Title of Project： Elucidation of the link of the gene, neural circuit, and behavior in the mouse olfactory system

Hitoshi Sakano
(The University of Tokyo, Graduate School of Science
Professor Emeritus)

Research Area：Neurophysiology
Keyword：neuron, synapse, and neural circuit

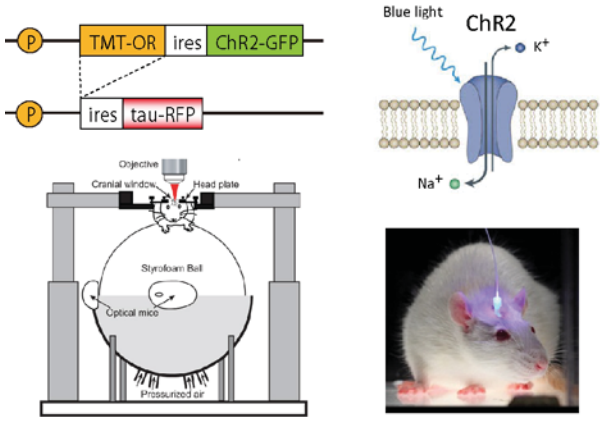
【Purpose and Background of the Research】

In the vertebrate nervous system, sensory information is spatially encoded in the brain, forming topographic maps that are fundamental for higher-order processing of sensory information. We will attempt to understand the link of the gene, neural circuit and behavior. Using the mouse olfactory system, we will try to understand how the two sensory decisions, one is by an innate hard-wired circuit and the other is by a memory-based learned circuit, are balanced and integrated in the central brain. This research will offer a new insight into the memory and consciousness of humans. Furthermore, it will shed light on the clinical application of mental disorders, such as autism and depression.

【Research Methods】

Specific aims and methods are as follows:
We will study the synapse formation of olfactory sensory neurons (OSNs) and mitral/tufted (M/T) cells, using various mutant mice in the lab, which affect glomerular formation and axonal projection of OSNs. We will also study how the M/T cells target to the olfactory cortex (OC). Axonal projection of M/T cells to the OC will be analyzed by injecting dye or trans-synaptic viruses. We will concentrate on the neural circuits for fox smell-induced fear responses. To analyze the neural circuits for fear responses,

Fig. 1 Genetic manipulations of olfactory neural circuits

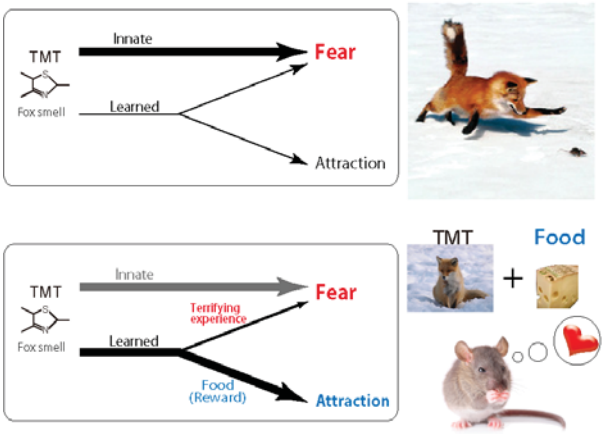


TMT-responsive OR genes will be cloned and then knocked-out or GFP-tagged. They will also be labeled with the channel-rhodopsin gene (ChR2) to perform the gain-of-function experiment for the TMT-responsive neural circuits.

【Expected Research Achievements and Scientific Significance】

The proposed research is expected to reveal how the M/T cells are instructed by OSNs, and how the axonal projection to the OC is regulated. This research will also clarify how the odor information is processed for a particular behavior in mammals. These studies will contribute to our understanding of axonal projection and neural circuit formation, not only in the olfactory system, but also in the mammalian brain in general.

Fig. 2 Innate vs. learned responses to fox smell, TMT



【Publication Relevant to the Project】

Mori, K. and Sakano, H.: How is the olfactory map formed and interpreted in the mammalian brain? *Ann. Rev. Neurosci.* **34**, 465-497 (2011).

