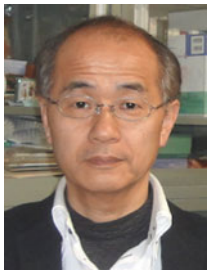

Title of Project : Development of basic technology of chemistry and biology for reducing damage by root parasitic weeds

Tadao Asami
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number : 18H05266 Researcher Number : 90231901
Keyword : root parasitic weeds, strigolactones, suicidal germination, inhibitor, receptor, biosynthesis

【Purpose and Background of the Research】

Strigolactones (SLs) are a plant hormone that regulates branching of plants. They also promote the germination of root parasitic plants that grow in the host plant's root, such as genus *Striga*. The root parasitic weeds infest cereals and non-cereals crops respectively, resulting 50-90% yield losses. Therefore, chemicals that regulate the functions of SLs will be very useful, so in this project we will try to develop several SL biosynthesis inhibitors, agonists and antagonists to reduce the damage by root parasitic weeds. Followings are important characters of chemicals: SL biosynthesis inhibitors must inhibit SL biosynthesis in host plants without morphological change of host plants, SL agonists must induce germination of root parasitic weeds without roots of host plants (suicidal germination) and antagonists must inhibit the perception of SLs in root parasitic weeds. To understand the mechanisms how these chemicals are perceived by their target proteins, we will also try to crystalize the complex of proteins and chemicals. As ethylene also induce suicidal germination, we will try to prepare ethylene agonists and antagonists, which will be used to facilitate the crystallization of ethylene receptors.

【Research Methods】

We have already reported a lead compound for SL biosynthesis inhibitor TIS103. In this project, we will carry out a structure-activity relationship study of TIS13 to discover more potent and specific SL biosynthesis inhibitor because TIS13 has a severe side effect at high concentrations. We will identify the target sites of the new potent inhibitors and prepare knockout mutants of the target proteins. We found that treatment of GAs also reduces the level of SLs. This means that GA can be used to protect plants from the attack of *Striga*. AC94377 and D67 are good candidates of GA agonists but their mode of binding to GA receptor is not clear. Here we will try to clarify 3D structures of complex between GA agonists and GA receptors, which will facilitate the design of new GA agonists.

To design suicidal germination inducers, we try to understand the mechanism of SL perception. At present two mechanisms, (A) and (B), are proposed as shown in Figure 1. We found receptor inhibitors that covalently bind to the catalytic site of SL receptors and inhibit the germination of *Striga* seeds induced by SLs. Among these covalent inhibitors, we found AGOL also covalently binds to catalytic site of SL receptors but shows agonistic activity. Investigation on the activation mechanism of SL signals by AGOL will make clear the mechanism of activation pathway (A). This research will facilitate the design of new suicidal germination inducers.

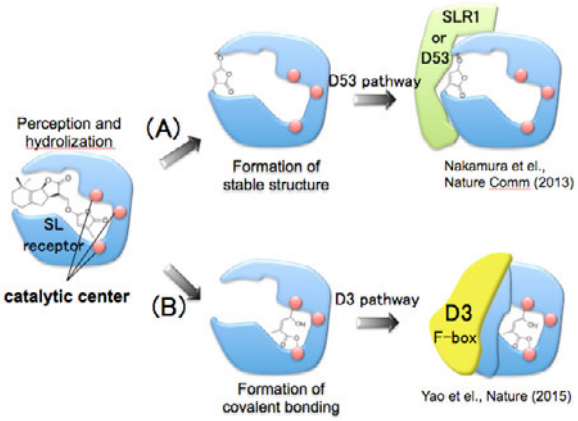

Figure 1. Perception of SL by SL receptor

【Expected Research Achievements and Scientific Significance】

- Practical use of developed chemicals in the infested fields
- Proposal of new mode of action of SLs

【Publications Relevant to the Project】

Nakamura H, et al., Molecular mechanism of strigolactone perception by DWARF14. ***Nature Comm***, **4**: 2613 (2013).
Zhou F et al., D14-SCFD3-dependent degradation of D53 regulates strigolactone signaling. ***Nature***, **504**: 406-410 (2013).

【Term of Project】 FY2018-2022

【Budget Allocation】 151,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://pgr.ch.a.u-tokyo.ac.jp/

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

Broad Section F

Title of Project : Identification of primer pheromones in mammals and elucidation of a neural basis for the pheromone action

Kazushige Touhara
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number : 18H05267 Researcher Number : 00280925
Keyword : Pheromone, olfaction, receptor, neural circuit, reproduction

【Purpose and Background of the Research】

Pheromones are categorized into two types; one that elicits specific behavior, calling a releaser pheromone, and another that causes physiological effects, calling a primer pheromone. Studies on a releaser pheromone have been much progressed at the level of molecule, receptor, and neural circuitry using insects and mice, whereas there are not many studies for mammals including human. In this study, we will identify primer pheromones in mice that induce synchronous estrus, accelerate puberty, or cause sexual suppression, and their receptors and neural circuits responsible for the effects. We will also look for primer pheromones in humans and identify a brain region involved in the physiology. The molecular and neural mechanisms underlying primer pheromone actions related to reproductive function will be revealed.

