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Comparison of Genome-wide Expression Patterns in Response to Heavy Metal Treatment in *Saccharomyces cerevisiae* 1) Cadmium and mercury

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Abstract

cDNA microarrays are very convenient tools that can be used to understand changes in genome-wide patterns of gene expression. The yeast DNA microarray contains 5880 ORF cDNA probes, which is almost whole genome, on a glass slide. We analyzed genome-wide transcript profiles following exposure to Cadmium (Cd) or Mercury (Hg) with the yeast DNA microarray, and while 22 genes were induced by both heavy metals, there were many discrepancies in those profiles. From these results, we concluded that yeast microarrays are valuable for bioassay of environmental agents.

Key Words: microarray, yeast, heavy metals, Cd, Hg

Area of interest: Genome Wide Experimental Data Analyses

Introduction

More than hundreds thousands of chemicals may exist and causes environmental stress to our natural world. New high throughput methods are urgently required to rapidly detect those dangerous chemicals so that some defense can be made against them. At present, DNA microarray technology enables genome-wide detection of cell response at the transcriptional level. Especially for yeast, almost the entire genomic cDNA probes can be spotted onto one glass slide enabling us to detect the entire response with only one DNA chip. Therefore we are planning to make new microarray-based bioassay systems to detect environmental chemicals for risk assessment. In

addition, although we have a stress indicator system that is monitored by the induction of β-galactosidase activities with the β-galactosidase structural gene located down stream of the *HSP104* promotor (1), we need new promoters responsive to new various potential bioreactive agents, and a microarray system is suitable to screen those promoters.

To establish a yeast DNA microarray for our purposes, we had analyzed the changes in gene expression underlying the yeast stress response to cadmium by a microarray of total mRNA. Cadmium is known to cause oxidative stress by changing intracellular glutathione levels (2-4). We have already reported that several common stress responsive genes, such as *HSP26*, *DDR48*, *HSP12*, and *GRE2*, were up-regulated more than 4-fold by cadmium. Furthermore not only the key enzyme gene of glutathione synthesis (*GSH1*) but also almost all transcripts of the enzymes involved in sulfur amino acid metabolism were greatly induced after exposure to cadmium (5).

In this study, we compare the genomic responses to two heavy metals, cadmium and mercury, and estimate the possibility of defining the differences in the mechanism of action in *Saccharomyces cerevisiae*.

Materials and Methods

Strain, growth conditions, and heavy metal stress conditions.

Saccharomyces cerevisiae S288C (α *SUC2 mal mel gal2 CUP1*), which was the strain used for DNA microarray analysis as the probes in a Kuhara DNA chip (DNA Chip Research Inc., Yokohama, kanagawa, Japan), and *S. cerevisiae* W303-1A β-gal (*ade2 his3 ura3 leu2 trp1* CIFI) were used for finding the conditions of cadmium treatment. Yeast cells were grown in YPD medium (2% polypeptone, 1% yeast extract, and 2% glucose) at 30°C. To know the effects of metals, we compared the gene expressions at the most stressful condition for each metal. Various concentrations of cadmium chloride and mercury chloride were added to logarithmic phase cells, and incubated at 30°C for 2 hours (one doubling time). The stress conditions were monitored by the induction of β-galactosidase activities with the strain W303-1A, where in the β-galactosidase structural gene is located down stream of the *HSP104* promotor as previously described (1). Yeast cells were incubated at the most effective conditions for 2h and harvested by centrifugation at 3000 x g. After washing with distilled water twice, the pellets were resuspended in 0.5 ml of Z buffer (21.5 g Na_2HPO_4 $12H_2O$, 6.2 g NaH_2PO_4 $2H_2O$, 0.75 g KCl, 0.246 g $MgSO_4$ $7H_2O$, 2.7 ml β-mercaptoethanol per liter, pH7.0) with glass beads, with the addition of 3 drops of chloroform and 2 drops of 0.1% SDS, followed by vortex mixing for 10 sec. The homogenate was incubated at 28°C for 5 min. The reaction was started by adding 0.1 ml of p-nitrophenyl β-D-galactopyranoside (ONPG). Reaction mixtures were incubated at 28°C for 15 min and stopped by adding 0.25 ml of 1M NaCO3. After removing the cells by centrifugation for 10 min, the absorbance was measured at 420 nm. β-Galactosidase activity was expressed per OD600 of assayed culture relative to that of untreated control cells.

