

## Cluster analysis and display of genome-wide expression profiles in dimethyl sulfoxide treatment

Yoshinori Murata, Yuko Momose<sup>a</sup>, Mika Hasegawa, Hitoshi Iwahashi\*,  
Yasuhiko Komatsu

*International Patent Organism Depositary, National Institute of Advanced Industrial Science Technology  
(AIST)*

*a) Research Institute of Biological Resources, National Institute of Advanced Industrial Science  
Technology (AIST)*

*\*E-mail: Iwahashi@aist.go.jp*

(Received October 12, 2001; accepted January 22, 2002; published online February 15, 2002)

### Abstract

We evaluated the effects of dimethyl sulfoxide (DMSO) on yeast cells by taking advantage of DNA microarrays and bioinformatics tools such as cluster analysis. There were 147 induced and 246 repressed genes in the expression profiles of 5,535 yeast genes by DNA microarray analysis. From the comparison of these expression profile changes in response to DMSO treatment with other publicly available expression data, the gene expression pattern of DMSO treatment resembled those of DTT and diamide. This suggests that DMSO causes damage similar to that caused by DTT and diamide. Further, DMSO inhibits protein folding and processing in the endoplasmic reticulum (ER), cell wall damage can result from the formation of improper disulfide bonds in the ER. The genes expressed during the DMSO treatment were also grouped in the cluster including the nonfermentable carbon sources, such as ethanol and galactose, during the stationary phase. This suggests that DMSO induces glucose depletion or starvation in yeast cells accompanied by new energy synthesis via an unknown metabolic pathway that utilizes DMSO as a carbon source.

**Key Words:** bioinformatics, dimethyl sulfoxide, DNA microarray, yeast

**Area of Interest:** Genome Wide Experimental Data Analysis

### 1. Introduction

Recently, the complete genomic sequences of many organisms have been published by the various genome sequencing projects [1][2][3][4]. Rapid advances of genome-scale sequencing have provided us with new information and driven the development of new analytical methods, such as DNA microarray analysis, to exploit this information. DNA microarrays, consisting of

thousands of individual cDNA sequences printed in a high-density array on a slide glass, provide a practical and economical tool for studying gene expression on a very large scale [5][6][7]. Large-scale expression profiling has emerged as a leading technology in the systematic analysis of cellular physiology [8]. Although the information obtained by DNA microarray analysis includes an immense number of data points for thousands or tens of thousands of genes; defining the role of each gene in these genomes will be a formidable task [9].

To extract meaningful information from the large amounts of data generated by DNA microarrays, there is an intense need for the development and application of bioinformatics tools, such as for the construction of a database in which all genes are given annotations and computational tools are available to recognize the transcriptional patterns of all genes [9][10]. Studies utilizing such bioinformatics tools to analyze the gene expression profiles under various conditions such as environmental changes [11][12], cell cycle stages [13], and sporulation [14] were recently reported. The budding yeast *Saccharomyces cerevisiae* has evolved autonomous mechanisms for adapting to drastic environmental changes. Yeasts regularly withstand fluctuations in the types and quantities of available nutrients, temperatures, and osmolarity as well as exposure to toxic chemicals, such as superoxide and H<sub>2</sub>O<sub>2</sub>. When these environmental conditions change abruptly, the cell rapidly adjusts its genomic expression program to maintain the optimal internal milieu. These genomic expression programs are unique for a variety of stresses. We compared the gene expression profile of DMSO treatments with those of some other stresses, such as heat shock, chemicals, and deleted nutrients, and examined the effects of dimethyl sulfoxide (DMSO) treatment as stress by using the tools of bioinformatics.

## 2. Material and Methods

### 2.1 Strain and culture conditions

*Saccharomyces cerevisiae* (S288C) was grown in YPD medium (1% Bacto Yeast Extract, 2% Polypeptone, 2% glucose) at 25°C (15). 10% DMSO, which represented the median growth inhibition constant (IC<sub>50</sub>), was added when the cell density reached 1.0 (*A*<sub>660</sub>), and after 2 h of incubation, the cells were harvested by centrifugation. Cultures treated with 0% and 10% DMSO were evaluated as control and sample, respectively.

### 2.2 Preparation of total RNA and mRNA

Total RNA was extracted by the hot-phenol method. Poly (A) +RNA was purified from total RNA with an Oligotex-dT30 mRNA purification kit (Takara Otsu, Shiga, Japan).

### 2.3 Preparation of labeled cDNA

Fluorescence-labeled cDNA probes were prepared from the mRNA pool by direct incorporation of fluorescent nucleotide analogues during the first-strand reverse transcriptase reaction. Each 20 µL of labeling solution consisted of 3 to 5 µg of poly (A)+RNA, 0.5 µg of oligo dT primer, 0.5 mM each of dATP, dCTP, and dGTP, 0.2 mM dTTP, 40 units of RNase inhibitor, 10 mM DTT, and 4 µL of 5x reaction buffer provided with SuperScript TM II (GIBCO BRL). For estimation of the induced genes, cDNA made from the poly (A)+ RNA of the control culture was

fluorescence-labeled with Cy3-UTP, and that of the DMSO-treated sample was labeled with Cy5-UTP (Amersham Pharmacia, Uppsala, Sweden). The solution was incubated at 70°C for 5 min and at 42°C for several minutes; then 200 U of SuperScript II Reverse Transcriptase (GIBCO BRL) was added, and reverse transcription was performed at 42°C for 2 h. Another 200 U of SuperScript II Reverse Transcriptase was added 1 h after the beginning of reverse transcription. The reverse transcription reaction was stopped by the addition of EDTA, and remaining RNA was dissolved by the addition of 0.5N NaOH for 1 h at 65°C. After neutralization with 1 M Tris-HCl, the Cy3- and Cy5-labeled cDNAs were mixed. The mixed cDNA probes were purified by using MICROCON YM-30 (amicon MILLIPORE) [15][16].

## 2.4 Hybridization and image scanning of the DNA microarrays

Competitive hybridizations were performed on a DNA microarray of the yeast *S. cerevisiae* (DNA Chip Research, Yokohama, Kanagawa, Japan) for 24 to 36 h at 65°C. After hybridization, the labeled microarrays were washed with x2 SSC, 0.1% SDS, x0.2 SSC, and 0.1% SDS, rinsed with x0.2 SSC and x0.05 SSC, and dried at room temperature. Subsequently, the labeled microarrays were scanned with a confocal laser ScanArray 4000 (GSI Lumonics, Billerica, MA, USA) system. The fluorescence intensities of the resulting image data were quantitated by using Genepix (version 3.0). The quantitated data were calculated and normalized by using GeneSpring version 4.07 (Silicon Genetics, Redwood City, CA, USA) [16].

