INTRODUCTION

Arsenic is a trace element ubiquitously present in the environment. The excessive intake of arsenic compounds causes health problems, such as skin pigmentation and cancers (Alam et al., 2002; Ratnaike, 2003). The molecular mechanisms underlying the manifestation of arsenic toxicity include the inhibition of enzyme activity through reactions with the thiol group of various enzymes (Petrick et al., 2001; Shi et al., 2004), increased production of reactive oxygen species (Jomova et al., 2011), and apoptosis induction through the activation of a signal transduction mechanism (Kumagai and Sumi, 2007; Sumi et al., 2010). However, the detailed mechanisms that mediate arsenic toxicity remain unclear.

The budding yeast, Saccharomyces cerevisiae, has been used as a eukaryotic model in various molecular biological studies (Breitkreutz et al., 2010; Tarassov et al., 2008). A gene deletion yeast library has been used for the screening of genes involved in sensitivity to various chemicals, such as arsenic compounds (Thorsen et al., 2009; Aouida et al., 2004; Zhou et al., 2009). However, genes essential for survival have barely been examined. Thus, we herein explored genes that influenced sensitivity to arsenite, a trivalent inorganic arsenic compound that is among those essential for yeast survival.

MATERIALS AND METHODS

Yeast strains and media

The Decreased Abundance by mRNA Perturbation (DAmP) yeast library (Open Biosystems, Huntsville, AL, USA) shows the decreased expression of each essential gene through mRNA perturbation (Breslow et al., 2008). This library contains essential gene hypomorphic alleles whereby the 3’ untranslated region (UTR) of each essential gene in the collection has been disrupted with an antibiotic resistance cassette. The DAmP yeast strains and the parental strain BY4741 (MATα, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0) were grown in yeast extract peptone-dextrose (YAPD) medium or in synthetic dextrose (SD) medium.

Screening for arsenite-hypersensitive yeast strains through the knockdown of essential genes

The DAmP yeast strains with knockdown of essential genes were cultured in YPAD medium (120 μl) in 96-well plates for 48 hr at 30°C. Each culture was diluted 1/40 with SD medium, and the aliquots (5 μl) were transferred to fresh SD medium (195 μl) containing 1.5 mM sodium arsenite in 96-well plates for 48 hr. Under these culture conditions, the parental strain BY4741 exhibited increased growth. After a 48 hr incubation, deletion mutants not exhibiting increased growth were identified as candidates for designation as arsenite-hypersensitive mutants.
Measurement of the sensitivity of yeast cells to arsenite

For spot assays using agar-solidified SD medium, a suspension of yeast cells was spotted onto a plate of agar-solidified SD medium with or without sodium arsenite. Plates were photographed after incubation for 48 hr at 30°C.

Confirmation of knockdown efficiency using quantitative real-time PCR

Isolation of total RNA from yeast cells was performed as described by Furuchi et al. (Furuchi et al., 2004). cDNAs were prepared from total RNA using the PrimeScript™ RT reagent kit (Takara, Shiga, Japan) (Hwang et al., 2011). We performed quantitative real-time PCR using the Thermal Cycler Dice Real Time system (Takara) and SYBR Premix EX Taq (Takara). The oligonucleotide sequences of the primers used for quantitative PCR were as follows: sense, 5'- CAAGCGTGTCGTGGAATATG -3', and antisense, 5'- CTTGATTTGTCTAGCCATGG -3' for YPT1; sense, 5'- ATCTGGTGCCGATATCGAAC -3', and antisense, 5'- GCCTGATAACACCAGCAC -3' for ERG8; sense, 5'- GAGGCAAGGGTTTCCATTG -3', and antisense, 5'- GCTTTAGCAGAACCTCCTTG -3' for RKI1; and sense, 5'- TTGGATTCCGGTGATGGTTACT -3', and antisense, 5'- TGAAGAAGATTGACGCGGTTTG -3' for ACT1. The mRNA levels were normalized to those of ACT1.

RESULTS AND DISCUSSION

Essential genes for yeast survival were screened to identify those involved in arsenite sensitivity. A yeast strain library, in which 878 of about 1,000 genes essential for the survival of budding yeast were suppressed through 3' UTR disruption, was used for screening. In this library, a kanamycin resistance gene is inserted between the termination codon and terminator of each gene in the genome to destabilize mRNA and suppress gene expression (Breslow et al., 2008). The knockdown yeast strains, in which the expression of each gene was suppressed, were cultured with arsenite at a concentration that allowed the growth of the parent strain BY4741. Then, arsenite-sensitive strains, which showed no growth even at 48 hr, were obtained. The screening demonstrated that yeasts with YPT1, ERG8, or RKI1 knockdown were more sensitive than wild-type yeast (Fig. 1A). Gene expression levels in the knockdown yeast strains were examined by real-time PCR. Gene expression levels were suppressed by about 70, 60, and 80% in YPT1, ERG8, and RKI1 knockdown yeast strains, respectively (Fig. 1B). The YPT1 gene encodes Rab family GTPase in the intracellular vesicular transport pathway (Benito-Moreno et al., 1994; Miaczynska et al., 2001). The ERG8 gene encodes phosphomevalonate kinase in the mevalonate pathway (Tsay and Robinson, 1991). The RKI1 gene encodes ribose-5-phosphate ketol-isomerase in the pentose phosphate pathway (Kondo et al., 2004). None of the genes identified by this screening have been examined regarding their relationships with arsenic toxicity. Thus, examining the relationships between these genes and arsenite toxicity may reveal unknown mechanisms of arsenic toxicity.

REFERENCES

Benito-Moreno, R.M., Miaczynska, M., Bauer, B.E., Schweyen, R.J. and Ragnini, A. (1994): Mr6p, the yeast homologue of the
Identification of three novel arsenite-resistant genes in budding yeast


