A study of plant adaptation to elevated CO_2 using CO_2 springs as a future ecosystem

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【Outline of survey】

Atmospheric CO_2 concentration is increasing and expected to double at the end of this century. Many studies have been conduted to understand plant responses to elevated CO_2. However, most of previous studies have used current plants that may adapt to current CO_2 concentration. We can expect that elevated CO_2 acts as a selective agent and plants that adapt to elevated CO_2 may evolve in future environments. The aim of the present study is to predict evolution of plants under future high-CO_2 world. We conduct ecophysiological and population-genetic studies for plants growing around CO_2 springs, where high CO_2 concentration has been maintained for long term. Furthermore, we conduct selection experiment to reproduce evolution under high CO_2 conditions.

【Expected results】

We will find advantageous and disadvantageous traits of plants under high CO_2 conditions and predict evolution of plants under future environment. This will contribute to prediction of future vegetation change and future ecosystem responses to global environmental change. Furthermore, our study will contribute to modelling of environmental response of plant growth. This model will be useful for understanding of evolutionary significance of plant traits and for improvement of agricultural yield.

【References by the principal investigator】

・Onoda Y, Hirose T, Hikosaka K (2007) Effect of elevated CO_2 on leaf starch, nitrogen and photosynthesis of plants growing at three natural CO_2 springs in Japan. *Ecological Research*, 22: 475-484.

・Miyagi KM, Kinugasa T, Hikosaka K, Hirose T (2007) Elevated CO_2 concentration, nitrogen use, and seed production in annual plants. *Global Change Biology*, 13: 2161-2170.

【Term of project】 FY2008− 2012	【Budget allocation】 80,100,000 yen (direct cost)

【Homepage address】 http://hostgk3.biology.tohoku.ac.jp/hikosaka/index.html

Role of PIP3 Transport in Regulation of Cell Polarity

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【Outline of survey】

PIP3 is involved in diverse cell biological phenomena such as proliferation and differentiation. Recent studies have revealed that PIP3 accumulates at the leading edges of migrating cells and at the growth cones of axons, where it plays crucial roles in the regulation of cell polarity. However, the molecular mechanism of how PIP3, which constitutes the membrane, accumulates at such specialized areas remains largely unknown. I identified a microtubule-based motor protein as a novel substrate for a polarity-regulating kinase. It was found that this motor protein accumulates at the growth cones as does PIP3. A foreign research group has suggested that a motor protein associates with and transports PIP3-containing lipid vesicles.
These new findings raise the possibility that the PIP3 accumulation occurs via the transport on the cytoskeleton. In this project, I am going to investigate the regulatory and functional mechanisms of motor proteins and clarify the importance of the PIP3 transport in cell polarity.

【Expected results】

Since the discovery of PIP3, most researchers have focused on the enzymes that generate or degrade PIP3. In this point, the idea that PIP3 is recruited by the transport on the cytoskeleton is a very interesting one and this study will certainly produce valuable results with a strong impact on cell biology. Malfunction of PIP3 regulation is known to be responsible for several human diseases such as cancers and diabetes, and thus this study may clarify the link of the cytoskeleton and the motor protein with human diseases.

【References by the principal investigator】

- Miki et al. (1998) Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. ***Nature*** 391, 93-96.
- Miki et al. (2000) IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. ***Nature*** 408, 732-735.
- Yamazaki et al. (2003) WAVE2 is required for directed cell migration and cardiovascular development. ***Nature*** 424, 452-456.
- Funato et al. (2006) The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-β-catenin signaling through Dishevelled. ***Nat. Cell Biol.*** 8, 501-508.

【Term of project】 FY2008-2012	【Budget allocation】 **70,200,000 yen** (direct cost)

【Homepage address】 http://www.protein.osaka-u.ac.jp/intra_signal/index.html

Cell-biological investigation of the stem cell system that supports the mammalian spermatogenesis

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（National Institutes of Natural Sciences ,National Institute for Basic Biology, Professor）

【Outline of survey】

Mammalians including humans exhibit a numerous daily sperm production for long reproduction period. This is supported by “stem cells” that supply differentiating progeny, while maintaining their own population. However, it is still largely a puzzle which cells the “stem cells” are among a numerous spermatogenic cells found in the testis, or, where and how the “stem cells” behave to ensure the continuous spermatogenesis.

