Title of Project : Designing the mammalian biological oscillators

Hiroki R. Ueda
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number : 18H05270 Researcher Number : 20373277
Keyword : Synthetic biology

【Purpose and Background of the Research】

We have demonstrated that the activity of Casein kinase I (CKI) δ/ε plays a critical role in the determination of the period length of mammalian circadian clocks, of which the transcription and translation loop was believed to be the core design principle of the oscillator. We revealed that CKIδ/ε's phosphorylation activity is almost constant over the physiological range of temperature and is partly responsible for the temperature compensation of mammalian circadian clocks, that is, the period length of the circadian clock does not depend on the environmental temperature (Refs 1-2). Therefore, part of the design principle of mammalian circadian oscillators lies in the phosphorylation reaction.

In vivo phosphorylation dynamics is reversibly controlled by the presence of dephosphorylation activity. The primary purpose of this study is to clarify the mechanisms responsible for the dephosphorylation reaction antagonizing the phosphorylation of CKIδ/ε in the control of mammalian circadian clocks.

【Research Methods】

We have established an in vitro system to reconstitute the CKIδ/ε phosphorylation reaction corresponding to the control of the mammalian circadian clocks. With this system, the dephosphorylation enzyme activity antagonizing phosphorylation by CKIδ/ε will be searched. Furthermore, how this dephosphorylation activity is controlled by the phase of the circadian clock will be investigated (Fig. 1). The significance of identified dephosphorylation mechanism in vivo will be rigorously tested by the circadian-functional complementary system in mice (Ref. 3).

We will then reconstitute the dephosphorylation mechanism in the in vitro CKIδ/ε assay system to ask whether the reconstituted reversible phosphorylation reaction shows the property as the oscillator of the mammalian circadian clocks. Given the fact that temperature-independent phosphorylation property is conserved in CKI homolog of yeast, which apparently shows no circadian clock function, we will also design the reversible phosphorylation system to ask whether the idea of phosphorylation-driven oscillators is applicable for the non-circadian biological oscillators.

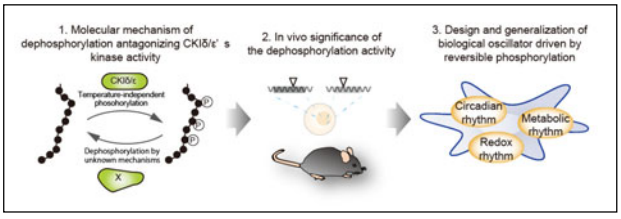


Figure 1 Oscillators driven by phosphorylation

【Expected Research Achievements and Scientific Significance】

This research will propose a new paradigm of the design principle of a mammalian oscillator by reversible phosphorylation. Also, by examining the possibility that reversible phosphorylation can drive biological oscillation involving CKI other than circadian clocks, the impact will be beyond the field of the circadian clock.

【Publications Relevant to the Project】

1. Isojima *et al.*, CKI ε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc. Natl. Acad. Sci. USA*, 106, 15744-15749 (2009)
2. Shinohara *et al.*, Temperature-Sensitive Substrate and Product Binding Underlie Temperature-Compensated Phosphorylation in the Clock. *Mol. Cell*, 67, 783-798 (2017)
3. Ode *et al.*, Knockout-rescue embryonic stem cell-derived mouse reveals circadian-period control by quality and quantity of CRY1. *Mol. Cell*, 65, 176-190 (2017)

【Term of Project】 FY2018-2022

【Budget Allocation】 154,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://sys-pharm.m.u-tokyo.ac.jp/index.html

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

Broad Section G

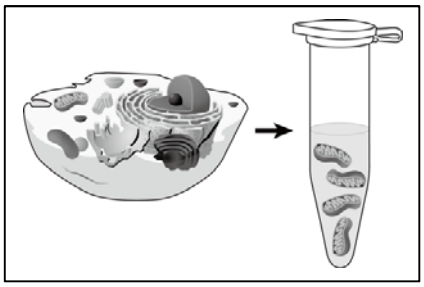
Title of Project : Biochemical approaches to understanding the reaction platforms of the piRNA pathway

Yukihide Tomari
(The University of Tokyo, Institute for Quantitative Biosciences, Professor)

Research Project Number : 18H05271 Researcher Number : 90447368
Keyword : piRNAs, small RNAs, reaction platform, RNA silencing, PIWI, Argonaute

