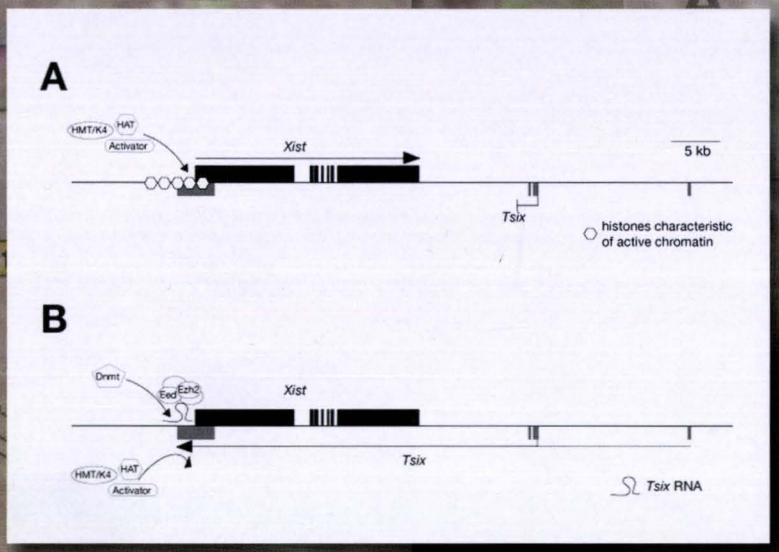
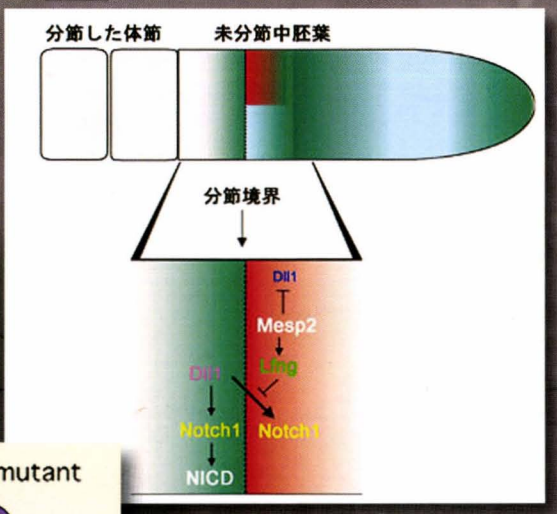
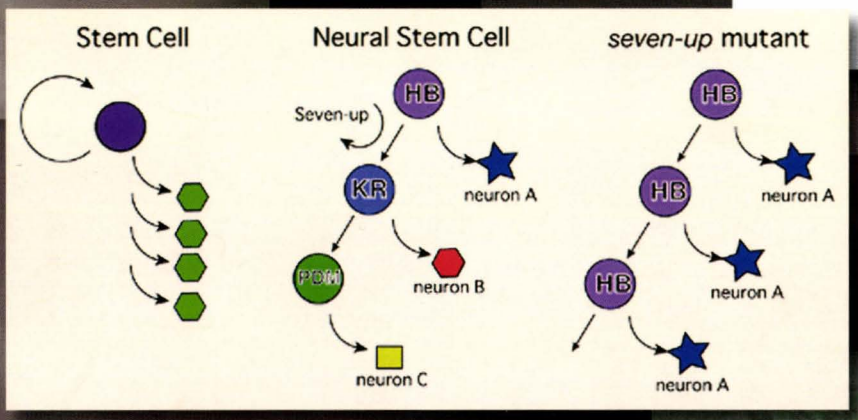
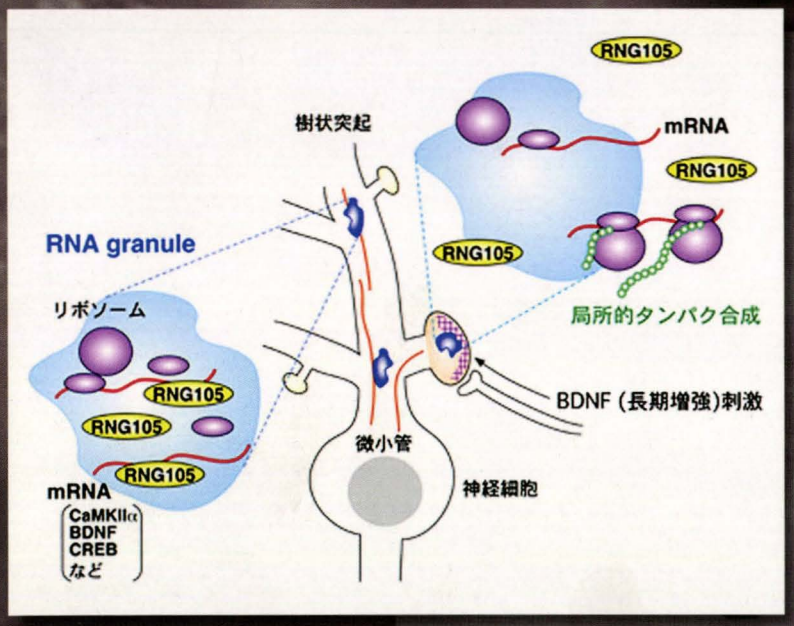
ANNUAL REPORT

