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

Broad Section G

Title of Project： **The prototype, and evolution, of the system which adapt plant growth to its environment through the signaling molecule, strigolactone.**

KYOZUKA Junko

(Tohoku University, Graduate School of Sciences, Professor)

Research Project Number: 20H05684 Researcher Number：90273838

Keyword：Plant hormone, Strigolactone, AM symbiosis, Rhizosphere signaling molecule

【Purpose and Background of the Research】

Strigolactones (SLs), carotenoid-derived signaling molecules, are unique. They play dual roles, as a class of phytohormones, regulating a wide spectrum of plant growth and developmental processes, and as allelochemicals, where they are secreted to the rhizosphere and induce symbiosis with soil arbuscular mycorrhizal (AM) fungi. This AM symbiosis can be traced back 450 million years. It is widely accepted that symbiosis with AM fungi was crucial for early plant terrestrial colonization (Fig. 1). Despite the importance of SLs in the evolution of land plants, their function outside seed plants is largely unknown.

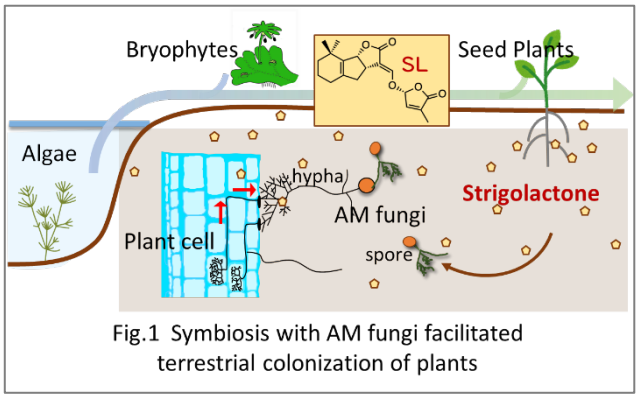


Fig.1 Symbiosis with AM fungi facilitated terrestrial colonization of plants

【Research Methods】

Our research methods include molecular genetics, imaging technologies using molecular markers and organic chemistry. Bryophytic plants are our main research material. Most liverwort species, one of the three groups of bryophytes, form a symbiotic interaction with AM fungi, while *Marchanthia polymorpha*, the model bryophyte species used in molecular genetic studies, does not. Therefore, we mainly use *M. paleacea*, which forms an AM symbiotic relationship, as well as *M. polymorpha*, and compare these two species (Fig. 2). We analyze the function of SLs in *M. paleacea* in detail and test the hypothesis that the function of the ancestral SL is as a rhizosphere-signaling chemical. In addition, we will verify that the novel SL we identified (Bryosimbiol) is an ancestral type of SL. We will reveal where and when it is synthesized, and secreted, in plants. We will also isolate the transporter of Bryosimbiol and clarify the mechanisms by which SL is externally secreted. The role of the KL signaling pathway, the original pathway from which the signaling pathway of SL as a plant hormone evolved, will be elucidated.

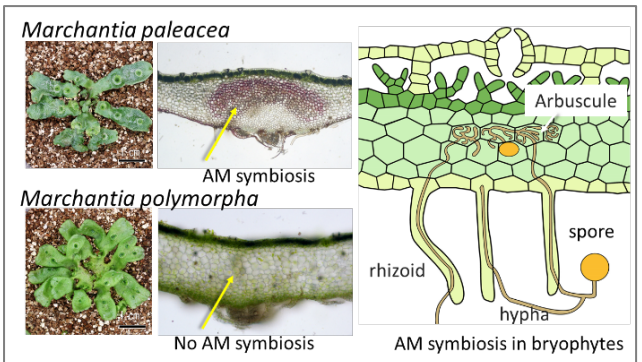


Fig. 2. AM symbiosis occurs in *M. paleacea*

【Expected Research Achievements and Scientific Significance】

This study is expected to reveal: **1.** the prototype, and evolution, of compound-mediated communication between organisms, **2.** mechanisms in the evolution of phytohormones, **3.** the origins of phytohormone synthesis and signal transduction, and the basis for diversification, and **4.** the prototype of the system which balance nutrient absorption and plant growth.

This study will provide a breakthrough in our knowledge of the regulation of nutrient uptake and growth through symbiosis with AM fungi, which enabled plants to flourish on land.