【Purpose and Background of the Research】

Since the discovery of RNA interference, our biochemical understanding of small RNAs has been greatly advanced. However, there remain many unknowns in the molecular mechanism of piRNAs, which play essential roles in protecting the germline cells from transposons. The biggest obstacle is that piRNAs require specific "reaction platforms" in cells for their biogenesis and function, and thus the natural activity can be easily lost in conventional biochemical approaches using soluble lysates prepared by high-speed centrifugation, purified recombinant proteins etc.
We have previously established a cell-free system that recapitulates a part of the piRNA biogenesis pathway using the whole mitochondrial fraction. In this project, we propose to further develop this unique in vitro system so as to precisely understand the biogenesis and function of the piRNA pathway at the molecular level.



Biochemical approaches to understanding the reaction platforms

【Research Methods】

In particular, we will focus on the following three questions.

1. How are piRNA intermediates loaded into PIWI proteins?
2. How are piRNA intermediates processed into mature piRNAs?
3. How are the piRNA-cleaved targets are properly handed over to the next PIWI protein?

We seek to extract the corresponding "reaction platforms" from cells to test tubes in their best intact forms, faithfully recapitulate the processes and monitor the on-site reactions. We will not only utilize biochemistry to dissect the reactions into fundamental steps, but also combine it with genome editing technologies, next-generation sequencing, bioinformatics etc.

【Expected Research Achievements and Scientific Significance】

Our molecular understanding of the piRNA pathway is still lacking in vague models. This project takes advantage of our unique knowledge and approaches to the characteristic features of piRNAs that depend on cellular "reaction platforms" and aims at breaking the deadlock in the field. The idea of "reaction platform"-focused biochemistry is not limited to the piRNA pathway but could also be applicable to various non-coding RNAs and other biological processes that relies on cellular platforms.

【Publications Relevant to the Project】

Structural basis for arginine methylation-independent recognition of PIWIL1 by TDRD2. Zhang H, Liu K, Izumi N, Huang H, Ding D, Ni Z, Sidhu SS, Chen C, *Tomari Y, *Min J. ***Proc Natl Acad Sci U S A.*** 2017 Nov 21;114(47):12483-12488.

Identification and functional analysis of the pre-piRNA 3′ Trimmer in silkworms. Izumi N, Shoji K, Sakaguchi Y, Honda S, Kirino Y, Suzuki T, Katsuma S, *Tomari Y. ***Cell.*** 2016 Feb 25;164(5):962-73.

3′-end formation of PIWI-interacting RNAs in vitro. Kawaoka S, Izumi N, *Katsuma S, *Tomari Y. ***Mol Cell.*** 2011 Sep 16;43(6):1015-22.

【Term of Project】 FY2018–2022

【Budget Allocation】 148,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.iam.u-tokyo.ac.jp/tomari/
tomari@iam.u-tokyo.ac.jp

Title of Project : Dynamic regulation of RNA modification and biological process

Tsutomu Suzuki
(The University of Tokyo, Graduate School of Engineering, Professor)

Research Project Number : 18H05272 Researcher Number : 20292782
Keyword : RNA modification, mRNA, tRNA, ribosome, metabolite

【Purpose and Background of the Research】

RNA molecule has been regarded as a regulatory element in gene expression at the levels of transcription and translation, and is associated with various biological processes. RNA molecules are decorated with a wide variety of chemical modifications that are introduced after transcription. This process is also referred to as “epitranscriptome” that generates an emerging field in life science. We found some instances of RNA modifications dynamically regulated by sensing cellular metabolites which are substrates of the RNA modifications. In this project, we aim to establish a novel concept of regulatory gene expression mediated by dynamic regulation of RNA modification by sensing cellular metabolic status.

【Research Methods】

Individual RNAs are isolated by our original method called ‘reciprocal circulating chromatography (RCC)’. A species and site of each modification in the isolated RNA are analyzed by a highly sensitive detection system for RNA molecules using mass spectrometry (RNA-MS). In this project, we focus on RNA modification dynamically regulated by various cellular conditions including environmental stresses and nutrient starvation by measuring frequency of RNA modification using RNA-MS. We also explore RNA-modifying enzymes, enabling us to study RNA modification biochemically as well as genetically.