To eliminate background noise, the median value of some genes (TE) included as negative controls was subtracted from the row values for each gene. The intensity of each gene was divided by its control channel value in each sample. When the control channel value was below 0.01, the data point was deleted. The 50th percentile of all measurements was used as a positive control for each sample; each measurement for each gene was divided by this synthetic positive control value, assuming that this was at least 0.01. The bottom tenth percentile was used as a test for correct background subtraction. This was never less than the negative value of the synthetic positive control. The fluorescence intensity of each spot on the images was subtracted from each background, and the ratios of intensity Cy5/Cy3 were calculated and normalized with a median value. These experiments were independently repeated six times. The genes with hybridization value ratios greater than 2.0 and less than 0.5 in at least five of the six experiments were considered to be, respectively, up-regulated and down-regulated by DMSO treatment. The ratio values used here are the average expression profiles from the six independent experiments with DMSO. All ratio values are log transformed (base 2) to represent inductions or repressions of identical magnitude as being numerically equal but with opposite signs.

## 2.5 Publicly available gene expression profile data

Gene expression data other than that for DMSO treatment were cited from <http://www-genome.stanford.edu/yeast-stress>. All data given for the DMSO treatment and the other publicly available expression data, such as that related to environmental stresses, alternative carbon sources, and stationary phase were organized into hierarchical clusters by GeneSpring ver 4.07.

## 2.6 Cluster analysis

Briefly, the clustering algorithm arranges experiments according to their similarity in expression profiles across all of the array experiments, such that treatments with similar expression patterns are clustered together. As shown in Figs. 2, 4, and 6, the data are graphically displayed so that each row of colored bars represents the variation in transcript levels for every gene in a given mRNA

sample, as detected on the array. The variations in abundance of transcripts for each gene are depicted by means of a color scale. The horizontal axis in the upper part of each figure indicates the gene's relative intensity on a continuous scale, red indicates overexpression to intensity + 6, a purple bar average expression, and blue indicates repression to intensity - 6. A dendrogram constructed during the clustering process depicts the relationships among experiments. Each bar crossing a set of branches forms a node of the intersecting branches. The lengths of the branches to the node represent the degree of similarity between experiments based on each expression profile. The experiments that display similar patterns of gene expression are grouped together on a contiguous branch of the dendrogram.

### 3. Results and Discussion

#### 3.1 Conditions for DMSO treatment

To determine the effects of DMSO in the budding yeast *S. cerevisiae* S288C we devised conditions that caused the inhibition of yeast cell growth after DMSO treatment. *S. cerevisiae* S288C, the strain used for DNA microarrays, was exposed to various concentrations of DMSO (data not shown). Since 10% DMSO was the half-maximal inhibitory concentration ( $IC_{50}$ ) for growth inhibition, this concentration was used for the assay of the genes induced by DMSO treatment (fig. 1 A and B). Also, the period of DMSO treatment was set at 2 h, the doubling time of yeast cells at 25°C [15]. All genes involved in the transcriptional response to DMSO treatments were observed by a whole-genome yeast microarray (Kuhara DNA chip). In the expression profiles of 5,535 genes by DMSO treatment, there were 147 and 246 genes with ratios of hybridization values greater than 2.0 and less than 0.5 estimated as the up-regulated and down-regulated genes, respectively. The genes involved in energy synthesis (aryl-alcohol dehydrogenase- related genes), metabolism, or cell rescue, defense, aging, and death, *etc.*, were induced in the up-regulated genes. In contrast, the down-regulated genes included those involved in energy synthesis (mitochondrial respiration- related genes), ion homeostasis, and protein synthesis.

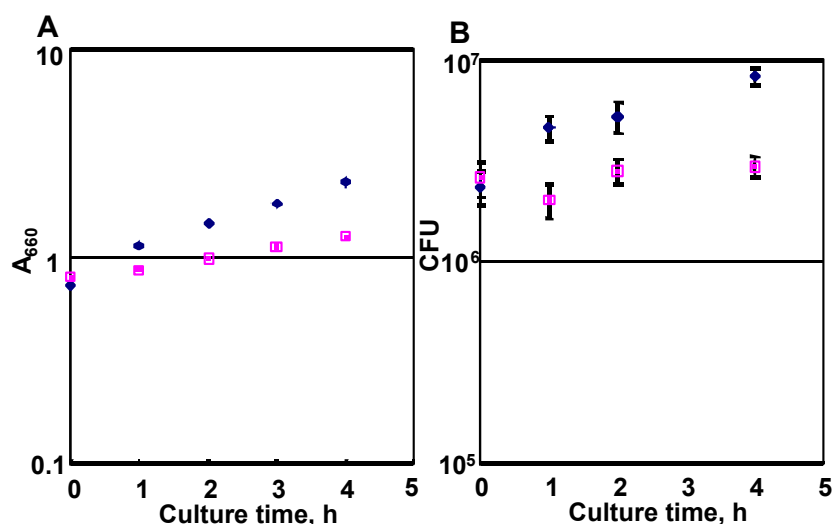


Fig. 1

(A) Effect of DMSO on the yeast growth. 10% DMSO indicated  $IC_{50}$  was added to the 2% polypeptone, 1% yeast extract, and 2% glucose medium. Yeast growth was monitored as the absorbance at 660 nm.

◆...Control, ■...10% DMSO

(B) Effect of DMSO on the viability of yeast cells. The colony forming units (CFU) were counted on an YPD agar plate. ♦..Control, ■..10% DMSO

### 3.2 Cluster analysis

We applied cluster analysis to an aggregation of data from various experiments with *S. cerevisiae* that examined the effect on gene expression of environmental changes, alternative carbon sources, stationary phase, and DMSO treatment (figs. 2, 4, and 6). These expressed genes have been functionally annotated in the Munich Information Center for Protein Sequences (MIPS; <http://www.mips.biochem.mpg.de/>) and the *Saccharomyces* Genome Database [17][18]. Although the gene expression images in figs. 2, 4, and 6 indicated the characteristic patterns for various conditions, the genes co-expressed across the sampled conditions are involved in common cellular processes. We tried to examine the characteristics of DMSO as an inducer of stress by the gene expression pattern and the co-expressed gene profiles across the various conditions. The detailed conditions for the environmental changes, stationary phase, and alternative carbon sources are attributed to Gasch A.P. *et al.* [12] and <http://www-genome.stanford.edu/yeast-stress>.

### 3.3 DMSO caused damage similar to that by DTT and diamide

The data for transcriptional genes responding to DMSO were compared with the data for environmental changes according to cluster analysis (fig. 2).

