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Elucidation of Copper and Asparagine Transport Systems in *Saccharomyces cerevisiae* KNU5377 Through Genome-Wide Transcriptional Analysis

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Abstract *Saccharomyces cerevisiae* KNU5377 has potential as an industrial strain that can ferment wasted paper for fuel ethanol at 40°C [15, 16]. To understand the characteristics of the strain, genome-wide expression was performed using DNA microarray technology. We compared the homology of the DNA microarray between genomic DNAs of *S. cerevisiae* KNU5377 and a control strain, *S. cerevisiae* S288C. Approximately 97% of the genes in *S. cerevisiae* KNU5377 were identified with those of the reference strain. YHR053c (*CUP1*), YLR155c (*ASP3*), and YDR038c (*ENA5*) showed lower homology than those of *S. cerevisiae* S288C. In particular, the differences in the regions of YHR053c and YLR155c were confirmed by Southern hybridization, but did not with that of the region of YDR038c. The expression level of mRNA in *S. cerevisiae* KNU5377 and S288C was also compared: the 550 ORFs of *S. cerevisiae* KNU5377 showed more than two-fold higher intensity than those of *S. cerevisiae* S288C. Among the 550 ORFs, 59 ORFs belonged to the groups of ribosomal proteins and mitochondrial ribosomal proteins, and 200 ORFs belonged to the group of cellular organization. *DIP5* and *GAP1* were the most highly expressed genes. These results suggest that upregulated *DIP5* and *GAP1* might take the place of *ASP3* and, additionally, the sensitivity against copper might be contributable to the lowest expression level of copper-binding metallothioneins encoded by *CUP1a* (YHR053c) and *CUP1b* (YHR055c) in *S. cerevisiae* KNU5377.

Key words: Microarray, *Saccharomyces cerevisiae*, asparagine transport, methallothionein, copper

DNA microarray technique is a powerful tool for simultaneous monitoring of gene expression for tens of thousands of genes under appropriate experimental conditions [6]. Many investigators have researched for genomic expression changes, specific cell cycle, and mutant studies under exposure to various environmental stresses [1, 8]. Time-course expression profiles of yeast cells exposed to various environmental changes have been also demonstrated [13]. Moreover, the statistical analysis and comparison of mRNA expression pattern in two related yeast strains using this technique under normal condition can identify the strain of which genes are expressed and which specific biochemical properties are endowed with these genes.

In particular, the technique has been used to analyze changes in transcript abundance in yeast cells responding to myriad environmental stressors including high temperature, hydrogen peroxide, the superoxide-generating drug menadione, hyper- and hypo-osmotic shock, and so on. These analyses of a large number of gene expression can allow us to illustrate stereotyped patterns of gene expression during the adaptation to stressful condition, and to compare and contrast the gene expression responses to different stresses [10].

Saccharomyces cerevisiae KNU5377 was isolated as a thermotolerant yeast that could produce fuel alcohol under stressful conditions, especially at a high temperature like 40°C [15, 16]. Owing to its tolerance, this strain is expected to have potential applications in the alcohol fermentation industry [28]. Therefore, the elucidation of the mechanisms responsible for thermotolerance might provide strategies for further improving its tolerance and industrial applications.

S. cerevisiae cells exposed to mild thermal stress synthesize stress proteins and undergo physiological changes, resulting in acquirement of thermotolerance at lethal temperatures. This phenomenon is known as acquired thermotolerance. On the other hand, if cells were tolerant to thermal stress without the induction of heat shock responses, it is called intrinsic thermotolerance [11, 12]. In our previous reports,

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a naturally isolated strain, *S. cerevisiae* KNU5377, exhibited tolerance under various stress conditions such as heat, sulfuric acid, and oxidant, with or without heat-shock responses [29]. Notably, this strain showed a well-modulated trehalose-related mechanism to accumulate more trehalose by controlling neutral trehalase activity after heat shock than a reference strain [22]. However, strain-specific features on the genomic background of *S. cerevisiae* KNU5377 are not well understood.

Yeast is an excellent model to characterize genomic expression programs, because of its intriguing biology. Microarray studies are rapidly applied to plant, animal, and microorganisms that remain in the early stages of characterization [9]. Information of yeast biology and functional annotations for each gene is presented in various databases available online, including the *Saccharomyces* Genome Database (SGD), the Munich Information Center Yeast Genome Database (MIPS), the Yeast Protein Databases (YPD and PPD), and others.

In this study, we compared the total genomic DNA of *S. cerevisiae* KNU5377 to that of *S. cerevisiae* S288C as a reference strain in order to demonstrate its inheritance. Additionally, genome-wide transcriptional analyses of approximately 6,200 ORFs of yeast total genome were performed under normal growth condition, which might illustrate characters leading to the strain-specific physiological and biochemical properties in *S. cerevisiae* KNU5377.

MATERIALS AND METHODS

Strains and Growth Conditions

Saccharomyces cerevisiae KNU5377 was isolated from Korean sewage soil [15, 16]. *S. cerevisiae* S288C (*MATá SUC2 mal mel gal2 CUP1*) was used as a reference strain, because most of the *S. cerevisiae* genomic sequence information that is accessible via databases has been obtained from the *S. cerevisiae* S288C [23]. Both strains were aerobically grown in nutrient rich YEPD medium (yeast extract 1%, peptone 2%, dextrose 2%) at 30°C [14, 18].

