Role of the TOR signaling pathway in the freeze-thaw stress response in yeast

Yoshiharu INOUE

Graduate School of Agriculture, Kyoto University

Research aims

Living organisms survive by adapting to changes in the environment. Changes in the temperature of the growth environment and changes in nutritional status are sensed by the corresponding sensors, and gene expression and metabolic regulation suitable for such an environment are performed. For example, the expression of heat shock proteins increases when the temperature of the growth environment is elevated, and the mechanism is well studied from *E. coli* to human. However, freeze-thaw stress is one of the most frequently encountered environmental stresses for cells in both natural and laboratory environments. Yeast encounter freeze-thaw stress in the industrial field also, such as in frozen dough. Nonetheless, studies on the freeze-thaw stress response are currently delayed compared to that on heat (high temperature) shock stress.

Yeast cells in the stationary phase of growth show higher freeze-thaw stress tolerance than cells in the logarithmic growth phase. Usually, yeast cells in the logarithmic growth phase contain 2 to 3 fragmented vacuoles. In contrast, in stationary phase cells, the morphology of the vacuole changes into one enlarged shape. In this study, we focused on the relationship between vacuolar morphology and freeze-thaw stress tolerance. However, another difference in the environment of yeast culture between the logarithmic growth phase and the stationary phase is the availability of nutrients. The TOR (target of rapamycin) pathway plays a pivotal role in transmitting nutrient signals in eukaryotes. The TOR signal is activated in the presence of sufficient nutrients but is inactivated when nutrients are depleted. The second purpose of this study was to explore the relationship between the TOR signaling pathway and the freeze-thaw stress response.

Methods

A freezing tolerance test was carried out as follows: yeast cells were cultured in YPD medium (2% glucose, 1% yeast extract, 2% peptone; pH 5.5) to log phase. After collecting cells by centrifugation, cells were washed with sterilized 50 mM potassium phosphate buffer (pH 6.5), quickly frozen at -30 ºC, and stored for 1-3 days. Cells were thawed at 25 ºC for 20 min in a heat block, diluted with sterilized 0.85% NaCl solution, and then spread on YPD agar plates. Cells were cultured at 28 ºC for several days to form

colonies, and the survival rate was compared with the survival rate of the samples before freezing.

Results

1. Vps34 and freeze-thaw stress resistance

One of the morphological features of yeast cells in the stationary phase of growth is the enlargement of vacuoles. Among the mutants defective in vacuolar shape, the mutants classified into class D (*vps8*Δ, *vps9*Δ, *vps15*Δ, *vps34*Δ, *vps45*Δ, *fab1*Δ, and *vac14*Δ) that have an enlarged vacuole even in the logarithmic growth phase, were subjected to the freezing tolerance test. As a result, cells carrying enlarged vacuoles did not necessarily acquire tolerance to freeze-thaw stress, but the mutants defective in phosphatidylinositol 3-kinase (Vps34) and its regulator protein (Vps15) showed significantly higher freeze-thaw stress tolerance, even in the logarithmic growth phase.

Next, to examine whether the lipid kinase activity of Vps34 was necessary for acquiring freeze-thaw stress tolerance, the freezing tolerance test was done using a $Vps34^{N736K}$ mutant defective in lipid kinase activity[1)]. The *vps34*Δ mutant carrying the vector showed high freeze-thaw stress tolerance, whereas the mutant with the wild-type *VPS34* gene lost freezing tolerance. The $Vps34^{N736K}$ mutant exhibited high freeze-thaw stress tolerance. Phosphatidylinositol-3-phosphate (PI3P) produced by the Vps34 reaction is converted to phosphatidylinositol-3,5-bisphosphate by the action of Fab1. Since the Fab1-deficient mutant did not exhibit freeze-thaw stress tolerance, PI3P seemed to be involved in the acquisition of freeze-thaw stress resistance.

2. TOR signaling and freeze-thaw stress resistance

One of the environmental changes in stationary phase yeast culture is a decrease (depletion) of nutrients inside and outside the cell. Thus, the freezing tolerance was compared after transferring logarithmic growth phase cells cultured in nutrient medium to medium lacking carbon or nitrogen sources. As a result, freeze-thaw stress tolerance was acquired when cells were transferred to the medium depleted of nutrients.

TOR signaling is a nutrient signaling pathway evolutionally conserved in eukaryotes. TOR is a Ser/Thr kinase and forms two functionally distinct TOR complexes (TORC1 and TORC2). Of these, the TOR complex that transmits nutrient signals is TORC1. TORC1 is activated under conditions where nutrients are sufficiently present but is inactivated when nutrients are depleted. Rapamycin is a specific inhibitor of TORC1. To explore the correlation between TORC1 inhibition and freeze-thaw stress tolerance, rapamycin was added to yeast culture medium in which a sufficient amount of nutrients was present, and then the freezing tolerance test was conducted. As a result, rapamycin-treated

cells acquired freeze-thaw stress tolerance. Taken together, these results suggest that that the inhibition of the TOR signaling pathway is involved in the acquisition of freezing tolerance.

3. Functional correlation between Vps34 and TORC1

Since both Vps34-deficiency and TORC1-dysfunction gave a common phenotype in terms of freezing tolerance, the possibility of a functional correlation between Vps34 and TORC1 was suspected. To investigate the effect of Vps34-deficiency on the kinase activity of TORC1, the phosphorylation state of Sch9, which is a substrate of TORC1, was examined. Sch9 was in a dephosphorylated state in the Vps34-deficient mutant, suggesting that Vps34 was involved in the activation of the TOR kinase.

TORC1 activation is regulated by two distinct pathways, i.e. a small G protein Gtr1-dependent manner and a vacuolar membrane-associated protein Pib2-dependent manner. Pib2 contains a PI3P-binding domain (FYVE domain)[2]. We examined the effect of deletion of Gtr1 and Pib2 on freeze-thaw stress resistance. A *pib2*Δ mutant did not show freezing tolerance, but a *gtr1*Δ mutant did; however, the extent of the freeze-thaw stress tolerance of the *gtr1*Δ mutant was not as high as that of the *vps34*Δ mutant. These results suggested that the TORC1 signaling pathway involved in freeze-thaw stress tolerance was dependent on Vps34 but was different from the Pib2 pathway.

Conclusion

Vps34 regulated TORC1 activity via PI3P, and TORC1 negatively regulated the freeze-thaw stress response in yeast. Thus, it seems that the TORC1 regulatory mechanism involved in the freeze-thaw stress response was regulated in a Pib2-independent manner.

References

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