【Expected Research Achievements and Scientific Significance】

It is widely known that gene expression is transcriptionally regulated by various cellular processes including development and differentiation in spaciotemporal manner. We have been focusing on dynamic alteration of RNA modification as a novel regulatory element in gene expression. This project aims to establish a novel concept of regulatory mechanism of gene expression by dynamic alteration of RNA modification by sensing cellular metabolic status. Especially, we study RNA modifications regulated by nutritional availability and respiratory conditions. We also study physiological importance of reversible RNA modifications. Understanding of molecular pathogenesis of RNA modopathies will contribute to medical and pharmaceutical applications.

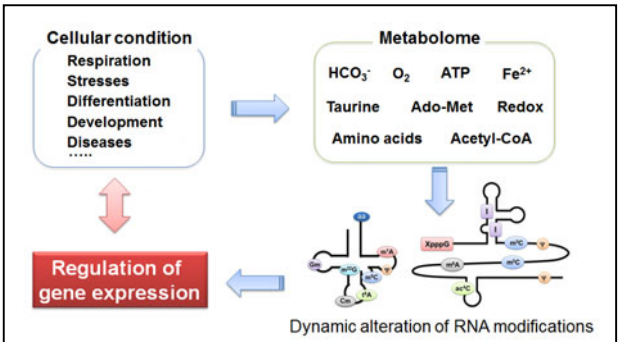


Figure Dynamic regulation of RNA modifications by sensing cellular metabolic status

【Publications Relevant to the Project】

- Taniguchi et al., Acetate-dependent tRNA acetylation required for decoding fidelity in protein synthesis. ***Nature Chem Biol.***, in press (2018)
- Lin et a., CO_2-sensitive tRNA modification associated with human mitochondrial disease. ***Nature Commun.***, 14, 9(1):1875 (2018)
- Nagao et al., Hydroxylation of a conserved tRNA modification establishes non-universal genetic code in echinoderm mitochondria. ***Nature Struct Mol Biol.***, 24, 778-782 (2017)
- Frye et al., RNA modifications: what have we learned and where are we headed? ***Nature Rev Genet.***, 17, 365-372 (2016)

【Term of Project】 FY2018-2022

【Budget Allocation】 149,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://rna.chem.t.u-tokyo.ac.jp/
ts@chembio.t.u-tokyo.ac.jp

Title of Project : Spectral opponency in photoreceptors : neuroethological analysis

Kentaro Arikawa
(SOKENDAI – The Graduate University for Advanced Studies, School of Advanced Sciences, Professor)

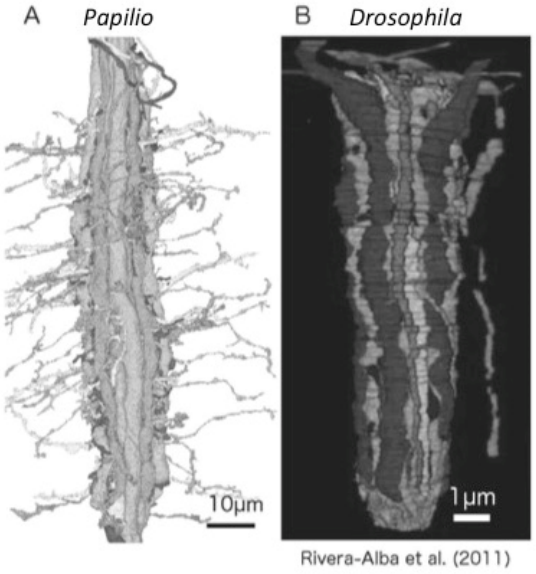
Research Project Number : 18H05273 Researcher Number : 20167232
Keyword : insect, color vision, photoreceptor, lamina, spectral opponency

【Purpose and Background of the Research】

We focus on the interphotoreceptor synapses in the lamina to study mechanisms of insect color vision. The interphotoreceptor synapses are presumably inhibitory, which we first identified in the butterfly, *Papilio xuthus*. *Papilio* has six distinct spectral receptors in the eye, and has long been a model species for color vision study. What would happen if spectrally distinct photoreceptors mutually inhibit? What is transmitted to second order neurons? Such photoreceptor interactions are absent in the lamina of *Drosophila*, which is perhaps related to their limited ability of discriminating colors. We hypothesize that the interphotoreceptor synapses provide crucial elements for color vision, and will analyse their function in the *Papilio* lamina. We will also perform comparative functional anatomy of the lamina in a variety of insects to address the question how color vision has evolved.