【Term of Project】 FY2012-FY2016
【Budget Allocation】 385, 000 Thousand Yen
【Homepage Address and Other Contact Information】

http://www.biochem.s.u-tokyo.ac.jp/sakano-lab/
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Title of Project : Comparative research on *Ardipithecus ramidus* and other fossil evidence: enhancing evolutionary morphological research

Gen Suwa
(The University of Tokyo, The University Museum, Professor)

Research Area : Biological Science
Keyword : Anthropology, Evolution

【Purpose and Background of the Research】

Knowledge of the biological origins and early history of humans and apes is crucial to a science-based understanding of humankind. The fossil record provides us with a crucial body of information which accumulates only from new discoveries and enables formulation and testing of evolutionary hypotheses. The aims of the present research project include 1) field research in the Ethiopian rift system, in attempting to contribute new fossil discoveries pertinent to ape-human divergence and their early histories; 2) morphological analysis on new and established collections, such as those of *Chororapithecus* and *Ardipithecus*, including analysis based on 3-dimensional scan data with a scope for testing key evolutionary hypotheses; 3) paleoanthropology activities related to the early Pleistocene Konso sites, including advancing research documentation of the existing collections; 4) strengthening the research basis for macroscopic evolutionary studies by enhancing 3 dimensional morphological archives.

【Research Methods】

Annual paleoanthropological field research will be planned with regards to the Chorora Formation area. In 2006/2007 we discovered a new species of ape, *Chororapithecus abysinnicus*, and since then have continued field work there. As a part of the present project, we aim to conduct field survey and excavations as may become required, with an eye towards further ape/hominid discoveries. The time period is ill-represented, so that acquisition of new fossils and establishing accurate chronologies are of prime importance. We will therefore conduct the necessary geological and geochronological field work and laboratory analyses.

We will conduct focused morphological analyses on *Chororapithecus*, *Ardipithecus ramidus*, later *Australopithecus* and early *Homo* species, as well as analytical research on the associated faunal, environmental and prehistoric evidence. Research directly involving fossils are primarily conducted at the paleoanthropological laboratory facilities, Addis Ababa, while fossil and modern collections at other locations will be accessed as appropriate for comparison.

Scanner devices will be combined to form a 3-dimensional morphological data source system that can be used in comparative analyses of fossils including those of *Chororapithecus* and *Ardipithecus ramidus*.

【Expected Research Achievements and Scientific Significance】

The gorilla clade hypothesis for *Chororapithecus* will be tested, enabling new insights into ape-human divergence. The evolutionary significance of the *Ar. ramidus* and other early hominid morphologies will be evaluated, and the respective hypotheses tested. A 3-dimensional morphological data source system will be established.

【Publications Relevant to the Project】

・Suwa G, RT Kono, Katoh S, Asfaw B, and Beyene Y (2007) A new species of great ape from the late Miocene epoch in Ethiopia. Nature 448: 921-924.
・Suwa G, Kono RT, Simpson S, Asfaw B, Lovejoy CO, White TD (2009) Paleobiological implications of the *Ardipithecus ramidus* dentition. Science 326: 94-99.
・Suwa G, Asfaw B, Kono RT, Kubo D, Lovejoy CO, White TD (2009) The *Ardipithecus ramidus* skull and its implications for hominid origins. Science 326: 68e1-e7.

【Term of Project】 FY2012–2016

【Budget Allocation】 376,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.um.u-tokyo.ac.jp/people/faculty_suwa.htm

【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

Title of Project : Circadian pacemaker of cyanobacteria by clock protein KaiC

Takao Kondo
(Nagoya University, Graduate School of Science, Division of Biological Science, Professor)

Research Area : Biology
Keyword : Circadian clock, clock protein, KaiC, ATPase

【Purpose and Background of the Research】

From bacteria and fungi to plants and animals, circadian clocks are ubiquitous endogenous biological timing mechanisms that adapt to daily alterations in environmental conditions.

We reconstituted the self-sustained circadian oscillation in phosphorylation state of the cyanobacterial clock protein KaiC by incubating it with KaiA protein, KaiB protein, and ATP. KaiC also has novel mechanisms for synchronization. Thus, the Kai protein clock is inheritantly designed as the master pacemaker of cyanobacterial circadian clock.

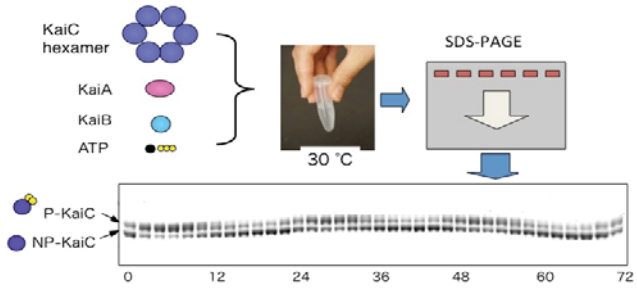

Fig. 1 In vitro reconstitution of circadian clock-2005

Moreover, we showed that KaiC possesses extremely weak but temperature-compensated ATPase activity (10-15 ATPs/day/KaiC) and that activities of wild-type KaiC and five period-mutant proteins are directly proportional to their *in vivo* circadian frequencies, indicating that the ATPase activity defines the circadian period. Based on these observations, we propose the KaiC ATPase activity as the most fundamental pacemaking reaction underlying circadian periodicity of cyanobacteria.