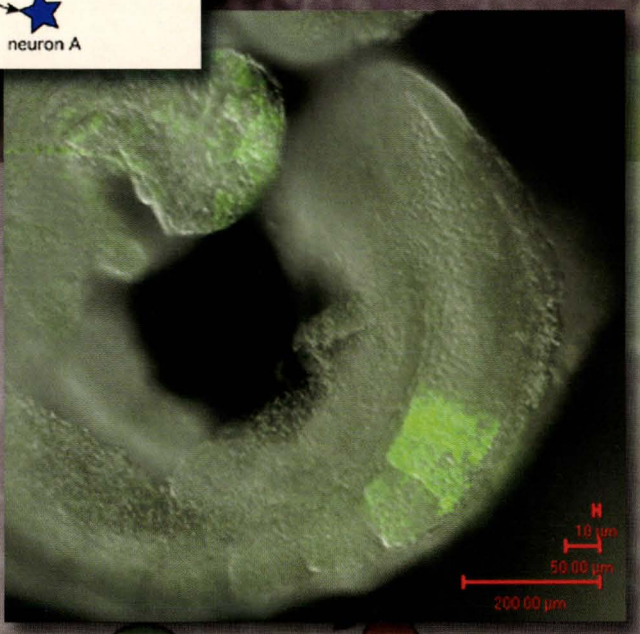
No.56
2005
ISSN 0077-4995





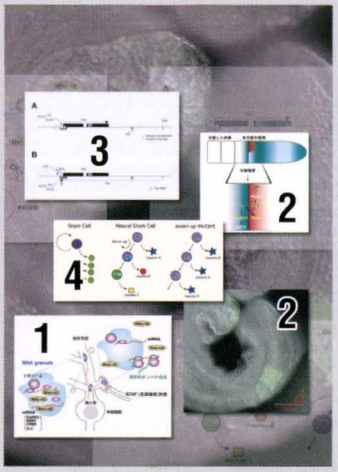






Research Organization of Information and Systems
NATIONAL INSTITUTE OF GENETICS
http://www.nig.ac.jp

Highlights of 2005 (http://www.nig.ac.jp/hot/index.html)



1. A novel RNA-binding protein in neuronal RNA granules: Regulatory machinery for local translation
   Shiina N, Shinkura K, Tokunaga M.
   J. Neurosci., *25 (17)*, 4420-4434, 2005.

2. The transcription factor Mesp2 establishes segmental borders by suppressing Notch activity.
   Morimoto M, Takahashi Y, Endo M, Saga Y.
   Nature, 435

3. Tsix Silences Xist through Modification of Chromatin Structure
   Takashi Sado, Yuko Hoki, and Hiroyuki Sasaki
   Developmental Cell, *9(1)*,in press,2005

4. Generation of neuronal diversity through switching genes expression in neural stem cells
   Kanai MI, Okabe M, Hiromi Y.
   Developmental Cell, *8(2)* 203-213, 2005

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C. DEPARTMENT OF DEVELOPMENTAL GENETICS

D. DEPARTMENT OF POPULATION GENETICS

E. DEPARTMENT OF INTEGRATED GENETICS

H. STRUCTURAL BIOLOGY CENTER

Introduction

The National Institute of Genetics (NIG) was established in 1949 as the central institute to study various aspects of genetics. It was reorganized in 1984 as an inter-university research institute to promote collaborations with researchers at universities. Since 1988, NIG has been participating in graduate education as the Department of Genetics of the Graduate University for Advanced Studies (SOKENDAI). NIG also serves as a center for various genetic resources such as mutant strains, clones and vectors, and houses DDBJ, the DNA Data Bank of Japan, and a DNA sequencing center.