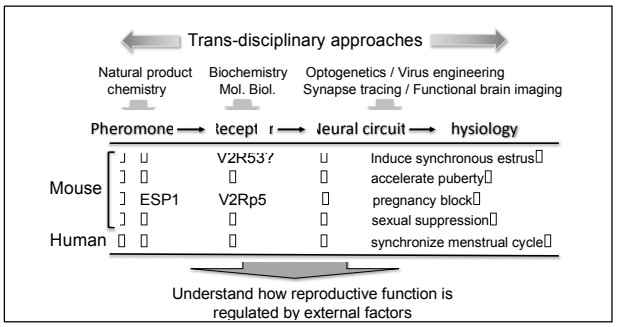

Figure Aims,

【Research Methods】

Regarding mouse primer pheromones, we will develop an *in vivo* Ca^{2+} imaging method using fiber photometry, purify active compound(s) from urine by HPLC, and determine the structure(s) based on GC-MS/LC-MS followed by chemical synthesis. We will reveal the receptors for the identified primer pheromone using a double in situ hybridization strategy from ~300 vomeronasal receptors. The neural circuitry underlying the pheromone action will be dissected by using virus technology and optogenetic and phamacogenetic approaches. To identify human pheromones, we will use non-invasive functional brain imaging techniques and measure autonomic nervous functions and endocrinological changes.

【Expected Research Achievements and Scientific Significance】

Mouse primer pheromones identified during '80 by Novotony group have been denied in 2011 by Stowers group. The human dormitory effect reported by McClintock in 1971 has not been characterized at the level of molecule. The only primer pheromone in mammals so far is a goat male pheromone identified by Murata et al. in 2014. Thus, revealing primer pheromones, their receptors, and responsible neural circuits gives a high impact in the field. Moreover, this will be the first molecular study on human pheromone. The approach is transdisciplinary in the field of chemistry-biology; from natural product chemistry, receptor signaling biology, to brain science. In practical senses, the expected results will become valuable information that helps various problems in reproductive functions in human and mammals.

【Publications Relevant to the Project】

・Hattori, T., Osakada, T., Masaoka, T., Oyama, R., Horio, N., Mogi, K., Nagasawa, M., Haga-Yamanaka, S., Touhara, K.* and Kikusui, T.* "Exocrine gland-secreting peptide 1 is a key chemosensory signal responsible for the Bruce effect in mice"
Current Biology 27, 3197-3201 (2017)
・Ishii, K., Osakada, T., Mori, H., Miyasaka, N., Yoshihara, Y., Miyamichi, K.*, and Touhara, K.* "A Labeled-Line Neural Circuit for Pheromone-Mediated Sexual Behaviors in Mice"
Neuron 95, 123-137 (2017)

【Term of Project】 FY2018-2022

【Budget Allocation】 147,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/biological-chemistry/

Title of Project : Antiaging system of long-lived termite kings

Kenji Matsuura
(Kyoto University, Graduate School of Agriculture, Professor)

Research Project Number : 18H05268 Researcher Number : 40379821
Keyword : longevity, antiaging, termite, metabolome, social insects

【Purpose and Background of the Research】

Eusocial insects, such as ants, bees, wasps, and termites, are characterized by a system of caste division (reproductive vs. non-reproductive individuals), where the lifespan of queens (and kings in termites) can reach 100 times longer than the average lifespan of non-social insects. Recent our study revealed that kings of some termite species including *Reticulitermes speratus* have extremely long life span, which is comparable to human longevity, due to their unique reproductive system AQS (Asexual Queen Succession).

Generally, longevity negatively correlates with reproduction. Most animals show a gradual decline in reproduction with age. Nevertheless, termite kings are the most sexually active individuals and also the most long-lived individuals among colony members. How can termite kings maintain high sexual activity over several decades without sacrificing longevity? Because of their unique characteristics, termite kings are expected to facilitate the discovery of the novel mechanisms underlying the extremely long longevity.

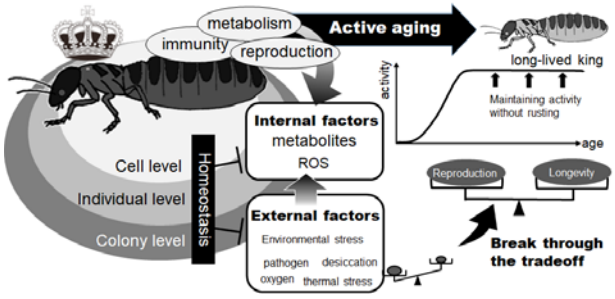

Figure 1. Research scheme

【Research Methods】

To identify metabolic pathway and the metabolic activity of the kings changing as aging, we perform metabolic flux analyses using doubly labeled water method and isotope distribution from 13C-labeled compounds.

Kings and queens have no symbiotic protozoa in their hindgut, and food supply is made exclusively through trophallaxis from workers. Based on our long-term behavioral monitoring, we found that workers provide a special royal food for kings and queens. We are going to identify the key compounds of the royal food by using MS/MS and also perform in vivo function analysis of the candidate compounds.

