Date: January, 2018
Institute Laboratories' Research Promotion Office RIKEN

Research Project Mid-term Evaluation Result

The following research project underwent a mid-term evaluation in accordance to Clause 10 and 11, Chapter 2 of the *Regulations for Research and Development Evaluations* (Regulation No. 74, October 1, 2003.)

Evaluation system:
Out of four reviewers, two experts from outside of RIKEN and two RIKEN Science Council Research Programs Committee members were appointed as reviewers for the following research project. The reviewers evaluated the project based on the reporting session held on November 7, 2017.

Reviewers list:

External experts (alphabetical order)

1) Kei-ichiro ISHIGURO, Professor, Kumamoto University
2) Yoshikazu OHYA, Professor, University of Tokyo

RIKEN Science Council Research Program committee member (alphabetical order)

3) Sidonia FAGARASAN, Team Leader, Laboratory for Mucosal Immunity, IMS
4) Atsuo OGURA, Director, Bioresource Engineering Division, BRC

Research project brief overview

Project name: Cellular Evolution: Karyogenesis and Diversification
Project Leader: Tatsuya HIRANO
Project duration: April, 2015～March, 2020（5 years）
Budget allocated: Total of 310,000 thousand Yen（ past 3 years）
Research overview:

Organisms currently living on Earth can be classified into two categories, prokaryotes and eukaryotes. The prokaryotic genome is surrounded directly by a cell membrane, whereas the eukaryotic genome is stored in a subcellular structure called the cell nucleus and is separated from the rest of the cell (i.e., the cytoplasm). From an evolutionary point of view, the acquisition of the nucleus, which led to the creation of eukaryotic cells, occurred approximately 2.0 billion years ago. This event (also known as “karyogenesis”) was one of the most important evolutionary events in the history of life, opening the path to subsequent multicellularization and diversification. The ambitious goal of the current proposal is to understand how the evolutionary event of karyogenesis, which was accompanied by a dramatic innovation of new biomaterials and programs, might have contributed to the creation of a wide variety of organisms that currently exist on Earth.

1. Comprehensive Evaluation (To be disclosed)

1) Evaluation on five-grade scale	S	A	B	C	D
(1) Research objective:	4	0	0	0	0
(2) Implementation of research plan:	1	3	0	0	0
(3) Research achievement:	2	2	0	0	0
(4) Future research plan:	1	2	1	0	0

S Outstanding / A Excellent / B Good / C Acceptable / D Not acceptable

2) Evaluation details (reviewer's number is different from the order of the above list)

＜Reviewer 1＞

(1) Research objective:

This is an ambitious research program, aiming to understand emergence of eukaryotic cells. The eukaryotic cells which have complex intracellular structure and function emerged more than a billion years ago. Since then, they led in a whole era for life on Earth, because these cells evolved multifariously and differentiated into huge number of multicellular organisms. Although emergence of eukaryotic cells is a big event in evolution, it remains to be unsolved how the eukaryotic cell itself evolved, how a quiet bacterium make this evolutionary jump to a more aggressive eukaryotic cell, and how the eukaryotic cell gained power to overcome prokaryotic cells. Drs. Hirano and Imamoto started the pioneering research project in 2015, aiming to address the above questions from the aspects of karyogenesis. Karyogenesis was one of the most important evolutionary events during the emergence of eukaryotic cells, enabling to keep and segregate huge amount of genetic information and opening the path to subsequent eukaryotic evolution and differentiation. One of the subproject of this project named "Evolution of Materials" studies mitotic chromosomal assembly and genome organization which is required for segregation of huge amount of genetic information typical to eukaryotic cells. The other subproject named "Evolution of Programs" studies machinery and regulation of nucleoplasmic transport which is typical phenomenon observed in the organisms with nuclear envelope. Research objective is quite unique and my overall evaluation is that this is very interesting topic both in cell biology as well as evolutional biology.

(2) Implementation of research plan

By newly incorporating the viewpoints of evolution, the team has been concentrating on specific topics for the past 3 years such as 1-1) Chromosomal and intra-nuclear structures, 1-2) 3 D-genome organization, 1-3) Cellular events outside of the nucleus3 D-genome organization, 2-1) nuclear transport and gene expression, 2-2) mathematical understanding of cellular and network structures, 2-3) development of multicellular organisms. These implementations are quite innovative and have no doubt been leading the community of cell biologists. Considering attention has been paid to collaborations between the teams.