【Publications Relevant to the Project】

- Kameoka H, **Kyozuka J** (2018) Spatial regulation of strigolactone function. J. Exp. Bot. 69:2255
- Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, **Kyozuka J**, Yamaguchi S. (2008) Inhibition of shoot branching by new terpenoid plant hormones. ***Nature*** 455: 195

【Term of Project】 FY2020-2024

【Budget Allocation】 151,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.lifesci.tohoku.ac.jp/PlantDev/

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

Broad Section G

Title of Project： From the structure-function relationship of dendritic spines to synaptic mechanobiology

KASAI Haruo
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number: 20H05685 Researcher Number：60224375
Keyword：Learning, Memory, Synapses, Cell motility. Mechanobiology

【Purpose and Background of the Research】

The depth penetration of the two-photon microscope allowed us to examine the structural plasticity of dendritic spines in 2000s. We have further developed the two-photon uncaging technique which allowed us to stimulate single submicron dendritic spine by glutamate, and found the spine sizes themselves changes and they are tightly related to the learning *in vivo*. In the present proposal, we will extend our investigations into the presynaptic terminals to test whether spine enlargement has direct mechanical effects on the presynaptic terminals. We first study rapid mechanical effects of spine enlargement, and then long-term effects by using the optical probes. In this manner, we developed the new field where short and long term mechanical action to presynatpic terminals are investigated.

【Research Methods】

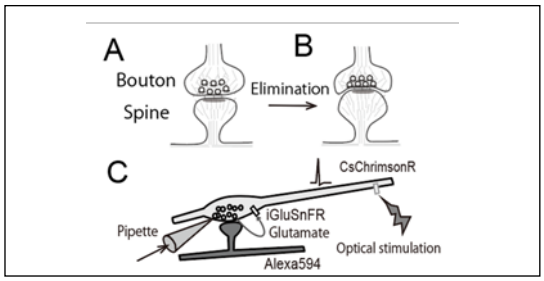


Figure 1 Mechanical interactions of synapses

Learning stimuli have been known to induce spine enlargements, which inevitably push the presynaptic terminal making synaptic contact with the spine. This type of effects has anticipated but never been experimentally examined due to the difficulty of the experiments. We are now challenging the issue, by using the two-photon uncaging, glutamate sensor protein, optogenetics, SNARE/FRET probe to quantify the engine of exocytosis, Q-dot coated glass pipettes, and founds that the pushing caused assembly of SNAREs, and facilitate evoked exocytosis. A similar effect can be seen with osmottic pressure, and learning-stimuli induced spine enlargement. Thus, axonal boutons have the pressure sensation and transduction (PREST) mechanisms with which synapses interact mechanically in addition to well-known chemical and electrical transmissions. Interestingly, the one minute pushing caused more than 20 min faciliatoty effects, and may act as working memory.

We have investigated the relationship between learning and behavior using the nucleus accumpens, and found that direct pathway mediate generalizing reward learning and the indirect pathway the discrimination learning, and these conditioned learning depend on the dopamine mediated spine enlargements in respective neurons using slice and in vivo preparation.

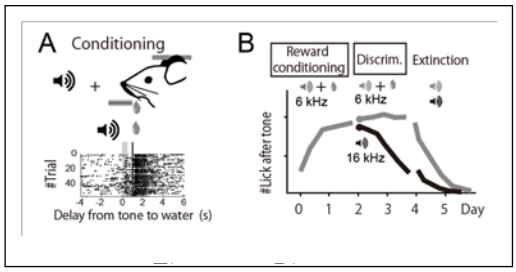


Figure 2 Generalization/discrimination in mice.

【Expected Research Achievements and Scientific Significance】

We will fast publish our amazing PREST mehanisms by completeing all the additonal experiments asked by reviewers. Then, we study the cellular and molecular bases of PREST using STED superresolution microspy to find either pharmacological or genetic intervention of PREST effects to identify the working memory role in vivo. We also obtain a new line of evidence for the synaptic bases of the conditioned learning.

【Publications Relevant to the Project】

- Takahashi, N., Sawada, W., Noguchi, J., Watanabe, S., Ucar, H., Hayashi-Takagi, A., Yagishita, S., Ohno, M., Tokumaru, H. & Kasai, H. (2015). Two-photon fluorescence lifetime imaging of primed SNARE complexes in presynaptic terminals and □ cells. *Nature Communications* 6:8531.
- Iino, Y., Sawada, T., Yamaguchi, Tajiri, M., K., Ishii, S., Kasai, H.* & Yagishita, S.* (2020) Dopamine D2 receptors in discrimination learning and spine enlargement. *Nature* 579: 555-560.

