Title of Project： RNA-based Synthetic Life Systems

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Research Project Number: 20H05626 Researcher Number：20423014
Keyword：Synthetic biology, RNA, Cell regulation, Artificial cell

【Purpose and Background of the Research】

Classical molecular biology assumes genes and proteins are the primary determinants of cell functions, but it is now increasingly recognized that RNA and RNA-protein (RNP) interactions have crucial roles as well (Fig. 1).

In this project, we will investigate how RNA and RNP interactions regulate gene expressions, and design functional RNA and RNP systems (e.g., synthetic circuits and synthetic organelles) to program cellular behavior.

Specifically, (1) we aim to comprehensively understand and reveal RNA/RNP interaction networks that contribute to the regulation of post-transcriptional gene expressions and the formation of RNA/RNP structures; (2) based on the acquired information of RNA/RNP interaction motifs that determine cellular networks, we aim to design synthetic RNA/RNP-based structures that control cell function; (3) we aim to generate synthetic RNA/RNP-based circuits and functional artificial cells for medical applications; and (4) we aim to create RNA-based protocell models to elucidate the origin of life. Through this research, we will provide a new concept, “synthetic life systems”, by generating living systems based on RNA and RNP.

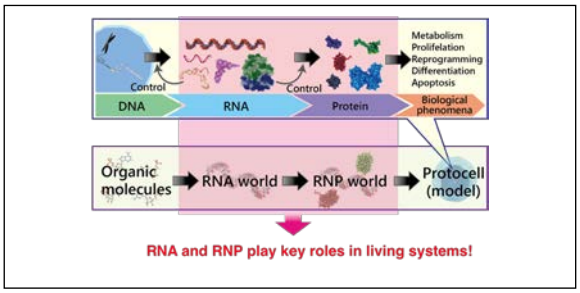

Figure 1 RNA and RNP-based Life Systems

【Research Methods】

In this research, we will utilize our unique RNA and RNP molecular design technology to elucidate unknown biological phenomena consisting of RNA and RNP interactions, and create functional artificial organelles and artificial cells based on that understanding. First, we will elucidate the design principle of RNP networks and RNP organelles by utilizing our unique RNA structure library technology that can extract RNA structural motifs from genome. Furthermore, we will design and construct artificial gene circuits and artificial organelles based on RNA and RNP, and create artificial cell models that will lead to elucidate the evolution of life systems.

【Expected Research Achievements and Scientific Significance】

By elucidating new RNA/RNP networks in cells and engineering them, we will develop innovative technologies that can be widely used in the next generation of science and medical applications. Furthermore, we will develop new methodologies based on the designed RNA/RNP to accelerate the emergence of synthetic living systems.

The overall work of this proposal will require technologies from different fields and will lead to the construction of synthetic RNA and RNP systems and artificial cells that will contribute to the understanding and regulation of living systems.

In the future, RNA-based, synthetic life systems are expected to have a wide range of applications in the medical, healthcare, environmental, and agricultural fields (Fig. 2).

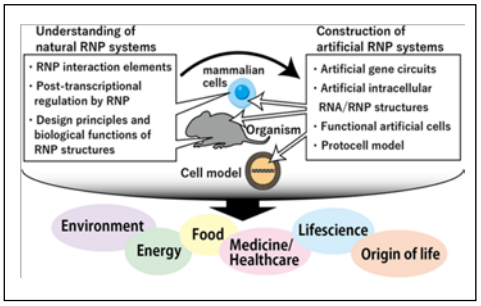

Figure 2 RNA-based Synthetic Systems & Outlook

【Publications Relevant to the Project】

・Endo K, Hayashi K, Saito H: Numerical operations in living cells by programmable RNA devices. ***Science Advances***, **5**(8):eaax0835, 2019
・Matsuura S, Ono H, Kawasaki S, Kuang Y, Fujita Y, Saito H: Synthetic RNA-based logic computation in mammalian cells. ***Nature Communications***, **9**:4847, 2018

【Term of Project】 FY2020-2024

【Budget Allocation】 289,100 Thousand Yen

【Homepage Address and Other Contact Information】
https://sites.google.com/view/hirohidesaitolabjp/home_en

【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

Title of Project： Unraveling the principles of microbiota function for rationally-designed biotherapeutics

HONDA Kenya
(Keio University, School of Medicine, Professor)