RNA Extraction

Both yeast strains were grown in YEPD medium at 30°C until the mid-log phase to OD_{600} of 1.0, and the cells were then collected by centrifugation at 3,000 rpm for 3 min at 4°C. Cell pellets were washed 3 times with saline (0.86% NaCl) and stored at −70°C until use. Total RNA was extracted from lyophilized cell pellets by the hot phenol method [24]. Cells were thawed on ice and then mixed with a solution containing 5 ml of hot phenol, 4.5 ml of Na-acetate buffer, and 0.5 ml of 10% SDS. The mixture was incubated at 65°C for 5 min with frequent vortex mixing, and the aqueous phase was separated from the organic phase by centrifugation. The aqueous layer was extracted once more with an equal volume of phenol/chloroform (1:1) and then with chloroform/isoamyl alcohol (24:1) before ethanol precipitation. The mRNA was purified from total RNA with an oligo (dT) selection step (Oligotex; QIAGEN, Chatsworth, CA, U.S.A.). Then, cDNA was synthesized by reverse-transcriptase.

Genomic DNA Extraction

Fifty ml of yeast cultures were grown in YEPD at 30°C with aeration until OD_{600} of 1.0. The cellular pellet was collected by centrifugation and washed twice with TE buffer (100 mM Tris-HCl, pH 8.0, and 10 mM EDTA) and then 3 ml of extraction buffer (100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2% SDS, and 1% 2-Mercaptoethanol), and 200 µl of zymolase 20,000 (2.5 mg/ml) were added to the washed pellet and incubated at 37°C for 1 h. The zymolase-treated cells were collected by centrifugation at 5,000 rpm for 5 min at 4°C. Three ml of TE buffer (50 mM Tris-HCl, pH 8.0, 50 mM EDTA) and 300 µl of 10% SDS were added to the collected cells and subsequently incubated at 65°C for 30 min. After centrifugation at 5,000 rpm for 10 min, the supernatant was transferred to a new tube containing 1 volume of phenol:chloroform (1:1). Eight µl of 50 µg/ml RNase A was added to the nucleic acid in the aqueous layer, and DNA was precipitated by mixing the upper solution with 2.5 volume of absolute ethanol and 0.1 volume of 3 M sodium acetate. Then, the DNA pellet was washed with 70% ethanol, air-dried, and resuspended in 200 µl of TE buffer.

Preparation of Probes

Probe with fluorescence dye from isolated mRNA was prepared as described previously [7]. Genomic DNA was labeled with a simple random-priming protocol based on BioPrime DNA Labeling system (Gibco/BRL) or a high concentration of Klenow fragment (Takara, Japan) with 20 µl of random primer/reaction buffer mix. After denaturation of genomic DNA in boiling-water for 5 min, it was placed on ice, and 5 µl of 10 µM dNTP mix, 3 µl of Cy5-dCTP (Amersham) or Cy3-dCTP, and 1 µl of Klenow Fragment (40–50 units/µl) were added. Cy5 and Cy3 were used to label cDNA of *S. cerevisiae* KNU5377 and that of S288C, respectively. It was then incubated at 37°C for 1–2 h, and the reaction was stopped by adding 5 µl of 0.5 M EDTA (pH 8.0) [20].

Hybridization with DNA Chip

For comparison of the gene expression pattern and genomic DNA homology between *S. cerevisiae* KNU5377 and *S. cerevisiae* S288C, DNA chip research INK (Yokohama, Japan) was used for both cDNA synthesized from mRNA and labeled genomic DNA. A mixture of Cy5- and Cy3-labeled probes was overlaid on the DNA chip, and they were incubated at 65°C for 48 h. After

hybridization, the solutions were removed, and the arrays were washed twice with 2× SSC/0.1% SDS for 20 min and then sequentially washed with 0.2× SSC/0.1% SDS, 0.2× SSC and 0.05× SSC, at room temperature (24 to 28°C) in the dark.

Southern Hybridization

Genomic DNA (10 μg) isolated from *S. cerevisiae* KNU5377 and *S. cerevisiae* S288C were digested with *Eco*RI (no-cut site in YHR053c, YLR155c, and YDR038c regions) and subjected to electrophoresis on 1.5% agarose gels. After electrophoresis, the gel was rinsed twice in distilled water and incubated at room temperature with gentle agitation in denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 30 min. This was followed by further incubation for 20 to 35 min with gentle agitation at room temperature in a neutralizing solution (1.5 M NaCl, 0.5 M Tris-HCl, pH 7.4). The gel was then transferred to 10× SSC for 10 min and capillary blotted overnight in 10× SSC. After blotting, the membrane was air-dried and baked for 2 h at 80°C to fix the membrane. Probes were labeled with ^{32}P-dCTP (20 μCi) using the Klenow fragment of DNA ploymerase (Takara Shuzo, Japan), according to the manufacturer's instructions. The labeled probes were purified on a Sephadex G-50 column and concentrated using a Microcon YM-30 concentrator (Amicon Millipore, Billerica, MA, U.S.A.). The membrane was hybridized with the probes using hybridization buffer (0.5 M church phosphate buffer, 1 mM EDTA, 7% SDS) according to the Church and Gilbert method [5].

Sensitivity Test to Copper

Both strains, *S. cerevisiae* KNU5377 and *S. cerevisiae* S288C, were grown in YEPD (pH 5.5) until the mid-log phase of an OD_{600} of 1.0 at 30°C with orbital shaker (200 rpm). Diluted cells (an OD_{600} of 0.3) were exposed to YEPD containing different concentrations of $CuCl_2$ (4 to 14 mM) for 0 to 4 h, and then 5 μl of each treated sample was spotted on the YEPD agar plate. After incubation at 30°C for 48 h, the plates were photographed.

Functional Characterization of Highly Expressed Genes by the Categories of MIPS

The biological characteristics of highly expressed ORFs were categorized according to the functional categories of the Munich International Center for Protein Sequences (MIPS) (http://mips.gsf.de/proj/yeast). In the MIPS database, yeast expression data have been released by several laboratories, and data from the projects will soon be made public: MIPS developed a processing routine for analyzing and presenting expression data from different sources. The genes were clustered according to their functional profiles.

