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Toxicity of Methanol and Formaldehyde Towards *Saccharomyces cerevisiae* as Assessed by DNA Microarray Analysis

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Abstract To assess the toxicity of the C1 compounds methanol and formaldehyde, gene expression profiles of treated baker's yeast were analyzed using DNA microarrays. Among approximately 6,000 open reading frames (ORFs), 314 were repressed and 375 were induced in response to methanol. The gene process category "energy" comprised the greatest number of induced genes while "protein synthesis" comprised the greatest number of repressed genes. Products of genes induced by methanol were mainly integral membrane proteins or were localized to the plasma membrane. A total of 622 and 610 ORFs were induced or repressed by formaldehyde, respectively. More than one-third of the genes found to be strongly repressed by formaldehyde belonged to the "protein synthesis" functional category. Conversely, genes in the subcategory of "nitrogen, sulfur, and selenium metabolism" within "metabolism" and in the category of "cell rescue, defense, and virulence" were up-regulated by exposure to formaldehyde. Our data suggest that membrane structure is a major target of methanol toxicity, while proteins were major targets of formaldehyde toxicity.

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Introduction

Methanol is a transparent, colorless liquid produced in very large volume. It is widely used as an industrial solvent and used in many consumer products, in adhesives, antifreeze, paints, and cleaning solutions, as an ingredient. Moreover, methanol has been proposed to be use as an alternative to fossil fuels. It is also detected as a by-product in sewage treatment, fermentation, and paper production. Vegetables and fruits contain methanol in some degree. There are some incident on surrogate alcohols (i.e., nonbeverage alcohols and illegally produced alcohols) and appears to be a major public health problem in some countries. Methanol is reported to be toxic even in low dose, like inhalation of carburetor cleaning fluid fumes [1]. Thus, all of us share some risks to expose to high dose of methanol accidentally, occupationally, or may be intentionally. It is reported that daily low level (at 1/4 LD50) exposure to methanol also influence a normal immune response on rat [2].

Similarly, formaldehyde is a common environmental agent found in exhaust gas and as a component in paint, glue, and many other medicinal and industrial products. Formaldehyde is also produced endogenously and exists in cells and tissues of our body; however, exposure to large amounts of formaldehyde vapor can irritate the nasal mucosa and may potentially be carcinogenic. It also gives a positive Ames test.

Methanol and formaldehyde are intermediates in the oxidation of methane and are referred to as C1 compounds. Formaldehyde is much more toxic than methanol [3]. LD_{50} values for formaldehyde and methanol by oral administration in the rat is 800 and 5,628 mg/kg, respectively. However, cytotoxicity of methanol has not been fully elucidated and it is not clear if the toxicity of methanol is mainly due to its possible conversion to formaldehyde by exposed cells.

Recently, toxicogenomics, which analyze global genetic response to toxic substances and elucidate the etiology has been developed. And budding yeast, *Saccharomyces cerevisiae* is one of the most studied model eukaryote and more than 70% of open reading frames (ORFs) are verified. The information of molecular function, biological process, cellular localization, related metabolic pathway, and others of each gene is collected, integrated, and updated. In this study, we employed DNA microarray analysis to explore and compare the toxicity of these two C1 compounds in *S. cerevisiae*.

Materials and Methods

Pre-cultures of *S. cerevisiae* S288C (*Mat alpha SUC2 mal mel gal2 CUP1*) were grown in YPD (2% polypeptone, 1% yeast extract, and 2% glucose) at 25 °C for 2–3 days. This strain was chosen because the DNA microarrays were produced using S288C DNA as the PCR template.

The pre-culture was diluted and grown overnight to an optical density (OD 660 nm) of 1.0. In the dose–response experiments, methanol or formaldehyde was added to the YPD cultures which were then grown for another 2 h along with control cultures without addition of these C1 compounds, respectively. Cells were harvested by centrifugation and stored at −80 °C until RNA was extracted.

