Construction of a ceramide production system using rescue mechanism against impaired biosynthesis of sphingolipids

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Research objective

Sphingolipids are essential membrane lipids in eukaryotes ranging from fungi to humans. Ceramides, which constitute the hydrophobic part of sphingolipids, exist in the human stratum corneum and contribute to the barrier function and moisturization of the skin. In recent years, attention has been focused on the use of ceramides in health foods, cosmetics, and pharmaceuticals. Budding yeast can be a good source of ceramides; however, ceramides are present only in trace amounts in natural resources, including yeast, because most of the synthesized ceramides are converted into complex sphingolipids containing a hydrophilic head group. Thus, although the conversion of ceramides to complex sphingolipids is necessary to obtain a larger amount of ceramides from yeast, doing so would substantially impair the metabolic balance of sphingolipids and can be lethal for the yeast. Therefore, the construction of a ceramide mass production system using yeast is difficult. Recently, we found that the activation of a specific signaling system (HOG pathway) rectified the growth defect caused by the repression of complex sphingolipid biosynthesis (1). In this study, we aim to establish a mass production system for ceramides by analyzing this rescue mechanism in detail.

Methods

To suppress the conversion of ceramides to complex sphingolipids in budding yeast, *AUR1*, which encodes for an IPC (the simplest structure of complex sphingolipids) synthase, was expressed under the control of a tetracycline-regulatable promoter (*tet-AUR1* strain). The expression of *AUR1* was repressed by the addition of doxycycline (Dox). DNA microarray analysis was used to perform a comprehensive search for genes whose transcription increased in a HOG-pathway-dependent manner under *AUR1*-repressive conditions.

Results

Identification of the final target factor of the HOG pathway

Transcriptome analysis led to the identification of 59 genes whose expression increased in a Hog1 (MAPK of the HOG pathway)-dependent manner under *AUR1*-repressive conditions. These candidate genes were separately overexpressed in *tet-AUR1* cells, and genes that were

able to rescue the impaired biosynthesis of complex sphingolipids were screened. As a result, *FMP48, UIP4, GIP2, TPS2*, and *MGA1* genes were identified. From these, *FMP48* and *TPS2* were identified as important candidate genes, because the deletion of one of these genes promoted growth defects due to *AUR1* repression (Fig. 1A). *FMP48* encodes a putative Ser/Thr kinase, and it was found that the overexpression of *FMP48* suppresses growth defects and increases mitochondria-derived reactive oxygen species (ROS), which are produced by *AUR1* repression (Fig. 1B). Comprehensive analysis of protein interactions showed that Fmp48 physically interacts with the transcriptional regulator Mks1 (2), and it was suggested that the effects of *FMP48* under *AUR1*-repressive conditions are mediated by *MKS1*.

TPS2 encodes trehalose-6-phosphate phosphatase involved in trehalose biosynthesis. Deletion of *TPS2* promoted growth inhibition caused by *AUR1* repression; however, deletion of the trehalose-6-phosphate synthase gene *TPS1* had no promoting effect. Thus, it is assumed that the rescue effect of *TPS2* is not mediated by the promotion of trehalose biosynthesis, but is exerted by regulating the amount of trehalose-6-phosphate, a precursor of trehalose. Trehalose-6-phosphate is a negative regulator of glycolysis. Therefore, glycolysis activity was evaluated by examining the production of ethanol in a fermentable medium. As a result, it was found that *AUR1* repression reduced the amount of ethanol produced per living cell, and this reduction was promoted by the deletion of *TPS2* or *HOG1*. These results suggest that *TPS2* is upregulated under *AUR1* repression and contributes to the suppression of the growth defects by regulating glycolysis.

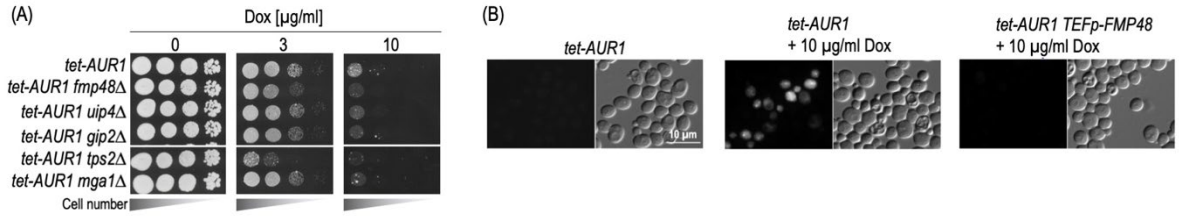

Fig. 1. (A) Effect of deletion of candidate genes that rescue growth defects caused by repression of complex sphingolipid biosynthesis (*AUR1* repression). (B) Effect of overexpression of *FMP48* (*TEFp-FMP48*) on increased ROS levels in *AUR1*-repressed cells. Detection of ROS was performed by staining with 2',7'-dichlorofruorescein diacetate, an indicator of ROS in cells.

Crosstalk of Protein kinase A and the HOG pathway under impaired biosynthesis of complex sphingolipids

Previously, we reported that the deletion of *RAS2*, which encodes a component of the cAMP/protein kinase A (PKA) signaling pathway, confers resistance to growth inhibition caused by *AUR1* repression (1). In the present study, we found that gene mutations (*pde2*Δ, *ira2*Δ), which cause the activation of the cAMP/PKA pathway, enhance growth inhibition

caused by *AUR1* repression (3) (Fig. 2A). To elucidate the function of the cAMP/PKA pathway under *AUR1*-repressive conditions, we screened multicopy suppressor genes that can suppress this enhancement of the growth inhibition. As a result, the *PBS2* gene encoding MAPKK of the HOG pathway was identified. We also found that the mutant strain with increased cAMP/PKA pathway activity decreased the phosphorylation of Hog1 under *AUR1*-repressive conditions (Fig. 2B). These results suggest that the cAMP/PKA pathway contributes to the proper activation of the HOG pathway under impaired biosynthesis of complex sphingolipids (3).

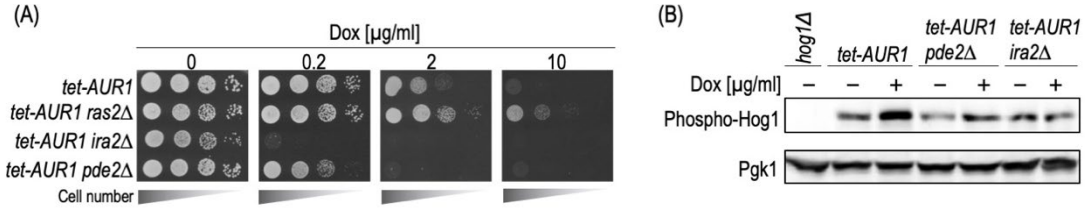

Fig. 2. (A) Effect of deletion of components of the cAMP/PKA pathway on growth defect caused by repression *AUR1*. (B) Effect of deletion of *PDE2* or *IRA2* on phosphorylation of Hog1 caused by *AUR1* repression.

Conclusion

This study revealed how yeast cells protect themselves against the impaired biosynthesis of complex sphingolipids. The TORC2-Ypk1 pathway is involved in the repair of complex sphingolipid biosynthetic pathways by activating the sphingolipid biosynthetic pathway; however, activation of the HOG pathway is not itself involved in the repair of the complex sphingolipid biosynthesis system. By combining these findings, we aim to develop mutant strains exhibiting resistance to complex sphingolipid reduction and abnormal ceramide accumulation in the future.

References

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