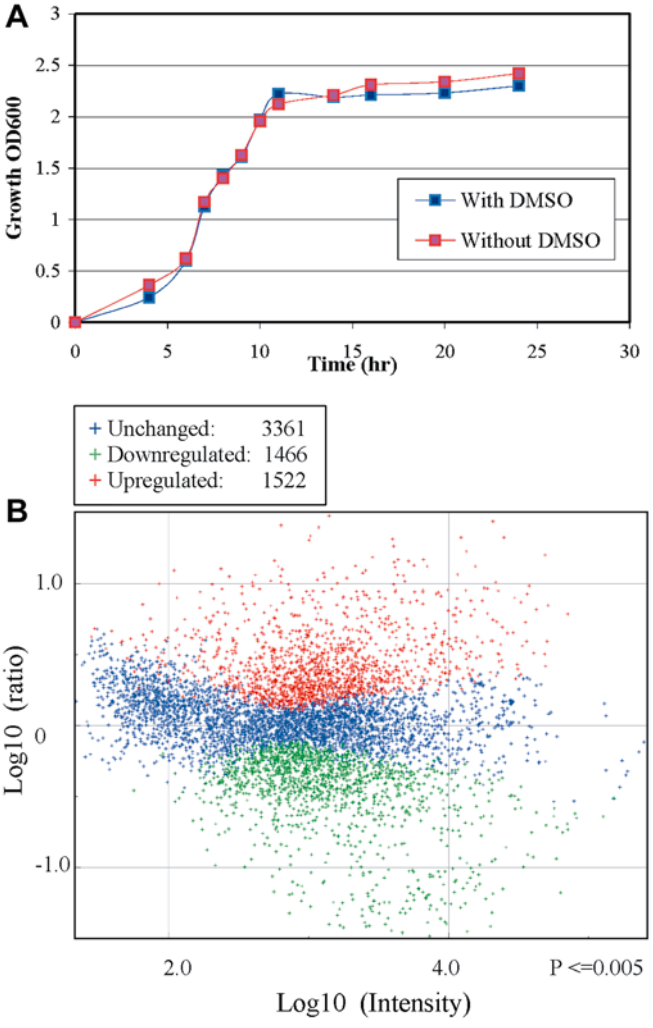

Fig. 1A, B Comparison of the growth kinetics and global gene expression profiles of yeast grown in medium with or without DMSO. **A** Growth kinetics of *Saccharomyces cerevisiae* BY4742; 1% (v/v) DMSO was added to the culture at the beginning of cultivation. **B** Global gene expression profiles: *red* DMSO up-regulated genes, *green* DMSO down-regulated genes, *blue* genes not responsive to DMSO treatment. $p <= 0.005$ was used as a cutoff value to determine the responsive genes

whereas 447 are unknown genes. To obtain a global view of the metabolic effects of DMSO, the function-known responsive genes were divided into functional classes, as shown in Fig. 2A. The responsive genes could be assigned to 21 function classes, including amino acid metabolism (5.34% of the responsive genes), carbohydrate metabolism (3.93%), and lipid metabolism (2.9%).

Comparison of genes responsive to DMSO treatment and general environmental stresses

In a recent study, Gasch et al. [11] explored genomic expression patterns in yeast responding to diverse environmental transitions. DNA microarrays were used to measure changes in transcript levels over time for almost

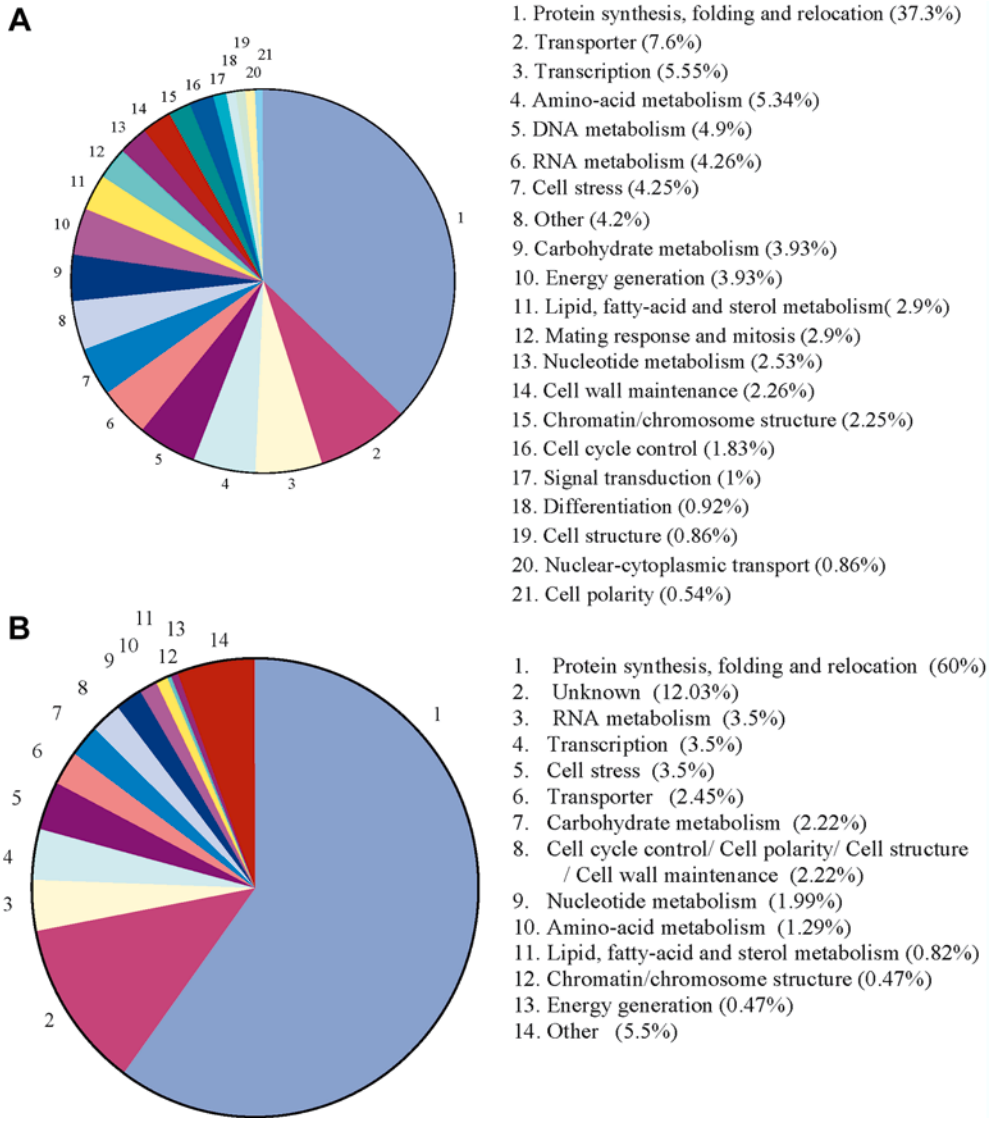

Fig. 2 A Pie chart grouping DMSO-responsive genes into functional classes. **B** Pie chart grouping the genes responsive to both DMSO treatment and general environmental stresses