Microarray experiments were carried out as described [4]. Poly (A)+ RNA was purified from three independent cultures of both control and treated cells. mRNA from C1

compound-treated cells was fluorescently labeled with Cy5-dUTP while mRNA from untreated cells was labeled with Cy3-dUTP. The two fluorescently labeled cDNAs were mixed and then hybridized to yeast microarrays (ver.2.0, DNA Chip Research, Inc., Yokohama, Japan) for 16–20 h at 65 °C. About 6,000 genes could be analyzed on these microarrays under these conditions [5]. Detected signals for each ORF were normalized by intensity-dependent (LOWESS) methods (http://www.silicongenetics.com/cgi/Sig.cgi/index.smf). Genes designated induced or repressed in treated cells (1) passed a one sample *t* test (*P* value cutoff 0.05) and (2) exhibited greater or less than twofold expression, respectively, relative to the control. Induced and repressed genes were characterized according to accepted gene ontology categories (MIPS, Munich Information Center for Protein Sequences, http://mips.gsf.de/ and Saccharomyces Genome Database, http://www.yeastgenome.org/). The data obtained in this experiment are available under accession number GSE9231 in the Gene Expression Omnibus Database (http://www.ncbi.nlm.nih.gov/geo/).

Clustering analyses were applied using GeneSpring (Ver.7.3.1). The settings for calculations were as follows: similarity was measured by Pearson's correlation, the separation ratio was 1.0, and the minimum distance was 0.001.

Results and Discussion

To elucidate the toxicity of methanol and formaldehyde, appropriate concentrations for microarray experiments had to be determined. From initial dose–response experiments, final concentrations of 1.23 M methanol and 1.8 mM formaldehyde were chosen, as these doses were found to significantly inhibit growth without killing cells. Generally, in lower concentrations of chemicals which have no effect on growth, we are not able to detect differences in gene expressions, and in higher concentrations which cause cellular death, we are not able to extract enough amount and quality of mRNA. In the condition above, it was sufficient to induce large changes in the gene expression profiles.

Methanol Toxicity

Methanol was found to repress 314 and to induce 375 ORFs. Table 1 shows the number of induced genes by category of biological function. Among 375 induced ORFs, 115 were categorized in "metabolism" and 60 of them within the subcategory of "C-compound and carbohydrate metabolism". Genes within the subcategory of "energy" (13.8%) comprised the greatest fraction. Functional categories for the repressed genes were also analyzed. "Protein synthesis (15.8%)" was found to comprise the largest category of repressed genes.

Within the category of "energy", 4 aldehyde dehydrogenases *ALD4* (major mitochondrial; 4.92-fold), *ALD3* (3.83-fold), *ALD2* (3.68-fold), and *ALD6* (major cytoplasmic; 2.11-fold), were strongly up-regulated by methanol treatment. Products of these genes may be involved in oxidizing the intermediate formaldehyde to formic acid. As shown below, these *ALD*s were also strongly induced by formaldehyde treatment. Other alcohol dehydrogenases, *ADH5* (1.69-fold), *ADH7* (1.65-fold), and *ADH2* (1.59-fold) were not induced more than twofold. Adh5p reduces acetaldehyde to ethanol during fermentation of glucose, while Adh2p oxidizes ethanol to acetaldehyde [6, 7]. *ADH7* is a non-essential gene that encodes a member of the cinnamyl alcohol dehydrogenase family [8]. These data suggest that yeast slowly metabolizes methanol to formaldehyde to avoid abrupt accumulation of the more toxic latter compound. The presumed methanol detoxification pathway via

Table 1 Categories of ORFs induced by methanol and formaldehyde treatment.

Gene categories	Methanol treatment		Formaldehyde treatment	
	Number of induced genes	Percentage in each category	Number of induced genes	Percentage in each category
Metabolism (1519)	115	7.6	175	11.5
Energy (369)	51	13.8	37	10.0
Cell cycle and DNA processing (1010)	21	2.1	67	6.6
Transcription (1078)	23	2.1	58	5.4
Protein synthesis (480)	2	0.4	9	1.9
Protein fate (1155)	31	2.7	134	11.6
Protein with binding function or cofactor requirement (1049)	31	3.0	102	9.7
Regulation of metabolism and protein function (249)	5	2.0	20	8.0
Cellular transport, transport facilitation, and transport routes (1042)	68	6.5	105	10.1
Cellular communication/signal transduction mechanism (234)	6	2.6	16	6.8
Cell rescue, defense and virulence (554)	50	9.0	123	22.2
Interaction with the cellular environment (463)	19	4.1	50	10.8
Transposable elements, viral, and plasmid proteins (120)	0	0.0	0	0.0
Cell fate (273)	5	1.8	20	7.3
Development (69)	3	4.4	6	8.7
Biogenesis of cellular components (863)	30	3.5	71	8.2
Cell type differentiation (452)	16	3.5	40	8.9
Unclassified proteins (1394)	98	7.0	133	9.5

Numbers in parentheses indicate total ORFs in each category

formaldehyde is shown in Fig. 1a. The glutathione-dependent branch is not considered induced.