Previous studies of our own have suggested that a particular subpopulation of transit amplifying cells retain the self-renewing potential, while do not self-renew in the normal spermatogenesis. This population may play an important backup role in replenishment of the occasional homeostatic stem cell loss, and thus ensure the spermatogenesis continuity. This research project will “look the shapes”, “clarify the locations”, “watch the movements” and “chase the fates” of these populations, taking advantages of a variety of experimental strategies. Subsequently, this study aims to reveal the entire composition and function of the “stem cell system” for the mammalian spermatogenesis.

【Expected results】

Results of this study would contribute toward a general understanding and control of the stem cell system, not only in spermatogenesis but also other system such as skin or blood stem cells. Revealing the mammalian spermatogenic stem cell system would contribute, on one hand, to the investigation and care of the male infertility in human. On the other hand, this would also lead to development of a novel contraceptive strategy against the global population problem.

【References by the principal investigator】

- T. Nakagawa, Y-i. Nabeshima and ***S. Yoshida**: Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis
  Developmental Cell 12, 195-206 **(2007)**
- ***S. Yoshida**, M. Sukeno and Y-i. Nabeshima: A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis
  Science 317, 1772-1776 **(2007)**

【Term of project】 FY2008−2012	【Budget allocation】 **79,500,000 yen** (direct cost)

【Homepage address】 http://lmls.med.kyoto-u.ac.jp

Molecular basis of self/non-self recognition in self-incompatibility on cruciferous plants

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【Outline of survey】

In higher plants, the genetic diversity is maintained by outcrossing. Self-incompatibility (SI) is one of outcrossing systems, which was written in C. Darwin's books. From my recent research, it has been revealed that *Brassica* SI, which is controlled by a single locus with multiple alleles, is regulated by allele-specific direct interaction between SP11 (male *S* determinant) and SRK (female *S* determinant). However, there is little information of the downstream signaling factor of SRK, at a moment. In this study, I aim to understand the intracellular signaling network of *Brassica* SI using two types of plant materials, self-compatible *B. rapa* and self-incompatible *Arabidopsis thaliana*, with multidisciplinary approach.

【Expected results】

SI is one of a model system of cell-cell communication in higher plants, and has been used in the production of F_1 hybrid variety in *Brassica* crops. Furthermore, a variety of genes encoding receptor kinase are contained in the genome of higher plants, although most of their functions were still unknown. This study will contribute to discover the downstream molecules of SI, and their intracellular signaling network and other orphan receptor kinase.

【References by the principal investigator】

- Takasaki, T., Hatakeyama, K., Suzuki, G., Watanabe, M., Isogai, A., and Hinata, K. (2000) SRK determines the *S* specificity of stigma in self-incompatible *Brassica*. Nature 403: 913-916.
- Murase, K., Shiba, H., Iwano, M., Che, F.-S., Watanabe, M., Isogai, A., and Takayama, S. (2004) A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. Science 303: 1516-1519.

【Term of project】 FY2008－2012	【Budget allocation】 80,000,000 yen (direct cost)

【Homepage address】 http://www.ige.tohoku.ac.jp/prg/watanabe/

Mechanisms for methylation imprinting establishment after fertilization

Keiji Tanimoto
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【Outline of survey】

Mammals inherit one genome from each parent and most genes are expressed from both alleles. Normal embryonic development requires both a male and female genome, because some genes remember their parental origin and are transcribed quite specifically from only one of the two parental alleles (genomic imprinting). In the Igf2/H19 locus, the Igf2 gene is transcribed when paternally inherited, while the H19 gene is maternally transcribed. Imprinted genes are often associated with differentially methylated regions (DMRs) that control monoallelic gene expression. It is generally accepted that DMRs establish their allelic methylation during gametogenesis. Since some DMRs are methylated during oogenesis and others during spermatogenesis, it is suggested that DMRs contain genetic “marks” that allow (or prevent) methylation acquisition at nearby CpG dinucleotides in only one of the gametes. In this study, I propose to search for the DNA sequence that is required and sufficient for establishment of methylation imprinting, through which further understanding of the molecular mechanism of genomic imprinting is anticipated.

【Expected results】

By examining YAC (yeast artificial chromosome) transgenic mice, I have shown that genomic imprinting could be recapitulated at a heterologous genomic locus by simply grafting the H19 DMR into an irrelevant (human beta-globin) genetic locus. Surprisingly, methylation imprinting was established after fertilization in these transgenic mice, which was different from what is observed at the endogenous Igf2/H19 locus. By determining the minimal cis DNA sequences within the H19 DMR that establish the parent of origin-dependent methylation pattern during the post-fertilization period in this experimental system, we expect to define the molecular entity that must be inherited.