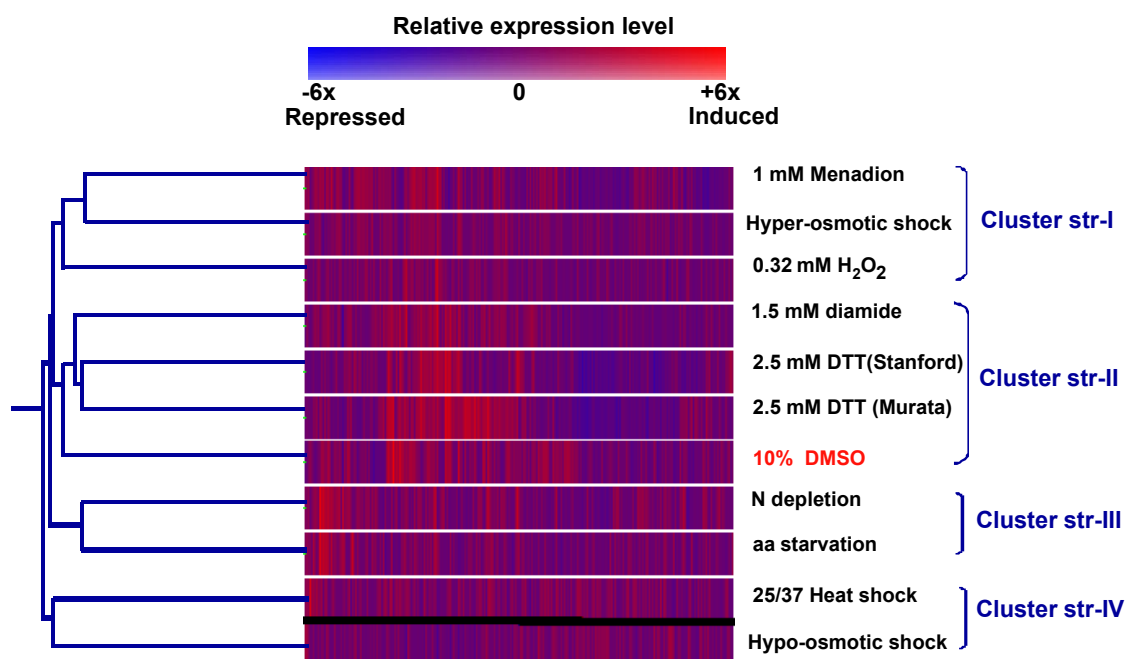


Fig. 2. The genes expressed by DMSO treatment were compared with the genes expressed in response to environmental stresses that were cited at <http://www-genome.stanford.edu/yeast-stress>. The samples exposed to environmental stresses were treated with each concentration of the following for 2 h; 0.3 mM H<sub>2</sub>O<sub>2</sub>, 1 mM menadione, 0M/1 M sorbitol (hyper-osmotic shock), 2.5 mM DTT, and 10% DMSO. Then, they were treated with 1.5 mM diamide for 90 min. Hypo-osmotic shock (1M/0M sorbitol) was applied as a transition from YPD supplemented with 1M sorbitol to YPD. Heat shock was applied as a transition from 25°C to 37°C for 1 h. The samples starved for nitrogen or amino acids were cultured in complete minimal medium (SCD) with limiting concentrations of ammonium sulfate or lacking amino acids for 2 h, respectively. DTT was attempted as a positive control for reappearance of the Stanford experimental

conditions (DTT Murata). The entire set of yeast genes identified by our analysis (5,535) was clustered with respect to the expression patterns of these genes in those experiments that followed the response of wild-type yeast to the environmental changes.

In these conditions, DTT was attempted as a positive control for recreation of the Stanford experimental conditions (DTT Murata). The gene expression profile for DMSO treatment was grouped in the cluster consisting of responses to DTT (Stanford and Murata) and diamide (fig. 2. cluster str-II). It is proposed that DTT inhibits protein folding in the endoplasmic reticulum (ER), by reducing the disulfide bonds in the protein structure [19]. Diamide, like DTT, induced genes involved in protein secretion and processing in the ER [12][20]. The transcriptional profile for the DMSO treatment showed induction of the ER organizational- and membrane- related genes, and the protein disulfide isomerase (PDI) catalyzed formation of disulfide bonds in protein folding (fig. 3A) [21].

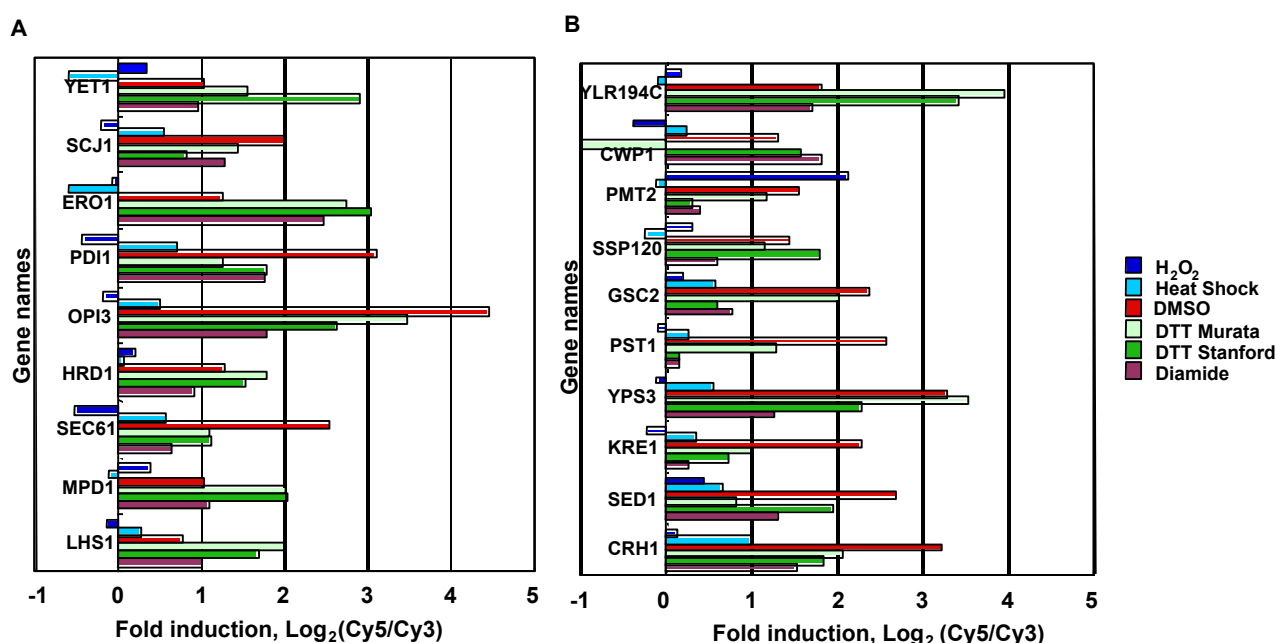


Fig. 3

(A) The genes involved in protein processing and folding in the ER. The genes co-expressed in DTT (Stanford and Murata), diamide, and DMSO treatment. The conditions of heat shock and H<sub>2</sub>O<sub>2</sub> were contrasted with DTT, diamide, and DMSO. YET1; yeast ER 25 kDa transmembrane protein, SCJ1; dnaJ homolog, ERO1; protein involved in protein disulfide bond formation in the ER, PDI1; protein disulfide isomerase, OPI3; methylene-fatty-acyl-phospholipid synthase, HRD1; protein required to degrade misfolded ER luminal and integral membrane proteins, SEC61; membrane component of ER protein translocation apparatus, MPD1; disulfide isomerase related protein, LHS1; Hsp70 family of molecular chaperones that localizes to the lumen of the ER. The fold values are log transformed (base 2) to represent indications (+) or repressions (-).