Two putative aryl-alcohol dehydrogenases, *AAD10* (2.97-fold) and *AAD6* (2.08-fold) were also up-regulated in the "energy" category. However, there is a very high nucleotide sequence similarity among all seven *AAD*s, *AAD3*, *AAD4*, *AAD6*, *AAD10*, *AAD14*, *AAD15*, and *AAD16*. While *AAD4* and *AAD6* were reported to be induced by oxidative stress in a Yap1p-dependent manner [9], they were not shown to contribute to alkyl-alcohol degradation.

Glucokinase, *GLK1* (2.26-fold), and hexokinase, *HXK1* (2.29-fold), were found to be up-regulated, suggesting that glycolysis was slowed or halted [10, 11]. Herrero and coworkers [12] reported that transcription of these genes was de-repressed when cells were grown on a non-fermentable carbon source. During methanol treatment, it is possible that cells are oxidizing the methanol as a carbon source.

In the category of "metabolism", just three ORFs, *MHT1* (2.12-fold), *MET13* (2.09-fold), and *MET17* (2.04-fold), within the subcategory of "metabolism of methionine and metabolism of cysteine" were up-regulated. This is the one of major differences found between methanol and formaldehyde toxicity. Far more ORFs involved in methionine and

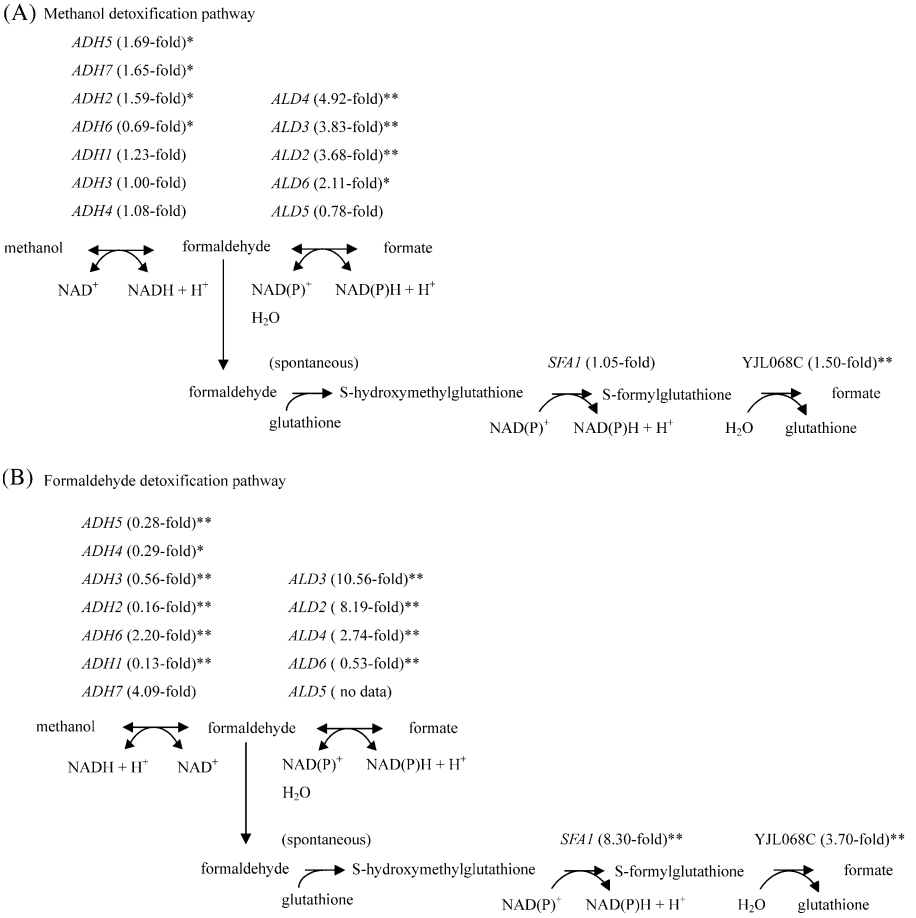

Fig. 1 Expected methanol and formaldehyde detoxification pathways in yeast. Induction and repression values of respective ORFs upon cell exposure to (**a**) methanol and (**b**) formaldehyde are shown in parentheses. *t* test *P* values less than 0.05 are indicated with a *single asterisk* (*); *t* test *P* value less than 0.01 are indicated with *two asterisks*

