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Original Article

A screening method for growth-dependent genes involved in the cytotoxicity of chemicals in *Saccharomyces cerevisiae*

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ABSTRACT — When considering mechanisms of toxicity development to chemical substances, one potentially important mechanism is the selective inhibition of proteins essential for cell growth (target molecules). In this study, to detect the target molecules of chemical substances, we established a method for comprehensively screening for essential proteins that confer resistance against chemical substances via overexpression in yeast. We used budding yeast, a common eukaryotic model organism, to produce yeast strains showing overexpression of different genes encoding essential proteins. This method was used to search for overexpressed genes conferring arsenite resistance in yeast, and as a result, we successfully identified ten types of new genes correlated with arsenite resistance.

Key words: Screening method, Essential genes, Arsenite, Resistance, Yeast

INTRODUCTION

Although environmental contamination resulting from the release of chemical substances has been increasing (Gueguen *et al.*, 2011; Jarup, 2003), there have been few chemical substances whose mechanisms of toxicity development have been clarified. It is possible that there are target molecules (essential proteins for growth) showing specificity to chemical substances *in vivo*, and that these chemical substances can selectively interfere with such target molecules and result in cellular toxicity.

In cells with increased quantities of target molecules, the level of a chemical substance needed to inhibit the activity of target molecules is higher than in normal cells. Consequently, cells showing overexpression of target molecules will demonstrate resistance against the chemical substance. Therefore, using budding yeast (*Saccharomyces cerevisiae*), we attempted to establish a method to comprehensively screen for essential proteins that confer resistance against chemical substances in cells via overexpression. Budding yeast has been widely used as a eukaryotic model organism in basic research since it is easily cultured and can be manipulated via genetic engineering (Friedberg, 1991; Trivedi *et al.*, 1985; Wells and Fridovich-Keil, 1996). In this study we considered yeast the most appropriate and useful organism for conducting this type of screening.

MATERIALS AND METHODS

Construction of a pKT10-based essential gene expression system

For construction of the essential gene vector, the essential gene was amplified via PCR, using the yeast open reading frame (ORF) collection (Open Biosystems, Huntsville, AL, USA) as the template. The following oligonucleotides were used as primers: 5'-GAAGGAATTATCAACAAGTT-3' and 5'-ACCAGCATAATCAGGAACATC-3'.

These primers were designed to amplify an ORF fused onto the C-terminus to a six-histidine tag (6×His) and a hemagglutination (HA) tag. The amplified DNA was subsequently inserted into the pKT10-GAPDH (*URA3*) expression vector. Essential gene expression vector libraries were introduced into the BY4742 strain (*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0*) using the lithium acetate procedure (Hwang *et al.*, 2013).

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Selection of essential genes that confer resistance to arsenite using a pkT10-based expression system

Yeast cells carrying pKT10 were cultured in synthetic dextrose (SD) liquid media without uracil (-Ura) in 96-well plates for 48 hr at 30°C. Each culture was diluted to 1/40 with SD medium, and the portions (5 μL) were transferred into 195 μL SD liquid media that contained 1.5 mM sodium arsenite in 96-well plates. After incubation for 48 hr, yeast strains that exhibited increased growth were identified as potential arsenite-resistant strains.

Selection of essential genes that confer resistance to arsenite using a BG1805-based expression system

Yeast cells carrying BG1805 were cultured in synthetic raffinose (SR) liquid media without uracil (-Ura) in 96-well plates for 48 hr at 30°C. Each culture was diluted to 1/40 with SR media and the portions (5 μL) were transferred into 175 μL synthetic galactose (SG) liquid media containing 2% raffinose (+Raf). After incubation for 2 hr, 20 μL of sodium arsenite (final 1.5 mM) was added to each culture. After incubation for 48 hr, yeast strains that exhibited increased growth were identified as potential arsenite-resistant strains.