every yeast gene, as cells responded to temperature shocks, hydrogen peroxide, the superoxide-generating drug menadione, the sulfhydryl-oxidizing agent diamide, the disulfide-reducing agent dithiothreitol, hyper- and hypo-osmotic shock, amino acid starvation, nitrogen source depletion, and progression into stationary phase. About 900 genes were found to have a similar drastic response to almost all of these environmental changes, and were identified as genes responsive to general environmental stress [11]. This set of general environmental-stress-responsive genes was extracted from the Stanford Genomic Resources Database (http://www-genome.stanford.edu/yeast_stress) and compared with the 1,338 DMSO-responsive genes. The results showed that 400 of the DMSO-responsive genes were also found among the genes responsive to general environmental stress, whereas 938 responsive genes were unique to the DMSO treatment.

According to results by Gasch et al. [11], the genes repressed by general environmental stress cluster into two groups, the first group consists of genes involved in growth-related processes, various aspects of RNA metabolism, nucleotide biosynthesis, secretion, and other metabolic processes. The second group consists almost entirely of genes encoding ribosomal proteins. The repression of ribosomal protein genes has been observed during multiple stress responses [35] and is regulated by the transcription factor Rap1p [20, 24]. Analyses of the 400 responsive genes common to DMSO treatment and general environmental stress showed that 60% of these genes were involved in protein synthesis folding and relocation function, and 3.5% were involved in RNA metabolism. However, a relatively small portion of DMSO-responsive genes involved in carbohydrate metabolism, amino acid metabolism, and lipid, fatty acid, and sterol metabolism are found among the genes responsive to general environmental stress (Fig. 2B), suggesting that DMSO treatment might have unique effects on those cellular metabolic functions in yeast.

Distribution of the responsive genes in metabolic pathways

Among the 1,338 genes regulated by DMSO treatment, 248 encoded enzymes that function in various metabolic pathways. According to the pathways compiled by Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.ad.jp/kegg), 53 metabolic pathway networks were regulated by DMSO treatment in yeast in which at least one gene was responsive. Among them, 30 pathways were also responsive to general environmental stress (Table 1). The responsive pathways include almost every aspect of cellular metabolism: carbohydrate, simple and complex lipids, amino acids, purines and pyrimidines, and vitamin metabolism. In most of the responsive metabolic pathway networks classified by KEGG, such as glycolysis and pentose phosphate metabolism, both up- and down-regulated responsive genes were found, suggesting the differential effects of DMSO on the metabolic network. However, the responsive genes in some pathway networks have consistent responses to DMSO treatment. For example, all the

Table 1 The distribution of responsive genes in metabolic pathways. The classification of pathways is according to KEGG: Kyoto Encyclopedia of Genes and Genomes (http://www.genome.ad.jp/kegg/).*Numbers in parentheses* Number of genes also responsive to general environmental stress

Pathway name	KEGG Map ID	Up-regulated genes	Down-regulated genes
Glycolysis/gluconeogenesis	map00010	6 (3)	8
Citrate cycle	map00020	3 (1)	
Pentose phosphate metabolism	map00030	4 (3)	5 (1)
Fructose and mannose metabolism	map00051	1 (1)	6 (1)
Galactose metabolism	map00052	2 (1)	3
Ascorbate and aldarate metabolism	map00053	1 (1)	
Fatty acid biosynthesis	map00062	1	12
Fatty acid metabolism	map00071	3 (1)	3
Sterol biosynthesis	map00100	2	
Bile acid biosynthesis	map00120	3	1
Ubiquinone biosynthesis	map00130		1
Oxidative phosphorylation	map00190	4	5
ATP synthesis	map00193	1	1
Urea cycle and metabolism of amino group	map00220	6	
Purine metabolism	map00230	13 (1)	10 (7)
Pyrimidine metabolism	map00240	3 (1)	9 (6)
Glutamate metabolism	map00251	7 (1)	2 (1)
Alanine and aspartate metabolism	map00252	7 (2)	1 (1)
Glycine, serine and threonine metabolism	map00260	7	4 (2)
Methionine metabolism	map00271	3	2 (1)
Cysteine metabolism	map00272	4	
Valine, leucine and isoleucine degradation	map00280	3 (1)	
Valine, leucine and isoleucine biosynthesis	map00290	3	
Lysine biosynthesis	map00300	3	1
Lysine degradation	map00310	3 (1)	1
Arginine and proline metabolism	map00330	6 (1)	
Histidine metabolism	map00340	7 (2)	
Tyrosine metabolism	map00350	3 (1)	1
Phenylalanine metabolism	map00360	4 (1)	
Tryptophan metabolism	map00380	4 (3)	2 (1)
Phenylalanine, tyrosine and tryptophan biosynthesis	map00400	11	2 (1)
β-Alanine metabolism	map00410	2 (2)	
Taurine and hypotaurine metabolism	map00430	1 (1)	
Selenoamino acid metabolism	map00450	5	2 (1)
Cyanoamino acid metabolism	map00460	2	
Glutathione metabolism	map00480	1	
Starch and sucrose metabolism	map00500	8 (6)	3
Glycoprotein degradation	map 00511	1	6
Nucleotide sugar metabolism	map00520	1	
Amino sugar metabolism	map00530	2	2
Glycerolipid metabolism	map00561	2 (1)	4
Sphingophospholipid biosynthesis	map00570		1
Phospholipid metabolism	map00580		1
Sphingoglycolipid biosynthesis	map00600		1
Pyruvate metabolism	map00620	7 (1)	3
Glyoxylate and dicarboxylate metabolism	map00630	3 (1)	3 (3)
Riboflavin metabolism	map00740	3	1
Vitamin B_6 metabolism	map00750	2	
Pantothenate and CoA metabolism	map00770	2	
Biotin metabolism	map00780	2	
Folate biosynthesis	map00790	1	2 (1)
Nitrogen metabolism	map00910	3	1 (1)
Sulfur metabolism	map00920	7	

responsive genes in the citrate cycle and amino acid biosynthetic pathways were up-regulated, whereas almost all the responsive genes in the fatty acid, sphingophospholipid, or sphingoglycolipid biosynthetic pathways were exclusively down-regulated.