【Research Methods】

We will take three approaches: *i*) spectral opponency in photoreceptors, *ii*) LMCs' spectral properties, the second order neurons in the lamina, *iii*) comparative anatomy of the lamia among insects. CRISPR-Cas9 method will be applied to produce genetically-modified *Papilio*, which will be compared with normal individuals to understand the basis of wavelength information processing in the lamina. The comparative anatomy will aim at collecting serial images sufficient for analyzing lamina circuit at the EM level. We will start analyzing the lamina of about 10 insect species where vision has been somewhat studied.



3D reconstruction of photoreceptors and LMCs in the lamina cartridge. Lateral processes are missing in *Drosophila*.

【Expected Research Achievements and Scientific Significance】

Color vision is wide spread among animals, and even insects often exhibit human-like color vision properties. However, insects' nervous systems are quite different from that of vertebrates, indicating that the similarity is due to convergent evolution. Pioneered by Karl von Frisch, study of insect color vision has been a main topic of neuroethology. Recent progress in this field is quite impressive in *Drosophila* where all the contemporary molecular biological techniques are available. However, color vision of these flies is quite limited, while butterflies are the champion animals in this regard. This project using butterflies will reveal the neuronal mechanisms underlying their sophisticated color vision, together with its evolutionary background, which would enlighten the essential parts for seeing colors.

【Publications Relevant to the Project】

- Arikawa. *J Physiol*, 16: 5457-64, 2017
- Perry *et al. Nature*, 535: 280–4, 2016
- Kinoshita, Arikawa. *J Comp Physiol A*, 200: 513-26, 2014
- Takemura, Arikawa. *J Comp Neurol*, 494: 663-72, 2006
- Takeuchi *et al. J Exp Biol*, 209: 2873-9, 2006

【Term of Project】

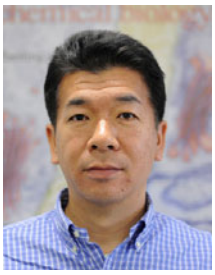
FY2018-2022

【Budget Allocation】

154,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.esb.soken.ac.jp/research/index.html#kentaro_arikawa



Title of Project : Molecular dissection of peptide signaling in plants

Yoshikatsu Matsubayashi
(Nagoya University, Graduate School of Science, Professor)

Research Project Number : 18H05274　Researcher Number : 00313974
Keyword : Peptide hormone, Receptor, *Arabidopsis*

【Purpose and Background of the Research】

Identification of hormones and their receptors in multicellular organisms is one of the most exciting research areas leading to breakthroughs in understanding how their growth and development are regulated. In particular, peptide signals offer advantages as cell-to-cell signals in that they have the most diversity in structure and function. Our goal in this project is to uncover the mechanisms by which plant development is regulated through identification of novel peptide signals and their receptors by using genome information, biochemical analysis and phenotypic observation.

【Research Methods】

Identification of novel peptide hormones by *in silico* screening:

By using *Arabidopsis* protein database, we will perform *in silico* screening of peptide hormone candidates based on the structural characteristics of the known peptide hormones. After structural elucidation by LC-MS/MS, we will identify receptors for peptides by exhaustive binding assay using receptor kinase expression library. Once we identified peptide ligand-receptor pairs, we will analyze their physiological roles in detail.

Ligand fishing using immobilized receptors:

We will use receptor-immobilized column to directly purify specific ligands in one step from the crude samples. We employ this approach to determine natural structures of peptide elicitors involved in disease resistance of plants.

Phloem-specific long distance mobile peptides:

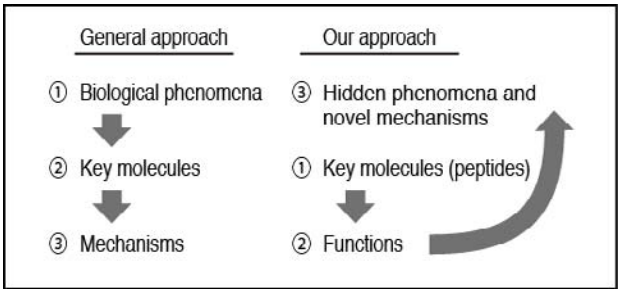


Figure 1　Outline of our experimental approach

Based on the tissue-specific microarray data, we have obtained several non-secreted peptides that show phloem-specific expression in leaves. They are strong candidates for shoot-to-root long distance mobile peptides. We will analyze their functions by combination of reverse genetics and biochemistry.