RESULTS

Characterization of *S. cerevisiae* KNU5377 Strain by the Expression Levels of mRNA

As the first step for characterization of *S. cerevisiae* KNU5377, we analyzed its mRNA expression level by comparing with a reference strain, *S. cerevisiae* S288C that was used as a template for the DNA microarray. The average ratio values of intensity were used for data analysis. Ratios of intensity over 2.0 and below 0.5 were regarded as upregulation and downregulation, respectively. Of approximately 6,000 open reading frames (ORFs) investigated, 5,914 ORFs (95%) showed detectable transcript change in the exponentially growing cell; 550 genes showed an over 2-fold highly expressed transcription level. The most highly expressed gene was *CTR3*, which is responsible for copper transport, followed by three kinds of hypothetical genes (Table 1). According to the classified 16 MIPS categories, 75 ORFs belonged to the category of protein synthesis. This number corresponds to 21% of the total number of genes in this category (Fig. 1). This category constituted mainly ribosomal proteins and mitochondrial ribosomal proteins. This also contained additional 200 ORFs and 31 ORFs, which belonged to categories of cellular organization and transport facilitation, respectively (Fig. 1). In the case of downregulated genes, the categories of transport facilitation (21 ORFs) and ionic homeostasis (11 ORFs) were included (Fig. 2).

Comparison of Genomic DNA Homology between *S. cerevisiae* KNU5377 and *S. cerevisiae* S288C

DNA microarray technology could be also used to compare genomic DNA homology of the two strains. After cultivation to the mid-log phase at 30°C, the genomic DNA was purified from the above two strains and hybridized to DNA chips as described in Materials and Methods. Among 5,556 ORFs, 4,659 ORFs of KNU5377 showed a 0.8-fold to 1.2-fold intensity, compared with those of the S288C strain. However, 63 ORFs showed lower relative intensity, including the regions of YDR038c (*ENA5*), YHR053c (*CUP1*), and YLR155c (*ASP3a*). These are within the 4th, 8th, and 12th chromosome of *S. ceresiviae* cells (Fig. 3).

Detection of Suspected Genes by Southern Hybridization

To confirm the difference of chromosomal structure by Southern hybridization, we used the candidate ORFs. The regions around YLR155c, 157c, 158c, 159w, 160c, and 161w including the genes of the *ASP3a*, *b*, *c*, and *d* were not detected in *S. cerevisiae* KNU5377 (Fig. 4A). Members of the list of the lowest relative intensity genes including YHR053c, 054c, 055c, and 056c were detected only in *S. cerevisiae* S288C (Fig. 4B-h, i, j, and k), and YHR057c with relative intensity of 1.3 was detected at the same level in both strains (Fig. 4B-l). These results are well coincident

Table 1. List of genes highly expressed in *S. cerevisiae* KNU5377. Among a ratio of over 2.0, the highest upregulated 40 ORFs were listed by descending order.

ORF name	Gene name	Induction value	SD value	Discription
YLR411w	*CTR3*	21.26	9.76	Copper transporter
YGR154c		16.84	8.33	Strong similarity to hypothetical proteins YKR076w and YMR251w
YIL169c		9.24	3.27	Similarity to glucan 1,4-alpha-glucosidase and YAR066w
YAR068w		6.97	1.52	Strong similarity to hypothetical protein YHR214w-a
YIL162w	*SUC2*	5.52	2.70	Invertase (sucrose hydrolyzing enzyme)
YJR048w	*CYC1*	5.10	1.93	Iso-1-cytochrome c
YGR292w	*MAL1*	4.74	2.84	Maltase (alpha-D-glucosidase)
YPL265w	*DIP5*	4.38	1.11	Dicarboxylic amino acid permease
YDL168w	*SFA1*	4.33	0.98	Long-chain alcohol dehydrogenase (glutathione-dependent formaldehyde dehydrogenase)
YHR214w		3.74	1.39	Hypothetical protein
YDR533c	*HSP31*	3.57	2.00	Possible chaperone and cysteine protease with similarity to *E. coli* Hsp31 and *S. cerevisiae* Hsp32p, Hsp33p, and Sno4p
YBR173c	*UMP1*	3.54	1.15	20S proteasome maturation factor
YGR287c		3.44	1.26	Hypothetical protein
YPR194c	*OPT2*	3.39	0.95	Oligopeptide transporter
YOR150w	*MRPL23*	3.10	0.39	Mitochondrial ribosomal protein of the large subunit
YMR024w	*MRPL3*	3.06	0.92	Mitochondrial ribosomal protein of the large subunit
YPL218w	*SAR1*	3.06	0.63	Secretion-associated, Ras-related. Component of COPII coat of vesicles; required for ER to Golgi protein transport; GTP-binding protein of the ARF family
YGR082w	*TOM20*	3.05	0.33	20 kDa mitochondrial outer membrane protein import receptor
YDL061c	*RPS29B*	3.02	1.80	Ribosomal protein S29B (S36B) (YS29)
YMR203w	*TOM40*	3.01	1.07	Mitochondrial outer membrane protein; forms the outer membrane import channel
YLR056w	*ERG3*	2.91	1.34	C-5 sterol desaturase
YER009w	*NTF2*	2.82	1.32	Nuclear envelope protein
YER044c	*ERG28*	2.78	1.15	Endoplasmic reticulum membrane protein
YKL087c	*CYT2*	2.72	0.67	Cytochrome c1 heme lyase
YEL050c	*RML2*	2.72	0.93	Ribosomal L2 protein, mitochondrial
YLR046c		2.70	0.70	Hypothetical protein
YJR047c	*ANB1*	2.70	0.74	Anaerobically expressed form of translation initiation factor eIF-5A
YNL069c	*RPL16B*	2.69	0.33	N-terminally acetylated protein component of the large (60S) ribosomal subunit
YJL221c	*FSP2*	2.64	1.68	Homology to maltase(alpha-D-glucosidase)
YCL040w	*GLK1*	2.60	0.53	Glucokinase
YOR286w	*FMP31*	2.59	0.89	The authentic, non-tagged protein was localized to the mitochondria
YLR008c	*PAM18*	2.59	0.83	Constituent of the mitochondrial import motor associated with the presequence translocase
YGL068w	*MNP1*	2.57	1.12	Putative mitochondrial-nucleoid specific ribosomal protein
YLR043c	*TRX1*	2.56	0.66	Thioredoxin
YIR021w	*MRS1*	2.56	0.60	RNA splicing protein of the mitochondrial carrier (MCF) family
YEL034w	*HYP2*	2.55	0.79	Translation initiation factor eIF-5A
YMR202w	*ERG2*	2.53	1.05	C-8 sterol isomerase
YDR304c	*CYP5*	2.53	0.66	Cyclophilin D, Peptidyl-prolyl cis-trans isomerase D
YER095w	*RAD51*	2.52	0.59	RecA homolog; Rad51p colocalizes to ~65 spots with Dmc1p prior to synapsis (independently of ZIP1 and DMC1), and interacts with Rad52p and Rad55p by two-hybrid analysis; human Rad51p homolog interacts with Brca2 protein which has been implicated in causing breast cancer
YDR115w		2.51	0.52	Similarity to bacterial ribosomal L34 proteins