Effects of DMSO on central carbohydrate and energy metabolism

As shown in Fig. 2, carbohydrate metabolism represented about 3.93% of the responsive genes. The enzyme-encoding genes responsive to DMSO are listed in Table 2. The results show that the presence of DMSO affected the expression of many genes in the central carbohydrate metabolism pathways. The pathway networks regulated by DMSO included glycolysis and gluconeogenesis, the citric acid cycle, the pentose phosphate pathway, and sugar metabolism pathways. In the metabolic pathway networks of glycolysis, pentose phosphate, and glyoxylate, responsive genes showed diverse patterns of expression . For example, YGL256W, encoding one of the five alcohol dehydrogenase isozymes in yeast, was down-regulated by 36.66-fold, while YBR145W and YMR303C, encoding another two alcohol dehydrogenase isozymes, were up-regulated by seven to eight-fold. In contrast, all the responsive genes in the glycolysis pathway showed consistent regulation patterns; hexokinase, 6-phosphofructokinase, and phosphoglycerate mutase were all down-regulated. In the metabolic pathway after pyruvate, pyruvate carboxylase, which catalyzes the reaction from pyruvate to oxaloacetate, three isozymes of citrate synthase and two isozymes of isocitrate dehydrogenase in the citrate cycle, were all up-regulated by three- to ten-fold. Two key enzymes in the glyoxylate pathway, malate synthase and isocitrate lyase, were also concurrently up-regulated. It is interesting that *Cit1*, which encodes the major mitochondrial citrate synthase of the TCA cycle, was also responsive to general environmental stress, whereas *Cit2*, encoding the peroxisomal citrate synthase, and *Cit3*, encoding the minor mitochondrial citrate synthase, were responsive only to DMSO treatment [22].

Twenty-five DMSO-responsive genes were involved in energy metabolism (Table 3). Among them, 15 were down-regulated 2.6- 5.2-fold. The down-regulated genes are divided into two functional groups. The first includes genes directly involved in ATP synthesis, such as YOL077W-A and YPL271W, encoding F1-ATP

Table 2 DMSO-responsive genes involved in carbohydrate metabolism. Genes also responsive to general environmental stress are *underlined*

Gene name	Function description	EC number	Fold changes
YGL256W	Alcohol dehydrogenase I	1.1.1.1	−36.66
YBR145W	Alcohol dehydrogenase II	1.1.1.1	7.21
YMR303C	Alcohol dehydrogenase III	1.1.1.1	8.28
YKL029C	Malate dehydrogenase (oxaloacetate decarboxylating).	1.1.1.38	2.54
YDL066W	Isocitrate dehydrogenase (NADP+) I	1.1.1.42	3.51
YLR174W	Isocitrate dehydrogenase (NADP+) II	1.1.1.42	4.57
YGR192C	Glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) I	1.2.1.12	−4.11
YJR009C	Glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) II	1.2.1.12	−3.89
YBR117C	Transketolase I	2.2.1.1	4.2
YLR354C	Transaldolase II	2.2.1.2	−2.54
YGL253W	Hexokinase	2.7.1.1	−8.76
YGR240C	6-Phosphofructokinase	2.7.1.11	−3.13
YGR087C	Pyruvate decarboxylase	4.1.1.1	9.62
YKL060C	Fructose-bisphosphate aldolase	4.1.2.13	−3.26
YER065C	Isocitrate lyase	4.1.3.1	21.14
YMR108W	Acetolactate synthase	4.1.3.18	3.9
YIR031C	Malate synthase I	4.1.3.2	3.21
YNL117W	Malate synthase II	4.1.3.2	29.71
YCR005C	Citrate (si)-synthase II	4.1.3.7	7.02
YPR001W	Citrate (si)-synthase III	4.1.3.7	10.18
YHR174W	Phosphopyruvate hydratase I	4.2.1.11	−4.38
YGR254W	Phosphopyruvate hydratase II	4.2.1.11	−4.18
YMR323W	Phosphopyruvate hydratase III	4.2.1.11	5.26
YJL121C	Ribulose-phosphate 3-epimerase	5.1.3.1	−4.54
YOL056W	Phosphoglycerate mutase	5.4.2.1	−3.03
YLR153C	Acetate-CoA ligase	6.2.1.1	−3.37
YGL062W	Pyruvate carboxylase	6.4.1.1	6.56
YGR256W	Phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	3.81
YOR374W	Aldehyde dehydrogenase (NAD+)	1.2.1.3	2.83
YMR169C	Aldehyde dehydrogenase (NAD(P)+)	1.2.1.5	2.75
YGR043C	Transaldolase III	2.2.1.2	5.56
YIL107C	6-Phosphofructo-2-kinase	2.7.1.105	2.77
YKL181W	Ribose-phosphate pyrophosphokinase	2.7.6.1	−3.19
YNR001C	Citrate (si)-synthase I	4.1.3.7	2.58
YMR105C	Phosphoglucomutase	5.4.2.2	3.76

Table 3 DMSO-responsive genes involved in energy metabolism. The genes responsive also to general environmental stress are *underlined*