Immunoblotting

Immunoblotting was performed as previously described (Takahashi *et al.*, 2005). Yeast cell lysates were prepared via homogenization with glass beads. Cell lysates were separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and transferred to an Immobilon-P transfer membrane (Millipore, Bedford, MA, USA). Immunoblotting was carried out using anti-HA monoclonal antibodies (Roche, Basel, Switzerland) and peroxidase-conjugated goat anti-rat immunoglobulins (Dako A/S, Glostrup, Denmark) as primary and secondary antibodies, respectively. Immunoreactive proteins were detected via a chemiluminescent system using Immobilon Western Chemiluminescent HRP Substrate (Millipore).

RESULTS AND DISCUSSION

The budding yeast genome encodes about 6,000 types of genes, of which approximately 1,000 genes are essential for yeast growth. Therefore, using budding yeast strains showing overexpression of genes essential for cell growth (essential gene-overexpressed yeast strain library), we aimed to establish a method to comprehensively screen for essential genes conferring resistance against chemical substances in yeast via overexpression.

First, we examined a screening method using a gene expression vector library (yeast ORF collection) by Open Biosystems, Inc. This yeast ORF collection is a library of *Escherichia coli* containing the BG1805 vector with each gene cloned from the yeast genome (about 5,500 types). To verify the usefulness of the BG1805 vector, we selected arsenite, an inorganic arsenic compound, as a test compound, and then transducted the BG1805 vector with the *ARR3* gene inserted (BG1805-*ARR3*) into yeast; *ARR3* is known to confer resistance against arsenite in yeast via overexpression (Maciaszczyk-Dziubinska *et al*., 2010). BG1805 contains the target gene, such as *ARR3*, integrated downstream of the *GAL1* promoter; expression of the target gene is induced by galactose. When expression of *ARR3* was induced during incubation of yeast containing BG1805-*ARR3* in the SG (-Ura) culture medium containing galactose, the sensitivity of yeast to arsenite was shown as almost the same as the control treatment (an empty vector introduced into yeast), demonstrating no resistance (Fig. 1A). The BG1805 vector is designed to add an approximately 19 kDa tag (6×His, HA, protease 3C and protein A (ZZ domain)) onto the C-terminus of the ORF. Because of the relatively large tag added, some functions of the gene product would possibly be lost. Thus, removing the sequence of protease 3C and protein A from the BG1805-*ARR3* vector, parts of *ARR3*, 6×His and HA were amplified via PCR to perform subcloning in pKT10, a plasmid having the *GAPDH* promoter constitutively functioning under the presence of glucose, to produce the pKT10 vector for *ARR3* overexpression (pKT10-*ARR3*). This *ARR3*-overexpressed yeast with the pKT10-*ARR3* vector introduced demonstrated higher resistance against arsenite compared to the control treatment, as shown in Fig. 1B. Next, for 961 types of essential genes in the BG1805 vector library, PCR amplification was performed only for the ORF as well as 6×His and HA in the BG1805 vector, to prepare the new gene expression vector by inserting it into the pKT10 vector. As a result, preparation of the pKT10 vector for some genes was not successful for various reasons, but the pKT10 vector was successfully produced with each of the 869 essential genes inserted. Having produced each gene expression vector using the BG1805-based expression system (961 types) and the pKT10-based expression system (869 types) introduced into BY4742 yeast strains, we obtained culturable yeasts of 961 strains and 809 strains, respectively. Yeasts with vectors introduced via the pKT10-based expression system were not culturable for 60 out of 869 types of essential genes. We then screened for essential genes that potentially conferred arsenite resistance on yeast, by examining each strain for sensitiv-