(B) The genes involved in cell wall biosynthesis. The genes co-expressed in DTT (Stanford and Murata), diamide and DMSO treatment. The conditions of heat shock and H<sub>2</sub>O<sub>2</sub> were contrasted with DTT, diamide and DMSO. YLR194C; hypothetical protein, CWP1; cell wall protein, PMT2; protein O-D-mannosyltransferase, SSP120; secretory protein, GSC2; catalytic component of 1,3-beta-D-glucan synthase, PST1; strong similarity to SPS2 protein, YPS3; GPI-anchored aspartyl protease 3, KRE1; cell wall beta-glucan assembly, SED1; abundant cell surface glycoprotein, CRH1; family of putative glycosidases might exert a common role in cell wall organization. The fold values are log transformed (base 2) to represent indications (+) or repressions (-).

It was suggested that DMSO inhibited protein secretion and processing in the ER. The genes involved in cell wall synthesis were induced by both DTT and diamide treatment [22]. In the DMSO treatment, the genes related to cell wall biosynthesis were also induced (fig. 3B). It was suggested that DMSO caused cell wall damage directly by the improper unfolding of the disulfide protein in the ER or cell wall.

### 3.4 DMSO caused glucose deletion or starvation such as in stationary phase

The transcriptional profiles for the DMSO treatment were compared with the gene expression profiles of cells grown with various carbon sources, ethanol, galactose, glucose, sucrose, and mannose. The gene expression profile of DMSO was located in cluster car-I and included ethanol and galactose (fig. 4).

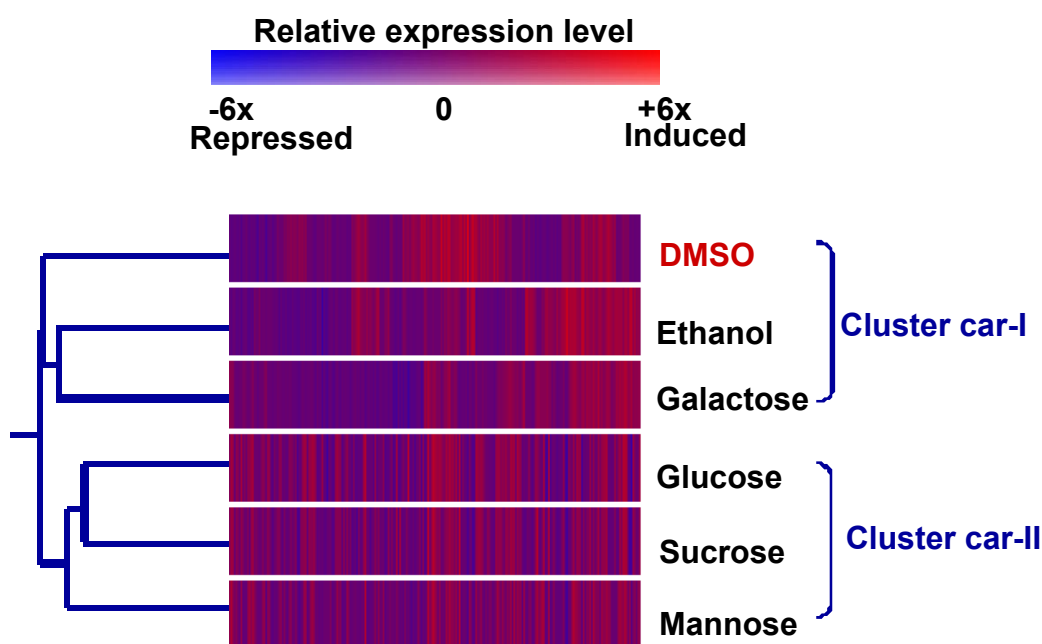


Fig. 4

Cluster image showing transcriptional profiles in response to steady-state growth on alternative carbon sources which were cited at <http://www-genome.stanford.edu/yeast-stress> and DMSO treatment. The samples other than DMSO were cultured in YP supplemented with 2% ethanol, galactose, glucose, sucrose, or mannose as carbon sources. The entire set of yeast genes identified by these analyses (5,535) was clustered on the basis of their expression patterns in those experiments that followed the response of wild-type yeast to alternative carbon sources.

The co-expressed genes for ethanol, galactose, and DMSO treatments were depicted in figs. 5A and B. The induced genes for ethanol, galactose, and DMSO treatment are shown in fig. 5A and the repressed genes are shown in fig. 5B. Ethanol and galactose are nonfermentable carbon sources. When the fermentable glucose is exhausted or depleted, the yeast cells turn to another nutrient, for example galactose or ethanol, as a nonfermentable carbon source for aerobic growth [23][24]. This ability to adopt nonfermentable growth is referred to as the diauxic shift [25][26]. For these reasons, it was considered that the DMSO treatment either caused glucose depletion and glucose starvation in the cells or that DMSO was utilized as a carbon source by the yeast cells, even under the conditions where the glucose contents in the YPD medium had remained at approximately 14 g

$L^{-1}$  after the DMSO treatment. Glucose starvation generally makes the yeast cells arrest in G1 or enter the stationary phase [27].