(3) Research achievement

The research team has produced high level outputs, in terms of both quality and quantity, in the past two years and half. Highlights include essential role of condensin II in reversible assembly of mitotic chromosomes, occurrence of mitotic chromosome assembly despite nucleosome depletion, reconstitution of mitotic chromatids with a minimum set of purified factors, super-resolution microscopy analysis of nuclear body organization, extensive cargo identification in different importin pathways and phylogenetic analysis of Hikeshi homologue.

(4) Future research plan

For the next 2 years Hirano's group plans to advance the ongoing studies to address the fundamental question of how eukaryotic condensins might functionally cooperate with nucleosomes to support large-scale mitotic chromosome assembly. Imamoto's group will elucidate the molecular mechanism of nuclear transport pathways. The plan is firm and will be efficiently performed, judging from the fact that the team has been successful so far. The goal of the team is very challenging, but at the same time, the achievement more related to evolution can be expected. In this context, it is important to further interact with evolutional biologists, although the team has already been making some efforts in this direction.

＜Reviewer 2＞

(1) Research objective

The aim of the pioneering project led by Tatsuya Hirano is to uncover the evolutionary aspects of karyogenesis-an essential process that happened approximately 2 billion years ago and that contributed to multicellularization, diversification and development of eukaryotic cells. The research focuses on two aspects; 1. evolution of materials-which mainly deals with representative ingredients-such as protein complexes organizing the genome, RNA-binding proteins etc) and 2. evolution of programs- using nuclear transport and gene expression profiles as examples. Such

research is essential for understanding fundamental processes in biology and expected to generate new knowledge on the evolution of eukaryotic cells. The proposal is excellent and such curiosity driven science research led by top scientists in the field of cellular biology should be fully supported by RIKEN.

(2) Implementation of research plan and (3) Research achievement

As mentioned above, the research involved two main subprojects, and although there were some deviations from the original plan-such as the change of the subproject 1 due to the move of leader Shin-ichi Nagagawa to Hokkaido University, the groups still maintain coherence and strength by the leadership of Dr. Hirano and addition of new members, such as Dr Hiratani and Dr. Iwasaki. That being said, it is expected that the interaction and full integration of new members into the subgroup 1 will bloom in the coming years.

The development of cell-free system by Hirano group in which mitotic chromatids were assembled in frog extracts starting from mouse sperm nuclei devoid of histone chaperone Asf1 was a big step toward answering an important question, namely if is it even possible to assemble chromatids without nucleosome. The work was published in a highly visible journal of Science. The group made also progress in understanding the role of condensins, and in collaboration with Imamoto group, of functional crosstalk between condensins and Ki-67, a peripheral chromosomal protein supporting the structural integrity of mitotic chomosomes. The involvement of Mochizuki' s group to boost understanding of condensing functions through mathematical modeling and computer simulation is very promising. The evolutionary insights into 3D-genome organization by Sasai's group is very interesting and their simulation, in collaboration with the newly recruited group of Hiratani likely to reveal the importance of epigenetic coding for determining the nuclear-scale structure of genomic DNA. More synergy is expected to develop in the near future for this part of subproject 1.

The asymmetric protein segregation during cell division studied by Tanaka's group is also very interesting, although we will probably see more about the perinuclear spaces as novel compartments for protein degradation and integration with other researchers involved in this subproject in the near future.

The Subproject 2 led by Dr. Imamoto is also very exciting. The assembled group aim at revealing the functional implications of the dynamic crosstalk between cytoplasm and nucleus, be fully exploiting their original and exciting findings on Hikeshi, Hsp70 and importins. Innovative cutting edge methods aim at identifying the cargo of importing for the nuclear delivery as well as the functional implications for such highly dynamic exchange at the nuclear cytoplasmic interface. The finding that Hikeshi is required for regulation of HSF1-a master regulator of protein homeostasis is very exciting. In addition the Sako's group aims among other to reveal the cytoplasm-nucleus oscillation of NF-kB transcription complexes in a well-established cell line. Mochizuki's group aims to mathematically model and establish the hierarchy, localization and adaptation in such chemical reaction networks-and it seems that they made progress in this direction.