Research Project Number: 20H05627 Researcher Number：60334231
Keyword：microbiota, vaccine, colorectal cancer, multidrug-resistant organisms, metabolic disease

【Purpose and Background of the Research】

Culture-independent metagenomic profiling of the microbiota by next-generation sequencing is powerful to associate certain microbiota with human health and disease, but unable to address the causality and directionality of the host-microbial relationship. In this context, we have established a top-down approach using anaerobic culture and gnotobiotic techniques, which enables us to narrow down the complex microbiota to individual bacteria or minimal consortia that causally induce a specific phenotype in the host. In this study, by applying our expertise, we aim to define the effector microbes and their metabolites with significant impact on health and disease in the context of; **vaccine responses** (**Aim 1**), **multidrug-resistant bacterial infections** (**Aim 2**), **colorectal cancer** (**Aim 3**), **metabolic disease** (**Aim 4**), and **aging** (**Aim 5**). In addition, we will explore **novel strategies for microbial manipulation** (**Aim 6**) by advancing the culture techniques and genetic tools to manipulate commensal genes and community. Ultimately, our goal is to decipher the basic principles that govern interactions between the microbiota and the host and to develop "rationally designed biotherapeutics" that can modify host physiology.

【Research Methods】

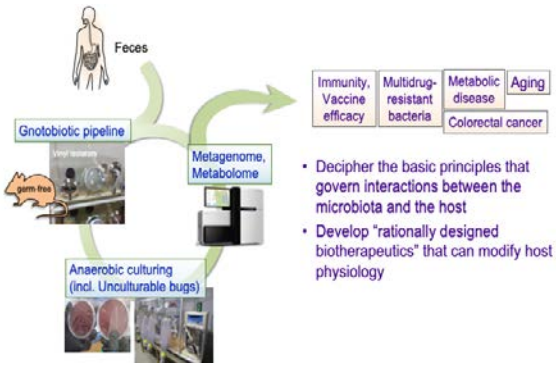

Figure 1 Identification of minimal effector bacterial consortia that causally induce a specific phenotype in the host

Aim 1. Vaccine responses: Fecal samples from individuals who have recovered from COVID-19 and showed effective antibody (Ab) responses will be orally administered to germ-free mice. We will immunize the mice with inactivated SARS-CoV2 and follow up on the group of mice which show effective Ab response. We will serially transplant their feces into another germ-free mice and isolate bacterial strains to obtain minimal effector strains.

Aim 2. Multidrug-resistant bacterial infection: From healthy volunteers, we previously isolated 37 strains that potently decolonize antimicrobial resistant *K. pneumoniae* strains. We will narrow down them to get minimum effectors and interrogate the mechanism of action.

Aim 3. Colorectal cancer (CRC): We have previously isolated 40 bacterial strains from the surface of surgical specimens of CRC patients. We will evaluate the influence of these isolates on the development of CRC using germ-free APC;K-ras mice and identify a minimal effector (oncogenic) consortium.

Aim 4. Metabolic disease: We found that fat browning (Beige cell induction) is induced by dietary intervention, which occurs in a microbiota-dependent manner. We will investigate the effector bacteria and the responsible molecules for the fat browning.

Aim 5. Aging: We found that centenarians have distinct gut microbiome enriched in microbes capable of generating unique secondary bile acids. We will identify and isolate responsible bile acid-metabolizing bacterial strains.

Aim 6. Novel strategies for microbial manipulation: We aim to develop effective methods to genetically manipulate commensals using artificial plasmid and CRISPR-Cas system. We will also develop methods to culture "Unculturable bacteria".

【Expected Research Achievements and Scientific Significance】

Our objective is to decode the convoluted cross-talk between the microbiota and host cells to understand the "causation" in various clinical contexts with a significant amount of unmet medical needs. The gnotobiotic pipeline is powerful for testing the community of microbes directly in vivo. Completion of this research proposal will not only address the principles of microbial functions but also lead to a series of transformative medicine.

【Publications Relevant to the Project】

· Tanoue T, Nature. 565(7741): 600-605. (2019)
· Atarashi K, Science. 358:359-365 (2017)
· Honda K, Nature. 535:75-84 (2016)
· Atarashi K, Cell. 163(2):367-80 (2015).