cysteine metabolism were up-regulated during formaldehyde treatment, as shown below. Seventeen ORFs, *SUT1*, *HSP12*, *OPI3*, *POT1*, *CAT2*, *POX1*, *GUT2*, *CRC1*, *TIP1*, *GUT1*, YDR018C, *GPT2*, *ATG15*, *FAA2*, YML131W, *PCS60*, and *AGP2*, within the subcategory of "lipid, fatty acid, and isoprenoid metabolism" were also found to be induced. This may suggest that cells are making more membrane components to overcome methanol toxicity.

Among the 375 genes induced by methanol treatment, Table 2 lists the most highly induced genes. Fifty were categorized within "cell rescue, defense, and virulence". As shown in Table 3, 12 genes were induced within the subcategory of "oxidative stress response" (21.8%) while ten were induced within the subcategory of "oxygen and radical detoxification" (38.5%). These data indicate that oxidative stress is a major consequence of exposure to methanol.

A number of studies have examined ethanol tolerance in yeast. It is conceivable that methanol elicits some of the same responses as this C2 alkanol. Ethanol-tolerant mutants of sake strains of yeast have been reported to be resistant to killer toxin and to a cell wall lytic

Table 2 Most highly induced ORFs by methanol.

Systematic name	Standard name	Expression ratio	Description
YGL158W	RCK1	21.50	Ser/thr protein kinase
YBR072W	HSP26	13.32	Heat shock protein
YDL218W		11.15	Weak similarity to hypothetical protein YNR061c
YOL052C-A	DDR2	10.74	Heat shock protein DDRA2
YKL217W	JEN1	10.62	Lactate and pyruvate permease
YNL195C		9.91	Conserved hypothetical protein
YKL187C		9.37	Mitochondrial membrane protein
YDR034W-B		9.33	Protein of unknown function
YPL223C	GRE1	8.89	Induced by osmotic stress
YOL084W	PHM7	7.49	Protein of unknown function, expression is regulated by phosphate levels
YMR175W	SIP18	7.27	Osmotic stress protein
YDR070C	FMP16	7.26	Found in mitochondrial Proteome
YEL070W	DSF1	7.18	Expression is glucose repressed by Mig1 and Mig2
YOR383C	FIT3	7.16	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor
YBR116C		6.99	Questionable protein
YHR139C	SPS100	6.89	Sporulation-specific wall maturation protein
YHR096C	HXT5	6.72	Hxt family protein with intrinsic hexose transport activity
YGR256W	GND2	6.48	Phosphogluconate dehydrogenase
YGR236C	SPG1	6.43	Protein required for survival at high temperature during stationary phase
YPR192W	AQY1	6.41	Water channel that mediates the transport of water across cell membranes

enzyme [13]. Both phenotypes are suggestive of an altered cell wall structure. It is has also been reported that the cell membrane is the primary target of ethanol toxicity and that tolerance can be partly explained in terms of membrane lipid composition [14–18].

The localization of the products of the methanol-induced genes was also analyzed according to MIPS. A relatively large percentage was localized to the "cell wall", "plasma membrane", and "integral membrane/endomembrane" (Table 4). Products of genes induced by formaldehyde were also found to be localized to the cell wall and membrane. However, a total of 622 genes were found to be induced by formaldehyde while 375 were induced by methanol. We define the "impact" of methanol and formaldehyde on a given cell component as the ratio of the number of component-associated genes induced to the total number of induced genes (Table 4). By this measure, methanol has a substantial impact on the cell wall and on membranes.

Some discrepancies were noted between this study and earlier reports on ethanol tolerance. Yazawa et al. [19] reported that *URA7* and *GAL6* (*LAP3*) null mutants exhibited greater tolerance to ethanol than the wild-type parent strain. In our study, *URA7* was also strongly repressed (0.20-fold), but *LAP3* was mildly induced (1.48-fold). Some studies have reported that ergosterol plays an important role in ethanol tolerance [20–22]. In the present study, no ORFs involved in ergosterol biosynthesis were found to be up-regulated more than twofold by methanol, from *ERG10* (0.82-fold; cytosolic acetyl-CoA C-

Table 3 Subcategories of ORFs in "cell rescue" category induced by methanol and formaldehyde treatment.