Gene name	Function description	Fold changes
YIL070C	Mitochondrial protein required for normal respiratory growth	−5.19
YER153C	Translational activator required for mitochondrial translation of COX3 mRNA	−4.51
YLR069C	Mitochondrial translation elongation factor G, promotes GTP-dependent translocation of nascent chain from A-site to P-site of ribosome	−4.33
YGR254W	Enolase 1 (2-phosphoglycerate dehydratase), converts 2-phospho-D-glycerate to phosphoenolpyruvate in glycolysis	−4.18
YBL030C	ADP/ATP carrier protein of the mitochondrial carrier family (MCF) of membrane transporters	−3.89
YJR144W	Mitochondrial genome maintenance protein	−3.77
YER154W	Protein required for assembly of F1F0-ATP synthase and cytochrome oxidase and for export of Cox2p termini through the inner mitochondrial membrane	−3.53
YGR062C	Protein required for activity of mitochondrial cytochrome oxidase	−3.12
YOL077W-A	Subunit k of F1-F0 ATP synthase complex, detected only in dimeric form of complex	−3.11
YGL129C	Component of the mitochondrial ribosomal small subunit, required for respiration and mitochondrial maintenance, putative ATPase	−3.06
YPL271W	ε-Subunit of F1-ATP synthase; one copy is found in each F1 oligomer	−2.9
YJL180C	F1-ATP synthase assembly protein	−2.89
YMR267W	Inorganic pyrophosphatase, mitochondrial	−2.75
YNL252C	Mitochondrial ribosomal protein of the large subunit	−2.74
YBR185C	Protein required for the assembly of mitochondrial respiratory complexes and for protein export from mitochondrial matrix	−2.72
YLR395C	Cytochrome *c* oxidase subunit VIII	−2.63
YPL252C	Protein with similarity to adrenodoxin and ferrodoxin	2.88
YDL067C	Cytochrome *c* oxidase subunit VIIA, essential component of the cytochrome *c* holoenzyme	2.92
YBL045C	Ubiquinol cytochrome *c* reductase core protein 1, component of ubiquinol cytochrome *c* reductase complex	2.95
YKL155C	Putative component of the mitochondrial ribosomal small subunit	3.35
YML054C	Cytochrome b^2 [L-(+)-lactate cytochrome *c* oxidoreductase], catalyzes the conversion of L-lactate to pyruvate	3.42
YKL150W	NADH-cytochrome b_5 reductase	3.81
YLL027W	Mitochondrial protein required for normal iron metabolism, involved in maturation of mitochondrial and cytosolic iron-sulfur proteins	4.08
Q0130	F0-ATP synthase subunit 9 (ATPase-associated proteolipid); mitochondrially encoded	4.14
YDL198C	Protein of the mitochondrial carrier (MCF) family that when overexpressed suppresses loss of Abf2p	7.55

synthase, and YLR395C, encoding cytochrome *c* oxidase; and genes involved in F1-ATP synthase assembly, such as YJL180C, which encodes a F1-ATP synthase assembly protein, and YER154W, which is required for assembly of the cytochrome oxidase complex and assembly or stability of F1F0-ATP synthase. The second group includes genes necessary for the maintenance of mitochondrial structure, such as YNL252C and YGL129C, encoding mitochondrial ribosomal proteins, and YJR144W, encoding a protein involved in repair of oxidatively damaged mitochondrial DNA. Nine genes involved in energy metabolism were up-regulated 2.8- to 7.5-fold by DMSO, which included genes encoding cytochrome b_2, NADH-ctochrome b_5 reductase, and F0-ATP synthase. Only one gene, YKL150W, encoding NADH-cytochrome b_5 reductase, was responsive to general environmental stress.

Effects of DMSO on lipid biosynthetic pathways

Microarray analyses revealed that a majority of genes involved in the biosynthesis of simple and complex lipids were down-regulated (Table 1 and Fig. 3) in response to DMSO treatment. Furthermore, none of the genes were responsive to general environmental stress, suggesting that regulation by DMSO was quite specific. The results showed that three key enzymes, acetyl-CoA carboxylase (*acc*1), fatty acid synthase 1 (*fas*1) and 2 (*fas*2), were down-regulated significantly by DMSO (five- to six-fold). YKL192C, encoding an acyl carrier protein (ACP) necessary for mitochondrial type II fatty acid synthase, was also 2.81-fold down-regulated. For sphingoglycolipid biosynthesis, serine C-palmitoyltransferase and 3-ketosphinganine reductase, which catalyze the first and second steps in the biosynthesis of a long-chain base component of sphingolipids, respectively, were down-regulated two- to three-fold. 1,2-Diacylglycerol ethanolaminephosphotransferase, which catalyzes synthesis of phosphatidylethanolamine from CDP-ethanolamine and diacylglycerol, were also down-regulated. Differential regulation patterns were also observed for the genes in the ergosterol biosynthetic pathway. Three of the six responsive genes, farnesyl pyrophosphate synthetase (*erg*20), S-adenosylmethionine δ-24-sterol-C-methyltransferase (*erg*6) and acetyl-CoA acetyltransferase (*erg*10), were down-regulated by DMSO. The results are partially in agreement with previous studies showing that the target of organic solvents like DMSO might be the cellular membrane by altering its lipid composition [23, 33]. However, previous studies seem to point to their physical effects on membrane lipid bilayers rather than their metabolic effects. Our results demonstrate that global down-regulation of