【Term of Project】 FY2020- 2024

【Budget Allocation】 150,700 Thousand Yen

【Homepage Address and Other Contact Information】

https://www.bm2.m.u-tokyo.ac.jp/
hkasai@m.u-tokyo.ac.jpp

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

Broad Section G

Title of Project： Regulation of Enhanceosome by Cohesin

SHIRAHIGE Katsuhiko
(The University of Tokyo, Institute for Quantitative Biosciences, Professor)

Research Project Number: 20H05686 Researcher Number：90273854
Keyword：Chromosome hyperstructure, transcriptional elongation, ATP motors, cohesins, enhanceosomes

【Purpose and Background of the Research】

Cohesin is a protein complex that plays a central role in the regulation of chromosomal high order structure in eukaryotic cells, and has recently been shown to have a motor activity that introduces a loop structure into DNA in an ATP-dependent manner. Although cohesins function as sister chromatid adhesins, we have shown that cohesins bind to insulator sequences and enhancer regions of genes, and that one of the roles of cohesins is to regulate the transcriptional elongation of genes. Their roles are closely related to known transcriptional elongation regulators such as bromodomain protein BRD4 and super elongation complex AFF4, suggesting that they play important roles in developmental and differentiation control and cancer malignant transformation. In recent years, it has been reported that an amorphous, dynamic, large protein network controlled by "liquid-liquid phase separation" is formed on some enhancer DNAs, and it is also required to reconsider the complex (enhanceosome) on the enhancer as a collection of weak interactions. The objective of the present application is to understand the regulation of transcription elongation by enhanceosomes in molecular terms, with a particular focus on cohesin, and to clarify its physiological significance.

【Research Methods】

The following four approaches are taken. (1) Elucidation of the cohesin function in the enhancer using in vitro reconstitution: We have already succeeded in reconstitution of the enhancer using HeLa nuclear extract. Quantitative Western blot and mass spectrometry are used to comprehensively analyze the amount of integration factors and modification of cohesin and NIPBL. This point is also evaluated, as there may be differences in DNA conformation by cohesin removal or mutation. The resulting enhanceosome is isolated and directly visualized with an atomic force microscope (high-speed AFM). Investigate whether the structure of the enhanceosome is dynamically altered or whether cohesin induces the change. (2) Functional analysis of cohesin through high-resolution visualization of the nuclear enhanceosome: To complement the results obtained in vitro in (1), analysis using a genomics technique targeting the intracellular enhancer is performed. (3) Comprehensive identification of proteinaceous and nonproteinaceous components of the enhanceosome: The enhanceosome is a large complex of diverse proteins. Because there is a high possibility that there are still unidentified enhanceosome components, a new method is used in combination with mass spectrometry to comprehensively identify the proteins present in the enhanceosome. (4) Cohesin modification and participation in transcription reaction: In the reaction system of (1), phosphorylation modification and acetylation modification of cohesin and NIPBL are examined, and the participation in the enhanceosome control is examined.

【Expected Research Achievements and Scientific Significance】

Mutations in cohesin/NIPBL, AFF4, and BRD4 are known to cause developmental abnormalities. It is also involved in carcinogenesis, mainly in the blood cell system. The regulation of transcription elongation by enhanceosomes is expected to play an essential role in the selection of differentiation pathways and the maintenance of differentiation status. The results of this study are expected to greatly contribute to understanding these diseases at the molecular level. Anticancer agents targeting epigenetic pathways have recently been developed and used. Transcriptional elongation control pathways may also be targets for anticancer drugs. The basic understanding of the enhanceosome provided by this research will be a foundation for future drug discovery.