Subcategories of "cell rescue"	Methanol treatment		Formaldehyde treatment	
	Number of induced genes	Percentage of induced genes	Number of induced genes	Percentage of induced genes
Cell rescue, defense and virulence (554)	50	9.0	123	22.2
Stress response (450)	41	9.1	107	23.8
Oxidative stress response (55)	12	21.8	30	54.6
Osmotic and salt stress response (59)	6	10.2	12	20.3
pH stress response (8)	1	12.5	1	12.5
Heat shock response (20)	4	20.0	6	30.0
Cold shock response (3)	0	0.0	0	0.0
Unfolded protein response (69)	5	7.3	11	15.9
DNA damage response (77)	1	1.3	7	9.1
Nutrient starvation response (15)	0	0.0	8	53.3
Electromagnetic waves stress response (2)	0	0.0	0	0.0
Disease, virulence, and defense (35)	3	8.6	7	20.0
Resistance proteins (33)	3	9.1	5	15.2
Virulence, disease factors (2)	0	0.0	1	50.0
Detoxification (117)	17	14.5	36	30.8
Detoxification involving cytochrome P450 (4)	1	25.0	0	0.0
Detoxification by modification (8)	3	37.5	6	75.0
Detoxification by export (3)	0	0.0	0	0.0
Oxygen and radical detoxification (26)	10	38.5	18	69.2
Degradation/modification of foreign compounds (1)	0	0.0	0	0.0
Degradation/modification of foreign polynucleotides (1)	0	0.0	0	0.0

Numbers in parentheses indicate total ORFs in each subcategory

acetyltransferase) to *ERG4* (1.11-fold; sterol C-24 reductase). Fujita et al. [23] compared yeast response to various straight-chain alcohols using cDNA microarrays and concluded that gene expression profiles differed as a function of the log P_{ow} values of the alcohols. They also reported genes essential for alcohol tolerance based on screening a library of deletion mutants [24]. Based on a comparison of their results with the present study, we conclude that toxicity induced by methanol differs from that induced by ethanol.

Formaldehyde Toxicity

Formaldehyde was found to induce 622 ORFs more than twofold and to repress 610 ORFs more than twofold, relative to the control. As shown in Table 1, 22.2% of the up-regulated genes belong to the category of "cell rescue, defense, and virulence" while 11.6% belong to the category of "protein fate". More than one-third of the genes (34.6%) in the category of "protein synthesis" were strongly repressed. However, repression of genes categorized in "protein synthesis" was often observed [5, 25–27] in other chemical treatments and changes

Table 4 Cellular localization of products of ORFs induced by methanol and formaldehyde.

Localization	Methanol treatment			Formaldehyde treatment		
	Number[a]	%[b]	Impact[c]	Number	%	Impact
Extracellular (54)	12	22.2	3.2	12	22.2	1.9
Bud (150)	5	3.3	1.3	7	4.7	1.1
Cell wall (44)	8	18.2	2.1	12	27.3	1.9
Cell periphery (216)	18	8.3	4.8	25	11.6	4.0
Plasma membrane (186)	27	14.5	7.2	18	9.7	2.9
Integral membrane/endomembranes (172)	27	15.7	7.2	32	18.6	5.1
Cytoplasm (2845)	166	5.8	44.3	339	11.9	54.5
Cytoskeleton (204)	4	2.0	1.1	15	7.4	2.4
ER (552)	28	5.1	7.5	87	15.8	14.0
Golge (158)	1	0.6	0.3	10	6.3	1.6
Transport vesicles (141)	1	0.7	0.3	10	7.1	1.6
Nucleus (2139)	81	3.8	21.6	210	9.8	33.8
Mitochondria (1047)	76	7.3	20.3	116	11.1	18.6
Peroxisome (52)	6	11.5	1.6	3	5.8	0.5
Endosome (58)	0	0.0	0.0	10	17.2	1.6
Vacuole (284)	17	6.0	4.5	43	15.1	6.9
Microsomes (5)	0	0.0	0.0	1	20.0	0.2
Lipid particles (27)	3	11.1	0.8	5	18.5	0.8
Punctate composite (140)	11	7.9	2.9	15	10.7	2.4
Ambiguous (237)	12	5.1	3.2	39	16.5	6.3

Number in parentheses indicate total ORFs in each localization or cell component

[a] Number of induced genes whose products are found associated with the indicated localization

[b] (Number of induced gene products associated with each localization)/(total number of gene products associated with the localization)×100

[c] Impact is defined as the (number of component-associated genes that induced)/(the total number of induced genes)×100

in growth environment. Sirisattha et al. [28] have pointed out that down-regulated genes might reflect the decreased growth rate. Also, Brauer et al. [29] demonstrated that differences in growth rates resulted in differences in gene expressions. And they consider the transcriptional response was closely correlated with the growth rate, not with cause of growth retardation. The repression of protein synthesis is a common observation in yeast which shows lower growth rate [4] and we cannot ascribe the repression to characteristic of formaldehyde toxicity.

