

P 66

Transcriptional Activator *CrT9* is Involved in Accumulating Chromium in *S. cerevisiae*

WON, Jong-Im · CHANG, Kwang-Suk · HWANG, Seong-Bin*

Dept. of Molecular Biology, Sejong University

Objectives

This study aims to clone and characterize a novel gene which is involved in chromium accumulation. For this, we isolated a chromium-tolerant yeast mutant and cloned a mutated gene from it.

Results and Discussion

1. Isolation of yeast mutant *CrT9* showing higher chromium tolerance, but lower accumulation of Cr

A chromium-tolerant yeast mutant *CrT9* was isolated after mutant pools of yeasts generated by mTn (mini transposon) insertion were screened on agar media containing 300 μ M potassium chromate on which the wild type of mutant pools (Y800) cannot survive. *CrT9* had a tolerance to only Cr not to other metals such as Cd, As and Fe, suggesting that *CrT9* has a metal-specificity to Cr. As a first step to investigate *CrT9*, the accumulation of Cr in cells was compared after growing WT (Y800) and *CrT9* in Cr-containing liquid culture for 24 hours. Interestingly the concentration of Cr in *CrT9* was only 26% of WT. This implies that the tolerance of *CrT9* mutant is derived

from low accumulation of Cr in cells, not from increased capacity to detoxify Cr.

2. *CrT9* mutant has a disruption at a gene encoding a transcriptional activator

To clone a gene mutated by transposon insertion in *CrT9*, IPCR (inverse PCR) was performed. Mutated gene was identified as a transcriptional activator (will be called *CrT9*), in which Tn was inserted in ORF. Northern hybridization showed that *CrT9* has a smaller size of mRNA than WT, which is due to Tn insertion. Therefore, the mutant phenotype of *CrT9* is truly due to the disruption of a gene *CrT9*, implying that *CrT9* might be involved in accumulating Cr in yeasts.

3. Transcriptional activator *CrT9* is involved in chromium accumulation in yeasts To confirm the role of transcriptional activator *CrT9* as a positive regulator in Cr accumulation, *CrT9* gene was overexpressed in *CrT9* mutant under a constitutive promoter. The *CrT9* expressing *CrT9* gene has lost tolerance to Cr, decreasing to level of WT.

Taken together, the transcriptional activator *CrT9* seems to play a positive role in accumulating chromium in yeasts.