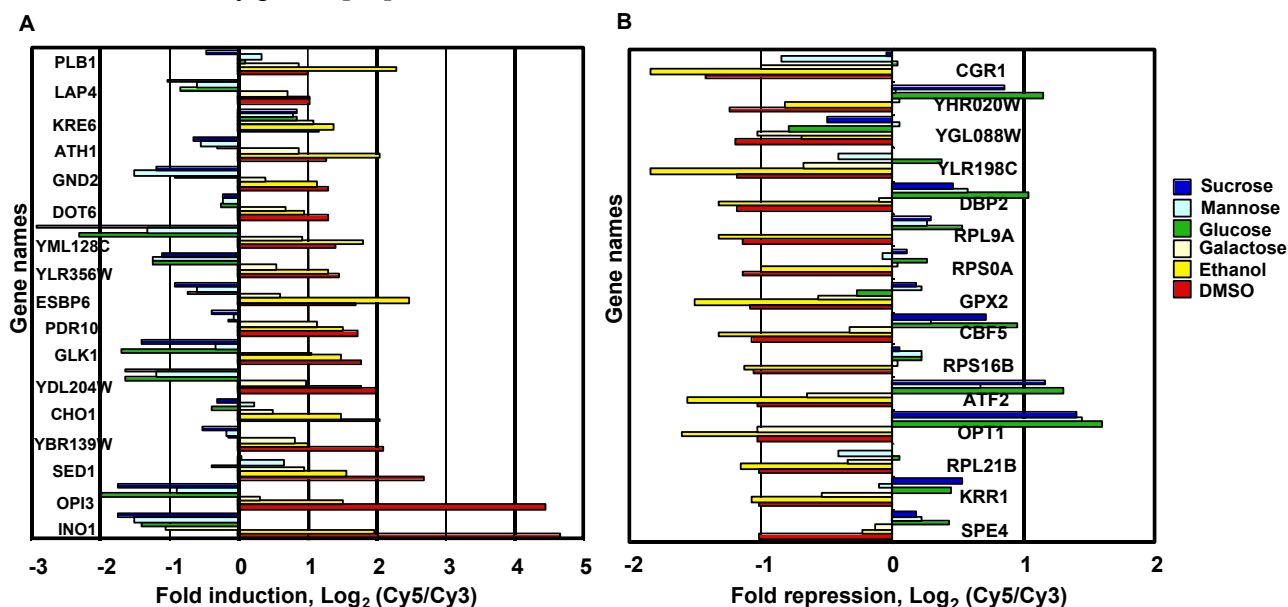


Fig. 5

(A) The genes induced in ethanol, galactose, and DMSO treatment. The conditions with glucose, sucrose, and mannose were contrasted with ethanol, galactose, and DMSO. PLB1; phospholipase B, LAP4; vacuolar aminopeptidase ysc1, KRE6; potential beta-glucan synthase, ATH1; acid trehalase, GND2; 6-phosphogluconate dehydrogenase, DOT6; nuclear protein with Myb domain involved in telomeric silencing, YML128C; meiotic sister-chromatid recombination, YLR356W; hypothetical ORF, ESBP6; putative monocarboxylate permease, PDR10; putative ABC transporter highly similar to Pdr5p, GLK1; glucokinase, YDL204W; hypothetical ORF, CHO1; phosphatidylserine synthase, YBR139W; probable serine-type carboxypeptidase, SED1; putative cell surface glycoprotein, OPI3; methylene-fatty-acyl-phospholipid synthase, INO1; L-myo-inositol-1-phosphate synthase. The fold values are log transformed (base 2) to represent indications (+) or repressions (-).

(B) The genes repressed in ethanol, galactose, and DMSO treatment. The conditions with glucose, sucrose, and mannose were contrasted with ethanol, galactose, and DMSO. CGR1; coiled-coil protein, YHR020W; aminoacyl tRNA-synthetase, YGL088W; hypothetical ORF, YLR198C; protein required for cell viability, DBP2; ATP-dependent RNA helicase of DEAD box family, RPL9A; ribosomal protein L9A, RPS0A; ribosomal protein S0A, GPX2; probable glutathione peroxidase, CBF5; major low affinity 55 kDa centromere, RPS16B; ribosomal protein S16B, ATF2; alcohol acetyltransferase, OPT1; oligopeptide transporter, RPL21B; ribosomal protein L21B, KRR1; cell division and spore germination, SPE4; spermine synthase. The fold values are log transformed (base 2) to represent indications (+) or repressions (-).

Thus, the gene expression pattern of the DMSO treatment was compared with those expression data for the various culture incubation periods from 0 h to 28 days. DMSO treatment responses were grouped in both of the clusters, sp-I containing the gene profiles at 12 h to 3 days and sp-II containing the gene profiles at 5 to 28 days of incubation, respectively (fig. 6).



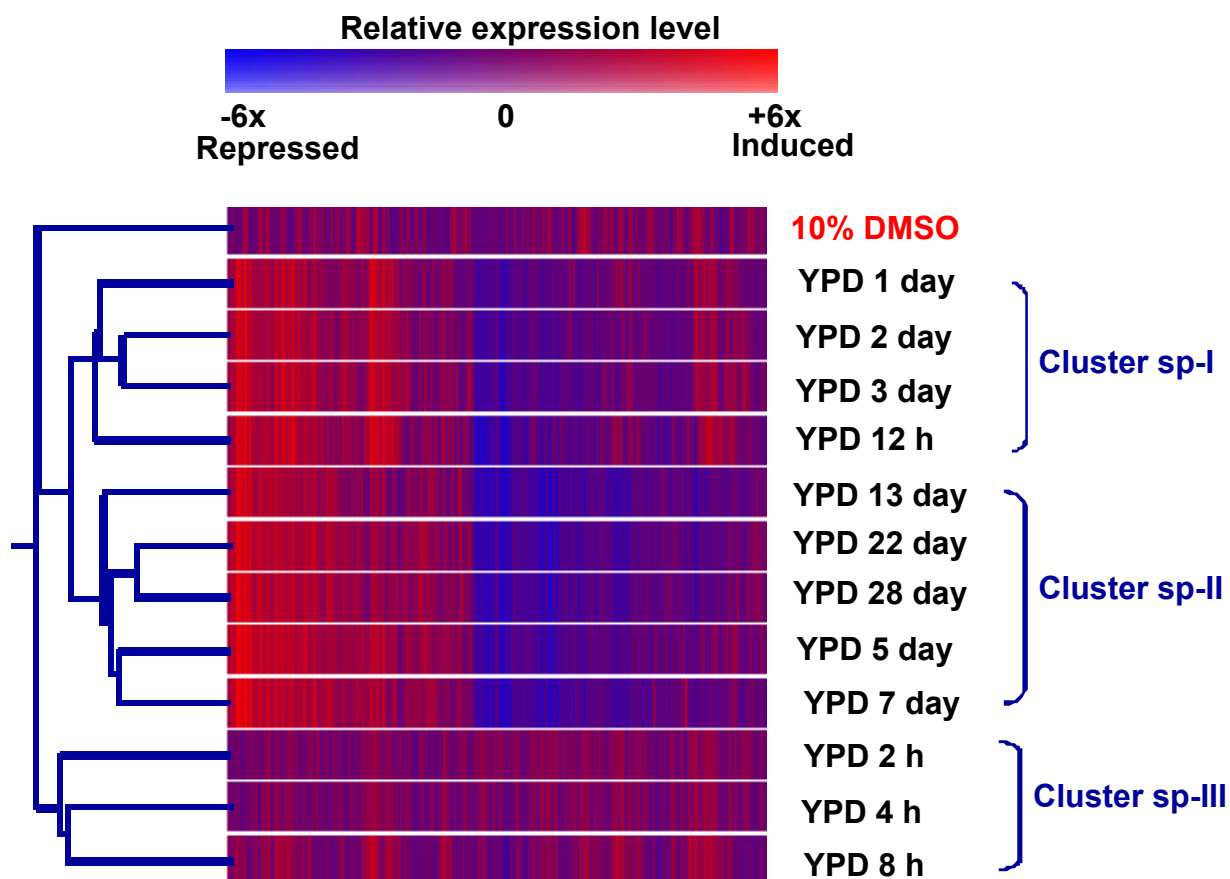


Fig. 6

The genes expressed in response to DMSO treatment were compared with the genes expressed at each time point of the YPD growth that were cited at <http://www-genome.stanford.edu/yeast-stress>. The samples other than DMSO were grown to  $A_{600}=0.3$  in YPD medium at 25°C and recovered at 2, 4, 8, and 12 h, 1, 2, 3, 5, 7, 13, 22, and 28 days. The entire set of yeast genes identified by these analyses (5,535) was clustered on the basis of their expression patterns in those experiments that followed the response of wild-type yeast to each time point in the culture changes.