【References by the principal investigator】

・Tanimoto, K., Sugiura, A., Omori, A., Felsenfeld, G., Engel, JD., and Fukamizu, A. "Human beta-globin locus control region HS5 contains CTCF- and developmental stage-dependent enhancer-blocking activity in erythroid cells" *Mol. Cell. Biol.* **23**, 8946-8952 (2003)

・Tanimoto, K., Shimotsuma, M., Matsuzaki, H., Omori, A., Bungert, J., Engel, JD., and Fukamizu, A. "Genomic imprinting recapitulated in the human beta-globin locus" *Proc. Natl. Acad. Sci. USA* **102**, 10250-10255 (2005)

【Term of project】 FY2008− 2012

【Budget allocation】 80,000,000 yen (direct cost)

【Homepage address】 http://akif2.tara.tsukuba.ac.jp/

Total Syntheses and New Biological Applications of Architecturally Complex Natural Products

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【Outline of survey】

Natural products have been tremendously important in biology and human medicine because of their power to modulate signal transductions of biological system. Three-dimensional structures of natural products are highly optimized for function through evolutionary processes; functional information is manifested by sophisticated assemblages of various ring systems and functional groups. Since the removal of sub-structures of the natural products often leads to significant losses of their activity, total syntheses of their entire structures with a precision at an atomic level are necessary to provide sufficient amounts of material required for biological and medical applications. Architecturally complex natural products with molecular weight over 1000 are capable of highly specific interactions with their target proteins. Therefore, they are powerful agents for selectively controlling intricate biological systems. In this research, we will develop new and efficient synthetic methodologies and strategies for the synthesis of highly complex compounds. We will then apply the synthetic natural products and designed artificial analogs for studying new functions and network of biological systems.

【Expected results】

The goal of our research program is efficient, practical and flexible syntheses of biologically important natural molecules. At the core of this research program is the development of new strategies for assembling architecturally complex natural products in a concise fashion. These synthetic developments would enable unified synthesis of new artificial analogs by modification of natural products templates. The new synthetic methods for the natural products and the synthetic analogs will allow us to tailor and enhance their druglike properties, to gain control over diverse signal transductions thereby offering new research methods for the study of life science.

【References by the principal researcher】

- M. Inoue, M. Hirama, et al. “Total Synthesis of Ciguatoxin and 51-HydroxyCTX3C,” *J. Am. Chem. Soc.* **2006**, *128*, 9352-9354.
- M. Inoue et al. “Total Synthesis and Bioactivity of an Unnatural Enantiomer of Merrilactone A: Development of an Enantioselective Desymmetrization Strategy,” *J. Org. Chem.* **2007**, *72*, 3065-3075.

【Term of project】 FY2008－2012	【Budget allocation】 **81,200,000 yen** (direct cost)

【Homepage address】 http://www.f.u-tokyo.ac.jp/~inoue/e_index.html

Simultaneous recording of conformational changes and ionic currents of single-molecular ion channels reveals the relationship between membrane potentials and motions of the channels

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【Outline of survey】

In cell membranes or microorganisms, membrane proteins are always affected by membrane potentials and its oscillations in their activities. How do they move and function in these physiological conditions? To answer this fundamental question I plan to develop a new measurement system using ion channels as a testing molecule. Ion channels are signal transduction molecules which transduce various stimuli, such as chemical substances in the body and membrane potentials, to electrical signals or ionic stream that runs across the membrane. In this transduction process, it had been predicted there were conformational changes for the openings and closings of their ion permeation pathway (gating). Our research group recently succeeded in recording these conformational changes in a single molecule as movies, which revealed that an ion channel twisted around the axis of the pathway upon gating. In this study, I will develop new measuring devices which enable simultaneous recording of the conformational changes and ionic currents through the channels by integrating the existing method for recording currents and our method for detecting motions.

【Expected results】

The results of this research will elucidate the mechanism of signal transduction in cells by revealing the relationship between the “function” and “motion” of channels. The simultaneous measurement of a single molecule, not averaged image of many molecules, will allow a detailed analysis of the transduction mechanism that is an essential part of biological processes. Furthermore, the developed measuring devices can be adapted to all the other membrane proteins including important targets of drugs. Thus, the results would contribute to the understanding of the mechanism of drug action and designing of a new drug by examining not only the “functions” and “structures” but “motions” of their target.