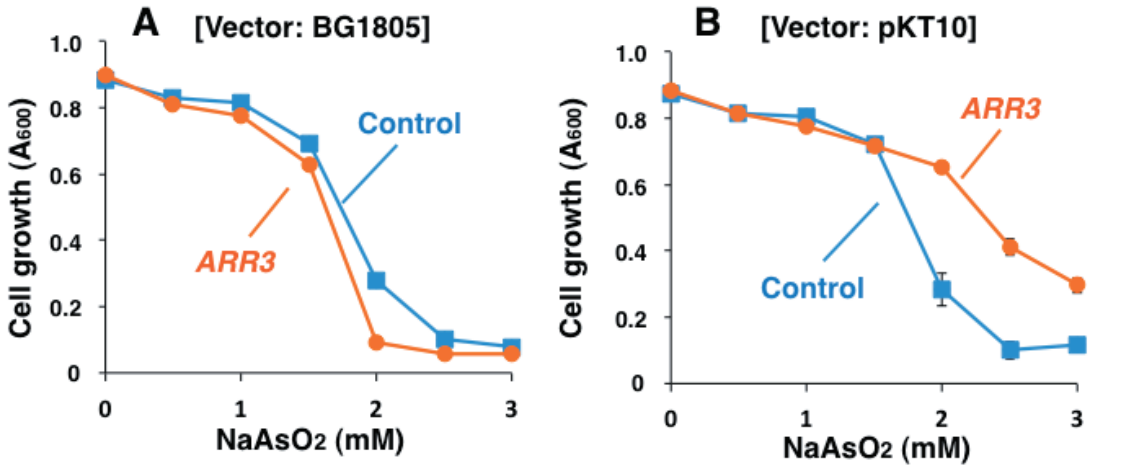

Fig. 1. Sensitivity to arsenite for yeast cells carrying BG1805-*ARR3* or pKT10-*ARR3*. Yeast cells (1 × 10^4 cells/ 200 µL) carrying BG1805-*ARR3* or BG1805 (control) were grown in synthetic galactose (SG) media containing 2% raffinose (+Raf) without uracil (-Ura) for 2 hr and added sodium arsenite (A). Yeast cells (1 × 10^4 cells/ 200 µL) carrying pKT10-*ARR3* or pKT10 (control) were grown in SD (-ura) liquid media with sodium arsenite (B). After a 48-hr incubation, absorbance at 600 nm was measured using a spectrophotometer. Each point represents the mean value of results from four cultures with SD (bars). The absence of a bar indicates that the SD falls within the symbol.

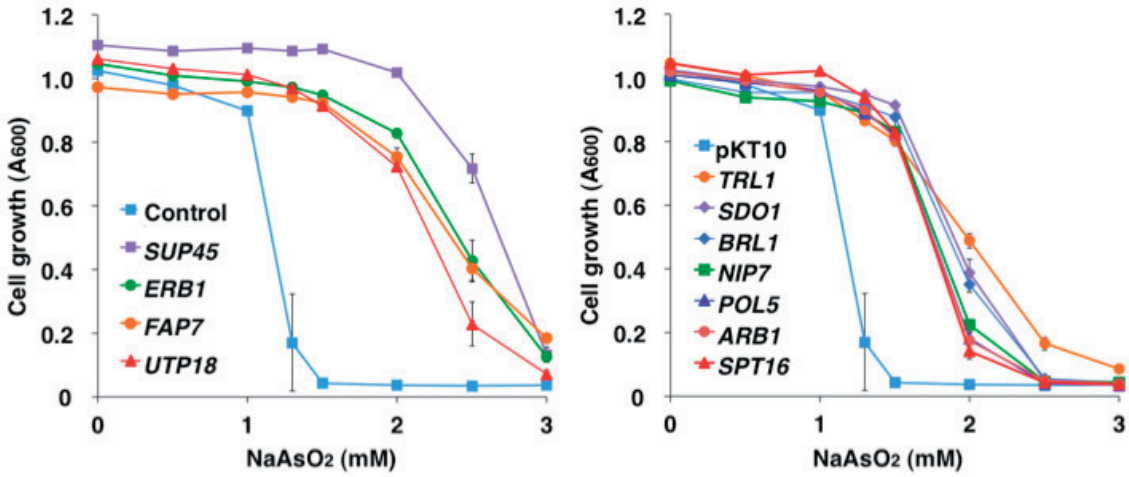

Fig. 2. Effects of overexpression of essential genes on the sensitivity of yeast cells to arsenite. Yeast cells carrying essential gene expression vectors or empty vectors (pKT10) were grown in synthetic dextrose (-Ura) media containing sodium arsenite for 48 hr. For further details, refer to Fig. 1 legend.

