Analyses of adaptation mechanism to low temperature and of low temperature-inducible genes in yeast

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Low temperature conditions are often applied for the traditional fermentation and beer brewing processes by yeast in Japan. However, the molecular process of yeast for the adaptation to low temperature is not well known. In this project, we tried to elucidate the molecular mechanism how yeast genes expressed in response to low temperature and what aspect is important in the adaptation to low temperature.

We used yeast *Saccharomyces cerevisiae* laboratory strains for all experiments, because of their genome information and accumulation of biochemical knowledge. We describe below the results of our research in this project.

1. Analysis of *LOT4/NRK1* promoter

LOT4/NRK1 was Isolated as by cDNA subtraction method[1] as a gene whose transcripts accumulated at low temperature. By deleting from the 5′ end of the *LOT4* promoter and by analyzing function of the deleted promoters in the Δlot4 mutant by northern analysis, we found that a region from 787 bp to 674 bp upstream of the translation initiation site was essential for the *LOT4* mRNA accumulation after the temperature downshift to 10°C. There were one Y-box (CCAAT or ATTGG) and two Yap1-binding sites (TTACTAA) in this region.

Y-box sequences were also present in promoters of other *LOT* genes. We replaced the respective Y-box sequences in the above region with GGGGG or CCCCC and examined effects of these alterations on the expression of *LOT4* gene. Because these alterations abolished accumulation of *LOT4* mRNA at low temperature, we concluded that Y-box is essential for the increased expression of *LOT4* after the temperature downshift. However, deletion of HAP2 which is known to bind to Y-boxes did not show any influences on the expression of *LOT4*, and hence other Y-box binding proteins seems to be present and working for the elevated expression of *LOT4* at low temperature.

Deletion of Yap1p binding site did have only small effect on the expression of *LOT4*, but over expression of *YAP1* under *GAL1* promoter increased the amount of transcripts of *LOT4*. Expression of *YAP1* itself increased at low temperature. Therefore, Yap1p has a certain role in the *LOT4* expression at low temperature.

2. Analysis of YAP1 promoter

When the promoter of *YAP1* was analyzed by deletion in a similar way to the case of *LOT4* promoter, we found that a region from 693 bp to 594 bp upstream of the translation initiation site was responsible for cold-inducible increase of *YAP1* mRNA. There are two Y-boxes and to change them to GGGGG or CCCCC inhibited the above increase. Thus Y-box sequence likely is important for the regulation of gene expression in response to cold treatment in yeast.

3. Relation of cold response to oxygen stress response

The finding that *YAP1* is a cold-responsive gene tempted us to analyze the expression of oxygen stress responsive genes after a temperature downshift, because Yap1p is a transcriptional activator for many genes that detoxify reactivated oxygen species (ROS). *SOD1* and *SOD2* are encoding superoxide dismutase that has a central role in the detoxification of ROS. *SOD1* mRNA but not *SOD2* mRNA was accumulated after a temperature downshift. SOD and catalase activities were increased by about 1.5 fold, respectively, after the temperature downshift. We also examined intracellular ROS concentration by DCF staining and found 1.5 fold increase in ROS after 2 h of a temperature downshift. These results indicate that the temperature downshift induces increases of ROS and ROS-detoxifying enzyme activities. If it does so, prior exposure to low temperature should increase the ability of yeast cells to adapt to oxygen stress. Actually incubation for 2 h at 10°C shortened by 4 h the lag time before starting growth in the presence of 1.8 mM H_2O_2 at 30°C. However, prior exposure to H_2O_2 never shortened the lag time after a downshift of growth temperature to 10°C. Thus the oxygen stress response could be a part of cold adaptation of yeast, but does not seem essential for cold response within a short term. However, it should have a critical role for the long term survival of yeast cells under the low temperature condition.

4. Genome-wide search of cold-responsive genes of yeast

In the beginning of this project, we intended to obtain cold-responsive gene by DNA tip analysis. However, several foreign and domestic laboratories started similar projects and it seemed difficult to extract essential genes within one year for cold adaptation from a number of candidates that will be identified by DNA tip analysis. Therefore, we changed our plan and

started to screen genes that are required for growth at 10°C from an EUROSCARF yeast disruption mutant collection that covers about 5000 nonessential genes. Until now, we identified 16 mutants that showed slower growth at 10°C than the parental wild type strain. Detailed analysis on their properties are now underway.

Reference

1) Chang, L., Ohta, A., Horiuchi, H., Takagi, M., and Imai, R. Multiple mechanisms regulate expression of low temperature responsive (LOT) genes in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.*, **283**: 531–535 (2001).