【Term of Project】 FY2020-2025

【Budget Allocation】 492,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.microbiolimmunol.med.keio.ac.jp/home.html

Title of Project： Regulation of synaptic and non-synaptic functions by extracellular scaffolding proteins

YUZAKI Michisuke
(Keio University, School of Medicine, Professor)

Research Project Number: 20H05628 Researcher Number：40365226
Keyword：Neuron, Synapse, Neural circuit

【Purpose and Background of the Research】

Neurons are interconnected by synapses, which are cell-cell adhesions formed by various synaptic organizers, to achieve fast neurotransmission. Since a broad range of neuropsychiatric and neurological disorders are caused by abnormal synaptic functions, understanding how synapses are formed, maintained and eliminated by synaptic organizers is one of the most important goals of basic and clinical neuroscience.

We have recently proposed a new class of synaptic organizers, termed extracellular scaffolding proteins (ESPs), which are secreted and serve as a scaffold at the synaptic cleft. In addition to fast neurotransmission, neurons communicate with each other and with peripheral tissues by “volume transmission,” in which modulatory neurotransmitters diffuse and reach their receptors located at distant targets. Interestingly, non-synaptic cell-adhesion structures mediated by certain ESPs are often found in neurons that achieve volume transmission.

The goal of this research is to clarify when, how, and why various ESPs regulate synaptic and non-synaptic cell adhesion. Guided by structural information, we also aim to develop synthetic connector molecules that will expand the range and affinity of trans-synaptic and non-synaptic interactions.

【Research Methods】

Complement family proteins (C1q, Cbln1-4, C1ql1-4) and neuronal pentraxins (NPs) belong to the ESP-type synaptic organizer. In this project, we will focus on C1q, Cbln2, Cbln4 and NPs, which are ESPs reported to play important roles in key neuronal circuits. We aim to identify their receptors and downstream signaling pathways.

We also aim to identify molecules that mediate non-synaptic cell-adhesion structures in three model brain regions (extended amygdala, striatum and cerebellum).

We have recently developed a synthetic synaptic connector, CPTX, combining structural elements from Cbln1 and NP1 (Figure 1). Application of CPTX to mouse models of cerebellar ataxia, Alzheimer's disease and spinal cord injury could successfully restore synapses and improve motor coordination, spatial and contextual memories, and locomotion associated with these disease models, respectively. We aim to develop new synthetic synaptic connectors that contain combinations of ESPs found in synaptic and non-synaptic cell adhesion structures.

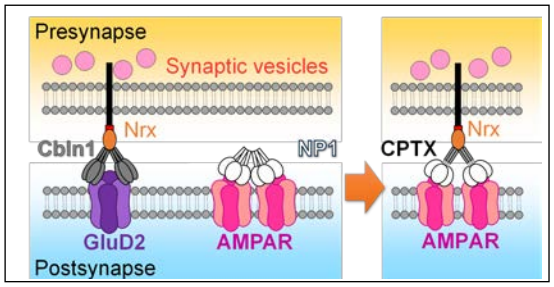

Figure 1 A synthetic synapse connector CPTX

【Expected Research Achievements and Scientific Significance】

Although the catalog of ESPs continues to grow, mechanisms by which ESPs regulate synaptic functions remain largely unclear. Identification of missing receptors and downstream signaling pathways for key ESPs will greatly advance our understanding of physiological and pathological synapse dynamics.

Modulatory transmitters, such as acetylcholine, dopamine and serotonin, achieve slow and diffuse volume transmission. In addition, autonomic nervous systems that regulate various target organs, such as heart and bowels, send signals by volume transmission. Identification of molecules that mediate non-synaptic cell-adhesion structures and volume transmission will be a major breakthrough in the field of modulatory neurotransmitters.

Structure-based design of new synthetic synaptic connectors is expected to pave the way for new treatments in neuropsychiatric or neurological disorders caused by synaptic abnormalities.

【Publications Relevant to the Project】

・ Suzuki K, Elegheert J, Song I, Sasakura H, (18 others), Yuzaki M. A synthetic synaptic organizer protein restores glutamatergic neuronal circuits. Science 369, eabb4853, 2020.
・ Yuzaki M. Two Classes of Secreted Synaptic Organizers in the Central Nervous System. Annu Rev Physiol 80:243-262, 2018.

【Term of Project】 FY2020-2024

【Budget Allocation】 463,200 Thousand Yen

【Homepage Address and Other Contact Information】
http://www.yuzaki-lab.org