The history of NIG overlaps the period of a revolution in the field of life science. Genetics is no longer a discipline to study the rules and mechanisms of heredity, but has become the basis for all fields of life science. Molecular techniques now allow us not only to decipher the entire genome sequence of organisms including humans, but also to understand the details of higher biological phenomena: cell differentiation, morphogenesis, brain function, and evolution — the history of life itself. Currently, 36 research groups are actively performing pioneering and cutting-edge researches in these fields at NIG.

Recent generation of massive information on biological systems and their environment calls for new directions in life sciences, such as bioinformatics, system-level analysis, and theoretical approaches to extract knowledge from databases. To this end NIG and three other national institutes, the National Institute of Informatics, The Institute of Statistical Mathematics and the National Institute of Polar Research have formed a new organization, the Research Organization of Information and Systems (ROIS) since April 2004, as a part of the reform of national universities and research institutes in Japan. Inter-institutional collaborations within the new organization are in progress.

We welcome your comments and suggestions on our research activities and endeavors.

Yuji Kohara, Director-General

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A. DEPARTMENT OF MOLECULAR GENETICS

A-a. Division of Molecular Genetics Tatsuo Fukagawa Group

Research Activities

(1) Identification and characterization of the CENP-H-I complex proteins in higher vertebrate cells.

Masahiro Okada, Tetsuya Hori, Mi-Sun Kwon, Aussie Suzuki, Mayumi Takahashi, and Tatsuo Fukagawa

The centromere plays a fundamental role in accurate chromosome segregation during mitosis and meiosis in eukaryotes. Its functions include sister chromatid adhesion and separation, microtubule attachment, chromosome movement and mitotic checkpoint control. Although chromosome segregation errors cause genetic diseases including some cancers, the mechanism by which centromeres interact with microtubules of the spindle apparatus during cell division is not fully understood.

To understand the function of the centromere, we were led to develop a genetic method that utilizes the hyper-recombinogenic chicken B lymphocyte cell line DT40. The high level of homologous recombination in DT40 cells permit efficient targeted disruption of genes of interest. We have improved this system and have created several cell lines with conditional knockouts of several centromere proteins to investigate the molecular mechanism of centromere assembly and function. We have proposed a model for kinetochore assembly in vertebrate cells (Fukagawa et al., EMBO J., 2001; Nishihashi et al., Dev. Cell, 2002; Fukagawa, Exp. Cell Res., 2004; Regnier et al., Mol. Cell. Biol., 2005; Mikami et al., Mol. Cell. Biol., 2005). In this model we explained that many unidentified proteins could be involved in kinetochore assembly. Therefore, we tried to identify new centromere proteins that associate with CENP-H or CENP-I using the proteomics approach. We prepared DT40 cell lines in which expression of CENP-H was replaced by expression of CENP-H-Flag or CENP-H-GFP (Mikami et al., Mol. Cell. Biol., 2005). We also prepared DT40 cell lines in which expression of CENP-I was replaced by expression of CENP-I-Flag or CENP-I-GFP. We extracted chromosome fraction from these cell lines and performed immunoprecipitation with anti-Flag or anti-GFP antibodies. Proteins co-precipitated with antibodies were analyzed by mass spectrometry, and we identified several polypeptides and cloned the corresponding cDNAs. As we confirmed that these proteins constitutively localize to centromeres in DT40 cells, we named them CENPs. We also performed immunoprecipitation experiments with human cells and identified similar proteins. In total, our purifications in chicken and human cells identified 11 interacting proteins (CENP-K, CENP-L, CENP-M, CENP-N, CENP-O, CENP-P, CENP-Q, CENP-R, CENP-50, CENP-H and CENP-I) that localize constitutively to centromeres as components of the CENP-H-I associated complex. Our analysis of these proteins using chicken DT40 knockouts and RNAi analysis in human cells demonstrates that the CENP-H-I complex can be divided into three functional sub-complexes, each of which is required for chromosome alignment and faithful chromosome segregation (Okada et al., Nature Cell Biology, 2006).