Royal chambers, where kings and queens are harbored, have a lower oxygen concentration in comparison with other parts of the termite nests. To investigate how the hypoxic condition influence on kings' reproduction and longevity, we perform comparative analysis of their metabolism by keeping kings under different oxygen conditions.

【Expected Research Achievements and Scientific Significance】

Having healthy active life for a long time is one ideal of the human society. Through this study, we will be able to find a number of novel factors underlying the extreme longevity of termite kings, which acquired such a long longevity in the history of evolution independently of mammals. Identification of novel molecular mechanism underlying the extremely long lifespan of social insect royals would have a high interdisciplinary impact in biology.

【Publications Relevant to the Project】

・Matsuura K. et al. (2018) A genomic imprinting model of termite caste determination: Not genetic but epigenetic inheritance influences offspring caste fate. . Am Nat 191: 677-690.
・Matsuura, K. et al. (2009) Queen succession through asexual reproduction in termites. Science 323:1687.

【Term of Project】 FY2018-2022

【Budget Allocation】 149,600 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 : Uncovering the secrets of lipid-transporting ABC proteins

Kazumitsu Ueda
(Kyoto University, Graduate School of Agriculture, Professor)

Research Project Number : 18H05269 Researcher Number : 10151789
Keyword : ABC proteins, cholesterol, transporter, atherosclerosis

【Purpose and Background of the Research】

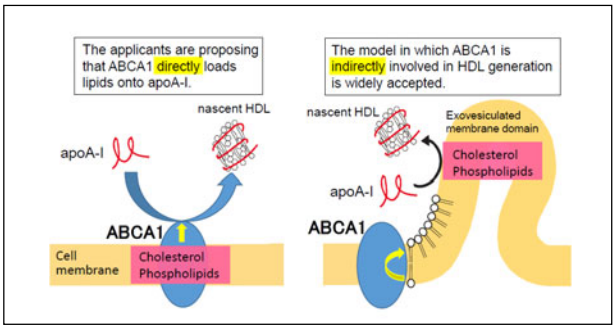
Genome analyses have revealed that 48 genes of the ATP-binding cassette (ABC) protein family are encoded on human chromosomes, and that defects in more than 20 family members are related to various diseases. Thus, ABC proteins play important roles for maintaining human health.

Ueda has been working on ABC proteins for 30 years, since the discovery of the multidrug transporter MDR1 (ABCB1), and recently succeeded in establishing a platform for ABC protein research by revealing the mechanism of MDR1 function based detailed structural analyses. In this project, we are aiming to reveal the mechanism of lipid-transporting ABC proteins, specifically ABCA1.

ABCA1 is a key transporter involved in the generation of high-density lipoprotein (HDL). However, the mechanism of HDL generation remains controversial. According to a widely accepted model for HDL generation, ABCA1 generates specific membrane domains (i.e., exovesiculated membrane domains) with outward phospholipid translocation activity, and apoA-I (a lipid acceptor in serum) spontaneously acquires lipids from these domains (Fig). Based on our biochemical and cell biological data, we propose "the direct loading model," a mechanism quite different from the conventional model.

【Research Methods】

To provide answers to unsolved questions in this field, we will integrate a variety of scientific and technical disciplines, including single molecule imaging, cryo-electron microscopy, high-speed AFM, high-resolution X-ray crystallography, and studies in model organisms in addition to conventional biochemistry and cell biology.



Models of HDL formation

We recently reported that ABCA1 is involved in the uneven distribution of cholesterol in the plasma membrane. Our ongoing studies will reveal the mechanism by which ABCA1 function is regulated. Lipid-transporting ABC proteins are thought to be involved in neurological diseases such as Alzheimer's. Our studies will reveal their roles in these disorders using model organisms.

【Expected Research Achievements and Scientific Significance】

Collapse of cholesterol homeostasis causes various diseases. However, the mechanism by which this homeostasis is maintained remains unclear, and the physiological role of cholesterol is not well understood. By revealing the mechanism underlying HDL generation and novel physiological functions of cholesterols, our study will facilitate the development of methods to cure and prevent diseases such as atherosclerosis, diabetes, and neurological diseases.

【Publications Relevant to the Project】

・Nagata KO, *et al.* ABCA1 dimer-monomer interconversion during HDL generation revealed by single-molecule imaging. **Proc. Natl. Acad. Sci. USA**, 110, 5034-5039 (2013)
・Liu SL, *et al.* Orthogonal lipid sensors identify transbilayer asymmetry of plasma membrane cholesterol. **Nature Chem Biol** 13, 268-274 (2017)
・Ishigami M, *et al.* Temporary sequestration of cholesterol and phosphatidylcholine within extracellular domains of ABCA1 during nascent HDL generation. **Sci Rep.** 8:6170 (2018)

【Term of Project】

FY2018-2022

【Budget Allocation】

148,900 Thousand Yen

【Homepage Address and Other Contact Information】

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