RNA preparation and hybridization to DNAchip

Total RNA was isolated by a hot-phenol method. Poly (A)+RNA was purified from total RNA with Oligotex-dT30 mRNA purification kit (Takara, Shiga, Japan). Fluorescently labeled

cDNA was synthesized as we mentioned before. CDNA made from poly (A)+ RNA of the control was fluorescently labeled with Cy3 (represented as green) and that of the metal treated sample was labeled with Cy5 (represented as red). For each labeling, 2-4μg of poly (A)+ RNA was used and the same amount of each poly (A)+RNA was used for one slide. The two labeled cDNA pools were mixed and hybridized simultaneously to a microarray. DNA chips of the yeast genome were purchased from DNA Chip Research Inc. and were hybridized with labeled cDNA probes under cover slides for 24-36 hr at 65^{o}C. Each set of hybridizations was performed with an independent RNA preparation. After hybridization, the labeled microarrays were washed and dried. Subsequently labeled microarrays were scanned with a confocal laser ScanArray 4000 (GSI Lumonics, MA, USA) system. Resulting image data were quantitated by the QuantArray Quantitative microarray Analysis application program (GSI Lumonics, MA, USA). From the fluorescence intensity of each spot on the images, each background was subtracted and the ratios of intensity Cy5/Cy3 were calculated and normalized with ACT1 as a positive control. Statistical analyses were carried out by using GeneSpring (Silicon Genetics, CA, USA).

Results and Discussion

Strain, growth conditions, and stress conditions

To determine the concentrations of these metals, we checked the activities of the promoter of HSP 104, which is one of the indicators of stress. The concentrations of these metals were determined to Cd and Hg, 0.3mM and 0.7mM, respectively, since the highest percentage of HSP104 promoter were induced for each metal at those conditions (Table I).

Table I Effect of different concentrations of heavy metals on the % induction of HSP104-*lac*Z

concentration (mM)	Cd	Hg
0.001	103%	
0.003		107%
0.004	93%	
0.008		111%
0.01	122%	
0.04	142%	
0.07		75%
0.1	242%	
0.2		76%
0.3	442%	
0.7		115%
1		49%

Yeast cells were incubated in the presence of different concentrations of Cd and Hg at 30^{o}C for 9h. The induction is expressed as the relative b-galactosidase induction ratio (3-9h) compared with the controls.

Global expression profile analysis in yeast cells treated with Cd or Hg

We repeated independent experiments four times and spotted against the normalized signal intensities of the controls (Fig.1). Data points in the top left region represent transcripts that were induced by heavy metals, and those in the bottom right region represent transcripts that were repressed by the treatments. As shown in Fig. 1, many more genes were induced by Cd treatment (A) than by Hg (B). Total number of the genes induced (Cy5/Cy3 ratio >2.0) by Cd was 356, while there were only 73 in Hg-treated cells ;the overall ratio induced by Cd was much higher than that by Hg. The number of the genes for which the Cy5/Cy3 ratio was over 5.0 for Cd was 21, whereas that for Hg was only 4.