A unique and very interesting question is addressed by Matsui-who elegantly try to answer the evolutionary meaningful question of how skin adapted from aquatic to terrestrial life. He is using incredibly solid knowledge of cellular and molecular biology in addition to cutting edge technique to address this fascinating issue. Clear progress with generation of tools was presented.

Although less clear presented was Kuratani's contribution, I believe that in addition to skin, the insights into the evolutionarily changes in musculoskeletal system in vertebrates will nicely fit the central theme of this project; cellular evolution. At a first glance that the project is not really study the cellular evolution in a conventional way. But this is exactly what I find exciting about the project-that researchers from different background work together in an rather unique way to uncover fundamental aspects of eukaryote evolution and the materials and programs that allow the development of organisms currently existing in our planet.

The research achievements, published results and ongoing works are highly evaluated by this reviewer.

(4) Future research plan

The research plans written by each participant are well organized. Given the expertise and commitment to best science of the researches involved in the project I also expect new exciting

developments not predicted. Perhaps more synergy will develop between and within the two subgroups.

＜Reviewer 3＞

(1) Research objective

The research objective focuses on how the acquisition of nucleus may have evolved genome organization, dynamic nuclear-cytoplasmic traffic, spatiotemporal uncoupling of RNA synthesis from translation and cellular signaling network from the aspect of karyogenesis, which is a fundamental issue yet to be well studied. Their viewpoint of “cellular evolution” is unique and unprecedented in the point that individual subprojects are synergistically integrated to understand how the evolutionary innovation of new biomaterials and programs might have contributed to diversification of cellular structure and systems upon karyogenesis. Although most of the current members in this project are not evolutionary specialists, this composition of research structure rather seems to propose a new direction towards scientific novelty. Thus, the research project has a potential to pioneer a new field beyond a mere evolutionary biology and consequently would raise a positive impact on cellular biology field. Such a bottom-up interdisciplinary project by RIKEN internal and external scientists is hardly supported by any external funding and should be further encouraged by RIKEN, so that they continue to play major leading roles in this new world-class research area.

(2) Implementation of research plan

The research project is well organized and balanced by two subgroups composed of researchers from inside and outside RIKEN, so that individual research activities are focusing on either point : biomaterials or cellular programs/systems, which evolutionarily emerged upon karyogenesis. The research subprojects have been progressing well along their initial proposal. In particular, it is worth noting that comparison of bacterial and eukaryotic condensins, conducted by subgroup 1 (Hirano’s group), not only will shed light on the evolutionary origin of segregation machineries but also help to understand how two distinct eukaryotic condensins contribute to genome organization in eukaryotes, which could extrapolate putative genome structure in currently-missing ancestor species before and after karyogenesis. In terms of scientific interaction, collaborations between individual groups are successfully facilitated both in the subproject 1 and 2, promoting interdisciplinary research project. Notably, mathematical analyses are extensively introduced by Mochizuki’s group whole through subproject 1 and 2, producing the underlying theoretical models for chromosome organization and cellular network structure. Furthermore, in both subgroups, single molecule studies have been or will be introduced by the collaborations with Sako’s group to deepen the individual projects, highlighting advantage of collaboration within RIKEN specialists. As far as I learned in the hearing, however, one minor concern is that the research activities of a few groups currently seem rather isolated or independent in the context of the research objective “cellular evolution”. Nevertheless, this issue would be overcome in their future research plan. Thus, overall implementation of research plan is satisfactory and highly evaluated.

(3) Research achievement

Overall research achievement at the midterm point is high enough, compared to their initial research proposal. In particular, extreme achievement has been done in the subprject1 (Hirano’s group), where they made a technical breakthrough for chromosome study. Because introduction of mutation in either of condensin subunits leads to impairment of its entire function, it would be difficult to analyze the roles of individual subunits by conventional genetic approach. To overcome this issue, Hirano’s group established a cell-free system that reconstitutes chromatid-like structure with limited number of components. Moreover, the development of the cell-free system not only allows dissection of individual mutant subunits of condensin but also assessment of minimal requirement for chromatid assembly, which led to surprising finding that chromatid-like structure is assembled even in the absence of nucleosome. Because condensin emerged before eukaryotic cell acquired the nucleosome, this discovery could provide intriguing insights into pivotal roles of condensins in evolution of genome organization upon nuclear compartmentalization. Thus, they made ground-breaking contributions to cell biology field.

