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A screening for essential cell growth-related genes involved in arsenite toxicity in *Saccharomyces cerevisiae*

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ABSTRACT — Genes that are essential for growth in yeast were screened to identify those involved in arsenite sensitivity. We found that the knockdown of *YPT1*, *ERG8*, or *RKI1* enhanced arsenite sensitivity in yeast.

Key words: Arsenite, Yeast, YPT1, ERG8, RKI1

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; Ratnaik, 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 (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*) were grown in yeast extract peptone-adenine-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 Prime-Script™ 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'-CAAGCGTGTCTGGAATATG-3', and antisense, 5'-CCTTGATTTGTCTAGCCATGG-3' for *YPT1*; sense, 5'-ATCTGGTGCCGATATCGAAC-3', and antisense, 5'-GCGTCATAACCACCAGCACC-3' for *ERG8*; sense, 5'-GAGGCAAGGTGTTCCCATTG-3', and antisense, 5'-GCTTTAGCAGAACCTCCTTGTC-3' for *RK11*; and sense, 5'-TTGGATTCCGGTGATGGT-GTTACT-3', and antisense, 5'-TGAAGAAGATTGAG-CAGCGGTTTG-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 *RK11* 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 *RK11* knockdown yeast strains, respectively (Fig. 1B). The *YPT1*

Fig. 1. Effects of knockdown of *YPT1*, *ERG8*, or *RK11* on the sensitivity of yeast cells to arsenite. (A) Yeast cells with knockdown (kd) of *YPT1*, *ERG8*, or *RK11* were serially diluted in liquid-SD medium and spotted on agar-solidified SD medium prepared with and without 1.5 mM arsenite. The plates were photographed after incubation for two days at 30°C. The data are representative of three independent experiments. (B) The mRNA levels for each of the three genes in the yeast cells were analyzed using quantitative PCR. For comparison of mRNA expression, the mRNA level for each gene was normalized to that of *ACT1*.

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 *RK11* 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.

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