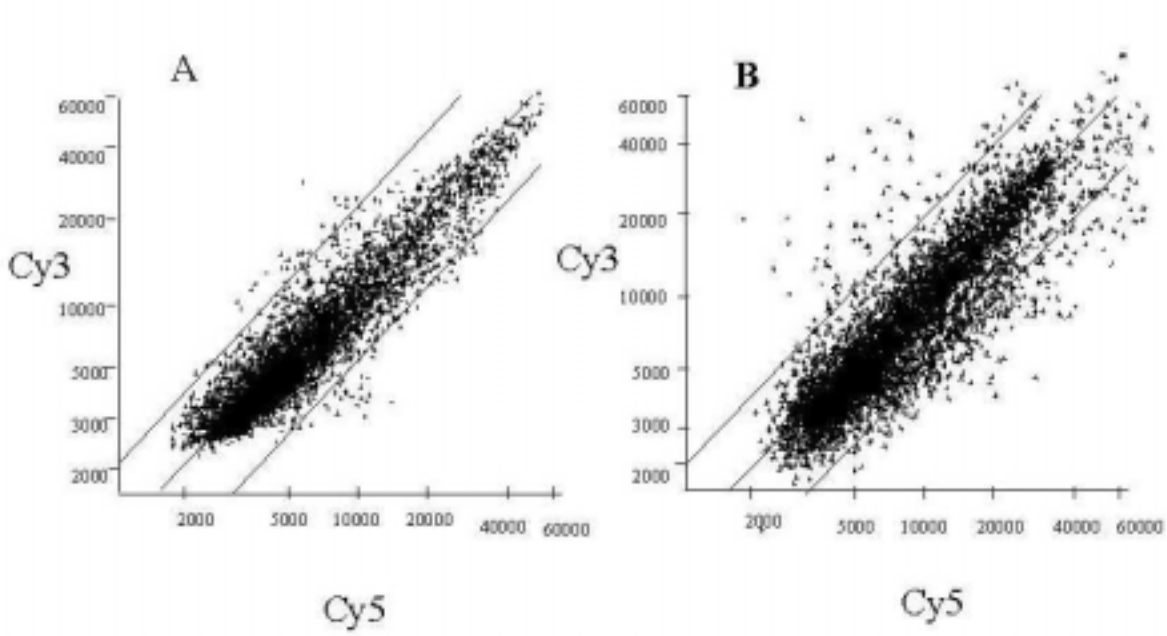

Fig.1. Comparison of log-log scatter plot of fluorescence measured for mRNA labeled with Cy3 (green) for the control and Cy5 (red) for metal treatment in competitive hybridization on a microarray containing 5880 ORFs. Lines represent a 2-fold difference in expression. A)Hg B) Cd

The percentages of categories of the genes induced by each metal were different (Fig2). These genes were annotated using functional categories assigned by the Munich Information Center for Protein Sequences (MIPS, http://www.mips.biochem.mpg.de/). There were differences in number and categories of genes induced by each metal. The most induced categories of Cd induction was cell rescue, defense, cell death and ageing, whereas that of Hg was energy and the percentages of each categories are much higher in Cd than in Hg.