ity to arsenite. As a result, two types of arsenite resistance genes, *FAP7* and *ERB1*, were identified through screening using BG1805-based expression system vectors (data not shown). In addition, during screening using pKT10-based expression system vectors, 11 types of arsenious acid resistance genes were identified, including *FAP7* and *ERB1* (Fig. 2). For these yeast strains showing arsenite resistance (11 types), expression of target gene products was detected in all strains via immunoblotting using anti-HA monoclonal antibodies (data not shown). The present study identified a role for the *FAP7* gene in conferring arsenite resistance in yeast, confirming findings from our previous study (Takahashi *et al.*, 2010). However, all other identified genes had not previously been reported to

Table 1. Essential genes whose overexpression causes resistance to arsenite.

Gene name	ORF	Function	Reference
SUP45	*YBR143C*	Polypeptide release factor (eRF1) in translation termination	(Stansfield *et al.*, 1995)
ERB1	*YMR049C*	Constituent of 66S pre-ribosomal particles	(Pestov *et al.*, 2001)
FAP7	*YDL166C*	Essential NTPase required for small ribosome subunit synthesis	(Granneman *et al.*, 2005)
UTP18	YJL069C	Small-subunit processome protein involved in pre-18S rRNA maturation	(Bernstein *et al.*, 2004)
TRL1	*YJL087C*	tRNA ligase required for tRNA splicing	(Abelson *et al.*, 1998)
SDO1	*YLR022C*	Guanine nucleotide exchange factor (GEF) involved in ribosome maturation	(Savchenko *et al.*, 2005)
NIP7	*YPL211W*	Nucleolar protein required for 60S ribosome subunit biogenesis	(Zanchin *et al.*, 1997)
ARB1	*YER036C*	ATPase of the ATP-binding cassette family involved in 40S and 60S ribosome biogenesis	(Dong *et al.*, 2005)
SPT16	*YGL207W*	Subunit of the heterodimeric FACT complex (Spt16p-Pob3p)	(Belotserkovskaya *et al.*, 2003)
BRL1	*YHR036W*	Essential nuclear envelope integral membrane protein	(Saitoh *et al.*, 2005)
POL5	*YEL055C*	DNA Polymerase φ	(Yang *et al.*, 2003)

have any relationship with the toxicity of chemical substances. From these results, our screening method using the pKT10-based vector expression system can be considered useful and reliable in identifying unknown essential genes associated with the development of toxicity to chemical substances.

Of the identified genes showing an association with arsenious acid resistance, *ERB1*, *UTP18*, *SDO1*, *NIP7*, *ARB1*, and *FAP7* are coding proteins that contribute to ribosome biosynthesis (Table 1) (Woolford and Baserga, 2013). In addition, *SPT16* is the gene encoding the subunit of the FACT complex relating to chromatin structural change and the *POL5* gene encodes DNA Polymerase φ; both genes are involved with rRNA transcription (Johnson *et al.*, 2013; Shimizu *et al.*, 2002), suggesting the possibility of involvement with ribosome biosynthesis. It was recently reported that arsenite can remarkably decrease intracellular levels of ribosomes in cultured human cells (Wehner *et al.*, 2010). Therefore, arsenite could possibly suppress functions of these gene products, resulting in inhibition of ribosome biosynthesis and cytotoxicity.

Using the screening method established in our study to identify unknown essential genes involved with the development of toxicity to chemical substances, we can expect to clarify the target molecules of chemical substances and the mechanism of toxicity development in future studies.

Conflict of interest---- The authors declare that there is no conflict of interest.

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