【Publications Relevant to the Project】

· Izumi K, Nakato R, ... Shirahige K*, Krantz ID* (*shared corresponding authors). Germline gain-of-function mutations in AFF4 cause a developmental syndrome functionally linking the super elongation complex and cohesin. **Nat Genet.** 47:338-344, (2015).
· Deardorff MA, Bando M, Nakato R,...Shirahige K.* HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. **Nature.** 489:313-317, (2012)

【Term of Project】 FY2020- 2024

【Budget Allocation】 151,800 Thousand Yen

【Homepage Address and Other Contact Information】
http://www.iam.u-tokyo.ac.jp/chromosomeinformatics/

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

Broad Section G

Title of Project：Analysis of signal transduction of stomatal movements and regulation of plant growth

KINOSHITA Toshinori

(Nagoya University, Institute of Transformative Bio-Molecules [WPI-ITbM], Professor)

Research Project Number: 20H05687 Researcher Number：50271101

Keyword：Stomata, Signal transduction, photosynthesis, growth, drought resistance

【Purpose and Background of the Research】

Stomata open in response to light, including blue and red light. Red light induces stomatal opening via photosynthesis in the mesophyll and guard cell chloroplasts. In contrast, blue light as a signal induces stomatal opening. Phototropins expressed in guard cells act as major blue light receptors for stomatal opening. Blue light-induced stomatal opening is mediated through activation of a plasma membrane (PM) H^+ pump, later identified as the PM H^+-ATPase, in guard cells. The blue light-activated pump provides driving force for stomatal opening concomitant with ion accumulation and cell volume increase in guard cells. However, the detail molecular mechanism of stomatal movements is still unknown.

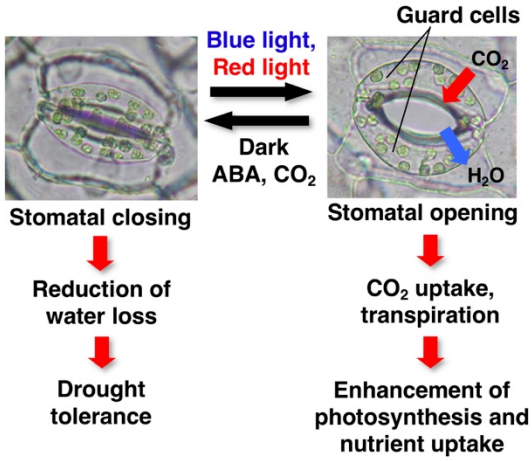


Figure 1 Stomatal movements and functions

【Research Methods】

To elucidate the signal transduction for stomatal movements, we are planning to perform 2 projects, as follows.

1, Identification of protein kinase and protein phosphatase, which regulate phosphorylation status of PM H^+-ATPase, in stomatal guard cells. We have screened protein kinase and protein phosphatase library and found several candidate inhibitors. We are going to identify these target components, which bind to these inhibitors, by genetic and biochemical approaches.

2, Identification of the novel signal components involved in stomatal movements. We have started comprehensive chemical screening to identify the chemicals, which affect stomatal opening and closing, and genetic screening to find the mutants, which defect light-induced stomatal opening. We are going to identify and clarify the function of these components in the signal transduction in stomatal guard cells.

Based on the results from these basic researches, we will try to manipulate stomatal aperture by transgenic techniques, but also chemical treatment and investigate stomatal phenotype, photosynthetic activity, plant growth, and drought resistance.

【Expected Research Achievements and Scientific Significance】

Stomata open in response to blue light to facilitate gas exchange between the plant and the atmosphere. This response is key to terrestrial plant life, as gas exchange is necessary not only for photosynthesis but also for water uptake from the roots. It is important to elucidate the signal transduction in stomatal guard cells in response to environmental signals for biological science.

Given the importance of stomatal function, future investigations will not only improve our understanding of the molecular mechanisms of signaling pathways in plants, but also provide important clues for agricultural strategies to improve photosynthetic or water use efficiency, leading to an increase in the biomass and harvest of crops.

【Publications Relevant to the Project】

・ Inoue S, Kinoshita T. (2017) Blue light regulation of stomatal opening and the plasma membrane H^+-ATPase. **Plant Physiology**, 174, 531-538.

・ Wang Y, Noguchi K, Ono N, Inoue S, Terashima I, Kinoshita T (2014) Overexpression of plasma membrane H^+-ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. **Proc. Natl. Acad. Sci. USA**, 111, 533-538.

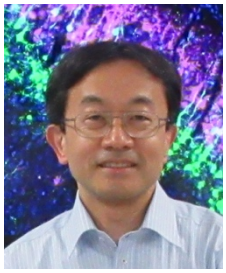
【Term of Project】 FY2020- 2024

【Budget Allocation】 143,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://plantphys.bio.nagoya-u.ac.jp

kinoshita@bio.nagoya-u.ac.jp



Title of Project： Mechanisms of corticogenesis in the developing brain

NAKAJIMA Kazunori
(Keio University, School of Medicine, Professor)

Research Project Number: 20H05688 Researcher Number：90280734
Keyword：cortex, development and differentiation, morphogenesis, neuron, glia

【Purpose and Background of the Research】

Mammalian brains have an important structure called the cortex on the surface of the brain, which is composed of innumerable neurons arranged in a layered structure.