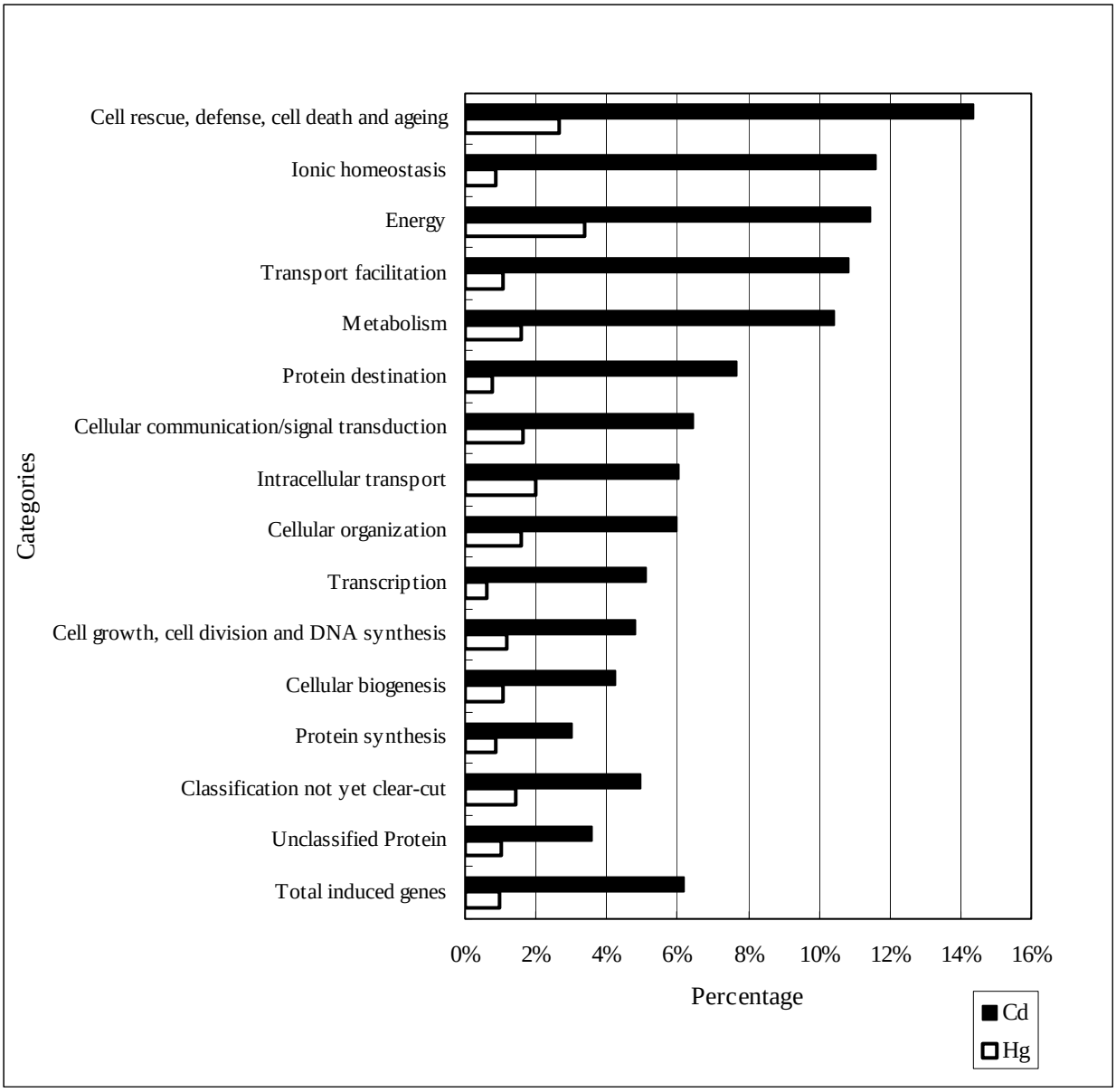

Fig. 2. Relative contents of the genes induced by Cd (A) and Hg (B).

Table II lists the genes that were induced by both metals with each gene's description and categories according to MIPS. There were 22 genes that induced both Cd and Hg and most of them were related to stress response and methionine amino acid metabolism. As shown in Fig.3, 9 genes of glutathione biosynthesis and its recycling system were induced by both metals, which were indicated by arrows in Fig.3). For example, *MET17p* is the first enzyme (O-Acetylhomoserine-O-Acetylserine Sulfhydrylase) of the sulfur salvage pathway and convert the pathway from usual sulfur amino acid pathway of methionine to glutathione synthesis. This result indicates that both of Cd and Hg induced glutathione biosynthesis.

The parts of gene expression patterns after treatment of Cd or Hg were showed in Table III. Although almost all genes involved in sulfur amino acid metabolism, glutathione synthesis, and amino acid transporters were activated (>2.0) by Cd (5), whereas Only 8 genes of the same

categories were activated by Hg and induction ratio of each gene were lower than Cd. There were also discrepancies of gene expression patterns between Cd and Hg in stress response categories. Only 8 genes were induced by Hg, whereas Cd induced 43 genes and all ratios were lower than Cd except that for one gene *SLT2*. YLL057C, which is in the subcategories of detoxification involving cytochrome C P450, showed the highest induction by Cd, while not by Hg.