【Expected Research Achievements and Scientific Significance】

Ligand-receptor pairs act as master switches of complex intracellular signaling that directly regulates plant growth and development. Molecular dissection of these signaling pathway greatly promote our understanding of plant growth mechanisms under fluctuating natural environment. Moreover, these signaling pathways are attractive targets for the development of novel plant growth regulators.

【Publications Relevant to the Project】

・Tabata R., Sumida K., Yoshii T., Ohyama K., Shinohara H., Matsubayashi Y. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* **346,** 343-346 (2014)
・Ohkubo Y., Tanaka M., Tabata R., Ogawa-Ohnishi M., Matsubayashi Y. Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. *Nature Plants* **3**, 17029 (2017)
・Nakayama T., Shinohara H., Tanaka M., Baba K., Ogawa-Ohnishi M., Matsubayashi Y. A peptide hormone required for Casparian strip diffusion-barrier formation in *Arabidopsis* roots. *Science* **355,** 284-286 (2017)

【Term of Project】 FY2018-2022

【Budget Allocation】 148,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.bio.nagoya-u.ac.jp/~b2/research4.html

Title of Project : Full elucidation of sorting mechanisms in and around the Golgi apparatus by super-resolution live imaging

Akihiko Nakano
(RIKEN Center for Advanced Photonics, Deputy Director)

Research Project Number : 18H05275 Researcher Number : 90142140
Keyword : membrane traffic, Golgi apparatus, sorting and transport

【Purpose and Background of the Research】

Understanding of the mechanisms of membrane trafficking is now being totally innovated by state-of-the-art super-resolution live imaging microscopy. We have recently developed a new method with an extremely high spatiotemporal resolution, which can track dynamic 4D behaviors of even vesicles in cytoplasm. With this technology, we will tackle fundamental questions underlying the transport processes in the secretory pathway, from the ER to the Golgi apparatus and further to the *trans*-Golgi network. Experts on yeast, plant and animal cells will compare corresponding transport processes and extract common mechanisms and different features and draw comprehensive models, which will lead to thorough understanding of molecular mechanisms.

【Research Methods】

By making full use of SCLIM2 we developed, we will investigate the following problems.
[Yeast cells] 1) cargo capture from the ER by *cis*-Golgi; 2) cargo delivery between Golgi cisternae; 3) spatiotemporal regulation of sorting in the TGN.
[Plant cells] 1) cargo capture from the ER by GECCO; 2) cargo delivery in the Golgi stack; 3) spatiotemporal regulation of sorting in the TGN.
[Animal cells] 1) cargo capture from the ER by ERGIC; 2) cargo delivery in the Golgi stack and the TGN; 3) roles of the Golgi in nerve axons.

【Expected Research Achievements and Scientific Significance】

SCLIM, super-resolution confocal live imaging microscopy, has been a powerful tool to examine dynamic processes of membrane trafficking. We have further improved its performance by raising the sensitivity and the speed of detection system (Figure 1). By single-photon counting and a new deconvolution algorithm we developed, it now enables us to visualize assembly of coat proteins on the organelle membranes, sorting and packaging of cargo, handover of cargo between compartments, etc. which we have been eager to unveil.

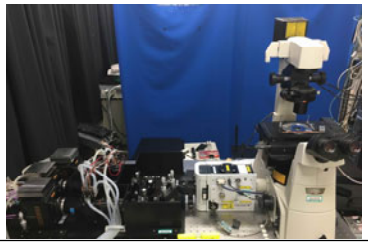


Figure 1 SCLIM2

【Publications Relevant to the Project】

- Ito, Y., Uemura, T., and Nakano, A. (2018). Golgi Entry Core Compartment functions as the COPII-independent scaffold for ER-Golgi transport in plant cells. *J. Cell Sci.* 131:jcs203893.
- Ishii, M., Suda, Y., Kurokawa, K., and Nakano, A. (2016). COPI is essential for Golgi cisternal maturation and dynamics. *J. Cell Sci.* 129:3251-3261.
- Kurokawa, K., Suda, Y. and Nakano, A. (2016). Sar1 localizes at the rims of COPII-coated membranes *in vivo*. *J. Cell Sci.* 129:3231-3237.
- Kurokawa, K., Okamoto, M., and Nakano, A. (2014). Contact of *cis*-Golgi with ER exit sites executes cargo capture and delivery from the ER. *Nat. Commun.* 5:3653.
- Uemura, T., Suda, Y., Ueda, T., and Nakano, A. (2014). Dynamic behavior of the *trans*-Golgi network in root tissues of Arabidopsis revealed by super-resolution live imaging. *Plant Cell Physiol.* 55:694-670.
- Suda, Y., Kurokawa, K., Hirata, R., and Nakano, A. (2013). Rab GAP cascade regulates dynamics of Ypt6 during the Golgi maturation. *Proc. Natl. Acad. Sci. U. S. A.* 110:18976-18981.