In this study, we propose to clarify the mechanism by which the three-dimensional basic structure of the cortex is constructed during development through interactions among the cells that compose it. First, we intend to clarify the mechanisms by which the cerebral cortical neurons migrate from their site of birth, appropriately differentiate into specific subtypes of neurons, and then are arranged in orderly layers near the brain surface. In addition, we shall attempt to clarify where and how astrocytes, which are more abundant than neurons in the brain, are produced, differentiated, and widely distributed in the cortex. Furthermore, we shall attempt to understand how the three-dimensional global morphological changes of the entire tissue are controlled by cell-cell interactions and the like.

【Research Methods】

Neurons in the cerebral cortex form a layered structure just beneath the surface of the brain in a so-called “inside-out.” Reelin, a molecule that controls the cell migration and positioning in this manner, is secreted by the Cajal-Retzius cells in the marginal zone on the surface of the brain. We previously determined that reelin alone regulates the arrangement of the cortical neurons in this “inside-out” manner, through experiments in which reelin was expressed ectopically. In this study, we shall attempt to identify the underlying molecular mechanisms. We also determined that the final differentiation fate of each neuron can be modified by extracellular factors, and shall attempt to identify the underlying mechanisms. Since we recently discovered that astrocytes show a completely different

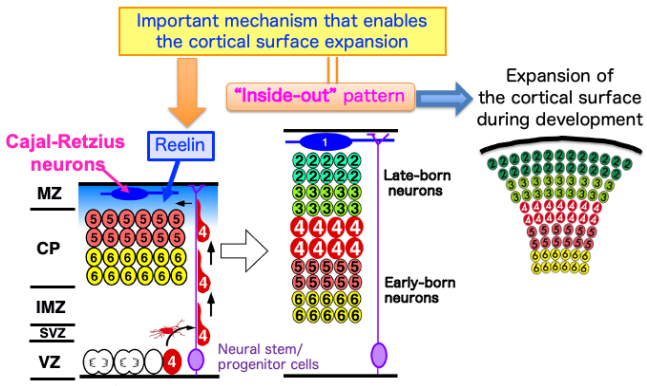


Figure 1 Development of cerebral cortex

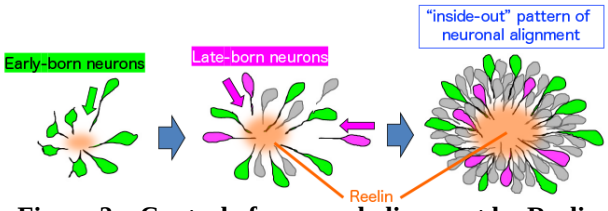


Figure 2 Control of neuronal alignment by Reelin

migration pattern from that of the cortical neurons, we will also explore the mechanisms underlying the regulation of the dynamics and differentiation of astrocytes. Reelin was also found to play an important role in the formation of the brain gyri/sulci, so that we propose to investigate the changes that reelin causes in its target cells and clarify the mechanisms that control the morphological changes of the entire tissue.

【Expected Research Achievements and Scientific Significance】

In recent years, attention has been focused on the possibility of minute abnormalities of the cortex that occur during development contributing to various neuropsychiatric disorders. In particular, abnormality of reelin regulation has been implicated in the development of schizophrenia, etc., and the results of this study might contribute to elucidation of the pathophysiology of these diseases.

【Publications Relevant to the Project】

・ Matsunaga, Y., Noda, M., Murakawa, H., Hayashi, K., Nagasaka, A., Inoue, S., Miyata, T., Miura, T., Kubo, K., and Nakajima, K. "Reelin transiently promotes N-cadherin-dependent neuronal adhesion during mouse cortical development.", ***Proc. Natl. Acad. Sci. U.S.A.***, 114 (8), 2048-2053 (2017).
・ Oishi, K., Aramaki, M., and Nakajima, K. “Mutually repressive interaction between Brn1/2 and Rorb contributes to establishment of neocortical layer 2/3 and layer 4.”, ***Proc. Natl. Acad. Sci. U.S.A.***, 113 (12), 3371-3376 (2016).