Table II. Functional classification of gene products activated by cadmium and mercury treatment

Gene Name	fold(Cd)	fold(Hg)	Gene description
Metabolism			
MET17	14.9	2.5	O-Acetylhomoserine-O-Acetylserine Sulfhydrylase
MET14	10.7	2.8	adenylylsulfate kinase
MET3	7.7	4.4	ATP sulfurylase
MET16	5.5	2.1	3'-phosphoadenylylsulfate reductase
MET10	4.6	2.1	sulfite reductase flavin-binding subunit (alpha subunit)
GSH1	4.0	2.5	gamma-glutamylcysteine synthetase
MET6	4.0	3.2	vitamin B12-(cobalamin)-independent isozyme of methionine synthase
GRE2	2.7	5.0	induced by osmotic stress
YML131W	3.3	2.5	similarity to human leukotriene b4 12-hydroxydehydrogenase
Transport facilitation			
SUL1	10.6	2.2	Putative sulfate permease
AGP3	8.4	2.0	Amino acid permease
Cell rescue, defense, cell death, and ageing			
YLL057C	17.4	5.1	similarity to E.coli dioxygenase
HSP26	10.0	3.6	heat shock protein 26
GRE1	9.2	3.1	Induced by osmotic stress
DDR48	3.8	2.5	flocculent specific protein
GSH1	4.0	2.5	gamma-glutamylcysteine synthetase
HSP30	3.3	2.2	Protein induced by heat shock, ethanol treatment, and entry into stationary phase
GTT2	3.0	2.3	Glutathione S-transferase
GRE2	2.7	2.4	induced by osmotic stress
Energy			
OYE3	3.4	2.6	NAD(P)H dehydrogenase
YNL331C	3.1	2.2	
Cellular organization			
YGP1	3.4	2.0	YGP1 encodes gp37, a glycoprotein synthesized in response to nutrient limitation
LAP4	3.2	2.2	vacuolar aminopeptidase ysc1

The ratios (treated/control) were averages of 4 independent experiments and the up-regulated genes were 2 fold higher than the control.