【References by the principal investigator】

・Shimizu, H., *et.al.* (2008) Global twisting motion of KcsA potassium channel upon gating. Cell 132, 67-78.

【Term of project】 FY2008－2012	【Budget allocation】 **70,600,000 yen** (direct cost)
【Homepage address】	None

Molecular mechanisms for the detection of microbes and cancer cells in innate immunity

Akinori Takaoka
(Hokkaido University, Institute for Genetic Medicine, Professor)

【Outline of survey】

Infctious disease is still a formidable issue, to which it could provide a key to find how we control microbial infection. We will address this issue by focusing particularly on "microbial sensing", the first line of host defense in triggering innate immune responses. Recent rapid progress in studies on innate immunity has facilitated the identification of various sensors, such as TLRs (Toll-like receptors), that detect microbe-specific components, and has elucidated their critical roles in the activation of dendritic cells. Recently, we have identified a candidate cytosolic DNA sensor called DAI (DNA-dependent activator of IRFs, previously known as DLM-1 or ZBP1). In addition, evidence has been provided regarding the existence of additional DNA sensor(s). In this study, our aim is to find a novel DNA sensing molecule(s), to determine which microbes can be recognized by them, and to elucidate a mechanism for the activation of these sensors and the related signaling pathways, leading to the induction of cytokine/chemokine genes in innate immune responses. In addition, we try to investigate the mechanism for the activation of innate immunity in the eradication of cancer cells, particularly in terms of a possible involvement of "DNA sensing" in this process.

【Expected results】

This study will contribute to further understanding of the microbial sensing mechanism for the activation of innate immune responses. In addition, it will be expected that this project may also provide a new insight into the mechanism for the pathogenesis of DNA-related diseases including autoimmune diseases and inflammatory diseases, and offer some therapeutic basis to those intractable diseases. Our research will further clarify a mechanism underlying the activity of DNA as a potent immunostimulant particularly for vaccination. The analyses for the recognition of cancer cells could provide a novel concept to the activation process of innate immunity against cancer.

【References by the principal investigator】

- Takaoka, A., Wang, Z., Choi, M.K., Yanai, H., Negishi, H., Ban, T., Lu, Y., Miyagishi, M., Kodama, T., Honda, K., Ohba, Y., and Taniguchi, T. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature*, **448**, 501-505, 2007.
- Takaoka, A., and Taniguchi, T. Cytosolic DNA recognition for triggering innate immune responses. *Adv. Drug Deliv. Rev.*, **60**, 847-857, 2008.

【Term of project】 FY2008− 2012	【Budget allocation】 77,200,000 yen (direct cost)

【Homepage address】 http://www.igm.hokudai.ac.jp/sci/

Mechanisms of chemotherapy resistance in human acute myelogenous leukemia (AML) stem cells

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【Outline of survey】

Acute myelogenous leukemia (AML) is one of the most common and intractable adult hematological malignancies that exhibits high relapse rate even following successful remission induction and hematopoietic stem cell transplantation. We have recently identified leukemic stem cells in AML that can self-renew, generate non-stem leukemic cells, and possess exclusive capacity to initiate leukemia in vivo. Transplantation of AML stem cells into NOD/SCID/IL2rgKO newborns successfully recapitulates human AML, which enables us to identify the role of leukemic stem cells in leukemogenesis and relapse. We aim to clarify the mechanisms of drug resistance underlying leukemia relapse by cell biological and global transcriptome analyses, with the ultimate goal of translating thus obtained research findings to the creation of novel therapeutic strategies for leukemia.

【Expected results】

The direct in vivo examination of stem cell properties such as stem-niche interaction, cell cycle quiescence, and drug efflux capacity as well as global gene expression profiling of leukemic stem cells enable us to identify the stem cell specific molecules and to develop therapeutic strategies to overcome AML relapse.

【References by the principal investigator】

- Ishikawa F, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone marrow endosteal region. ***Nature Biotechnology*** 25:1315-21, 2007.
- Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. ***Nature Reviews Immunol*** , 7:118-130, 2007.
- Ishikawa F, et al. Development of functional human blood and immune systems in NOD/SCID/IL2rg chain null mice. ***Blood*** 106:1565-1573, 2005.