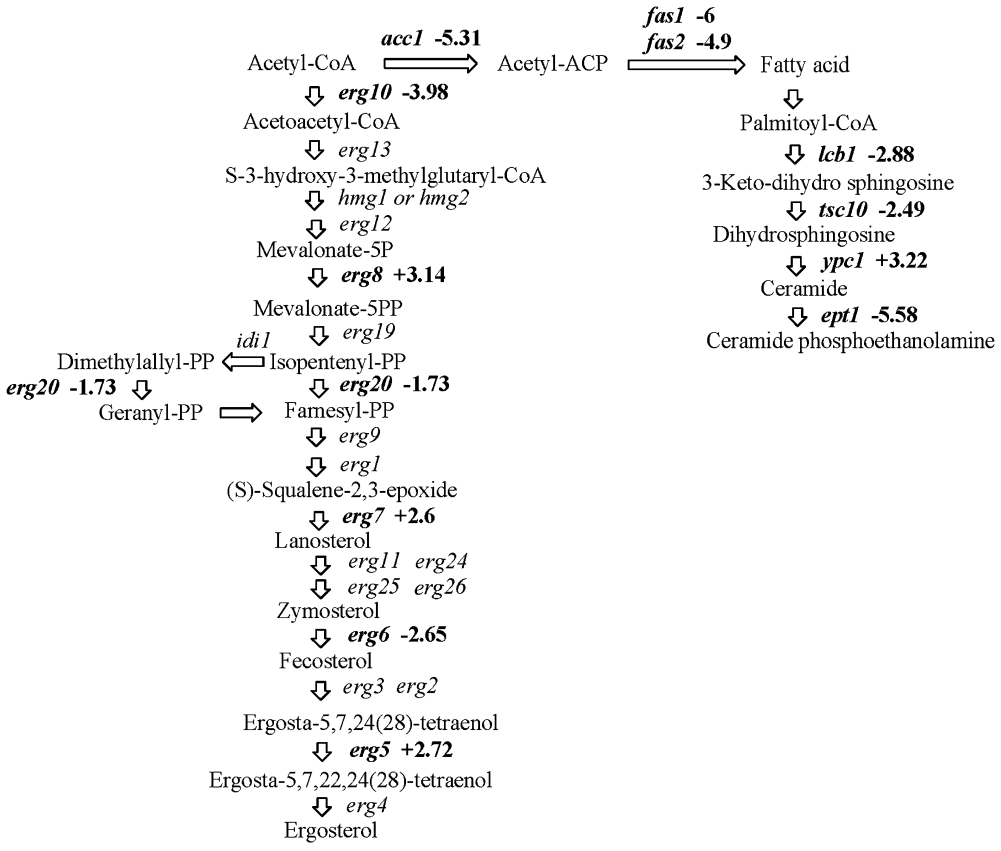

Fig. 3 Effects of DMSO treatment on the lipid biosynthetic pathway. The responsive genes are indicated as SGD names in *italic*. The responsive genes are indicated in *bold* followed by the -fold changes. The SGD names are available from http://www-sequence.stanford.edu/

lipid biosynthetic pathways might also be a major factor contributing to loss of integrity of the cellular membrane.

Effects of DMSO on amino acid metabolism pathways

The major amino acid biosynthetic pathways were significantly up-regulated by DMSO (Fig. 4), very similar to the general cellular metabolic responses under amino acid starvation [27]. Five enzymes in the histidine biosynthetic pathway, ATP phosphoribosyltransferase (*his*1), phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphohydrolase/histidinol dehydrogenase complex (*his*4), histidinol-phosphate aminotransferase (*his*5), which catalyze the first, second, third, eighth and tenth steps of the pathway, respectively, were up-regulated three- to 13-fold. In the serine/glycine/cysteine biosynthetic pathway, 3-phosphoserine transaminase (*ser*1), serine hydroxymethyltransferase (*shm*2), and glycine decarboxylase (*gcv*1 and *gcv*2) were up-regulated three- to 11-fold. In the aromatic amino acid pathway, the arom pentafunctional enzyme (*aro*1), 2-dehydro-3-deoxyphosphoheptonate aldolase (*aro*3), anthranilate synthase (*trp*3), anthranilate phosphoribosyltransferase (*trp*4), and histidinol-phosphate aminotransferase (*his*5) were up-regulated two to 13-fold. In the biosynthetic pathways starting from pyruvate to alanine, valine, leucine and isoleucine, alanine aminotransferase (*L2518*), acetolactate synthase (*ilv*2), 2-isopropylmalate synthase (*leu*4), and cytosolic branched-chain amino acid transaminase (*bat*2) were up-regulated three- to six-fold. In the aspartate pathway, the first two enzymes of the pathway, aspartate kinase (*hom*3) and aspartate-semialdehyde dehydrogenase (*hom*2), which lead to the biosyntheses of lysine, methionine, isoleucine, threonine, glycine, leucine and valine, were up-regulated three- to four-fold. Two other enzymes in the aspartate pathway, homoserine *O*-acetyltransferase (*met*2) cystathionine γ-synthase (*str*2), were also up-regulated seven- to 11-fold. In the urea cycle and arginine biosynthetic pathway, five enzymes, argininosuccinate synthetase (*arg*1), ornithine carbamyltransferase (*arg*3), argininosuccinate lyase (*arg*4), acetylglutamate kinase (*arg*5), and acetylornithine aminotransferase (*arg*8), were up-regulated seven- to 15-fold.

Effects of DMSO on purine and pyrimidine metabolism pathways

Thirty-one genes in the purine and pyrimidine metabolism pathways were responsive to DMSO treatment, among them, 13 DMSO-responsive genes were also regulated by general environmental stress [11], as shown in Table 4. Interestingly, the responsive genes in the purine metabolism pathway clustered into two categories. Twelve genes involved in the biosynthesis from 5-phosphoribosyl diphosphate, a product of the