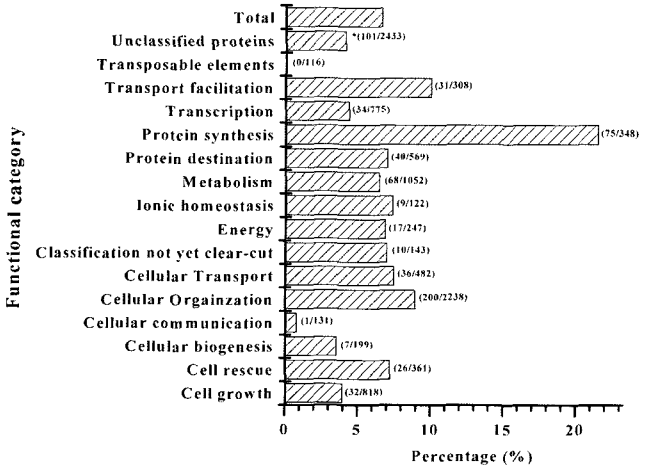

Fig. 1. Functional classification of ORFs in *S. cerevisiae* KNU5377 expressed over two-fold higher than those in *S. cerevisiae* S288C.

mRNA extracted from exponential-phase cultures of KNU5377 was labeled with Cy5 and those from S288C as control strain with Cy3, whose relative intensity of Cy5/Cy3 was calculated and filtered out below 2-fold one. On the 16 yeast functional categories, protein synthesis constituted the highest intensity. A percentage was defined as ORF number of the total number of the relevant functional category. *Number of 2-fold high expressed genes/total number included in each functional category.

with that of the DNA microarray (Fig. 3). However, against our expectations (Fig. 4C), YDR037w, 038c, 039c, and 040c were all detected in both strains, belonging to the lowest intensity group of DNA hybridization.

Although similar relative intensities detected by microarray analysis were shown in *S. cerevisiae* KNU5377, we could

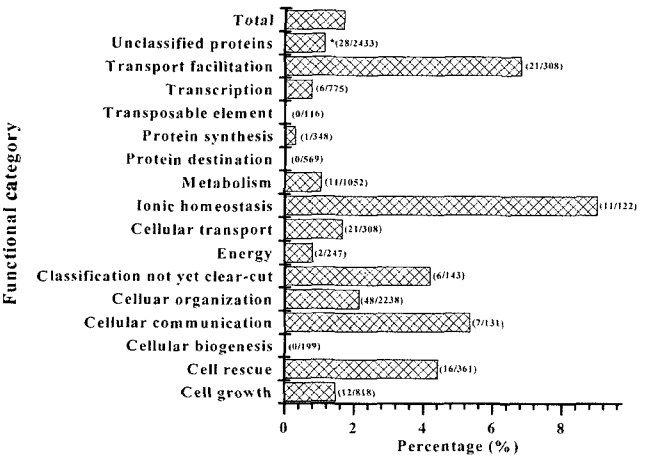

Fig. 2. Global gene expression profiling of less expressed ORFs in *S. cerevisiae* KNU5377 than that in *S. cerevisiae* S288C.

Chromosomal DNA isolated from *S. cerevisiae* KNU5377 was labeled with Cy5, and that of *S. cerevisiae* S288C was labeled with Cy3, whose labeled DNA samples were hybridized to one DNA chip. About 6,200 ORFs were distributed into 16 groups according to the relative intensity. *Number of weakly expressed genes/total number included in each functional category.