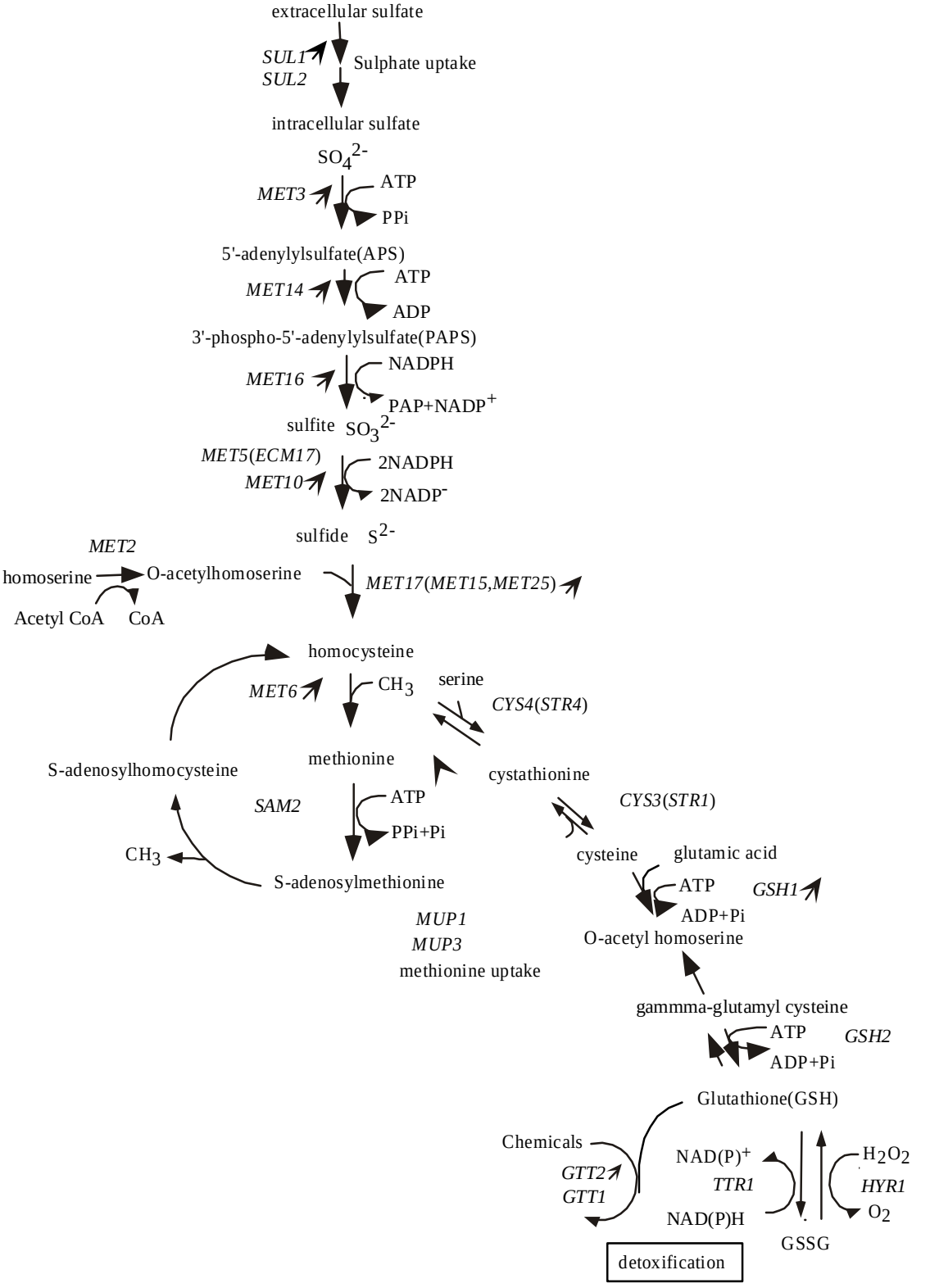

Fig.3. Schematic representation of the biological steps involved in sulfur assimilation and biosynthesis of methionine, SAM, cysteine and the sulfur salvage pathway of which the final product is glutathione, and the glutathione recycling cycle in Saccharomyces cerevisiae and the genes that encoding the enzymes and transcription factors. The genes up-regulated by Cd and Hg were indicated by arrows.