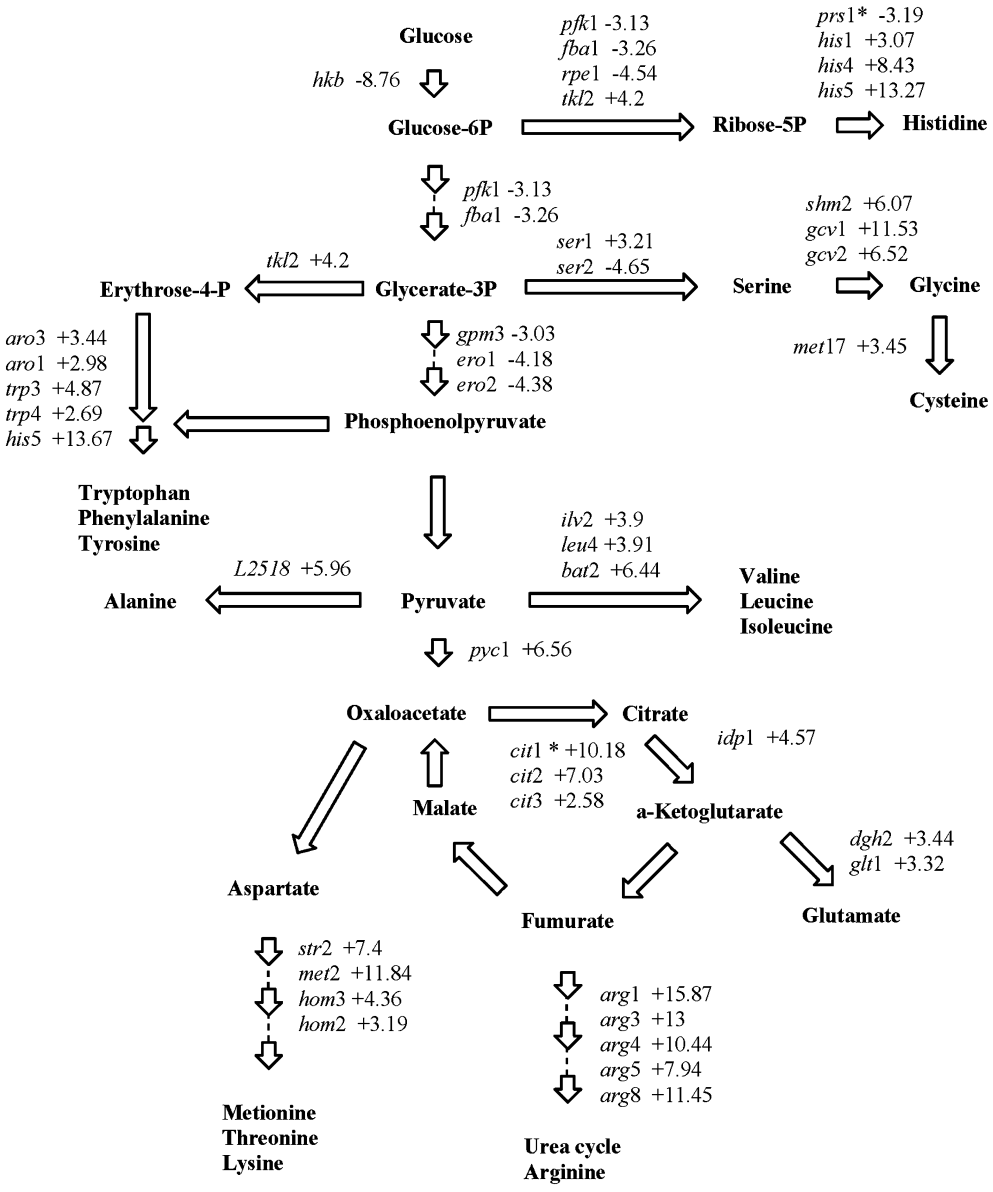

Fig. 4 Effects of DMSO treatment on amino acid metabolism pathways. The responsive genes are indicated as SGD names, followed by the -fold changes. The SGD names are available from http://www-sequence.stanford.edu/. Genes also responsive to general environmental stress are marked by an *asterisk*

pentose phosphate pathway, to inosine monophosphate (IMP) were up-regulated, whereas eight genes involved in the transfer of IMP into adenine, deoxyadenosine, and guanine were down-regulated. In the pyrimidine metabolism pathway, more genes were found to be down-regulated than up-regulated after DMSO treatment. Two genes, orotate phosphoribosyltransferase II and carbamoyl-phosphate synthase, were up-regulated, while six genes, dihydroorotate oxidase, ribonucleoside-diphosphate reductase, thymidylate synthase, orotate phosphoribosyltransferase I, dUTP pyrophosphatase, and CTP synthase, were down-regulated.

Effects of DMSO on vitamin biosynthetic pathways

Since vitamins function as cofactors for various enzymes of intermediary metabolism, it was anticipated that DMSO treatment would also affect expression of the genes involved in vitamin or cofactor biosynthesis. Microarray analyses showed that DMSO treatment affected the expression of several genes involved in riboflavin, pyridoxal phosphate, pantothenate and coenzyme A, biotin, and folate biosynthesis. Ten genes were induced by DMSO treatment, including riboflavin synthase, acid phosphatase, and GTP cyclohydrolase I, in the riboflavin biosynthetic pathway; pyridoxamine-phosphate oxidase and phosphoserine aminotransferase in the vitamin B_6 biosynthetic pathway; branched-chain amino acid aminotransferase and acetolactate synthase in the pantothenate and CoA biosynthetic pathway; desthiobiotin synthase and adenosylmethionine-8-amino-7-oxononanoate aminotransferase in the biotin biosynthetic pathway; and GTP cyclohydrolase I in the folate biosynthetic pathway. Only two genes involved in folate biosynthesis, thymidylate synthase and formate-

Table 4 DMSO-responsive genes involved in purine andpyrimidine metabolism. * The genes responsive also to general environmentalstress are *underlined*

Gene name	Function description	EC number	Fold changes
YDR441C	Adenine phosphoribosyltransferase II	2.4.2.7	−3.16
YJR105W	Adenosine kinase	2.7.1.20	−3.79
YDR226W	Adenylate kinase	2.7.4.3	−3.36
YNL220W	Adenylosuccinate synthase	6.3.4.4	2.88
YKL001C	Adenylylsulfate kinase	2.7.1.25	2.95
YMR300C	Amidophosphoribosyltransferase I	2.4.2.14	6.7
YDR530C	ATP adenylyltransferase	2.7.7.53	3.06
YOR303W	Carbamoyl-phosphate synthase subunit I	6.3.5.5	5.19
YJR109C	Carbamoyl-phosphate synthase subunit II	6.3.5.5	4.21
YKL216W	Dihydroorotate oxidase	1.3.3.1	−8.18
YMR120C	IMP cyclohydrolase	3.5.4.10	4.47
YOR128C	Phosphoribosylaminoimidazole carboxylase	4.1.1.21	9.33
YMR120C	Phosphoribosylaminoimidazolecarboxamide formyltransferase	2.1.2.3	4.47
YAR015W	Phosphoribosylaminoimidazole-succinocarboxamide synthase	6.3.2.6	3.74
YDR408C	Phosphoribosylglycinamide formyltransferase	2.1.2.2	2.81
YJR010W	Sulfate adenylyltransferase	2.7.7.4	5.23
YOR074C	Thymidylate synthase	2.1.1.45	−5.4
YIR032C	Ureidoglycolate hydrolase	3.5.3.19	6.09
YGL248W	3′,5′-cyclic-nucleotide phosphodiesterase	3.1.4.17	5.64
YML022W	Adenine phosphoribosyltransferase I	2.4.2.7	−4.28
YNL141W	Adenosine deaminase	3.5.4.4	−2.7
YBL039C	CTP synthase	6.3.4.2	−6.97
YBR252W	dUTP pyrophosphatase	3.6.1.23	−3.35
YDR454C	Guanylate kinase	2.7.4.8	−3.93
YAR073W	IMP dehydrogenase I	1.1.1.205	−4.86
YHR216W	IMP dehydrogenase II	1.1.1.205	−7.09
YML056C	IMP dehydrogenase III	1.1.1.205	−4.59
YML106W	Orotate phosphoribosyltransferase	2.4.2.10	−3.02
YMR271C	Orotate phosphoribosyltransferase II	2.4.2.10	8.75
YER070W	Ribonucleoside-diphosphate reductase	1.17.4.1	−5.18
YKL181W	Ribose-phosphate pyrophosphokinase	2.7.6.1	−3.19

tetrahydrofolate ligase, were down-regulated by DMSO treatment.