【Term of Project】 FY2018-2022

【Budget Allocation】 148,300 Thousand Yen

【Homepage Address and Other Contact Information】

https://rap.riken.jp/en/labs/sprg/lcmirt/

Title of Project : Molecular mechanisms of condensins I and II

Tatsuya Hirano
(RIKEN, Cluster for Pioneering Research, Chief Scientist)

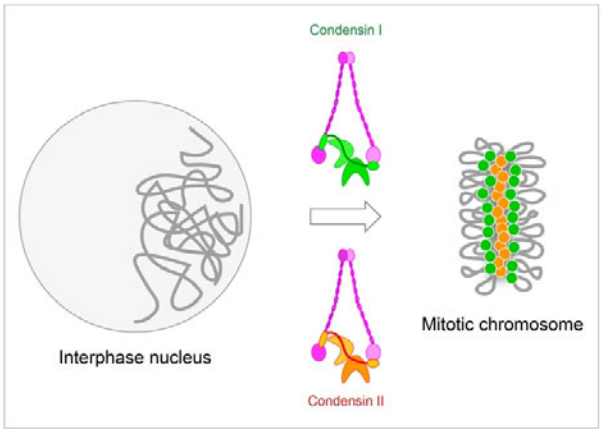
Research Project Number : 18H05276 Researcher Number : 50212171
Keyword : Biochemistry, Cell Biology, Mathematical Biology, Chromosomes, Cell Division

【Purpose and Background of the Research】

The assembly of mitotic chromosomes is an essential process for the faithful segregation of duplicated genetic materials into daughter cells. Our group discovered two types of protein complexes, now known as condensins I and II, that play central roles in this process, and made substantial contributions to our understanding of their cellular functions and molecular mechanisms of action. More recently, we have succeeded in reconstituting a mitotic chromosome structure with purified protein components including condensin I, and further demonstrated that a chromosome-like structure can be assembled even in the near-absence of nucleosomes in a condensin-dependent manner. The goal of this research project is to elucidate the molecular mechanisms of condensins I and II by combining two complementary approaches, namely, biochemistry and mathematical modeling (Figure below).

【Research Methods】

(1) We will reconstitute condensins I and II from their recombinant subunits, purify them, and test their ability to assemble chromosomes in *Xenopus* egg cell-free extracts. In addition to the wild-type holocomplex, holocomplexes harboring point mutations and subcomplexes lacking one or two of the regulatory subunits will be tested to understand how the two condensin complexes might work and collaborate with each other.
(2) We will establish a protocol in which the recombinant complexes can be activated in vitro by Cdk1-mediated phosphorylation, and thoroughly compare the biochemical activities of condensin I with those of condensin II.
(3) We will take an approach of mathematical modeling and computer simulation to get deeper insights into the action of condensins I and II. Such a theoretical approach will not only complement the experimental approach, but also provide us with hints about designing a new set of innovative experiments.



【Expected Research Achievements and Scientific Significance】

The question of how mitotic chromosomes might assemble is arguably one of the biggest questions left in the field of modern cell biology. It is anticipated that this research project will help uncover a whole molecular picture of how condensins I and II cooperate to assemble mitotic chromosomes at a mechanistic level. The outcome of this project will have a broad impact on our understanding of how anomalies of chromosome architecture cause human diseases including cancers and birth defects.

【Publications Relevant to the Project】

・Kinoshita, K., T. J. Kobayashi, and T. Hirano. (2015). Balancing acts of two HEAT subunits of condensin I support dynamic assembly of chromosome axes. *Dev. Cell.* 33:94-106.
・Hirano, T. (2016). Condensin-based chromosome organization from bacteria to vertebrates. *Cell.* 164:847-857.

【Term of Project】 FY2018-2022

【Budget Allocation】 148,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/chromdyna/