Research area in Strategic Objective *"Development of innovative cell manipulation technologies and elucidation of cellular regulatory mechanisms"*

Cell Control

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Overview

The aim of this research area is to achieve innovative technological advancements that impact a wide range of life sciences through interactive research that seeks to manipulate and understand cell control mechanisms.

Here, "cell" in "cell control" is regarded as an element that constitutes a multi-cellular system such as individual organisms and artificial organs, or as an entire system made up of subcellular components such as organelles. In contrast to conventional analytical methods, for example, electrophoresis that requires grinding more than one million cells for use as samples, advanced techniques with high spatiotemporal resolution, including single-cell omics analysis and bio-imaging techniques, safeguard the personality and integrity of individual cells. As a result, these advanced techniques have yielded more detailed and multifaceted data on cell control mechanisms than before. As if in step with this explosive growth in data volume, fundamental technology for artificial intelligence has been disseminated, allowing for quick data analysis.

However, an increase in the amount of data does not necessarily lead to a better understanding of cell control mechanisms. To uncover causal relationships governing a complex system, it is useful to manipulate the function of one particular element to examine the behavior of the system as a whole or of other elements. From the perspective of technological development, this research area focuses on the innovative manipulation of cell control mechanisms (hereinafter referred to as "cell control"). While recognizing the need to quantitate the manipulation of objects that inevitably occurs in analysis, we are keenly pursuing the development of technology that allows researchers to manipulate objects at will. It is indeed important to persistently pursue the growth potential of existing technology. If genome editing and opto- and chemo-genetics are improved by some state-of-the-art technology, for example, reasonable progress will be made toward increasing the precision and diversification of cell control. In addition, the development of both hardware and software will be necessary to support cell control. It is highly recommended that attempts be made to realize breakthroughs through exhaustive crossovers with different technologies. Furthermore, it is necessary to create element technologies from scratch to accomplish new cell manipulation methods to explore new aspects of cell control mechanisms.

Cells are full of mysteries waiting to be uncovered. Cells will probably stay clear of researchers who completely rely on entrenched textbook knowledge. On the other hand, cells will disclose the soul of cell control mechanisms to researchers who possess inquisitive and malleable minds. Here we will view cell control as playing with and in cells. In a playful environment, we would like researchers to challenge mysterious cells with no fear of failure. We hope that the spiral of manipulation and understanding that emerges from playing cells will grow positively while interacting with fields beyond this research area, and will create a new vortex somewhere, no matter how small it may be. By actively incorporating unforeseen developments, each team is expected to revise its set goals as it undertakes the actual process of research, and this research area will grow in such a flexible way.

<From the Deputy Research Director: Summary of PRESTO Research (Supplementary Information)

Based on the CREST "Cell Control" policy, PRESTO expects ambitious and innovative proposals that aim to generate new knowledge on cell regulation mechanisms and develop innovative cell manipulation techniques (including the development of manipulation tools). In recent years, various technologies for cell regulation (e.g., epigenome editing) have been competitively developed based on optogenetics and genome editing. The development of these novel technologies is not merely a combination of elemental technologies, but requires the identification of functional molecules from unknown microbial species, structural biology approaches, and the advancement of functions incorporating evolutionary engineering. In addition to the use of novel cell manipulation technologies, data-driven approaches such as AI and big data analysis, and approaches that integrate mathematical model analysis are also important to elucidate cellular control mechanisms, which will open the way to control technologies that can be applied in various fields.

<u>Research supervisor's policy on call for application, selection, and management of the research area</u>

1. Expected goals and examples of research projects

Please be sure to read the CREST "Cell Control" research summary policy at the end of this document.

We expect proposals for the following items, singly or in combination, as they relate to control and understanding of the mechanisms of cellular regulation.

(1) Development of cell control tools and cell control technology

(2) Understanding of cell control mechanisms

(3) Development of cell control techniques in individuals

(4) Development of cell manipulation technology for social implementation

For (1) and (2), we envision research aimed at the development and advancement of novel technologies for control target molecules and organelles. Here, we will target the development of novel and innovative control tools that go beyond existing optogenetics and genome editing technologies. The challenge to develop control tools beyond CRISPR-Cas9 and rhodopsin is particularly important. While the combination of existing technologies is important, we expect the creation of highly original technologies for basic science and industrial fields. In the development of cell control technology, it is also important to seek control methods based on the elucidation of the diverse functions possessed by various organisms (microorganisms). For example, biological species (including microorganisms) that exhibit high radiation tolerance have the ability to accurately repair fragmented genomes. Focusing on such functions, we expect to develop new cell control technologies based on the identification of functional molecules involved in DNA repair and their regulatory mechanisms. In addition, technologies to control organelles such as mitochondria and chloroplasts are positioned as important issues in this PRESTO.

For (3), we aim to develop technologies to utilize the cells produced based on the development in (1) and the findings in (2) in individuals and to realize cell regulation by modifying the cells within individuals. Artificial chromosome technology and synthetic biology are especially important for this development. Currently, effective chromosome modification techniques have not been established in many species. Therefore, it is necessary to develop a regulatory system in which multiple regulatory tools and probes are expressed within an individual by developing new technologies in fertilized eggs and stem cells. The development of delivery methods for control tools and probes is also an important issue for intra-individual modification.

Regarding (4), since cell control technology has a significant impact in the industrial field, it is important to conduct research and development with an awareness of social implementation in the fields of biomanufacturing, agriculture, and drug discovery and treatment. It is also important for researchers to be aware of intellectual property and social acceptability issues, and to grasp social needs from the basic technology development stage.

2. Research Period and Research Funding

The research period is limited to three and a half years. The maximum initial research funding is 30-40 million yen (direct costs) per proposal. Please note that the research expenses may be adjusted upon selection.

