Generation of high-lipid producing yeast cells using the endoplasmic reticulum-stress response

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Research objective

In general, eukaryotic cells contain the endoplasmic reticulum (ER), which is a suc-shaped organelle with a flat or tubular morphology and covered with a single phospholipid bilayer. The role of the ER includes the formation of higher-order structures of secretory proteins and cell surface proteins, and the biosynthesis of lipids. Molecular chaperones and related proteins reside in the ER lumen, and lipid metabolism/synthase family proteins are present on the ER surface. mRNAs encoding secretory proteins and cell surface proteins are translated by ribosomes attached to the ER surface, and nascent polypeptides are folded in the ER. The failure of these processes is referred to as ER stress, and cells evoke an ER stress response to cope with serious cellular damage.

For the ER stress response in yeast cells, the ER transmembrane protein Ire1 acts as a sensor that detects denatured proteins accumulated in the ER lumen and activates an intracellular signal transduction pathway[1)]. The cytosolic domain of Ire1 has a protein kinase region and an endoribonuclease region. In response to ER stress, Ire1 induces autophosphorylation, which leads to its activation as an endoribonuclease. In *Saccharomyces cerevisiae*, the target of Ire1 as an endoribonuclease is the mRNA transcript of *HAC1 gene*. The *HAC1* mRNA, namely the immature *HAC1* mRNA, has an intron and is not translated into a protein. In contrast, the activation of Ire1 proteins induces splicing of the *HAC1* mRNA and translation into the transcription factor protein Hac1. The Hac1 protein t induces the transcription of ER-located molecular chaperones and phospholipid biosynthetic enzymes, leading to the reactivation of ER functions and enlargement of the ER.

In the present study, we addressed the following issues, which are deeply related to ER stress and ER stress response in yeast cells.

(1) Biological significance of Ire1 regulation by its kinase region

(2) Effect of molecular oxygen on ER stress and ER stress response in *S. cerevisiae*

(3) ER stress response in the methanol-utilizing yeast *Pichia pastoris* (*Komagataella phaffii*).

(4) Application of the *S. cerevisiae* strain that constitutively expresses mature *HAC1* mRNA.

Methods

The reverse transcriptase (RT)-PCR technique was used to monitor the splicing of *HAC1* mRNA, and PolyT primers were used in the RT reaction. For PCR, we used a *HAC1*-specific primer set that amplifies the non-spliced and spliced sequences as different-sized DNA products.

The genetic manipulation of *S. cerevisiae* and *P. pastoris*, and the analyses of nucleic acid, protein, and lipidic samples were performed as described in the literature[2)-3)].

Results

(1) Even when Ire1 carried a mutation that abolished its requirement for the luminal domain-dependent detection of ER-accumulated unfolded proteins for its activation, the *HAC1*-mRNA splicing was not constitutive but was dependent on the nutrient condition. Contrarily, the *HAC1*-mRNA splicing was constitutively evoked when another mutation was introduced into the kinase region of its cytosolic domain[2)].

(2) When *S. cerevisiae* cells were cultured under anaerobic conditions, the *HAC1* mRNA splicing level induced by the conventional ER stress inducers tunicamycin and dithiothreitol was higher than that induced under aeration conditions. The same pattern was observed when mitochondrial respiration was halted by the rho0 mitochondrial-gene deletion mutation[4)].

(3) Unlike *S. cerevisiae*, in *P. pastoris*, *HAC1* mRNA splicing was observed at high levels even under normal culture conditions. When the *P. pastoris Ire1* gene was introduced into *S. cerevisiae*, *HAC1* mRNA splicing was not induced under normal conditions. Therefore, *P. pastoris* cells were likely to be under ER stress even under normal culture conditions. The protein secretion level of *P. pastoris* was significantly higher than that of *S. cerevisiae*. Moreover, ER stress response-target genes of *IRE1* and *HAC1*-gene deletion mutants of *P. pastoris* that were analyzed using RNAseq have been listed.

(4) The constitutive and high expression of the spliced *HAC1* mRNA in *S. cerevisiae* induced the expression of lipid biosynthetic enzymes, leading to the accumulation of phospholipids and triglycerides. It also increased the secretion of free fatty acids. However, overexpression of spliced *HAC1* mRNA drastically retarded cellular growth. To cope with this problem, fast growing spliced *HAC1* mRNA-expressing cells were screened. The mutant carried a loss-of-function mutation in the histone deacetylase gene *HDA3*.

Conclusion

Here, we showed that the protein kinase region of yeast Ire1 was involved in the regulation of ER stress responses by nutrient conditions. Additionally, in *S. cerevisiae*, molecular oxygen attenuated ER stress independent of mitochondrial respiration. Unlike *S. cerevisiae*, *P. pastoris* robustly secreted proteins, leading to potent ER stress, even under normal conditions.

By combining the constitutive and high expression of the spliced *HAC1* mRNA and the loss-of-function mutation of the *HDA3* gene, *S. cerevisiae* strains that produced abundant lipidic molecules without severe growth retardation (Fig. 1) were obtained.

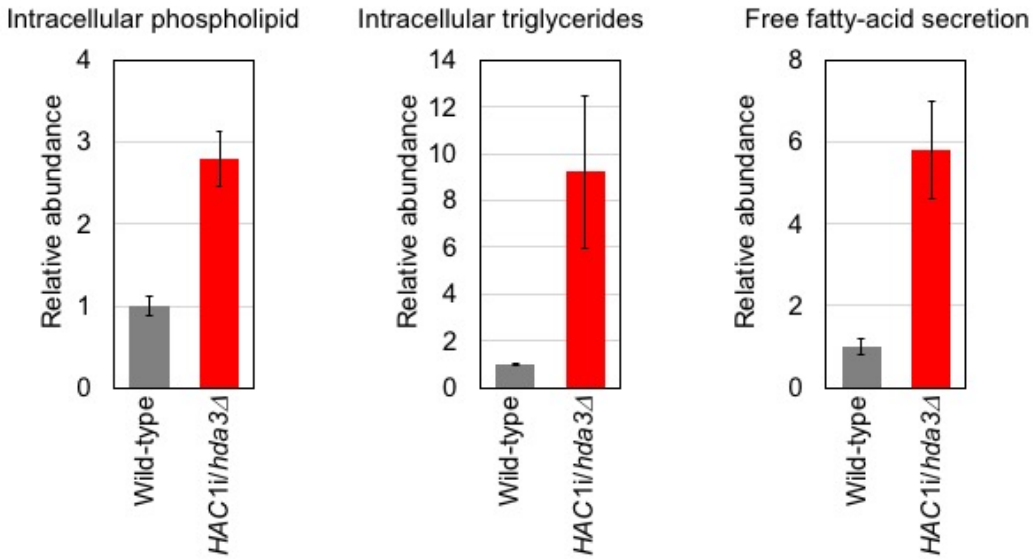

Fig. 1 Production of lipidic molecules in yeast cells. Wild type and mutant (high expression of the spliced *HAC1* mRNA and *HDA3* deletion (*HAC1i/hda3*□) *S. cerevisiae* cells were cultured at 30 °C in SD medium and were checked for lipid contents.

References

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