These results indicated that the gene expression in response to the DMSO treatment was more similar to the transcriptional profiles at the stationary phase (12 h to 3 days and 5 to 28 days) than at the exponential phase of growth (2, 4, and 8 h). It was suggested that the DMSO treatment induced a culture state similar to the stationary phase, because the culture at 2 days had already entered the stationary phase [28]. Some of the co-induced genes in the stationary phase are depicted in fig. 7. The genes involved in cell wall and cell membrane biosynthesis were mainly induced in the stationary phase and by DMSO treatment. The high induction of SNZ1 also was characteristic to the DMSO treatment and stationary phase (fig. 7). SNZ1 is a member of a highly conserved gene family and is induced in the stationary phase or under nutrient limitation [29][30].

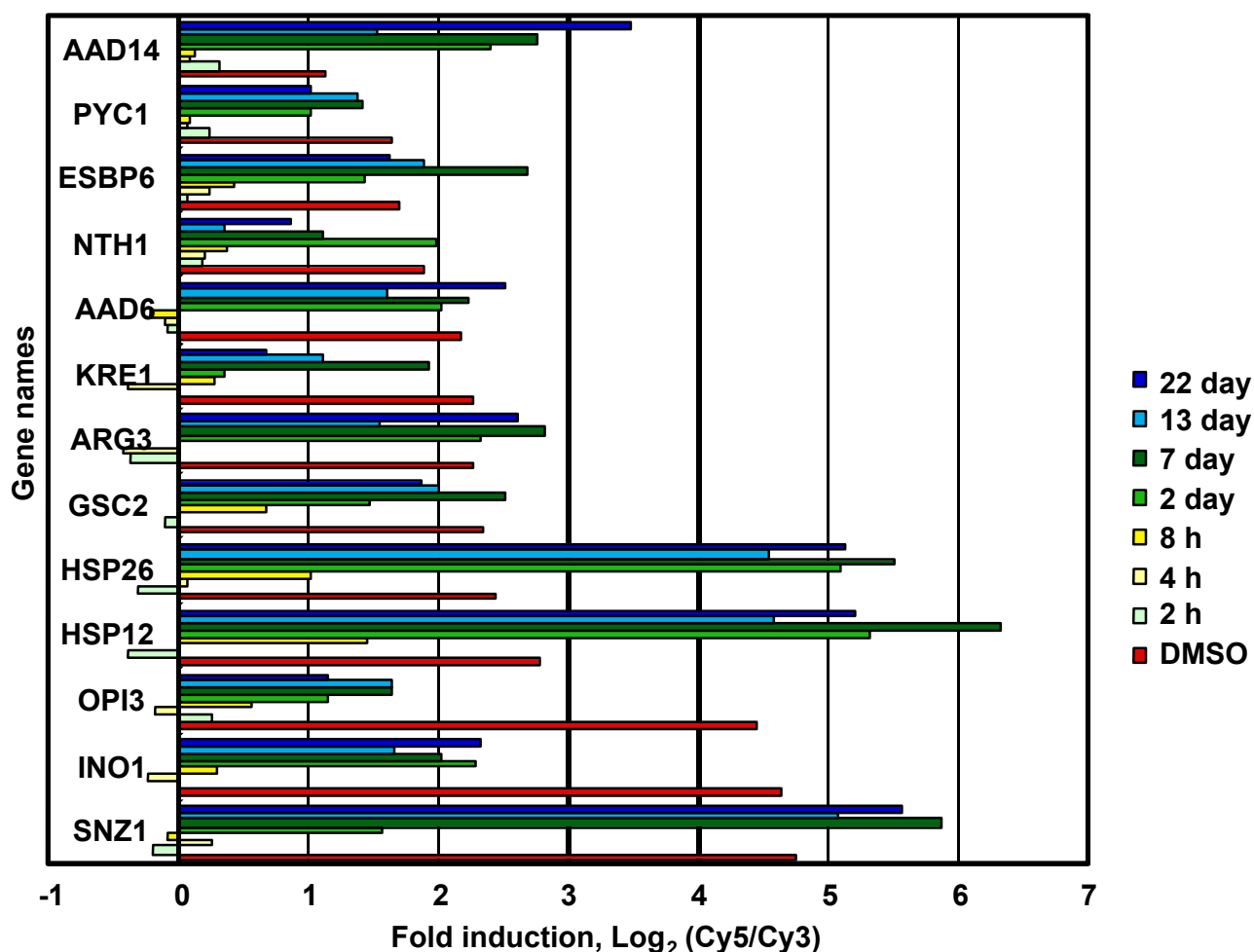


Fig. 7

The genes induced in stationary phase (2, 7, 13, and 22 days) and DMSO treatment. The conditions of co-expressed genes in exponential phase (2, 4, and 8 h) were contrasted with stationary phase. AAD14; hypothetical aryl-alcohol dehydrogenase, PYC1; pyruvate carboxylase, ESBP6; putative monocarboxylate permease, NTH1; neutral trehalase, AAD6; hypothetical aryl-alcohol dehydrogenase, KRE1; cell wall beta-glucan assembly, ARG3; ornithine carbamoyltransferase, GSC2; catalytic component of 1,3-beta-D-glucan synthase, HSP26; heat shock protein 26, HSP12; 12 kDa heat shock protein, OPI3; methylene-fatty-acyl-phospholipid synthase, INO1; L-myo-inositol-1-phosphate synthase, SNZ1; stationary phase-induced gene family. The fold values are log transformed (base 2) to represent indications (+) or repressions (-).

The glucose contained in YPD started to be consumed with the rise in cell density at  $A_{600}$  (from the cell density at 13.5 h), and was exhausted completely at 18.5 h, in the stationary phase [19][31]. Under DMSO treatment, NTH1, which mediated the hydrolysis reaction from trehalose to glucose, was induced (Fig. 7), and GSY1 and GLC3, which mediated the glycogen branching reaction, were repressed (data not shown). These results agreed with the suggestion that the DMSO treatment induced glucose depletion and the stationary phase (fig. 4 cluster car-I and fig. 6). Although mRNAs involved in respiratory metabolism were up-regulated for adaptive responses to the transition from fermentable to nonfermentable growth in the stationary phase [32], in the case of DMSO treatment, the genes involved in energy synthesis, such as in respiration and mitochondrial organization were repressed (fig. 8A).