Table III Comparison of gene expression profiling of Cd or Hg treatment

ORF code	Gene Name	Cd	Hg	ORF code	Gene Name	Cd	Hg
Sulfur amino acid metabolism and transporters				YDR406W	PDR15	2.6	1.2
including glutathione biosynthesis				YLR375W	STP3	2.5	1.0
YLR303W	MET17	11.8	2.5	YKR093W	PTR2	2.5	1.5
YKL001C	MET14	10.7	2.8	YML123C	PHO84	2.5	1.8
YBR294W	SUL1	10.6	2.2	Stress response			
YJR010W	MET3	7.7	4.4	YLL057C		17.4	5.1
YPR167C	MET16	5.5	2.1	YBR072W	HSP26	10.0	3.6
YDR253C	MET32	4.9	1.3	YPL223C	GRE1	9.2	3.1
YHL036W	MUP3	4.8	1.8	YFL014W	HSP12	4.6	1.3
YFR030W	MET10	4.6	1.9	YJL101C	GSH1	4.0	2.5
YHR112C		4.5	1.1	YNL239W	LAP3	3.8	1.4
YNL277W	MET2	4.3	1.9	YGR209C	TRX2	3.7	1.1
YLR092W	SUL2	4.3	2.3	YNL160W	YGP1	3.4	1.8
YGL184C		4.2	2.2	YMR173W	DDR48	3.3	2.0
YER091C	MET6	4.0	3.2	YCR021C	HSP30	3.3	1.9
YJL101C	GSH1	4.0	2.5	YOR285W		3.2	0.9
YLL058W		3.9	1.1	YDL229W	SSB1	3.1	1.0
YGR055W	MUP1	3.8	1.3	YDL022W	GPD1	3.1	0.7
YKR069W	MET1	3.5	1.5	YLL060C	GTT2	3.0	2.3
YFL030W		3.4	0.9	YCR044C	COS16	2.9	1.2
YIR017C	MET28	3.2	1.0	YDR258C	HSP78	2.9	1.2
YAL012W	CYS3	3.1	1.4	YJL160C		2.9	1.5
YJR139C	HOM6	3.0	1.1	YDR477W	SNF1	2.9	1.7
YJR137C	ECM17/MET5	2.9	1.5	YIR037W	HYR1	2.9	1.0
YIL046W	MET30	2.8	1.1	YOL158C		2.8	0.9
YGR155W	CYS4	2.6	1.2	YER042W	MXR1	2.8	1.4
YOL064C	MET22	2.6	1.0	YDR513W	TTR1	2.8	1.1
YPL038W	MET31	2.5	1.1	YJL128C	PBS2	2.8	1.2
YDR502C	SAM2	2.3	1.1	YNR075W	COS10	2.8	1.6
YJR060W	CBF1	2.2	1.1	YHR008C	SOD2	2.7	1.3
Transporters except sulfur amino acid and				YKL073W	LHS1	2.7	1.5
C-compound transporters				YOL151W	GRE2	2.7	2.4
YLL055W		9.5	1.3	YBR070C	SAT2	2.6	1.2
YIL166C		6.4	1.2	YLR109W		2.6	0.8
YHL047C		6.3	1.0	YCL035C	GRX1	2.5	1.2
YFL055W	AGP3	5.9	1.6	YIR038C	GTT1	2.5	1.1
YBR296C	PHO89	5.4	1.3	YLL026W	HSP104	2.5	1.1
YAL067C	SEO1	5.2	1.0	YKL088W		2.5	1.2
YNL259C	ATX1	4.2	1.0	YML054C	CYB2	2.5	1.2
YHL040C	ARN1	3.9	1.1	YBR054W	YRO2	2.5	1.2
YNL142W	MEP2	3.5	1.3	YHR030C	SLT2	1.5	2.4
YKL198C	PTK1/STK1	3.4	1.3	YDR263C	DIN7	2.4	1.2
YDR170C	SEC7	3.4	1.5	YBR169C	SSE2	2.4	1.3
YJL212C	APT1	3.2	1.1	YHR053C	CUP1	2.3	1.2
YMR058W	FET3	3.1	1.0	YER103W	SSA4	2.3	1.4
YCR098C	GIT1	3.1	0.9	YDL168W	SFA1	2.2	1.1
YLR360W	VPS38	3.1	1.1	YOR027W	STI1	2.2	1.6
YDR080W	VPS41	3.0	1.4	YML028W	TSA1	2.1	1.0
YCL025C	AGP1	3.0	0.9	tRNA			
YBR217W		3.0	1.2	YLR136C	TIS11	4.1	1.8
YKR039W	GAP1	3.0	1.3	YGR171C	MSM1	2.6	1.3
YEL065W	SIT1	2.9	1.2	YDR268W	MSW1	2.6	1.4
YDR270W	CCC2	2.9	1.1	nitrogen metabolism			
YMR301C	ATM1	2.8	1.2	YHR176W	FMO	3.6	1.0
YPL274W		2.8	1.0	YOR226C		3.1	1.2
YLL044W	YBT1	2.8	1.0	YJL060W		2.9	1.7

The numbers indicate the ratios (treated/control) were averages of 4 independent experiments.

With these results, we were successful in analyzing genomic responses to cadmium or mercury by the yeast DNA microarray, and the ratios of HSP104 induction were similar to those of the promoter assay. We are going to profile the genomic responses of other chemicals from now on to make databases for new bioassay systems.

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酵母DNAマイクロアレイを用いた、重金属で誘導される遺伝子発現の比較　1）カドミウムと水銀

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要旨

酵母のほぼ全遺伝子5880個のORFを含むDNAマイクロアレイ（ORF　5880）を用いてカドミウムと水銀で誘導される遺伝子発現の比較を行った。どちらの金属もHSP104によるストレス誘導プロモーターアッセイで最高の値をとる濃度、カドミウム0.3mM、水銀0.7mMで30℃2時間処理後のmRNAを用いた。カドミウムは水銀と比べて多くの遺伝子を高い割合で誘導していた。水銀とカドミウムに共通に誘導される遺伝子は22個あった。カドミウムと水銀2つの金属による遺伝子誘導発現のパターンにはいくつかの相違があり、それを基に今後新たな化学物資毒物評価システムにマイクロアレイが用いられる可能性が示唆された。

キーワード: マイクロアレイ、酵母、重金属、カドミウム、水銀

領域区分：ゲノムワイドな実験データの解析