【Research Methods】

How KaiC protein that forms hexamer attains such extraordinary characteristics that apparently contradict with the normal chemical reaction? We proposed the intramolecular negative-feedback regulation of ATPase activity could generate tension inside the KaiC hexamer to suppress the activity and to gain circadian period determination. In this study, we will analyze the biochemical and genetical approaches to the ATPase activity of KaiC to explain a time-keeping function of KaiC. We also try to understand function of KaiC at atomic level by introducing dynamic structural biology.

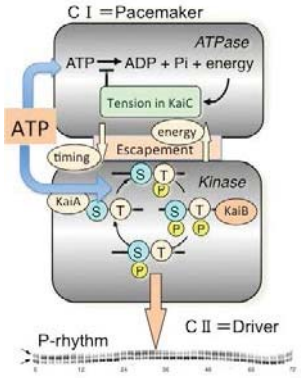

Fig. 2 KaiC clock model

【Expected Research Achievements and Scientific Significance】

1) This project could explain one of the final questions of circadian biology, that is, to explain how living organisms remember 24 h period and how it was stablilized against temperature. 2) This study could reveal the circadian clock of many eukaryote, because biochemical process by protein activity are recently reported to affect circadian period.
3) This study might find novel function of proteins that is not included current list of protein function, such as enzyme, motion, chemical reactor, etc.

【Publications Relevant to the Project】

・*Nakajima M, et al. Science 308, 414-5 (2005)*
・*Terauchi K, et al. Proc. Natl Acad. Sci. USA. 104, 16377-81 (2007)*

【Term of Project】 FY2012-2016

【Budget Allocation】 315, 500 Thousand Yen

【Homepage Address and Other Contact Information】

http://clock.bio.nagoya-u.ac.jp/web/index.htm

【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

Title of Project： Molecular Mechanism of Florigen Action and Application of Florigen in Crop Improvement

Ko Shimamoto
(Nara Institute of Science and Technology, Graduate School of Biological Sciences, Professor)

Research Area：Plant molecular genetics, Plant physiology, Plant breeding
Keyword：Florigen, Rice, Genes, Bioimaging, Structural biology, Gene targeting

【Purpose and Background of the Research】

Florigen is a molecular switch for flowering in plants, which is generated in leaves and moves up into the shoot apex to initiate flowering. The molecular nature of florigen has been unknown for over 75 years; however, our recent study showed that the protein encoded by *Hd3a/FT* gene is florigen (Tamaki et al, ***Science***, **2007**). Hd3a is expressed in leaf vasculature, then transported into shoot apex to induce flowering in rice.

Most recently we have demonstrated that Hd3a florige protein forms a protein complex with 14-3-3 protein and OsFD1, a transcription factor important for flowering in rice. The hexamer containing two proteins each of these three components is termed Florigen Activation Complex (FAC) and we determined itsvcrystal structure (Figure 1, Taoka et al., ***Nature***, **2011a**)

We demonstrated that Hd3a can induce potato tuber formation as a mobile tuberization signal (collaboration with Dr. S. Prat, Spain, Navarro et al., ***Nature*** **, 2011b**). This finding will provide an unexpected function of florigen as the diverse developmental cue beyond flowering.

We plan to study two objectives; 1) identify molecular mechanisms of florigen function, and 2) study crop productivity by controlling florigen activity.

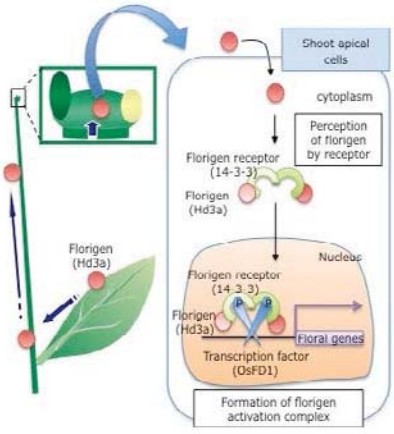

Figure 1　Model of florigen function

【Methods】

1. Analysis of molecular mechanisms for florigen activation complex function by biochemical and molecular genetic strategies.
2. Analysis of vegetative to reproductive phase transition in the shoot apical meristem by next generation sequencing technologies and live imaging.
3. Studies of crop productivity by manipulating florigen expression and activity by gene targeting.

