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

Broad Section F

Title of Project：Study on the mechanism of nutrient recognition and coordination of nutrient response in plants

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Research Project Number：19H05637　Researcher Number：80242163
Keywords：ribosome, plasma membrane, cell wall, mineral nutrients, growth analysis, modeling

【Purpose and Background of the Research】

Historically, fertilization contributed greatly to global food production and is also necessary in modern agriculture to achieve high yields. Fertilization, on the other hand, faces problem of eutrophication and limitation of resources. Importance of fertilization is based on the limited ability of plants to absorb nutrients from soils.

Plants evolved in the natural soil with mostly nutrient poor soils and carries ability to adapt low-nutrient environments. This ability, however, has a certain limitation, and if we can improve this ability, it contributes realization of "Low input agriculture." For this, it is important to understand plant mechanisms to adapt low-nutrient environments.

To respond to nutritional conditions, it is essential to sense the nutrient levels in cells and in the environments. Based on the sensing, multiple processes including nutrient transport, metabolism and growth are regulated in a coordinated manner to achieve response as an organism. Our previous study identified mechanism to sense nutrients in cytoplasm which induce regulation of gene expression. Nutrient sensing can also happen in plasma membrane and cell walls. In this project we study mechanisms of nutrient sensing in different cellular compartments and describe multiple phenomena that associates with sensing. Such observation will lead us to comprehensively understand plant responses to low nutrient environments.

【Research Methods】

We previously demonstrated that nutrient sensing occurs in the process of translation of *NIP5;1*, a gene encoding boron transporter (Figure 1, Tanaka et al 2016).

In this project, details of the nutrient sensing mechanisms including structural analysis of ribosome and biochemical analysis will be conducted to elucidate molecular basis of recognition.

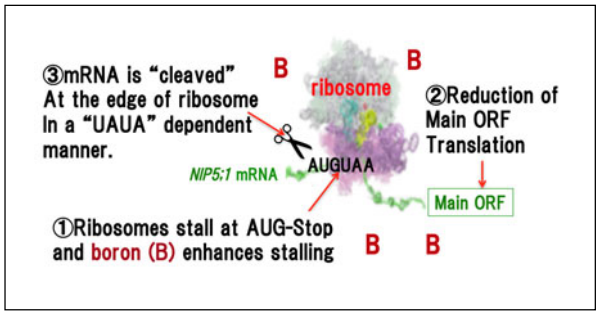

Figure 1 Regulation of boron dependent *NIP5;1* expression

Plasma membrane and cell wall are also the subcellular locations where nutrients are recognized. Transporters and polysaccharides chemically interact with nutrients and possibly function for nutrient recognition. Effects of mutations on transporters and/or genes affecting polysaccharides accumulation will be used to examine their effects on transporter expression, nutrient distribution, growth and gene expression. Such analysis will leads to comprehensive understanding of nutrient response in plants.

【Expected Research Achievements and Scientific Significance】

Our study is unique in that nutrient recognition is studied in three different compartments in plants and expected to lead to comprehensive understanding of nutrient response in plants. The outcome of our project will provide useful information for sustainable agriculture in the future.

【Publications Relevant to the Project】

-Tanaka, M., Sotta, N., Yamazumi, Y., Yamashita, Y., Miwa, K., Murota, K., Chiba, Y., Hirai, MY., Akiyama, T., Onouchi, H., Naito, S. & Fujiwara, T "The Minimum Open Reading Frame, AUG-Stop, Induces Boron-Dependent Ribosome Stalling and mRNA Degradation" Plant Cell 28: 2830–2849 (2016) doi: org/10.1105/tpc.16.00481.

-Sotta, N., Duncan, S., Tanaka, M., Sato, T., Marée, A. F.,Fujiwara, T., & Grieneisen, V. A. "Rapid transporter regulation prevents substrate flow traffic jams in boron transport." eLife 6:e27038 (2017) doi: 10.7554/eLife.27038

【Term of Project】FY2019-2023

【Budget Allocation】153,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/syokuei/index_e.html

Title of Project：Molecular basis of bulk transport machinery playing key roles in lipid secretion in plant cells

YAZAKI Kazufumi
(Kyoto University, Research Institute for Sustainable Humanosphere, Professor)

Research Project Number：19H05638　Researcher Number：00191099
Keyword：Lipid secretion, Plant cell, Bulk transport, Secondary metabolism, Shikonin

【Purpose and Background of the Research】

Plants secrete a large number of lipophilic metabolites, both polymers and low molecular weight substances, such as wax/suberins and terpenoid compounds, respectively. The latter includes many biologically active compounds like taxol and shikonin, which are mostly accumulated outside the cells after biosynthesis, i.e.: in apoplastic spaces. However, it is not well known how such lipophilic compounds are accumulated in oil droplets surrounded by the lipid monolayer like oil bodies, how they recognize the plasma membrane, or how they can go across the plasma membrane to be largely accumulated in the apoplast.