【Term of Project】 FY2020- 2024

【Budget Allocation】 151,300 Thousand Yen

【Homepage Address and Other Contact Information】

https://www.nakajimalab.com
kazunori@keio.jp

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

Broad Section G

Title of Project： **Molecular basis of the protein trafficking system for mitochondrial biogenesis and functional maintenance**

ENDO Toshiya
(Kyoto Sangyo University, Faculty of Life Sciences, Professor)

Research Project Number: 20H05689 Researcher Number：70152014
Keyword：mitochondria, translocator, cryo-electron microscopy, protein trafficking

【Purpose and Background of the Research】

Mitochondria have central roles in cellular energy production, metabolic pathways, signaling, and programmed cell death in eukaryotic cells. Biogenesis and maintenance of mitochondria require transport of over 1,000 different mitochondrial proteins from the cytosol to pre-existing mitochondria, and detection and removal of defective mitochondrial proteins. We found that the mitochondrial protein transport system is controlled by dynamic re-organization of the components of the translocator machineries, and protein quality control (QC) operates not only as degradation but also a proofreading to re-deliver mistargeted proteins to the ER. On the basis of these findings, we will perform cryo-electron microscopy (EM) based structural analyses and biochemical and cell biology analyses of the yeast mitochondrial protein trafficking and related QC systems. We will also search for factors facilitating expansion of mitochondria. By doing so, we aim to understand the molecular mechanisms of the fundamental question of how mitochondria are made and maintained in the cell.

【Research Methods】

The questions to be answered in this project are as follows: (1) What is the structural basis of the mechanism for the TOM complex, the outer membrane (OM) translocator, to import over 1,000 different proteins by its subunit re-organization? (2) How does the SAM complex, another OM translocator, facilitate β-barrel formation and OM integration of substrate proteins by dynamic subunit exchange? (3) How does Msp1, an AAA-ATPase of the OM, recognize mistargeted proteins, extract them from the outer membrane, and re-deliver them to the ER, and how

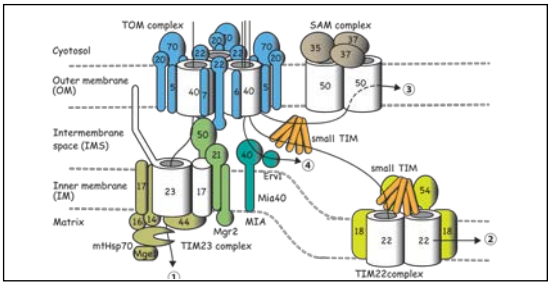


Figure 1 Mitochondrial protein trafficking; pathways and translocators

can we generalize the concept of the “proofreading of the protein trafficking” in terms of substrates and organelles? (4) How do the TOM-Ubx2 complex and Msp1 handle mitochondrial precursor proteins accumulated at the OM due to functional failure of the mitochondrial protein trafficking? (5) What are the factors and pathways that control expansion of pre-existing mitochondria? To answer these questions, we will perform structure-biology analyses of the translocators and related factors, together with *in vivo* and *in vitro* biochemical, cell biology analyses, and yeast molecular genetic analyses.

【Expected Research Achievements and Scientific Significance】

This project will reveal dynamic and high-resolution structures of the proteins involved in mitochondrial protein trafficking, which will allow us to understand the basic principle of the operation and regulation of the mitochondrial protein trafficking systems as a whole. In addition, the entire picture will be revealed for the new concept of re-trial or proofreading of the protein trafficking. Identification of the factors promoting expansion of mitochondria could be linked to new concept of mitochondrial biogenesis. The expected outcome of this project will lead to development of a novel strategy for human health maintenance and preventative as well as therapeutic treatments of aging and aging-related diseases. Furthermore, the obtained results are not limited to mitochondria, but will have a broad impact on protein trafficking to other organelles and on the construction of the intracellular membrane structures in general.

【Publications Relevant to the Project】

- Araiso, Y. *et al*. Structure of the mitochondrial import gate reveals distinct preprotein paths. ***Nature*** 575, 395-401 (2019).
- Matsumoto, S. *et al*. Msp1 clears mistargeted proteins by facilitating their transfer from mitochondria to the ER. ***Mol. Cell*** 76, 191-205 (2019).

