【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project：Integrated understanding of nitric oxide in yeasts and fungi and its application to microbial breeding and drug development

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【Purpose and Background of the Research】

Nitric oxide (NO) is a signaling molecule involved in the regulation of many biological processes and NO is produced by NO synthase (NOS) in mammals. Research on NO in the yeast *Saccharomyces cerevisiae*, which is important as a model for higher eukaryotes and in fermentation industry, do not make progress due to the lack of mammalian NOS orthologues in the genome.

We found that NO is synthesized through the flavoprotein Tah18-dependent NOS activity in yeast and that NO confers high-temperature tolerance on yeast via the transcription factor Mac1-mediated activation of the Cu,Zn-superoxide dismutase Sod1. We also proposed a novel regulatory mechanism of NO synthesis mediated by the Tah18-Dre2 complex. Furthermore, it was shown that he dual functions (cell protection vs. cell death) of NO found in higher eukaryotes also occur in yeast (Figure 1).

In this study, for understanding of molecular functions of NO in yeasts and fungi, we will analyze the synthetic mechanisms and the physiological roles of NO. The effects of NO on fermentation ability of yeasts and on growth, infection and biologically active substances production in fungi will be investigated for contribution to breeding of industrial yeasts and development of antifungal agent.

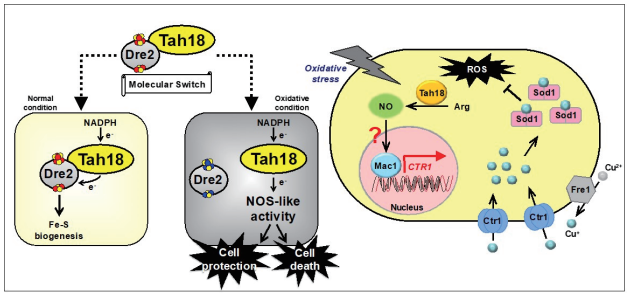

Figure 1 Model of NO synthesis (left) and stress tolerance by NO (right) in yeast

【Research Methods】

1) Elucidation of molecular functions of NO in yeast: We will analyze the expression of Tah18-dependent NOS-like activity, including identification of the oxygenase-like protein, and the function of the mammalian Ndor1 and Ciapin1, which are homologous to Tah18 and Dre2, respectively. We will also identify the NO-targeted proteins with *S*-nitrosylation and nitration using the biotin switch and western blotting methods combined with LC-MS. Furthermore, we will understand the molecular mechanism and physiological significance of the dual functions of NO.

2) Functional analysis of NO in industrial yeasts and its application to fermentative production: We will construct industrial yeast strains with modified expression of NO-related genes (overexpression, knockout), examine the effect of NO on fermentation ability, and challenge the breeding of strains with increased fermentation ability.

3) Functional analysis of NO in fungi and search for drug target molecules: We will focus on the molecular functions of NO, particularly NO-related genes, secondary metabolism and NO tolerance, examine the effect of NO on growth, infection and biologically active substances production and identify target genes for antifungal drug in both model and pathogenic fungi.

【Expected Research Achievements and Scientific Significance】

1) Accumulation of basic knowledge on NO: Our study will contribute to understanding of molecular functions of NO acquired by yeasts and fungi as a survival strategy under various environments. In addition, a series of the studies on yeasts and fungi as a model for higher eukaryotes may lead to the discovery of mechanisms of NO-mediated pathogenesis and NO generation in plants.

2) Applications to industrial yeasts and fungi: By regulating intracellular NO synthesis, improvement of fermentative production will be promising in industrial yeasts. Moreover, elucidation of molecular functions of NO and regulatory mechanisms of secondary metabolism in pathogenic fungi will lead to development of antifungal agent and discovery of biologically active substances.

【Publications Relevant to the Project】

- Yoshikawa Y, *et al.* Regulatory mechanism of the flavoprotein Tah18-dependent nitric oxide synthesis and cell death in yeast. *Nitric Oxide*, **57**, 85-91 (2016).
- Nasuno R, *et al.* Nitric oxide-mediated antioxidative mechanism in yeast through the activation of the transcription factor Mac1. *PLoS One*, **9**, e113788 (2014).
- Zhou S, *et al.* NO-inducible nitrosothionein mediates NO removal in tandem with thioredoxin/ *Nat. Chem. Biol.*, **9**, 657-663 (2013).

【Term of Project】

FY2019-2023

【Budget Allocation】

153,800 Thousand Yen

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