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Overexpression of *RPN8, SKP1, MIA40* or *MES1* increases resistance to cadmium in *Saccharomyces cerevisiae*

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ABSTRACT — We screened for genes associated with cadmium resistance among genes essential for cell growth in yeast. Four novel genes, *RPN8*, *SKP1*, *MIA40* and *MES1*, were identified as genes providing cadmium resistance to yeast via overexpression.

Key words: Cadmium, Yeast, *RPN8*, *SKP1*, *MIA40*, *MES1*

INTRODUCTION

Cadmium is one of the harmful heavy metals commonly found in the environment. Proximal tubule disorder is well known to be the major chronic toxicity of cadmium (Jarup *et al*., 1998; Nordberg, 2009). Endoplasmic reticulum stress and apoptosis induction via mitochondria have been reported as the mechanism of cadmium toxicity at the molecular level (Gobe and Crane, 2010; Thevenod and Lee, 2013); however, precise details regarding the development mechanism of this toxicity remain unclear.

Budding yeast (*Saccharomyces cerevisiae*) is a versatile eukaryote model used in many molecular biology studies. Making use of gene deletion libraries and gene expression libraries for yeast, genes affecting the determination of cadmium sensitivity have been screened (Hwang *et al*., 2009; Thorsen *et al*., 2009). However, few screenings to date have targeted genes essential for cell growth in yeast. In this study, we conducted screenings for gene clusters affecting the sensitivity of yeast to cadmium, using our yeast strain library (Zhu *et al*., 2014) designed to overexpress each gene essential for yeast cell growth.

MATERIALS AND METHODS

Screening for cadmium-resistant yeast strains with overexpression of essential genes

S. cerevisiae BY4742 strain (*MAT*α, *his3*Δ*1*, *leu2*Δ*0*, *lys2*Δ*0*, *ura3*Δ*0*) was transformed with an essential gene expression library using the lithium acetate procedure (Takahashi *et al.*, 2011). The library was designed for protein overexpression of 869 essential genes related to cell growth. These genes are controlled by the *GAPDH* promoter in the *URA3*-based high-copy plasmid pKT10. Details of the construction of an essential gene expression library are described elsewhere. Yeast cells with overexpression of essential genes were cultured in SD (-ura) liquid media (120 μL) in 96-well plates for 48 hr at 30°C. Each culture was diluted 1/40 with SD media and the aliquots (5 μL) were transferred to fresh SD media (195 μL) containing 50 μM cadmium chloride in 96-well plates for 48 hr. This concentration of cadmium inhibits the growth of wild-type BY4742 cells. After 48 hr incubation, yeast cells exhibiting increased growth were identified as candidates for cadmium resistant yeast cells.

Measurement of the sensitivity of yeast cells to cadmium

The effects of cadmium on yeast strains were quantified during growth of cells in SD media. A suspension of cells (1×10^4 cells) was cultured in a 200 μL aliquot of fresh media that contained cadmium chloride at the concentration indicated. After 48 hr, the absorbance at 600 nm (A600) was measured, using spectrophotometry as an index of cell growth.

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RESULTS AND DISCUSSION

We screened for genes affecting the sensitivity of yeast to cadmium, using our yeast strain library (Zhu *et al*., 2014) designed to overexpress each of the 809 genes (among approximately 1,000 genes) considered essential for the growth of yeast cells. Each yeast strain, with each essential gene overexpressed, was individually cultured for 48 hr in the presence of cadmium chloride levels that typically inhibit the growth of control wild-type yeast. We then considered those cultures showing cell growth as candidates for cadmium resistant strains. We found that yeast strains showing overexpression of *RPN8*, *SKP1*, *MIA40* and *MES1* genes exhibited increased cadmium resistance as compared to the control yeast strain (Fig. 1).

SKP1 is the gene encoding the subunit of the SCF ubiquitin ligase complex (Craig and Tyers, 1999). Since the literature suggests that cadmium promotes disassembly of the SCF complex (Yen *et al*., 2012, 2005), overexpression of *SKP1* may reduce the degree of SCF complex disassembly following cadmium exposure, thereby leading to attenuation of cadmium toxicity. *RPN8* is a gene encoding a non-ATPase regulatory subunit of the 26S proteasome (Finley *et al*., 1998), *MES1* encodes a methionyl-tRNA synthetase (Chatton *et al*., 1987) and *MIA40* encodes a mitochondrial oxidoreductase (Chacinska *et al*., 2004). None of these remaining three genes have been examined for associations with cadmium toxicity. Since all four gene products identified in this study are essential for cell growth, it is possible that cadmium would result in cytotoxicity by restraining the mechanism of these gene products. By examining the relationship between these identified genes and cadmium toxicity, we hope to further elucidate the molecular mechanism of cadmium toxicity and define potential defensive mechanisms against this toxicity.

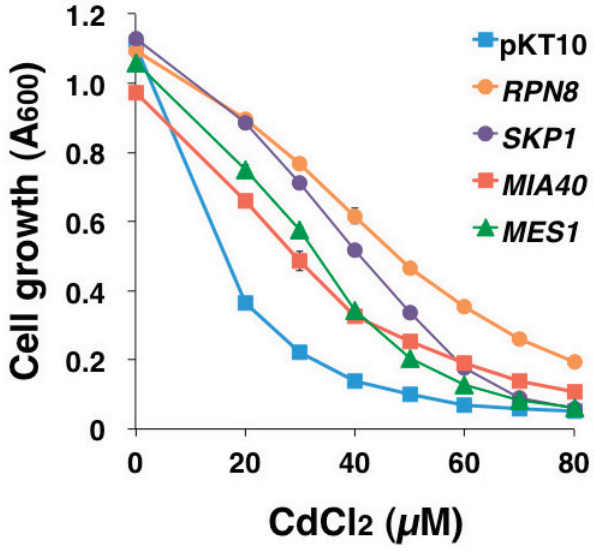

Fig. 1. Effects of overexpression of *RPN8*, *SKP1*, *MIA40* or *MES1* on the sensitivity of yeast cells to cadmium. Yeast cells carrying pKT10-RPN8, pKT10-SKP1, pKT10-MIA40, pKT10-MES1 or pKT10 were grown in SD (-ura) liquid media with cadmium chloride. After 48 hr incubation, absorbance at 600 nm was measured using a spectrophotometer. Each point and bar represents the mean value and SD of results from three cultures. The absence of a bar indicates that the SD falls within the symbol.

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

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