【Term of Project】 FY2020- 2024

【Budget Allocation】 151,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://endolab.jp/wp/
tendo@cc.kyoto-su.ac.jp

Title of Project： Structural basis of higher-order complexes connecting transcription and its related functions

SEKINE Shun-ichi
(RIKEN, Center for Biosystems Dynamics Research, Team Leader)

Research Project Number: 20H05690 Researcher Number：50321774
Keyword：RNA polymerase, Transcription factors, Nucleosomes, Ribosomes, Cryo-electron microscopy

【Purpose and Background of the Research】

RNA polymerase is a large protein complex, which governs transcription of genetic information in DNA into RNA. It interacts with various molecules in cells, and serves as a hub for many biological processes. In eukaryotes, transcription by RNA polymerase II is tightly linked to important biological functions such as epigenetics, signal transduction, and mRNA processing. In prokaryotes, transcribing RNA polymerase interacts with a translating ribosome, and thereby, transcription and translation are coordinated. However, structural basis of the complexes formed at the interface between transcription and its related functions are largely unknown. Recent advances in cryo-electron microscopy (cryo-EM) methodologies made it possible to address such higher-order complex structures. In this study, through investigating such higher-order complex structures mainly by cryo-EM, we aim to elucidate not only the basic transcription mechanism, but also structural foundations of interplay and coordination between transcription and its related essential biological processes.

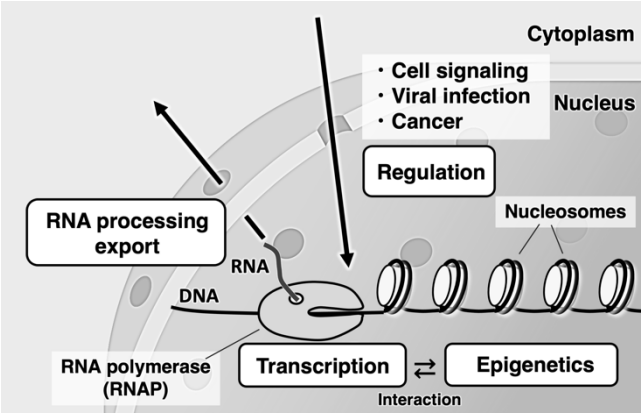


Figure 1. Interplay between transcription and various biological processes/functions

【Research Methods】

We will focus on the molecular mechanisms of the relationship between transcription and epigenetics, transcriptional regulation in higher eukaryotes, the coordination between transcription and mRNA processing, and transcription-translation coupling in bacteria. Key higher-order complexes involved in these phenomena will be prepared, and their structures will be analyzed by cryo-EM. In combination with biochemical and mutational analyses, we will elucidate the mechanisms of interplay, coordination, and regulation of transcription and its surrounding processes at near-atomic resolution.

【Expected Research Achievements and Scientific Significance】

Little is known about how biological macromolecules interact with each other and form higher-order complexes in cells to support cellular functions. In this study, by analyzing the structures of higher-order complexes, we will elucidate the molecular mechanisms of interconnection between transcription and other important biological functions (epigenetics, gene regulation, mRNA processing, translation, etc.), which will shed new lights on the mechanism of chromatin transcription and regulation. The study of transcriptional regulation in higher eukaryotes will also be expected to provide important insights into the disease mechanisms such as virual infection and cancer.

【Publications Relevant to the Project】

・Ehara, H.,* Kujirai, T.,* Fujino, Y., Shirouzu, M., Kurumizaka, H.† and Sekine, S.† “Structural insight into nucleosome transcription by RNA polymerase II with elongation factors”, ***Science*** *363*, 744-747 (2019).
・Kujirai, T.,* Ehara, T.,* Fujino, Y., Shirouzu, M., Sekine, S.† and Kurumizaka, H.† “Structural basis of the nucleosome transition during RNA polymerase II passage”, ***Science*** *362*, 595-598 (2018).
・Ehara, H., Yokoyama, T., Shigematsu, H., Yokoyama, S., Shirouzu, M. and Sekine, S.† “Structure of the complete elongation complex of RNA polymerase II with basal factors”, ***Science*** *357*, 921-924 (2017).

【Term of Project】

FY2020- 2024

【Budget Allocation】

145,500 Thousand Yen

【Homepage Address and Other Contact Information】

https://www.bdr.riken.jp/jp/research/labs/sekine-s/index.html
shunichi.sekine@riken.jp