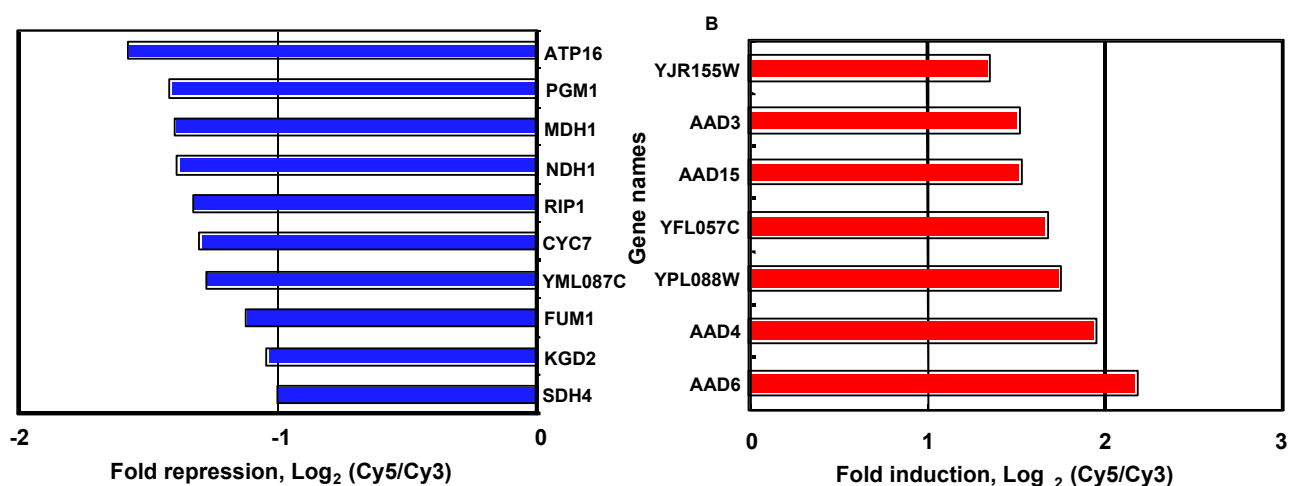


Fig. 8

(A) The genes involved in respiration and mitochondrial organization repressed by DMSO treatments.

ATP16; ATP synthase delta subunit, PGM1; phosphoglycerate mutase, MDH1; mitochondrial malate dehydrogenase, NDH1; mitochondrial cytosolically directed NADH dehydrogenase, RIP1; Rieske iron-sulfur protein of the mitochondrial cytochrome *bc1* complex, CYC7; iso-2-cytochrome *c*, YML087C; similarity to cytochrome-*b5*- and nitrate reductases, FUM1; mitochondrial and cytoplasmic fumarase, KGD2; dihydrolipoyl transsuccinylase component of alpha-ketoglutarate dehydrogenase complex in mitochondria, SDH4; succinate dehydrogenase membrane anchor subunit. The fold values are log transformed (base 2) to represent repressions (-).

(B) The genes had high similarity with aryl-alcohol dehydrogenase induced by DMSO treatments.

YJR155W; strong similarity to aryl-alcohol dehydrogenase, AAD3; hypothetical aryl-alcohol dehydrogenase, AAD15; putative aryl alcohol dehydrogenase, YFL057C; strong similarity to aryl-alcohol dehydrogenases, YPL088W; similarity to aryl-alcohol dehydrogenases, AAD4; strong similarity to aryl-alcohol dehydrogenase, AAD6; strong similarity to aryl-alcohol dehydrogenase. The fold values are log transformed (base 2) to represent indications (+).

Thus, it is possible that yeast cells had a different energy generation pathway with in the stationary phase during glucose depletion when the yeast cells were treated with DMSO. The genes having a high similarity to the aryl-alcohol dehydrogenase (AAD) gene of the lignin-degrading fungus *P. chrysosporium* [33][34] were induced by the DMSO treatment (fig. 8B). Although AAD is induced in yeast in response to oxidative stress by chemicals such as diamide, its obvious function in yeast is still unknown in detail [35]. Because the genes having a high degree of similarity with AAD are grouped in the functional category for energy synthesis in MIPS, it is considered that such genes might be related to energy synthesis in DMSO treatments. A wide variety of organisms, including prokaryotes and eucaryotes, are capable of reducing DMSO to dimethyl sulfide (DMS) [36][37][38]. It was considered that a new metabolic pathway was induced to allow the utilization of DMSO as a carbon source.

#### 4. Conclusions

We tried to estimate the effects of DMSO on yeast cells, *S. cerevisiae*, by using DNA microarrays and bioinformatics tools for cluster analysis.

Because the gene transcriptional pattern for DMSO resembled those for DTT and diamide (fig. 2 cluster str-II), it was suggested that DMSO caused damage similar to that by DTT and diamide.

The results reported here show that DMSO inhibited protein folding and processing in the ER following the oxidation modification of protein sulfhydryl and ultimately caused cell wall damage due to the formation of improper disulfide bonds, similarly as did DTT and diamide (fig. 3A and 3B).

The gene expression profiles obtained by DMSO treatment were grouped in the cluster including the nonfermentable carbon sources, such as ethanol and galactose, and the stationary phase (fig. 4 cluster car-I, fig. 6). This indicates that DMSO caused glucose depletion or starvation in yeast cells, but we could not detect the induction of genes related to respiration and mitochondrial organization (fig. 8A). Thus, we can consider that a different means of energy synthesis with those conditions and new metabolic pathways were induced by DMSO treatment to permit yeast cells to utilize DMSO as a carbon source.

## References

- [1] K. Mayer, O. White, M. Bevan, K. Lemcke, T. H. Creasy, C. Bielke, B. Haas, D. Haase, R. Maiti, S. Rudd, J. Peterson, H. Schoof, D. Frishman, B. Morgenstern, P. Zaccaria, M. Ermolaeva, M. Pertea, J. Quackenbush, N. Volfovsky, D. Wu, et al., *Nature*, **408**, 796-815 (2000).
- [2] C. Jacq, J. Alt-Morbe, B. Andre, W. Arnold, A. Bahr, J. P. Ballesta, M. Bargues, L. Baron, A. Becker, N. Biteau, H. Blocker, C. Blugeon, J. Boskovic, P. Brandt, M. Bruckner, M. J. Buitrago, F. Coster, T. Delaveau, F. del Rey, B. Dujon, L. G. Eide, J. M. Garcia-Cantalejo, A. Goffeau, A. Gomez-Peris, P. Zaccaria, et al., *Nature*, **387**, 75-78. (1997).
- [3] M. Johnston, L. Hillier, L. Riles, K. Albermann, B. Andre, W. Ansorge, V. Benes, M. Bruckner, H. Delius, E. Dubois, A. Dusterhoft, K. D. Entian, M. Floeth, A. Goffeau, U. Hebling, K. Heumann, D. Heuss-Neitzel, H. Hilbert, F. Hilger, K. Kleine, P. Kotter, E. J. Louis, F. Messenguy, H. W. Mewes, J. D. Hoheisel, et al., *Nature*, **387**, 87-90 (1997).
- [4] F. Kunst, N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Connerton, A. Danchin, et al., *Nature*, **390**, 249-256 (1997).
- [5] J. DeRisi, L. Penland, P. O. Brown, M. L. Bittner, P. S. Meltzer, M. Ray, Y. Chen, Y. A. Su, and J. M. Trent, *Nat. Genet.*, **14**, 457-460 (1996).
- [6] D. A. Lashkari, J. L. DeRisi, J. H. McCusker, A. F. Namath, C. Gentile, S. Y. Hwang, P. O. Brown, and R. W. Davis, *Proc. Natl. Acad. Sci. U S A*, **94**, 13057-13062 (1997).
- [7] D. A. Lashkari, J. H. McCusker, and R. W. Davis, *Proc. Natl. Acad. Sci. U S A*, **94**, 8945-8947 (1997).
- [8] R. A. Young, *Cell*, **102**, 9-15 (2000).
- [9] M. B. Eisen, P. T. Spellman, P. O. Brown, and D. Botstein, *Proc. Natl. Acad. Sci. U S A*, **95**, 14863-14868 (1998).
- [10] C. S. Brown, P. C. Goodwin, and P. K. Sorger, *Proc. Natl. Acad. Sci. U S A*, **98**, 8944-8949 (2001).
- [11] H. C. Causton, B. Ren, S. S. Koh, C. T. Harbison, E. Kanin, E. G. Jennings, T. I. Lee, H. L. True, E. S. Lander, and R. A. Young, *Mol. Biol. Cell*, **12**, 323-337 (2001).
- [12] A. P. Gasch, P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown, *Mol. Biol. Cell*, **11**, 4241-4257 (2000).
- [13] P. T. Spellman, G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders, M. B. Eisen, P. O.

