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Study on the yeast signaling pathways responsive to stresses and starvation

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Research aims

Yeasts are superior host microorganisms suitable for various bioproduction, which have been widely used for fermentation and brew processes. This study addressed stress-sensing and regulation mechanisms of two stress-responsive pathways that we have been studying, aiming at the establishment of the theoretical basis to control the cellular responses to stresses and starvation, which potentially impair productivity in bioproduction processes using yeasts. For the osmotic stress-responsive HOG pathway, mutants were isolated and characterized that activate the pathway even under low osmolarity conditions, to clarify what and how the sensing machinery monitors as an index of osmotic stress. For the starvation-responsive TOR pathway, novel regulatory mechanisms were explored by screening for new regulatory factors. Additionally, the bases for the different sensitivity to rapamycin, a specific inhibitor of TOR, among various cells were also addressed.

Methods

Osmotic stress-responsive HOG pathway

In a host strain for the yeast two-hybrid system expressing a DNA-binding domain-Hog1 fusion protein, the expression of the reporter gene becomes responsive to osmotic stress[1]. From this reporter strain, mutants were isolated in which the HOG pathway is activated even under low osmolarity conditions. Then the mutated genes were identified by plasmid complementation. The activation of the HOG pathway was monitored by immunoblotting using an anti-phospho Hog1 antibody. Myriocin and aureobasidin A were used to inhibit the biosynthesis of sphingolipids.

Starvation stress-responsive TOR pathway

Cells expressing the activated TOR mutant grow poorly under nutrient-limited conditions due to inappropriately high activation of the TOR pathway. In order to identify novel negative regulators of the TOR pathway, genes were screened for that rescue the poor growth when over-expressed. The localization of the components of the TOR complex was monitored using functional GFP-fusion constructs. The activation status of the TOR pathway was monitored by immunoblotting using a phospho-specific antibody against Sch9, a major substrate of TOR. Mutation frequency was measured at the *CAN1* locus by monitoring canavanine resistance.

The growth of fission yeast was monitored on media containing various concentrations of caffeine and rapamycin in combination. TOR was purified from fission yeast cells using an epitope tag added to TOR, and its in vitro kinase activity was measured using 4E-BP1 as the substrate. To examine the rapamycin-sensitivity, rapamycin and GST-FKBP12 were included in the kinase assay.

Results

The osmotic stress-sensing mechanism of the HOG pathway[2)]

From a reporter strain to monitor the activation of the HOG pathway, mutants were isolated that constitutively activate the pathway even under low osmolarity conditions. Among the mutants, one that showed clear activation and temperature sensitive growth was subjected for cloning of the responsible gene. It turned out to be an *lcb2* mutant. *LCB2* encodes a subunit of serine palmitoyltransferase, which catalyzes the first step of the sphingolipid biosynthesis. It was confirmed that cells with the *lcb2* mutation constitutively activate Hog1 phosphorylation even under low osmolarity conditions.

Then, we found that it was decrease in the levels of the complex sphingolipids that induces activation of the HOG pathway, by using inhibitors and mutants of the sphingolipid biosynthesis. Furthermore, inhibition of the ergosterol biosynthesis also induced activation of the pathway. Sphingolipids and ergosterol are tightly packed together in cell membranes, resulting in the formation of membrane domains called rafts. Sensing components of the HOG pathway, Sln1 and Sho1, were distributed in detergent-resistant membranes, and therefore appeared to localize to rafts. This distribution of Sho1 was enhanced upon not only osmotic stress but decrease in sphingolipid levels. In contrast, the distribution of Sln1 under the same conditions did not significantly change, but the electrophoretic mobility of Sln1 on blue native PAGE changed from that of multimer to monomer.

A novel regulatory mechanism of the TOR pathway mediated by stress granules[3)]

Stress granules (SGs) are cytoplasmic foci transiently formed in response to stresses, which contain nontranslating mRNA and translation factors. Overexpression of Pbp1, a component of SG, was found to reduce the activity of TOR. Then, we found that the TOR complex is sequestered

into SG, when SG formation is induced by heat stress. It was known that the activity of the TOR pathway is transiently inhibited in cells exposed to heat stress. We also found that the reactivation kinetics of the TOR pathway during the recovery phase from heat stress was accelerated when SG formation was inhibited by cycloheximide treatment. This indicates that TOR sequestration into SGs inhibits premature activation of the TOR pathway during the recovery phase. This model was also confirmed using a mutant TOR complex that is reluctant to SG sequestration. Furthermore, the mechanism contributes to reducing heat-induced mutations.

Bases for cellular rapamycin-sensitivity[4]

In many organisms including the yeast *Saccharomyces cerevisiae*, cell growth is sensitive to rapamycin treatment. In contrast, it is not the case in fission yeast, *Schizosaccharomyces pombe*, and the bases for this rapamycin-resistance have attracted wide interests. Recently, it was reported that rapamycin only partially inhibits the activity of TOR from the observations using novel mTOR inhibitors that directly inhibit mTOR's kinase activity. It is therefore possible that fission yeast cells are rapamycin-resistant just because the residual TOR activity after inhibition by rapamycin is enough to support the growth. To test the possibility, we examined the kinase activity of fission yeast TOR with an in vitro assay and found that it was indeed inhibited by rapamycin of similar concentrations that inhibit TOR in other organisms. As the kinase activity of TOR is inhibited also by caffeine, caffeine treatment may further reduce the remaining TOR activity after inhibition by rapamycin. The growth of fission yeast was indeed inhibited by rapamycin in the presence of a moderate concentration of caffeine, which by itself did not affect the growth. This inhibition of growth was shown to be mediated by TOR inhibition by using TOR mutants resistant to each drug. Moreover, starvation responses including G1 arrest, transcription of starvation responsive genes, and autophagy, which are not induced by rapamycin treatment alone, were induced by rapamycin plus caffeine treatment.

Conclusion

The osmotic stress-sensing mechanism of the HOG pathway[2]

Sphingolipids regulate the osmosensing machinery of the HOG pathway, probably because the sensing components localize to rafts and the change in their localization and/or association in response to osmotic stress underlies their stress-sensing mechanism.

A novel regulatory mechanism of the TOR pathway mediated by stress granules[3]

The TOR complex is sequestered to stress granules, and which determines the reactivation kinetics of the TOR pathway during the recovery phase from the stress. As this regulation in response to heat stress contributes to reducing heat-induced genomic mutations, its physiological role can be to balance between repair and growth depending on the severity of the stress.

Bases for cellular rapamycin-sensitivity[4]

The bases for the difference in rapamycin-sensitivity among different cells have been elusive. We found that it depends on the different cellular responses to equally reduced TOR activity, but not on the different rapamycin-sensitivity of the TOR complex itself. Moreover, difference in the rapamycin-sensitivity of various physiological events controlled by TOR was shown to be just quantitative rather than qualitative.

References

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