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

Broad Section F

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)

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【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.

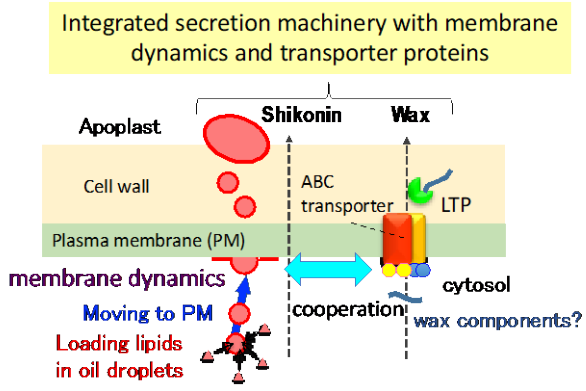

Figure 1　Bulk transport of lipid molecules

【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】

http://www.rish.kyoto-u.ac.jp/lpge/index.html
yazaki@rish.kyoto-u.ac.jp