Effects of DMSO on the genes involved in cell structure maintenance

Organic solvents are believed to physically disrupt the microbial membrane [1], suggesting that exposure to DMSO will induce some genes involved in cell structure maintenance. Microarray analyses showed that 17 genes in this functional category were regulated by DMSO (Table 5). Most of them were involved in the functions of actin and tubulin. YFL037W and YML085C encode tubulin proteins required for mitosis and karyogamy. YOR239W, encoding an actin-filament binding protein, was down-regulated 2.65-fold; YIL034C, encoding an actin-capping protein, was down-regulated 2.58-fold. YDR212W, YJR064W, YJL014W, YDL143W, YJL111W, and YIL142W are involved in actin and tubulin folding; these genes were down-regulated two- to eight-fold by DMSO treatment. YIL138C, a gene that may play an important role in polarity establishment, was down-regulated 2.82-fold. YNL180C, encoding a member of the *rho* family, was up-regulated 2.85-fold; this gene may be involved in the control of actin cytoskeleton dynamics in response to extracellular signals. YPL269W was up-regulated 4.51-fold; it encodes a protein of the cell cortex required for the congression (nuclear migration) step of karyogamy, involved in the proper orientation of cytoplasmic microtubules.

Effects of DMSO on signal transduction systems

Nineteen genes involved in signal transduction were responsive to DMSO treatment (Table 6). Among them, 15 were not responsive to general environmental stress. Twelve were up-regulated 2.5- to 5.7-fold whereas seven genes were down-regulated 2.5- to 5.4-fold. YGL248W, encoding a 3′, 5′-cyclic-nucleotide phosphodiesterase, was up-regulated 5.64-fold. This gene functions specifically in controlling agonist-induced cAMP signaling [21]. YCR073C, encoding map kinase kinase kinase (MAPKKK), with strong similarity to Ssk2p, was up-regulated 3.76-fold. The gene is thought to be activated (along with Ssk2p) by the upstream factor Ssk1p and to phosphorylate the downstream MAP kinase kinase (MAPKK) Pbs2p in the high-osmolarity signal transduction pathway. YOL100W and YMR104C, encoding two serine/threonine protein kinases, were both up-regulated 3.51 fold.

Discussion

Recent advances in functional genomic technologies such as DNA microarrays provide a unique way to

Table 5 DMSO-responsive genes involved in cell structure maintenance. The genes responsive also to general environmentalstress are *underlined*

Gene name	Function description	Fold changes
YIL142W	Component of chaperonin-containing T-complex (TCP ring complex, TRiC); homologous to mouse CCT2	−2.58
YDL143W	Component of chaperonin-containing T-complex; (TCP ring complex, TRiC), homologous to mouse CCT4	−2.97
YOR239W	Protein that binds actin filaments	−2.65
YNL180C	Protein of unknown function, member of the rho family in the ras superfamily	2.85
YJR064W	Component of chaperonin-containing T-complex (TCP ring complex and TRiC); homologous to mammalian CCT family of chaperonin proteins	−4.11
YIL034C	Actin-capping protein, β-subunit	−2.58
YBR081C	Component of the nucleosomal histone acetyltransferase (Spt-Ada-Gcn5-Acetyltransferase or SAGA) complex	4.36
YJL111W	Component of Chaperonin-containing T-complex (TCP ring complex, TRiC), has similarity to Tcp1p	−2.62
YIL138C	Tropomyosin isoform 2, coiled-coil protein	−2.82
YDR212W	Component of chaperonin-containing T-complex (TCP ring complex, TRiC), homologous to mouse TCP1/CCT1	−4.26
YML085C	Tubulin α-1chain, required for mitosis and karyogamy	−2.72
YFL037W	Tubulin β-chain, required for mitosis and karyogamy	−8.64
YBR294W	Sulfate permease (high-affinity sulfate transporter), member of the sulfate permease family of membrane transporters	12.37
YJL014W	Component of chaperonin-containing T-complex (TCP ring complex, TRiC); homologous to mouse CCT3	−3.97
YPL269W	Protein of the cell cortex required for the congression (nuclear migration) step of karyogamy, involved in proper orientation of cytoplasmic microtubules	4.51
YBR106W	Membrane protein involved in inorganic phosphate transport	−3.41
YNL283C	Protein required for maintenance of cell wall integrity	−2.5

Table 6 DMSO-responsive genes involved in signal transduction. * The genes responsive also to general environmental stress are *underlinedl*

Gene name	Function description	Fold changes
YER120W	Type II integral ER membrane protein involved in the activation of INO1 eexpression	−5.41
YPL093W	Putative essential nucleolar GTP-binding protein, has similarity to *Halobacterium cutirubrum* GTP-binding protein (SP:P17103)	−4.11
YGR054W	Protein with a possible role in signal transduction, has significant similarity to C. elegans E04D5.1 protein	−3.45
YER118C	Osmosensor in the HOG1 MAP kinase, high-osmolarity signal transduction pathway, has an SH3 domain	−3.29
YGL115W	Protein involved in derepression of glucose-repressed genes, acts with Snf1p	−2.69
YBR164C	GTP-binding protein of the arf-sar family in the ras superfamily, possibly involved in signal transduction	−2.62
YNL283C	Protein required for maintenance of cell wall integrity, glucose-induced activation of plasma membrane H + -ATPase, and for the stress response	−2.5
YDR006C	Protein that when overexpressed suppresses mutations of cAMP-dependent protein kinase	2.59
YML016C	Protein serine/threonine phosphatase required for normal osmoregulation, member of the PPP family of protein phosphatases and related to PP1 phosphatases	3.02
YDL035C	G protein-coupled receptor coupled to Gpa2p, involved in the pathway of pseudohyphal differentiation in response to nutrient starvation	3.1
YJR152W	Allantoate and ureidosuccinate permease, member of the allantoate family of the major facilitator superfamily (MFS)	3.37
YOL100W	Serine/threonine protein kinase with similarity to mammalian 3-phosphoinositide-dependent protein kinase	3.5
YMR104C	Serine/threonine protein kinase with similarity to Ypk1p	3.54
YGR070W	GDP-GTP exchange factor for Rho1p	3.58
YCR073C	Map kinase kinase kinase (MAPKKK) with strong similarity to Ssk2p, participates in the high-osmolarity signal transduction pathway	3.76
YJR110W	Phosphatidylinositol 3-phosphate phosphatase with similarity to protein tyrosine phosphatases, has similarity to human myotubularin MTM1	3.88
YNL142W	Ammonia permease of low capacity and high affinity, involved generation of pseudohyphal differentiation in response to ammonium starvation	4.37
YGR043C	Protein of unknown function, may be involved in signal transduction, has strong similarity to Tal1p	5.56
YGL248W	3′,5′-Cyclic-nucleotide phosphodiesterase, low affinity	5.64