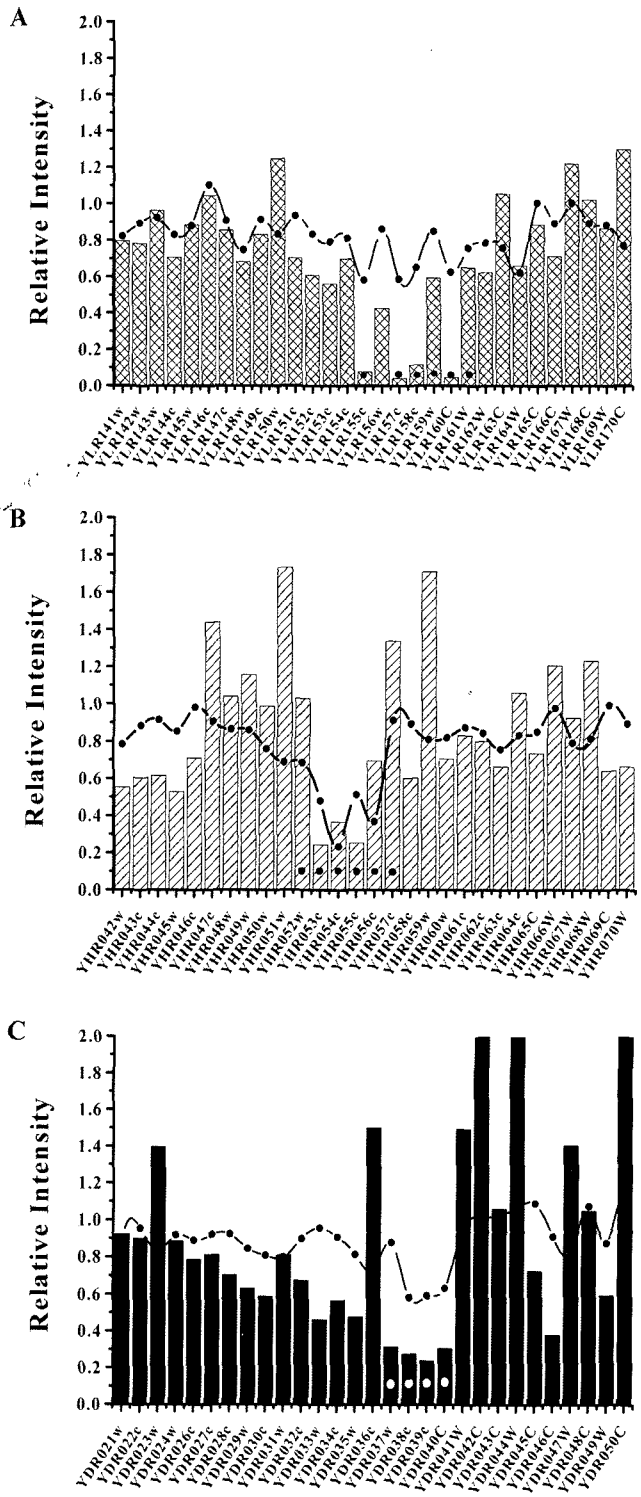

Fig. 3. Comparison between mRNA expression and genomic DNA hybridization.

In YLR155c (A), YHR054c (B), and YDR038c (C) regions, the relative intensity of mRNA expression was compared by genomic DNA hybridizaiton. Line and bar graphs indicate the relative intensity of genomic DNA and mRNA expression levels, respectively. ORFs, marked as spots in the middle of the bar graph, were confirmed by Southern hybridization.

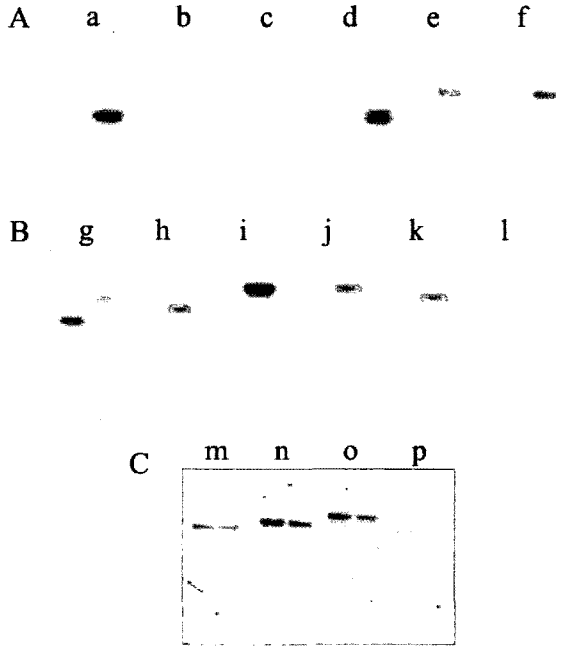

Fig. 4. Southern analysis confirmed the regions of the lowest relative intensity.
Genomic DNAs isolated from *S. cerevisiae* KNU5377 (left side on each panel) and S288C (right side on each panel) were digested with *Eco*RI and prepared for Southern blotting. Amplified probes for YLR155c regions (A), including YLR155c (a), YLR157c (b), YLR158c (c), YLR160c (d), YLR159w (e), and YLR161w (f), were hybridized onto the membrane. Two YHR054c (B) and YDR038c (C) regions were also analyzed. Lanes marked with g, h, i, j, k, l, m, n, o, and p indicate YHR052w, YHR053c, YHR054c, YHR055c, YHR056c, YHR057c, YDR037w, YDR038c, YDR039c, and YDR040c, respectively.