Five methionine biosynthesis genes, *MET17*, *MET3*, *MET16*, *MET10*, and *MET6*, and two methionine permease genes, *MUP3*, and *MUP1*, were included among the 20 most highly induced genes (Table 5). *ADH1*, encoding alcohol dehydrogenase that is responsible for converting acetaldehyde to ethanol, was strongly repressed 0.13-fold. It has been reported that overexpression of *ADH1* in a wild-type strain rendered it hyper-resistant to formaldehyde [30]. Four other genes involved in aldehyde metabolism, *AAD16*, *AAD6*, *AAD14*, and *AAD3*, were also highly induced. Most of the genes needed for methionine biosynthesis are also needed for glutathione biosynthesis, and almost all of the latter genes were up-regulated. Twenty ORFs in the category of "metabolism of methionine" (35

Table 5 Most highly induced ORFs by formaldehyde treatment.

Systematic name	Standard name	Expression ratio	Description
YDL218W		331.79	Weak similarity to hypothetical protein YNR061c
YHR139C	SPS100	118.03	Sporulation-specific wall maturation protein
YKL071W		104.47	Protein of unknown function, cytoplasmic
YBR008C	FLR1	90.54	Putative H+ antiporter regulated by yAP-1
YLR303W	MET17	73.41	O-acetylhomoserine sulfhydrylase
YFL057C	AAD16	72.23	Aryl-alcohol dehydrogenase
YFL056C	AAD6	49.33	Putative aryl-alcohol dehydrogenase, stress response
YHR096C	HXT5	36.73	Hxt family protein with hexose transport activity
YNL331C	AAD14	35.02	Putative aryl-alcohol dehydrogenase
YCR107W	AAD3	34.22	Aryl-alcohol dehydrogenase
YJR010W	MET3	27.72	Sulfate adenylyltransferase
YPR167C	MET16	26.12	3'-phosphoadenylylsulfate reductase
YLL060C	GTT2	23.75	Glutathione S-transferase
YCR102C		23.61	Similarity to Zinc-type alcohol dehydrogenase
YFR030W	MET10	23.11	Sulfite reductase flavin-binding subunit
YHL036W	MUP3	22.55	Low-affinity methionine permease
YLR460C		21.46	Protein of unknown function
YGR055W	MUP1	21.07	High affinity methionine permease
YGR197C	SNG1	21.06	Probable transporter, resistance to nitrosoguanidine
YER091C	MET6	20.61	5-methyltetrahydropteroyltriglutamate—homocysteine methyltransferase

entries) were up-regulated. On the other hand, only three ORFs in this same category were found to be up-regulated by treatment with methanol.

Thus, glutathione appears to play a very important role in formaldehyde detoxification. The glutathione-dependent formaldehyde dehydrogenase gene *SFA1* [31] was found to be strongly up-regulated, 8.30-fold (Fig. 1b), while the gene encoding S-formylglutathione hydrolase, YJL068C [32], whose product regenerates glutathione, was also up-regulated (3.70-fold). Expression of *ADH6* was induced 2.20-fold by formaldehyde treatment (Fig. 1b). However, it is not clear whether cells were able to alleviate the toxicity of formaldehyde efficiently by reducing it to methanol because Adh6p is a medium-chain alcohol dehydrogenase [33].

Within the category of "cell rescue", more than half of the genes belonging to the subcategories of "oxidative stress response", and "oxygen and radical detoxification" were induced (Table 3). As noted above, one of the major radical scavengers, glutathione, is synthesized via methionine and cysteine biosynthesis.

Within the "protein fate" category, 32 ORFs (36.0%) in the "protein processing (proteolytic)" subcategory and 63 ORFs (24.6%) in the "protein/peptide degradation" subcategory were induced (Table 6). These data suggest that formaldehyde treatment interferes with protein synthesis by blocking ribosome assembly and that denatured proteins were degraded by proteolysis. In contrast, only 32 ORFs (2.8%) in the "protein fate" category were induced and just 15.8% of the genes in the "protein synthesis" category were repressed by methanol treatment.