explore metabolic responses on a genomic scale. We investigated the global metabolic responses of *S. cerevisiae* to DMSO treatment using DNA microarray techniques [38]. Although DMSO had no effect on cell growth kinetics, the microarray results showed that cellular metabolism responded significantly to the presence of DMSO, with the expression of about 1,338 genes involved in many aspects of cellular metabolism being affected. Among these, 938 genes were not responsive to general environmental stress, such as heat shock and amino acid starvation [11]. The results from the yeast/DMSO model study indicate that organic

solvents might have rather global effects on cellular metabolism.

The lipid biosynthetic pathways were found to be the metabolic network most significantly down-regulated by DMSO treatment. This result partially confirms previous studies suggesting that organic solvents like DMSO exert their effects on cellular membranes by altering the lipid composition [24, 33]. However, the results suggested that alteration of the lipid composition of cellular membranes by organic solvent might not be limited to direct physical attack, such as dissolution of membrane lipids and dissociation of membrane components [4]. Instead, organic solvents might also have an indirect effect on membrane composition by altering the synthesis levels of various essential components of cellular membrane lipids. Recently, Bammert and Fostel studied the gene expression pattern of the ergosterol biosynthetic pathway in *S. cerevisiae* following treatment with azole or genetic alterations [6]. Their results showed that, although the biosynthesis of ergosterol was reduced by the drug inhibition treatment as well as by gene disruptions in *erg*2, *erg*5 and *erg*6, nine other genes involved in the ergosterol biosynthetic pathway from acyl-CoA responded with increased transcript levels. Among them, increased transcript levels of *erg*19 and erg3 were found in response to all azole inhibition experiments in the mutants [6]. Compared with azole drugs, which specifically target certain enzymes in the ergosterol pathway, DMSO treatment seems to have more global effects on lipid biosynthesis; about half of the genes in the ergosterol, sphingoglycolipid and fatty acid biosynthetic pathways were down-regulated.

Recently, Natarajan et al. conducted a genome-wide analysis of the gene expression profiles in response to histidine starvation imposed by 3-aminotriazole (3AT) in *S. cerevisiae* S288c [27]. They found that the synthesis of Gcn4p was induced by a lack of histidine, and Gcn4p further induced a much larger set of genes, encompassing 10% or more of the yeast genome. Profiling of a *gcn*4 strain and a constitutively induced mutant showed that Gcn4p is required for full induction by 3AT of at least 539 genes, termed Gcn4p targets, which included almost all the genes encoding amino acid precursors and genes in every amino acid biosynthetic pathway except that of cysteine. Through the Gcn4p-Gcn2p regulatory network, histidine starvation induced not only the pathway for histidine biosynthesis, but also those for all other amino acids. There is increasing evidence that Gcn4p is induced under conditions of starvation or stress besides amino acid deprivation. Results showing that the expression levels of the majority of the genes in the amino acid biosynthetic pathways were up-regulated by DMSO treatment suggests that a similar transcriptional regulation network might exist in response to DMSO treatment. However, microarray showed that the expression levels of the Gcn4p and Gcn2 genes were not affected by DMSO (data not shown), implying that the expression of amino acid biosynthetic genes in response to DMSO treatment might have a mechanism of regulation different than that of Gcn4p and Gcn2p.

In a recent study by Mirua et al. [23], mRNA differential display was employed to study the genes responsible for isooctane resistance in *S. cerevisiae* KK-211, which was isolated by the long-term bioprocess of stereoselective reduction in isooctane and shows extremely high tolerance to the solvent. On the differential display fingerprints, the expression of 14 genes was induced, while the expression of 16 genes was decreased in strain KK-211 cultivated with isooctane. Among them, only two genes were also found responsive to DMSO treatment, *JEN*1 and *KAP*123, which encode a pyruvate and lactate/H^+ symporter and a karyopherin involved in nuclear import of ribosomal proteins, respectively. In addition, *JEN*1 was up-regulated 13.25-fold, while *KAP*123 was down-regulated 2.94-fold. The results imply that yeast might have a variety of resistance mechanisms for different organic solvents.

The exposure of *S. cerevisiae* to mild stress conditions triggers a set of cell responses that presumably allow the cells to cope with a more severe stress of the same or of a different type [7]. The complex metabolic responses involve aspects of cell sensing, signal transduction, transcriptional and posttranslational control, protein-targeting to organelles, accumulation of protectants, and activity of repair functions [5]. The exact interpretation of the metabolic consequences caused by stress will allow the genes involved in stress tolerance to be identified and engineered strains with elevated tolerances to be developed. In one study, Chen and Piper [10] found that ubiquitin was induced by diverse stresses in yeast, probably as a result of the need for more extensive protein turnover by the ubiquitination system in stressed cells. By overexpressing the polyubiquitin gene (GUB) under a galactose-inducible promoter, the engineered yeast had slightly increased ethanol and osmostress tolerances, and tolerance of the amino acid analogue canavanine was markedly increased [10]. In the present study, YBR294W, encoding a sulfate permease (membrane transporter), was significantly up-regulated by DMSO treatment. Interestingly, the gene has previously been shown to be responsible for sulfite resistance in *S. cerevisiae* [9]. Although additional, independent experiments must be done to further confirm the biological significance inferred from the microarray data, our experiments provide the first data-set to explore metabolic responses to organic solvents at the genome level. The primary data analyses demonstrate that application of microarray technology allows better interpretation of the metabolic response, and the information obtained will be crucial for identification of genes responsible for organic-solvent tolerance and for construction of engineered yeast strains expressing these genes.

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