In this study, we utilize a model system to characterize the molecular mechanism of lipid secretion, i.e.: the shikonin production system by *Lithospermum erythrorhizon*. This is an herbal medicinal plant, from which a high shikonin-producing cell line was established. This line produces more than 10% of shikonin derivatives. There are several advantages in utilizing these plant cell cultures, for instance the visibility of the lipid (shikonin) as a red pigment, the strict regulation of shikonin production and the availability of cell mass due to the cultured cells. Using this system, we aim to elucidate the molecular basis of lipid secretion from plant cells.

【Research Methods】

To uncover the molecular mechanism of lipid secretion, we first listed genes, which were selectively expressed in shikonin-producing conditions, as being relevant for shikonin production. Among them, subcellular localization was analyzed to narrow down the candidate genes putatively involved in lipid secretion from *L. erythrorhizon* cells. The involvement of individual genes/proteins in the production and secretion of shikonin, will be evaluated by virus-induced gene silencing (VIGS), which will take advantage of the high throughput, despite the transient expression. The strong candidates will be then subjected to genome editing to produce knockout hairy roots, which will then be analyzed by transparent electron microscopy. Fluorescence tag for candidate proteins will also be used to trace the subcellular movement of these proteins accompanied with shikonin molecules. Protein-protein interaction will then be evaluated to figure out the entire bulk transport machinery.

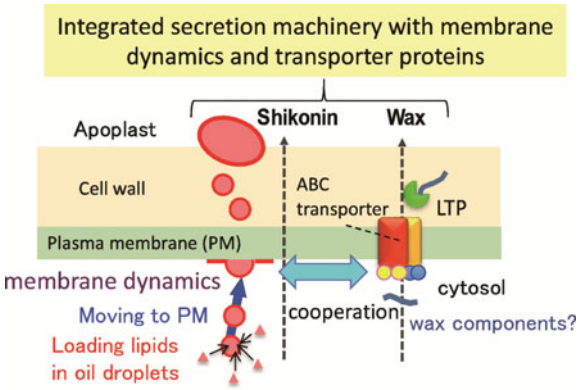

Figure 1　Bulk transport of lipid molecules

【Expected Research Achievements and Scientific Significance】

Plants accumulate many valuable lipophilic natural compounds in apoplastic spaces, like subcuticular cavities of glandular trichomes and resin ducts, whilst the secretion mechanisms are largely unknown. Elucidation of the molecular mechanism of lipid secretion from plant cells, will enable us to understand the survival strategy of land plants that prevents dryness and is also expected to provide the technical basis for the production of valuable secondary metabolites, e.g.: monoterpenoids as fragrances, as well as vincristine and paclitaxel as anticancer drugs.

【Publications Relevant to the Project】

・Bowman JL, et al., Insights into land plant evolution garnered from the *Marchantia polymorpha* genome, ***Cell***, 171(2): 287-304.e15 (2017).
・Tatsumi, K., et al., Characterization of shikonin secretion in *Lithospermum erythrorhizon* hairy roots as a model of lipid-soluble metabolite secretion from plants, ***Frontiers Plant Sci.*** 7, 1066 (2016).
・Morita, M., et al., Vacuolar transport of nicotine is mediated by a novel multidrug and toxic compound extrusion (MATE) transporter in *Nicotiana tabacum*. ***Proc. Natl. Acad. Sci. USA***, 106, 2447-2452 (2009).

【Term of Project】

FY2019-2023

【Budget Allocation】

127,400 Thousand Yen

【Homepage Address and Other Contact Information】

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【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

TAKAGI Hiroshi
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Research Project Number：19H05639　Researcher Number：50275088
Keyword：Nitric oxide, Yeast, Fungi, Synthetic regulation, Physiological function

【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.