Table 6 Subcategories of methanol and formaldehyde-induced genes in "protein fate" category.

Subcategories of "protein fate"	Methanol treatment		Formaldehyde treatment	
	No. of induced genes	Percentage of induced genes	No. of induced genes	Percentage of induced genes
Protein fate (folding, modification, destination) (1155)	32	2.8	134	11.6
Protein folding and stabilization (93)	2	2.2	17	18.3
Protein targeting, sorting, and translocation (281)	4	1.4	20	7.1
Protein modification (616)	15	2.4	76	12.3
With fatty acids (33)	1	3.0	1	3.0
With sugar residues (69)	0	0.0	1	1.5
By phosphorylation, de-phosphorylation, auto-phosphorylation (186)	6	3.2	14	7.5
By acetylation, de-acetylation (69)	1	1.4	2	2.9
By ubiquitination, de-ubiquitination (79)	1	1.3	15	19.0
By ubiquitin-related proteins (21)	0	0.0	3	14.3
Post-translational modification of amino acids (26)	0	0.0	1	3.9
Protein processing (proteolytic) (89)	5	5.6	32	36.0
Assembly of protein complexes (199)	5	2.5	19	9.6
Protein/peptide degradation (256)	11	4.3	63	24.6
Cytoplasmic and nuclear (188)	7	3.7	51	27.1
Lysosomal and vacuolar (26)	2	7.7	6	23.1

Thirteen ORFs in the subcategory of "DNA recombination and DNA repair" within the category of "cell cycle and DNA processing" were induced and seven ORFs were mildly induced (1.5–2.0 fold) by formaldehyde treatment, whereas only five ORFs in this subcategory were induced by methanol treatment (Table 7).

Methanol vs. Formaldehyde Toxicity

One hundred and thirty-six ORFs were repressed by both methanol and formaldehyde and 39 of them were in the category of protein synthesis (8.1%). One hundred and twenty-six ORFs were induced by both C1 compounds, including putative aryl-alcohol dehydrogenases, *AAD6* and *AAD10*, aldehyde dehydrogenases, *ALD2*, *ALD3*, and *ALD4*, glutaredoxin, *GRX1*, glutathione S-transferase, *GTT1*, heat shock proteins, *HSP12*, *HSP26*, *HSP30*, and *HSP31*, Hxt family proteins with intrinsic hexose transport activity, *HXT5* and *HXT9*, superoxide dismutases, *SOD1* and *SOD2*, among others. However, among the more than 20 ORFs associated with the yeast proteasome that were up-regulated by formaldehyde only *UBC5* was up-regulated by methanol.

A total of 21 genes were induced >4-fold by both treatments. Among them, nine are associated with lipids, membranes, and the cell wall. *HSP12* and *HSP26* are known to be involved in ethanol tolerance in a *gal6* null mutant and their expression was reported to be

Table 7 DNA repair genes induced by methanol and formaldehyde treatment.

Systematic name	Standard name	Fold induction	Description
Methanol treatment			
YFL014W	HSP12	5.87	Heat shock protein
YGR144W	THI4	3.09	Protein involved in thiamine biosynthesis and DNA repair
YOR346W	REV1	2.48	DNA repair protein
YML058W-A	HUG1	2.41	Hydroxyurea and UV and gamma radiation induced
YDR030C	RAD28	2.08	Protein involved in transcription-coupled repair
Formaldehyde treatment			
YFL014W	HSP12	8.57	Heat shock protein
YIL143C	SSL2	4.18	DNA helicase
YER142C	MAG1	4.03	3-methyladenine DNA glycosylase
YGL163C	RAD54	4.00	DNA-dependent ATPase of the Snf2p family
YMR173W	DDR48	3.74	Heat shock protein
YGL058W	RAD6	3.12	E2 ubiquitin-conjugating enzyme
YKL025C	PAN3	3.09	Component of the Pab1p-dependent poly(A) ribonuclease
YAR007C	RFA1	2.92	DNA replication factor A, 69 kDa subunit
YNL312W	RFA2	2.74	DNA replication factor A, 36 kDa subunit
YML032C	RAD52	2.72	Recombination and DNA repair protein
YDR227W	SIR4	2.65	Silencing regulatory and DNA repair protein
YKL213C	DOA1	2.21	Protein involved in ubiquitin-dependent proteolysis
YEL037C	RAD23	2.13	Nucleotide excision repair protein

up-regulated by ethanol stress [34, 35]. In the present study, both genes were also up-regulated by methanol and formaldehyde treatment.