Comparative MD simulation, carried out by Sasai’s group, led to an evolutionary insight into how

3D genome organization evolved from unicellular to multicellular organisms. This new biophysical method would further help to understand the mechanisms of A/B compartment reorganization during ESC differentiation, which shows correlation with DNA replication timing found by a new technique scRepli-seq of Hiratani's group. Tanaka's group extensively revealed new asymmetrically segregated proteins, raising the insight how eukaryotic organism evolved selective asymmetric segregation of proteins and the way to deal with aggregates.
Comprehensive cargo identification for Importin family, conducted by subgroup 2 (Imamoto's group), provided the finding that the Importin carriers are linked to distinct biological functions by their cargoes, raising evolutionary insights into diversity of nuclear-cytoplasmic transport and signaling. Furthermore, they provided an evolutionary insight how eukaryotic cells might have acquired Hikeshi/Hsp70-HSF1mediated gene regulatory system for stress response. Their approach stands at one of the important core subprojects to consider about diverse cellular functions inside and outside of the nucleus. Sako's group proposed a model that karyogenesis evolved the switch-like signal transduction response by nuclear translocation of ERK protein, which would have been evolved to adapt to nuclear compartmentalization. Matsui's group are trying to elucidate nuclear degradation mechanism during cornification in epidermis with newly established live imaging method, having preliminary data on relationship among nuclear degradation, epigenetic regulation and NPC distribution. Thus, overall their project has shown several novelties in terms of scientific discoveries and innovation of new technique.

(4) Future research plan

The future plans in the two subprojects are reasonably presented and well organized. It is appropriate that Dr. Kuratani joined the project, which expands their projects to understanding the diversification of multicellular organisms. However, one concern is that interdisciplinary research path between karyogenesis and diversification of multicellular organisms is yet to be well strengthened. Thus, it would be appreciated if further scientists with strong evolutionary biology background joined the project to facilitate overall research plan. This would be important to accomplish their ambitious goal of elucidation of "cellular evolution" upon karyogenesis. Since significant achievements are undoubtedly expected both in the subgroups, it is highly recommended to proceed towards the future plans.

＜Reviewer 4＞

(1) Research objective

The object of this project is to explore the molecular mechanisms underlying karyogenesis of cells, one of the most important and dynamic events in the history of life. The object is described clearly and its biological significance is understandable to us. As the karyogenesis is a series of complex processes, its underlying mechanisms can be clarified fully only with a tremendous amount of scientific data. In this context, Dr. Hirano focuses appropriately on some important phenomena of karyogenesis, most of which are originally the main subjects, or themes, of the researchers involved in this project. Therefore, we may expect that some specific knowledge, rather than general information, of karyogenesis will be deepened by this project. I evaluated the aim of this project from this viewpoint.

(2) Implementation of research plan

To achieve the project goal efficiently, the project is composed of two subprojects: "Evolution of Materials" led by Dr. Hirano himself and "Evolution of Programs" led by Dr. Imamoto. These two subprojects are not separated completely, but can be mutually collaborated as appropriate. The collaborations are well organized within the subprojects.

(3) Research achievement

As mentioned above, the specific subjects in this project are mostly the continuation of the preexisting research subjects of the researchers. As expected, the world-recognized scientists in this project have been successful in making high-class achievements during these three years. Dr. Hirano's and Dr. Imamoto's publications are especially outstanding and should be respected. Condensins are the molecules present in both prokaryotes and eukaryotes and should say

something about the evolution of karyogenesis. Dr. Hirano is about to achieve this point. Dr. Imamoto's Hikeshi story is expanding and provides evidence for involvement of the Hikeshi family of proteins in many biological activities. I hope that their roles related to karyogenesis will be sought more stringently. All other members also published high-impact papers or are planning to publish important papers based on their achievements. They are all promising. The only drawback I found was the paucity of collaboration papers by the members. I understand that it may be difficult to publish collaboration papers in the first half of the project term, but as "synergy" and "interdisciplinary" are the most important keywords of the Pioneering Project, we should expect such outcomes. There are several key persons, including Drs. Sasai, Mochizuki, and Sako, who may connect systemically the expertise of members in this project. This is the strong point of the project and would ultimately make it more successful.

(4) Future research plan

The future research plans presented are practical and realistic because most have been thoroughly well prepared or even already started. It is pleasing that many of the plans were made in collaboration across the members.

RIKEN Science Council Research Programs Committee