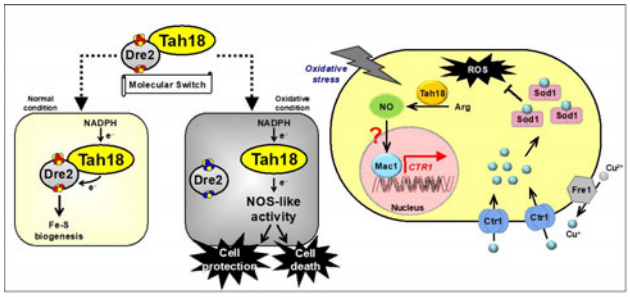

Figure 1 Model of NO synthesis (left) and stress tolerance by NO (right) in yeast

【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

【Homepage Address and Other Contact Information】

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Title of Project：Innovative chemical genetics on novel function of endogenous metabolites

YOSHIDA Minoru
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Research Project Number：19H05640　Researcher Number：80191617
Keyword：Endogenous metabolite, Chemical genetics, Drug target, Posttranslational modification, Metabolic pheromone

【Purpose and Background of the Research】

Many metabolites have cellular function independent of their metabolic roles by acting as cofactors or inhibitors for posttranslational modification enzymes (Fig. 1). Therefore, it seems possible that metabolites in common metabolic pathways possess unexpected activity, and that their dynamic fluctuation upon environmental changes greatly affects the destiny of life through affecting their adaptation and homeostasis. Although fluctuation of metabolites can be analyzed by metabolome, their biological function has been poorly understood because of difficulties in activity measurements. This study aims at elucidating novel function of metabolites by molecular and chemical genetics.

【Research Methods】

In this research, we will uncover new function of metabolites using fission yeast and animal cells with our original screening systems. To this end, we will expand our metabolite compound library.

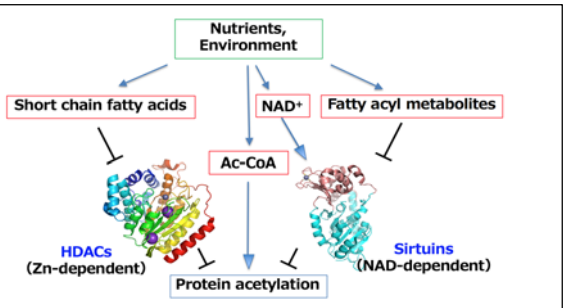

Fig. 1. Protein acetylation regulated by metabolites

(1) Chemical genetics for energy metabolism
We previously showed that SIRT2 has defatty-acylase activity, which requires formation of a large hydrophobic pocket to accommodate the substrate fatty-acyl lysine. However, once the defatty-acylation reaction occurs, it loses deacetylase activity. This is probably because *O*-acyl-ADP ribose, the product of defatty-acylation, binds the hydrophobic pocket thereby inhibiting deacetylase activity. On the other hand, the fatty-acyl lysine substrate may kick it out from the pocket, allowing the next cycle of catalysis. Here we will elucidate the molecular mechanism for the conversion of enzyme activity from deacetylation to defatty-acylation by using synthetic derivatives of *O*-acyl-ADP ribose. In addition, we will analyze the mode of action of a natural product derivative named TLAM, which activates mitochondria respiration and suppresses the Warburg effect in cancer cells.

(2) Chemical genetics for hypoxia response
The eukaryotic translation factor eIF5A is subject to hypusination, a unique posttranslational modification. Hypothesis that hypusination of eIF5A acts as a sensor of hypoxia at the translation level will be examined. Furthermore, we will investigate why defective hypusination under the hypoxic conditions downregulates mitochondrial protein synthesis by ribosome profiling.
(3) Chemical genetics for amino acid metabolism
Based on our previous discovery of a fission yeast pheromone that induces cancellation of nitrogen catabolite repression, we will identify novel signaling small molecules by searching for mutants whose growth can be recovered in the vicinity of the wild-type cell colony.
(4) Chemical genetics for lipid metabolism
We will elucidate the mechanism of cell growth inhibition by fatty acids with odd number carbons or marine microbial lipids by identifying genes that alter their sensitivity.

【Expected Research Achievements and Scientific Significance】

Fluctuation of metabolites upon environmental changes regulates homeostasis through altered posttranslational modification such as acetylation. Uncovering of hidden function of metabolites will lead to the development of novel medical or material production technologies.

【Publications Relevant to the Project】

- Sun *et al.* Identification of novel secreted fatty acids that regulate nitrogen catabolite repression in fission yeast. *Sci. Rep*. 6: 20856, 2016.
- Ito *et al.* The subcellular localization and activity of cortactin is regulated by acetylation and interaction with Keap1. *Sci. Signal.* 8: ra120, 2015.
- Nishimura *et al.* Marine antifungal theonellamides target 3beta-hydroxysterol to activate Rho1 signaling. *Nat. Chem. Biol.* 6: 519-526, 2010.

【Term of Project】

FY2019-2023

【Budget Allocation】

154,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/research/labs/csrs/chem_genom/
http://www2.riken.jp/SPD/CG/index.html