Gasch et al. [36] found two clusters of genes which were up-regulated or down-regulated, respectively and named them the “environmental stress response (ESR) genes”.

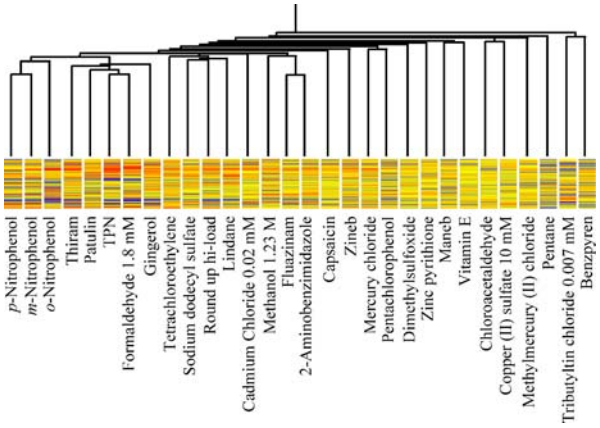

Fig. 2 Cluster analysis of the expression profiles after methanol and formaldehyde treatment. Hierarchical cluster analysis was performed using GeneSpring as described in the text

These ESR genes commonly shift their expression responses regardless of stress conditions. And many of the ESR-induced and ESR-repressed genes were correlated with growth rate [29]. However, among 375-induced ORFs in methanol treatment, and among 622-induced ORFs in formaldehyde treatment, only about 100 genes were growth rate correlated and ESR-induced genes, respectively (data not shown). These findings suggest that most of the up-regulated ORFs in C1 compound treatments were thought to be mainly compound specific, but not dependent on growth rate.

Cluster analysis was used to characterize the toxicity of these C1 compounds (Fig. 2) relative to previous DNA microarray data generated in our laboratory for heavy metals, detergents, food additives, herbicides, and physiologically active substances. The gene expression profile for formaldehyde was not grouped in a same cluster that included methanol but was grouped in a cluster that included two fungicides, tetrachloroisophthalonitrile, and thiuram, and a mycotoxin, patulin. Patulin and Thiuram also activated protein degradation and DNA repair genes [4, 27, 37]. These chemicals and the mycotoxin are known to attack thiol and amino groups and to promote protein denaturation [38–40].

Conclusions

Formaldehyde is much more toxic than methanol, based on its inhibition of yeast growth at a much lower concentration than methanol. The concentration of methanol and formaldehyde used in the present study was 1.23 M and 1.8 mM, respectively. As shown in Fig. 1, methanol strongly induced *ALD*s, in contrast to modest or no induction of *ADHs*, presumably to mediate rapid elimination of the formaldehyde derived from the metabolism of methanol. This is a remarkable and very rational toxicity-evading mechanism to minimize concentrations of a highly toxic metabolic intermediate.

As shown in Table 7, formaldehyde significantly stimulated genes involved in DNA repair. It is well known that formaldehyde is mutagenic to experimental animals [41], bacteria, and to lower eukaryotes [42]. Exposure to formaldehyde leads to the formation of DNA-protein crosslinks as the major form of DNA damage [43, 44]. While methanol and formaldehyde induced 126 and repressed 136 of the same ORFs, respectively, the toxicities of two chemicals were found to differ somewhat. In this study, a haploid strain *S. cerevisiae* S288C was used. The toxicity due to formaldehyde may be exacerbated in a haploid compared to a diploid strain. And this may have contributed to the differences in toxicity observed between the methanol and formaldehyde.

Cluster analysis highlighted these differences. We interpret the microarray results as follows. Methanol caused oxidative stress and also seems to affect membrane integrity. Formaldehyde denatures proteins and DNA via oxidization. To minimize the toxicity of formaldehyde, whether added directly or generated via oxidation of added methanol, yeast synthesizes glutathione, degrades denatured proteins, and repairs damaged DNA. Formaldehyde is a much worse toxicant than methanol, as evidenced by the induction of aldehyde-oxidizing genes upon exposure to methanol as a novel mechanism for minimizing concentrations of methanol-generated formaldehyde.

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