- Brown, D. Botstein, and B. Futcher, *Mol. Biol. Cell*, **9**, 3273-3297 (1998).
- [14] S. Chu, J. DeRisi, M. Eisen, J. Mulholland, D. Botstein, P. O. Brown, and I. Herskowitz, *Science*, **282**, 699-705 (1998).
- [15] Y. Momose, E. Kitagawa, and H. Iwahashi, *Chem-Bio Informatics J.*, **1**, 41-50 (2001).
- [16] Y. Momose and H. Iwahashi, *Environ. Toxicol. Chem.*, **20**, 2353-2360 (2001).
- [17] C. A. Ball, K. Dolinski, S. S. Dwight, M. A. Harris, L. Issel-Tarver, A. Kasarskis, C. R. Scafe, G. Sherlock, G. Binkley, H. Jin, M. Kaloper, S. D. Orr, M. Schroeder, S. Weng, Y. Zhu, D. Botstein, and J. M. Cherry, *Nucleic Acids Res.*, **28**, 77-80 (2000).
- [18] J. M. Cherry, C. Ball, S. Weng, G. Juvik, R. Schmidt, C. Adler, B. Dunn, S. Dwight, L. Riles, R. K. Mortimer, and D. Botstein, *Nature*, **387**, 67-73 (1997).
- [19] K. J. Travers, C. K. Patil, L. Wodicka, D. J. Lockhart, J. S. Weissman, and P. Walter, *Cell*, **101**, 249-258 (2000).
- [20] J. S. Cox, C. E. Shamu, and P. Walter, *Cell*, **73**, 1197-1206 (1993).
- [21] P. Norgaard, V. Westphal, C. Tachibana, L. Alsoe, B. Holst, and J. R. Winther, *J. Cell. Biol.*, **152**, 553-562 (2001).
- [22] C. Cappellaro, V. Morsa, and W. Tanner, *J. Bacteriol.*, **180**, 5030-5037 (1998).
- [23] E. L. Braun, E. K. Fuge, P. A. Padilla, and M. Werner-Washburne, *J. Bacteriol.*, **178**, 6865-6872 (1996).
- [24] M. Johnston, *Trends Genet.*, **15**, 29-33 (1999).
- [25] J. L. DeRisi, V. R. Iyer, and P. O. Brown, *Science*, **278**, 680-686 (1997).
- [26] P. J. Cullen and G. F. Sprague, Jr., *Proc. Natl. Acad. Sci. U S A*, **97**, 13619-13624 (2000).
- [27] S. C. Howard, Y. W. Chang, Y. V. Budovskaya, and P. K. Herman, *Genetics*, **159**, 77-89 (2001).
- [28] E. K. Fuge, L. Braun, and M. Werner-Washburne, *J. Bacteriol.*, **176**, 5802-5813 (1994).
- [29] E. L. Braun, E. K. Fuge, P. A. Padilla and M. Werner-Washburne, *J. Bacteriol.*, **178**, 6865-6872 (1996).
- [30] P. A. Padilla, E. K. Fuge, M. E. Crawford, A. Errett and M. Werner-Washburne, *J. Bacteriol.*, **180**, 5718-5726 (1998).
- [31] S. Puig and J. E. Perez-Ortin, *Yeast* **16**, 139-148 (2000).
- [32] K. M. Kuhn, J. L. DeRisi, P. O. Brown, and P. Sarnow, *Mol. Cell. Biol.*, **21**, 916-927 (2001).
- [33] A. Muheim, R. Waldner, D. Sanglard, J. Reiser, H. E. Schoemaker, and M. S. Leisola, *Eur. J. Biochem.*, **195**, 369-375 (1991).
- [34] J. Reiser, A. Muheim, M. Hardegger, G. Frank, and A. Fiechter, *J. Biol. Chem.*, **269**, 28152-28159 (1994).
- [35] D. Delneri, D. C. Gardner, and S. G. Oliver, *Genetics*, **153**, 1591-1600 (1999).
- [36] P. T. Bilous and J. H. Weiner, *J. Bacteriol.*, **162**, 1151-1155 (1985).
- [37] J. Hansen, *Appl. Environ. Microbiol.*, **65**, 3915-3919 (1999).
- [38] J. Moskovitz, B. S. Berlett, J. M. Poston, and E. R. Stadtman, *Proc. Natl. Acad. Sci. U S A*, **94**, 9585-9589 (1997).

## DMSO 処理におけるゲノム発現プロファイルのクラスター解析

村田善則 百瀬祐子<sup>a</sup> 長谷川実加 岩橋均<sup>\*</sup> 小松泰彦

独立行政法人 産業技術総合研究所 特許生物寄託センター

a. 独立行政法人 産業技術総合研究所 生物遺伝子資源

E-mail: iwahashi@aist.go.jp

### 要旨

バイオインフォマティクス・ツールを用いて酵母における DMSO の影響を調べた。酵母 DNA マイクロアレイによる全遺伝子発現プロファイルと各ストレスや培養条件の違いによる遺伝子発現データをクラスター解析により比較した。酵母 DNA マイクロアレイを用いて DMSO により発現した遺伝子 5,535 個のうち、発現が上昇した遺伝子は、147 個、発現が減少した遺伝子は、246 個であった。DMSO の遺伝子発現パターンを各種ストレスと比較したところ、DMSO は DTT と diamide の遺伝子発現と同じクラスターに分類された。また、DMSO 処理により細胞壁の生合成に関わる遺伝子発現が誘導された。これらの結果から、DMSO は DTT や diamide と同様に、ER におけるタンパク質のフォールディングとプロセッシングを阻害し、不適切なジスルフィド結合の形成の結果として細胞壁にダメージを与えることが示唆された。また、DMSO はエタノールやガラクトースを炭素源とした場合と培養の定常期における遺伝子発現を含む同じクラスターに分類されたことから、DMSO 処理により、グルコースの欠乏あるいは枯渇を促されることが考えられた。DMSO 処理によりミトコンドリアを介する呼吸系が抑制されていたことから、DMSO を炭素源として利用する新たなエネルギー合成系や代謝経路の存在が示唆された。

**キーワード：**バイオインフォマティクス、dimethyl sulfoxide (DMSO)、DNA マイクロアレイ、酵母

**領域区分：**ゲノムワイドな実験データの解析