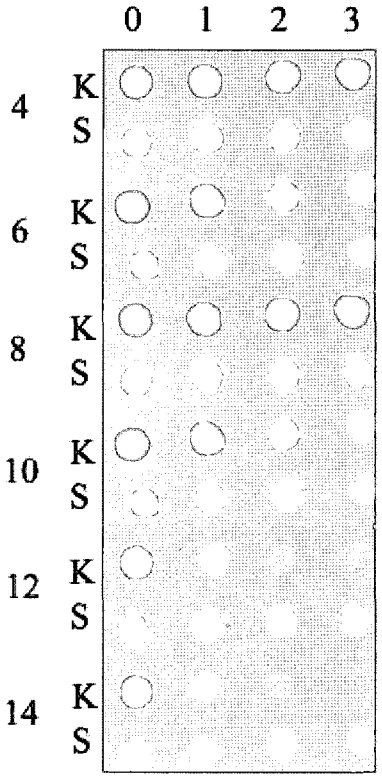

Fig. 5. Sensitivity test against copper.
S. cerevisiae KNU5377 (K) and *S. cerevisiae* S288C (S) cells from the exponentially growing phase (OD_{600}=1) were spotted onto the YEPD agar plates after exposure to 4 mM (A), 6 mM (B), 8 mM (C), 10 mM (D), 12 mM (E), and 14 mM (F) $CuCl_2$ concentrations for indicated times (0, 1, 2, and 3 h). Growth was monitored after 48 h at 30°C.

not detect the similar levels of positive signal by Southern blot. These differences might have been derived from low homology and high copy number of the region, when YDR038c in *S. cerevisiae* KNU5377 was compared with reference strain. Thus, we concluded that the regions of YLR155c (*ASP3a*) and YHR053c (*CUP1*) might be deleted in *S. cerevisiae* KNU5377 or have very low homology to that of *S. cerevisiae* S288C.

Asparagine Transport Mechanism of *S. cerevisiae* KNU5377

In *S. cerevisiae*, there are four L-asparaginase II genes, named *Asp3a*, *b*, *c*, and *d*, which are located in YLR155c, YLR157c, YLR158c, and YLR160c, respectively. None of these genes were detected in *S. cerevisiae* KNU5377 (Fig. 3A and Fig. 4A). These enzymes are located in the cell wall and mediate conversion of asparagine to aspartic acid by producing three different kinds of intermediates during nitrogen metabolism (http://mips.gsf.de/proj/yeast). It is possible that two alternatives, namely dicarboxylic amino acid permease (*DIP5*) and general amino acid permease (*GAP1*), take the place of L-asparaginases (Fig. 6).

According to DNA microarray analysis of mRNA, *DIP5* and *GAP1* of KNU5377 showed 4.4-fold and 2.0-fold higher expression levels than those of *S. cerevisiae* S288C (Fig. 6).

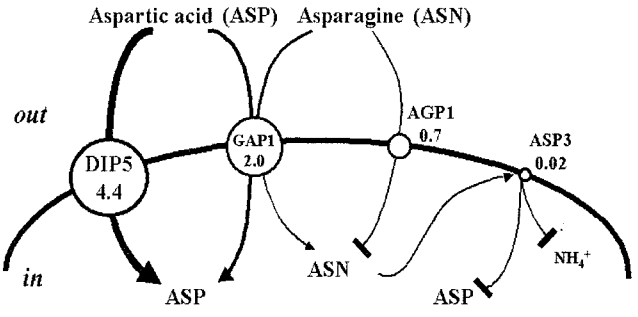

Fig. 6. Scheme of asparagine transport mechanism in *S. cerevisiae* KNU5377.
Asparagine did not seem to be hydrolyzed to aspartic acid and ammonium ion through *ASP3a*, *b*, *c*, and *d* in *S. cerevisiae* KNU5377. Alternatively, environmental aspartic acid and asparagines might be transported through *DIP5* and *GAP1*. Numbers indicate mRNA expression level under normal condition.

Table 2. List of genes lowly expressed in *S. cerevisiae* KNU5377. Of 82 genes with a value of less than 0.45, the lowest relative intensity of genomic DNA were summarized.

ORF name	Gene name	Induction value	SD value	Discription
YKL178c	*STE3*	0.033	0.013	A factor receptor
YML123c	*PHO84*	0.043	0.010	Inorganic phosphate transporter, transmembrane protein
YLR160c	*ASP3*	0.043	0.016	Nitrogen catabolite-regulated cell-wall L-asparaginase II
YLR157c	*ASP3*	0.051	0.020	Nitrogen catabolite-regulated cell-wall L-asparaginase II
YLR155c	*ASP3*	0.106	0.025	Nitrogen catabolite-regulated cell-wall L-asparaginase II
YLR040c		0.119	0.067	Hypothetical protein
YCR021c	*HSP30*	0.138	0.055	Protein induced by heat shock, ethanol treatment, and entry into stationary phase; located in plasma membrane
YLR158c	*ASP3*	0.139	0.059	Nitrogen catabolite-regulated cell-wall L-asparaginase II
YJL217w		0.148	0.036	Hypothetical protein
YNR075w	*COS10*	0.160	0.070	Protein with strong similarity to subtelomerically-encoded proteins such as Cos5p, Ybr302p, Cos3p, Cos1p, Cos4p, Cos8p, Cos6p, Cos9p
YMR319c	*FET4*	0.177	0.087	Low-affinity Fe(II) transport protein
YPL019c	*VTC3*	0.183	0.036	Phosphate metabolism; transcription is regulated by PHO system; polyphosphate synthetase (putative)
YOL158c	*ENB1*	0.185	0.128	Endosomal ferric enterobactin transporter
YDR481c	*PHO8*	0.206	0.048	Repressible alkaline phosphatase
YBR092c	*PHO3*	0.208	0.064	Acid phosphatase, constitutive
YOR153w	*PDR5*	0.234	0.125	Multidrug resistance transporter
YMR027w		0.245	0.058	High-level expression reduced Ty3 transposition
YHR055c	*CUP1*	0.246	0.214	Copper-binding metallothionein
YBR093c	*PHO5*	0.257	0.099	Acid phosphatase, repressible
YGR044c	*RME1*	0.264	0.061	Zinc finger protein; negative regulator of meiosis; directly repressed by a1-a2 regulator
YFL032w		0.274	0.061	Hypothetical protein
TDR039c	*ENA2*	0.288	0.069	Plasma membrane protein; putative Na+ pump; P-type ATPase
YKR042w	*UTH1*	0.289	0.062	YOUTH, involved in determining yeast longevity
YNR074c	*CPD1*	0.291	0.102	Putative reductase
YHR053c	*CUP1*	0.293	0.220	Copper-binding metallothionein
YFL014w	*HSP12*	0.294	0.178	12 kDa heat-shock protein
YOL164w		0.297	0.092	Hypothetical protein
YIL121w	*QDR2*	0.300	0.093	Multidrug transporter responsible for resistance to quinidine and barban; member of a family of drug:proton antiporters; plasma membrane transporter
YGL089c	*MF-Alpha2*	0.300	0.129	Alpha mating factor
YFL031w	*HAC1*	0.300	0.079	Basic ZIP (basic-leucine zipper) protein
YAR071w	*PHO11*	0.303	0.132	Acid phosphatase, secreted
YER073w	*ALD5*	0.304	0.187	Mitochondrial aldehyde dehydrogenase that is activated by K+ and utilizes NADP+ as the preferred coenzyme
YOR338w		0.309	0.070	Hypothetical protein
YMR320w		0.310	0.088	Hypothetical protein
YHR215w	*PHO12*	0.311	0.173	Acid phosphatase, nearly identical to Pho11p
YKL185w	*ASH1*	0.315	0.057	Zinc-finger inhibitor of HO transcription
YLR265c	*NEJ1*	0.331	0.074	Nonhomologous End-Joining regulator 1
YDR038c	*ENA5*	0.333	0.137	P-type ATPase involved in Na+ efflux
YKL177w		0.334	0.148	Hypothetical protein
YHR136c	*SPL2*	0.336	0.134	17 kDa protein
YPL250c	*ICY2*	0.341	0.233	Protein that interacts with the cytoskeleton and is involved in chromatin organization and nuclear transport, interacts genetically with TCP1 and ICY1; potential Cdc28p substrate
YOR273c	*TPO4*	0.344	0.124	Polyamine transport protein