【Term of project】 FY2008－2012	【Budget allocation】 **65,700,000 yen** (direct cost)

【Homepage address】 http://web.rcai.riken.jp/en/labo/human/index.html

Establishment of autoimmune disease therapies based on the elucidation of target genes

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【Outline of survey】

Most of the genes involved in autoimmune diseases remain unclear despite a variety of basic immunological and genetic studies. It is crucial to clarify the complex regulatory mechanisms that maintain the homeostasis of the immune system as well as acquire immune tolerance in order to establish therapeutic systems to treat autoimmune diseases. We plan to identify crucial genes that cause autoimmune diseases by genome wide screens in this study. In addition, we plan to clarify the roles of Notch signaling that is associated with T cell-mediated autoimmunity.

【Expected results】

The discovery of target genes that play key role in the etiology of autoimmune diseases would help establish targeted therapies to treat autoimmune diseases. Furthermore, such studies might help identify a novel regulatory mechanism to control immune homeostasis. Dissecting the specific roles of Notch in the immune system or elucidating the relationship between Notch and other regulatory systems would contribute not only to a better understanding of complex immune networks but also provide a new approach to modulate the immune systems.

【References by the principal investigator】

(1) Kijima M, et al. Dendritic cell-mediated NK cell activation is controlled by Jagged2-Notch interaction. Proc Natl Acad Sci USA 105: 7010-7015 (2008)
(2) Maekawa Y, et al. Delta1-Notch3 interactions bias the functional differentiation of activated CD4+ T-cells. Immunity 19:549-59 (2003).
(3) Yasutomo K, et al. Mutation of DNASE1 in people with systemic lupus erythematosus. Nat Genet. 28:313-4 (2001)
(4) Yasutomo K, et al. The duration of antigen receptor signalling determines CD4+ versus CD8+ T-cell lineage fate. Nature.404:506-10 (2000)

【Term of project】 FY2008 － 2012	【Budget allocation】 81,200,000 yen (direct cost)

【Homepage address】

http://immunology.hosp.med.tokushima-u.ac.jp/immunology/system/top/index.php

Analysis and regulation of tooth morphogenesis

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(Tohoku University, Graduate School of Dentistry, Professor)

【Outline of survey】

In tooth morphogenesis, the dental epithelium and mesenchyme interact reciprocally for growth and differentiation to form the proper number and shapes of teeth. Previously, about twenty genes expressed in tooth specifically were identified by microarray and computer based differential display methods. Among of these genes, gap junctional molecule Gja1 is expressed in tooth germ. Gja1 null-mouse showed disorganization of ameloblasts. Epiprofin (Epfn) is tooth specific transcription factor. Epfn mutant showed increase number of tooth. Mutant mouse of down-stream signal molecules for ectodysplatin A showed decrease tooth width. These results indicated that these tooth specific molecules regulate tooth number and shape. We will analyze their gene function and molecular mechanism of abnormal tooth formation in their mutant mice.

【Expected results】

By this project, we will gain several novel molecular mechanisms for the determination of tooth size and shape. These discovered mechanisms will help to understand the morphogenesis of not only tooth, but also the organs formed by epithelial-mesenchymal interaction. It is possible to organize the artificial tooth, which has proper size and shape, using their information about tooth morphogenesis.

【References by the principal investigator】

・Yoshizaki K, Yamamoto S, Yamada A, Yuasa K, Iwamoto T, Fukumoto E, Harada H, Saito M, Nakasima A, Nonaka K, Yamada Y & Fukumoto S. Neurotrophic factor NT-4 regulates ameloblastin expression via full-length TrkB. **J Biol Chem** 283, 3385-3391, (2008).
・Fukumoto S, Miner JH, Ida H, Fukumoto E, Yuasa K, Miyazaki H, Hoffman MP & Yamada Y. Laminin alpha5 is required for dental epithelium growth and polarity and the development of tooth bud and shape. **J Biol Chem** 281, 5008-5016, (2006).
・Fukumoto S, KIba T, Hall B, Iehara N, Nakamura T, Longenecker G, Krebsbach PH, Nanci A, Kulkarni AB & Yamada Y. Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblast. **J Cell Biol** 167, 973-983, (2004).

【Term of project】 FY2008－2012	【Budget allocation】 **78,100,000 yen** (direct cost)
【Homepage address】	None