【Expected Research Achievements and Scientific Significance】

A major challenge is to study molecular mechanisms by which florigen is transported into shoot apex and induces floral phase transition in shoot apical cells. This will open up the new research field of plant biology such as the regulation of plant development through mobile protein signal.

Our recent findings indicate that florigen controls diverse developmental events beyond flowering. Thus, we will study how florigen exerts this interesting multi-functional cellular activity and try to apply it for crop improvement in the future.

【Publications Relevant to the Project】

- Taoka, K.-I., et al. (2011a) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. ***Nature***, 476:332-335.
- Navarro, C. et al. (2011b) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. ***Nature*** 478:119-122.
- Tamaki S., et al (2007) Hd3a protein is a mobile flowering signal in rice. ***Science*** 316:1033-103.

【Term of Project】 FY2012-2016

【Budget Allocation】 438,000 Thousand Yen

【Homepage Address】

http://bsw3.naist.jp/simamoto/simamoto.html

Title of Project : Elucidation of the mechanisms of water-splitting in photosystem II

Jian-Ren Shen
(Okayama University, Graduate School of Natural Science and Technology, Professor)

Research Area : Biology, Biophysics
Keyword : Photosynthesis, Membrane proteins, Light-energy conversion, Water-splitting

【Purpose and Background of the Research】

The purpose of the present study is to elucidate the mechanism of light-induced water-splitting reaction catalyzed by photosystem II (PSII), the largest mystery remained unsolved in oxygenic photosynthesis, by means of a combination of structural biology, structural and functional characterization of various mutants, infra-red spectroscopy, electron spin resonance (EPR) measurement, and quantum mechanical (QM) and molecular mechanical (MM) calculations.

In oxygenic photosynthesis, PSII catalyzes light-induced water-splitting, leading to the evolution of dioxygen, protons and electrons. PSII is a multi-subunit membrane protein complex consisting of 20 subunits and a number of cofactors, with a total molecular mass of 350 kDa. We have succeeded in obtaining high quality crystals of PSII from a thermophilic cyanobacterium, and analyzed its structure at a 1.9 Å resolution. The structure we obtained, however, corresponds to the S_1-state in the S-state cycle (Fig. 1) of the water-splitting reaction. In order to fully unravel the mechanism of the water-splitting reaction, it is essential to solve the structures of the reaction intermediates, and to elucidate the structural and energetic changes accompanying each step of the reaction, along with the roles of individual subunits. We will accomplish these goals by using a combination of advanced technologies described above.

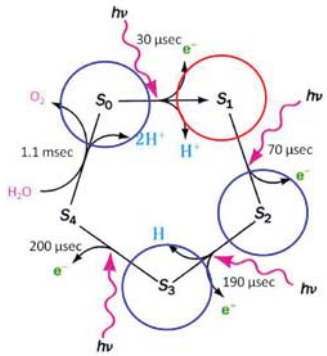

Fig. 1. S-state model of the water-splitting reaction taking place in PSII.

【Research Methods】

The research methods will be based mainly on crystal structural analysis of various reaction intermediates and mutants of PSII at atomic resolutions, but will also include visible, infra-red spectroscopy, EPR, QM/MM calculations, and various functional analysis of the mutants.

【Expected Research Achievements and Scientific Significance】

Water-splitting and oxygen evolution have been cited as the last and most important mystery in photosynthesis; the elucidation of this natural system will yield valuable information for not only natural but also artificial photosynthesis, since the natural system is highly efficient in utilizing visible light and uses only abundant, non-toxic metals for the catalytic reactions. PSII is the largest membrane protein complex whose structure has been solved beyond 2.0 Å resolution so far. Its refined structural and functional studies will inspire higher resolution structural studies of a vast number of other membrane proteins and their complexes, the structure of most of them, even if solved, have remained at a "medium" resolution.

【Publications Relevant to the Project】

Umena Y., Kawakami K., *Shen J.-R., *Kamiya N. Crystal structure of oxygen-evolving photosystem II at 1.9 Å resolution. *Nature* **473**, 55-60, 2011.

Kawakami K., Umena Y., Kamiya N., Shen J.-R. Location of chloride and its possible functions in oxygen-evolving photosystem II revealed by X-ray crystallography. *Proc. Natl. Acad. Sci. USA* **106**, 8567-8572, 2009.

【Term of Project】

FY2012-2016

【Budget Allocation】

399, 500 Thousand Yen

【Homepage Address and Other Contact Information】

http:// http://www.biol.okayama-u.ac.jp/shen2/ﾄｯﾌﾟ.htm
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