Table 2. Continued.

ORF name	Gene name	Induction value	SD value	Discription
YNL066w	*SUN4*	0.346	0.075	Protein involved in the aging process
YER037w	*PHM8*	0.347	0.104	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p
YOR303w	*CPA1*	0.350	0.174	Carbamoyl phosphate synthetase, arginine specific
YCR039c	*ALPHA2*	0.352	0.164	Mating type regulatory protein, expressed copy at MAT locus
YER150w	*SPI1*	0.361	0.101	Protein with similarity to Sed1p
YOR302w		0.362	0.180	CPA1 uORF, Arginine attenuator peptide, regulates translation of the CPA1 mRNA
YOL152w	*FRE7*	0.364	0.147	Putative ferric reductase with similarity to Fre2p
YGL255w	*ZRT1*	0.368	0.174	High-affinity zinc transporter of the plasma membrane
YBL016w	*FUS3*	0.368	0.149	Cdc2+\/CDC28 related kinase with positive role in conjugation
YDR040c	*ENA1*	0.370	0.125	Plasma membrane protein; putative Na+ pump; P-type ATPase; Ca2+ ATPase
YER130c		0.373	0.089	Hypothetical protein
YJR004c	*SAG1*	0.374	0.341	Alpha-agglutinin
YFL004w	*VTC2*	0.374	0.066	Phosphate metabolism; transcription is regulated by PHO system; polyphosphate synthetase (putative)
YBR158w	*CST13*	0.380	0.169	Chromosome stability protein
YJL012c	*VTC4*	0.383	0.097	Phosphate metabolism; transcription is regulated by PHO system; polyphosphate synthetase (putative)
YBR293w		0.383	0.224	Similarity to multidrug resistance proteins
YDR037w	*KRS1*	0.383	0.141	Lysyl-tRNA synthetase
YJL116c	*NCA3*	0.386	0.111	With NCA2
YPR156c	*TPO3*	0.389	0.175	Polyamine transport protein
YGR250c		0.390	0.057	Hypothetical protein
YHR138c		0.392	0.129	Hypothetical protein
YNL087w	*TCB2*	0.406	0.086	Bud-specific protein with a potential role in membrane trafficking
YOL059w	*GPD2*	0.410	0.092	Glycerol-3-phosphate dehydrogenase (NAD+)
YDR127w	*ARO1*	0.413	0.133	Pentafunctional arom polypeptide (contains: 3-dehydroquinate synthase, 3-dehydroquinate dehydratase (3-dehydroquinase), shikimate 5-dehydrogenase, shikimate kinase, and epsp synthase)
YLR414c		0.415	0.111	Protein of unknown function
YFR056c		0.416	0.080	Hypothetical protein
YOL016c	*CMK2*	0.422	0.078	Calmodulin-dependent protein kinase
YOL109w	*ZEO1*	0.424	0.299	Peripheral membrane protein of the plasma membrane that interacts with Mid2p
YOL130w	*ALR1*	0.428	0.064	Aluminium Resistance 1
YBR169c	*SSE2*	0.429	0.147	HSP70 family member, highly homologous to Sse1p
YBR069c	*VAP1*	0.430	0.036	Amino acid permease
YOR212w	*STE4*	0.440	0.163	Beta subunit of G protein coupled to mating factor receptor
YBR182c	*SMP1*	0.441	0.146	MADS-box transcription factor
YLR237w	*THI7*	0.446	0.033	Thiamine transporter
YJR015w		0.447	0.118	Hypothetical protein
YHR018c	*ARG4*	0.449	0.383	Argininosuccinate lyase
YHR054c		0.450	0.210	Hypothetical protein
YNL046w		0.451	0.121	Hypothetical protein
YPR127w		0.455	0.133	Hypothetical protein
YOR161c	*PNS1*	0.458	0.194	Protein of unknown function
YDR046c	*BAP3*	0.465	0.144	Valine transporter
YGR032w	*GSC2*	0.474	0.090	Catalytic component of 1,3-beta-D-glucan synthase
YHR071w	*PCL5*	0.477	0.461	PHO85 cyclin
YNL104c	*LEU4*	0.479	0.202	Alpha-isopropylmalate synthase (2-isopropylmalate synthase)
YLR286c	*CTS1*	0.496	0.198	Endochitinase
YBR068c	*BAP2*	0.496	0.205	Probable amino acid permease for leucine, valine, and isoleucine