(2) Functional analysis of CENP-50 that is required for recovery from spindle damage.

Tetsuya Hori, Masahiro Okada, Tomoko Motohashi, Kazuko Suzuki, and Tatsuo Fukagawa

We identified CENP-50 as a kinetochore component. We found that CENP-50 is a constitutive component of the centromere that colocalizes with CENP-A and CENP-H throughout the cell cycle in vertebrate cells. To determine the precise function of CENP-50, we examined its role in centromere function by generating a loss-of-function mutant in the chicken DT40 cell line. The CENP-50 knockout was not lethal; however, the growth rate of cells with this mutation was slower than that of wild-type cells. We observed that the time for CENP-50-deficient cells to complete mitosis is longer than that for wild-type cells. Centromeric localization of CENP-50 was abolished in both CENP-H- and CENP-I-deficient cells. Co-immunoprecipitation experiments revealed that

CENP-50 interacts with the CENP-H-I complex and the Nuf2-Hec1 complex in chicken DT40 cells. We also observed severe mitotic defects in CENP-50-deficient cells with apparent premature sister chromatid separation when the mitotic checkpoint was activated, indicating that CENP-50 is required for recovery from spindle damage (Minoshima, Hori et al., Mol. Cell. Biol., 2005).

Although the CENP-50 knockout was not lethal at cellular level, it is interesting to investigate function of CENP-50 at individual level. Therefore, we are making and characterizing knockout mice of CENP-50. We also discovered that expression of a brain specific gene was reduced in CENP-50-deficient cells.

(3) Functional analysis of the CENP-C complex, which associates with the Mis12 complex.

Mi-Sun Kwon, Masahiro Okada, Tetsuya Hori, and Tatsuo Fukagawa

CENP-C, an essential inner kinetochore component, is essential for faithful chromosome segregation. We previously reported a creation of conditional knockout DT40 cell line of CENP-C with eastergen receptor (ER) system. Since a target protein is inactivated by post-translational regulation in the ER system, it would be better to use the system in which a target protein is inactivated at expression level such as a tetracycle-inducible system. In this project we created a CENP-C conditional knockout cell line with the tetracycle-inducible system. Consistent with prior results, CENP-C inactivation cause mitotic delay. However, living cell analysis showed that CENP-C knockout cells progressed to next cell cycle without normal cell division following the mitotic delay and interphase cells had di-nuclei before subsequent cell death. We detected significant reduction of signal intensities of the Mis12 complex proteins and the Nuf2 complex proteins at centromeres in CENP-C-deficient cells. We also found ~50% of CENP-C-deficient cells had no Mad2 signals even after treatment of a spindle poison, suggesting that CENP-C-deficient cells compromise spindle checkpoint function. We also tried to identify CENP-C interacting proteins and isolated some candidates. We are now characterizing them.

(4) Functional roles of the RNAi machinery in verterbrate centromeres

Tomoko Motohashi, and Tatsuo Fukagawa

RNAi-mediated silencing of gene expression occurs when double-stranded RNAs (dsRNAs) are cleaved by Dicer into 21- to 23-nt small interfering RNAs (siRNAs). These siRNAs guide a multicomponent nuclease, RNA-induced silencing complex (RISC), to degrade specific mRNAs. Although the Dicer-mediated gene-silencing is evolutionarily conserved system, the biological functions of the RNAi machinery are not fully understood. Genetic strategies have been used to examine the biological functions of the RNAi machinery in *C. elegans, Arabidopsis, Drosophila* and fungi. There are also many reports that the RNAi machinery via RNA-induced initiation of transcriptional silencing (RITS) complex is related to heterochromation formation and chromosome segregation in fission yeast. However, it was unclear whether the RNAi machinery is associated with chromosome segregation in vertebrate cells. To examine the biological function of the RNAi-related pathway in vertebrate cells, we generated and characterized a conditional loss-of-function mutant of Dicer in a chicken-human hybrid DT40 cell line that contains human chromosome 21 (Fukagawa et al, Nature Cell Biol., 2004). Loss of Dicer leads to cell death with accumulation of abnormal mitotic cells that show premature sister chromatid separation. Aberrant accumulation of transcripts from α-satellite sequences, which consist of human centromeric repeat DNAs, was detected in Dicer-deficient cells. We then started to examine expression profile of non-coding region using high-density DNA-microarray, when expression of Dicer was lost. We identified several non-coding RNAs from this method. We are characterizing biological function these RNAs. We also created conditional knockout cells for other components involved in RNAi machinery such as *Ago*-family proteins. Phenotype analyses of *Ago*-deficient cells were started. We would like to comprehensive understand relationships RNAi machinery with centromere function.