3. Other consideration

This PRESTO is an individual research project open only for the fiscal year 2024.

[Reference: Research Supervisor's Policy for Selection of CREST "Cell Control"].

1. Background

The position of cell control technology in modern life science has been described earlier in the Overview. Genome editing and optogenetics are examples of cutting-edge cell control technology. What is common about their origins is that whereas Japanese scientists have made significant contributions to the discovery of fundamental phenomena and substances, it is Western scientists who have assumed the flagship position with the introduction of new concepts. By encouraging the back-and-forth and fusion between basic and applied sciences in Japan strategically, and by supporting imaginative research projects unconditionally, it will be possible for Japanese scientists to create technological innovations FOR the world. We contemplate such an innovation in the developing field of cell control.

2. Expected goals and examples of research projects

We are not specifying goals in the first year of the call. Nevertheless, in a year or so, we expect to shape our ideas into a few themes. At this point in time, the following categories may reflect the content of cell control, but research proposals can be made irrespective of these categories.

(1) Development of advanced technology for controlling cells in a multicellular society (organisms, organoids, etc.)
(2) Development of advanced technology for controlling subcellular components
(3) Development of truly innovative technology for cell control
(4) Quantification of classic cell control
(5) Research on social demands for cell control technology

Category (1) concerns technology for manipulating cell behaviors and is expected to receive the most proposals. Category (2) refers to technology for manipulating specific organelles or molecules in a cell and is expected to have the second largest number of proposals after (1). Categories (1) and (2) differ only in the setting of the system boundary and may overlap with each other substantially. We expect that a pivot will be set in (1) and/or (2) for crossovers with different areas to propose multidisciplinary research projects. We look forward to ambitious research proposals that attempt to unravel the mysteries of cell control mechanisms through practical development of the above technology. We will expand target cells to all living organisms. In fact, the control mechanisms of CRISPR-Cas9 and microbial rhodopsin in (archaeal) bacteria and algae remain a great mystery.

Category (3) has an infinity of possibilities. On the other hand, category (4) may be far from state-of-the-art technology, and category (5) may be an issue that should be addressed by this research area as a whole. A slightly biased explanation of these three categories is provided below. Although we do not know if we will receive such research proposals, we believe that, if selected, they would become distinctive features of this research area. Of course, we would welcome other unique proposals that do not belong to categories (1) to (5).

(3) Development of truly innovative technology for cell control

From time immemorial, living organisms have been exposed to electromagnetic waves, particle beams, sound waves, pressure, electric currents, heat, desiccation, and unfamiliar gases and compounds. To survive, they have been equipped with robust cellular systems to protect themselves against such external factors. In addition, they have created sophisticated devices that either utilize these external factors or produce them. Moreover, they have created clever devices in their sustained engagement with heterogeneous life forms, such as infection, parasitism, and symbiosis. Many of the in vogue "cell control methods" involve the replication of such devices across different kingdoms. The devices have been refined and diversified for the sake of human beings. The greater the ectopicity of replication is, the easier it is to achieve bio-orthogonality, but the more difficult it is to design an efficient expression system. We believe that the growth potential of cell control technology is immeasurable. To date, we have not been able to utilize or even know about most of the devices that exist in life on Earth. It may be possible to recruit interested taxonomists who attempt to provide a prophetic overview of potentially existing devices that can be used or modified for cell control. Furthermore, from the birth of life to the present, there must have been a huge number of life forms that once flourished and were armed with splendid devices but became extinct for some reason (and never fossilized). Our dream is to recover such lost devices through computer simulations.

(4) Quantification of classical cell control

As a matter of terminology, the term "cell control" may refer to the manual and mechanical operations of cells. Such operations include poking, stroking, and grasping individual cells, or sowing, collecting, and lysing groups of cells. Cultured cells reside in an artificial environment and are always subjected to a variety of manipulations. The following are just two simple questions that may occur to many cell biologists. First, when a gene is mechanically introduced into the cell nucleus, under what condition should a glass tube be inserted to minimize DNA damage? Second, which collection process is most stressful to cultured cells? Is it scraping or centrifuging? It is important to quantify both manipulation and reaction of cells. By examining the input/output relationship, we should be able to know more about the origin of dispersion of experimental data. While this type of measurement has been proposed along with the demand to control the proliferation/differentiation of iPS cells and stem cells, it is time to advocate the establishment of new methods that now involve bio-imaging, robotics, precision engineering, materials science, etc. for that purpose. The unique culture of craftsmanship in Japan is expected to benefit us.

(5) Research on social demands for cell control technology

It will be necessary to understand international conventions/treaties and agreements to promote global social implementation of cell control technology. This is because the fruits of this research area will include products of genetic recombination and genome editing. Accordingly, we will discuss, for example, how to confront the Cartagena Protocol, which is based on the Convention on Biological Diversity (Cartagena Act in Japan). Notable is the fact that the United States is the only advanced nation that has not yet ratified this treaty. This points to the complexity of this global issue. Back to your local community, let us assume that a transgenic fish has accidentally escaped from your laboratory into a nearby river. It should be important to simulate its survival and impact on the surrounding ecosystem beforehand using various parameters.

3. Research periods and research funds

The research period is up to five and a half years. The maximum initial research cost is 300 million yen (direct costs) per proposal. Considering the importance of flexibility, a small team structure (mini-CREST) will be established. Support for research acceleration will be provided during the research period, if necessary. Please note that research cost may be adjusted upon selection.

4. Other consideration

CREST is a team-type research program. We encourage the establishment of a system in which researchers participating in the team can utilize their strengths to create synergy. There may be cases where adjustments are made to cover imperfections through collaboration with other researchers in and outside this research area.