Sensitivity Test to Copper

CUP1a and *CUP1b*, cysteine-rich and copper-binding metallothioneins encoded by the genes of the YHR053c and YHR055c regions, showed the lowest relative intensity of genomic DNA hybridization (Fig. 3B): The relative expression level of *CUP1a* and *CUP1b* was 0.25–0.3 (Table 2), and the relative intensity of genomic DNA was about 0.5 (Fig. 3B). Additionally, those genes were faintly detected by Southern analysis (Fig. 4B-h and j).Therefore, the resistance of KNU5377 to copper was assayed by treatment of its exponentially growing cells with $CuCl_2$ as described in Materials and Methods. The result indicated that the reference strain S288C at 14 mM $CuCl_2$ could survive for 2 h, whereas KNU5377 could survive for only 1 h at 12 mM (Fig. 5).

DISCUSSION

In our study, we characterized the genome-wide profiling of the strain of *S. cerevisiae* KNU5377, and found it different from that of S288C type culture. These data are expected to provide powerful and important clues for this strain breeding in industrial application. Therefore, it is quite possible that we can construct a strategy for the breeding of this strain according to its special genetic background using genome-wide characterization.

Genes related to protein synthesis and fate were strongly upregulated in *S. cerevisiae* KNU5377, suggesting that this stain may have strong capacity to synthesize some proteins, thereby quickly enabling cells to adjust their genomic expression program against environmental changes. Therefore, *S. cerevisiae* KNU5377 might be able to rapidly adapt to new conditions and effectively maintain their ability to survive without growth arrest [25].

Broadly, there are two systems that affect the uptake of all naturally occurring amino acids in yeast [26]: one is referred to as the general amino acid permease, or GAP, and the other system is related with DIP5, dicarboxylic amino acid permease, which displays the specificity for one or a small number of related amino acids, and is moderately regulated by nitrogen source [3]. L-Asparaginase II genes, named *Asp3a*, *b*, *c*, and *d*, were not detected in *S. cerevisiae* KNU5377 (Fig. 3A and Fig. 4A), indicating that this strain should have different transport systems for amino acid uptake. Historically, *S. cerevisiae* strains isolated from the nature have been grouped into the copper resistance; therefore, all of the Cu-resistant strains were named as CUP1 strains. The strain of *S. cerevisiae* S288C, a reference in this study, had been grouped as the copper-resistant strain. However, our study revealed that *S. cerevisiae* KNU5377 should be included in the copper-sensitive group, because both intensity of genomic DNA hybridization and relative expression level of *CUP1a* and *CUP1b* were extremely low in *S. cerevisiae* KNU5377 (Table 2, Fig. 3B).

It is very interesting to find that the most highly expressed gene in this KNU5377 strain was *CTR3* and this strain did not have sufficient metallothioneins. This mechanism remains unclear, suggesting that the genes related to uptake of copper ions were highly expressed, but metallothionein might not completely trap the bound copper ions. According to the strain-specific 21-fold higher expression of *Ctr3* in *S. cerevisiae* KNU5377 than the reference type strain *S. cerevisiae* S288C, copper ions should be well transported into the cell by this transport protein. In *S. cerevisiae* KNU5377, high affinity of a plasma membrane copper ion permease, *Ctr3*, was definitely inhibited under copper deficient conditions, whose product was also depressed in cellular uptake of ions [27]. Although copper-binding metallothionein does not play enough a role to detoxify excess copper, CTR3 was highly expressed under not-stressful copper condition, suggesting that the basal level of copper in *S. cerevisiae* KNU5377 was steadily maintained by its role as a cofactor for copper-metalloenzyme such as cytochrome c oxidase and superoxide dismutase [19]. However, the sensitivity of *S. cerevisiae* KNU5377 against cellular copper level under stressful condition might be derived from an insufficient copper detoxification system of CUP1, which plays an important role in protection under copper cytotoxicity.

In the present study, *S. cerevisiae* KNU5377 was found to have transporter systems for nutrient uptake different from that of the reference *S. cerevisiae* S288C. The PHO regulatory pathway, regulated by extracellular inorganic phosphate concentration, is involved in the acquisition of phosphate, which is an essential nutrient in the cell. *S. cerevisiae* KNU5377 has low mRNA level of inorganic phosphate transporter [4], PHO84, which is regulated by the PHO regulation system responding to extracellular phosphate level [2, 4, 21]. In addition, Fe transport protein, which may easily enable *S. cerevisiae* KNU5377 to resist various stresses, also occurs during ethanol production [17, 25].

It is said that medical care is now in the stage of "Order made". Similarly, we can also say that breeding is in the stage of "order-made-breeding"; for example, for ethanol production by endowing a thermo- or cold-tolerance to wine yeast, or cryotolerance or osmotolerance to baker's yeast. Therefore, our data are undoubtedly expected to give an important clue for improving the resistance of common yeasts against various stress conditions.

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