PUBLICATIONS

Papers

1. Mikami, Y., Hori, T., Kimura, H., Fukagawa T. (2005) The functional region of CENP-H interacts with the Nuf2 complex that localizes to centromere during mitosis, **Mol. Cell. Biol.**, *25, (5)* 1958 - 1970

2. Minoshima, Y., Hori, T., Okada, M., Kimura, H., Haraguchi, T., Hiraoka, Y., Bao, Y. C., Kawashima, T., Kitamura, T., Fukagawa, T. (2005) The constitutive centromere component CENP-50 is required for recovery from spindle damage, **Mol. Cell. Biol.** *25,* 10315 - 10328

3. Motohashi, T., Shimojima, T., Fukagawa, T., Maenaka, K., Park, E. Y. (2005) Efficient large-scale protein production of larvae and pupae of silkworm by Bombyx mori nuclear polyhedrosis virus bacmid system, **Biochem. Biophys. Res. Commun.**, *326, (3)* 564 - 569

4. Regnier, V., Vagnarelli, P., Fukagawa, T., Zerjal, T., Burns, E., Trouche, D., Earnshaw, W., Brown, W. (2005) CENP-A is required for accurate chromosome segregation and sustained kinetochore association of BubR1, **Mol. Cell. Biol.** *25,* 3967 - 3981

5. Yamauchi, A., Ichimiya, T., Inoue, K., Taguchi, Y., Matsunaga, N., Koyanagi, S., Fukagawa, T., Aramaki, H., Higuchi, S., Ohdo, S. (2005) Cell-cycle-dependent pharmacology of methotrexate in HL-60, **J. Pharmacol. Sci.**, *99,* 335 - 341

EDUCATION

1. 深川竜郎　高等動物におけるセントロメアおよびヘテロクロマチンの分子構築　京都大学ウイルス研究所セミナー　京都大学・ウイルス研究所

2. 深川竜郎　高等動物におけるセントロメアおよびヘテロクロマチンの分子構築　協和発酵バイオフロンティア研究所

SOCIAL CONTRIBUTIONS AND OTHERS

特許

特願2005-101959，セントロメアに局在するタンパク質，岡田聖裕，深川竜郎，大学共同利用機関法人情報・システム研究機構

各種受賞

深川竜郎　文部科学大臣表彰，若手科学者賞

A-b. Division of Mutagenesis
Fumiaki Yamao Group

The higher-order structure of chromatin essential for the chromosomal integrity is maintained via a variety of the post-translational modifications. The preservation of the chromatin structure during replication of chromosomes is another aspect of the epigenetic inheritance of the structural information. We focused on a replication factors involved in the chromatin structure maintenance, possibly by regulating the modifications of the chromatin proteins during the chromosomal duplication.

Research Activities

(1) Multifunctional role of Mcl1/Slr3 in DNA replication, repair and chromatin structure.

Toyoaki Natsume, Yasuhiro Tsutsui and Fumiaki Yamao

Slr3 in fission yeast is a member of eukaryotic family of WD repeat proteins and important for genome stability. *slr3* gene was originally identified as mutants showing synthetic lethality with Rad2 defect. Fission yeast Rad2 is a FEN-1 family nuclease processing Okazaki fragment during lagging strand DNA synthesis. Since recombination activity is essential for survival of cells defective in Rad2 function, genes that show, when mutated, synthetic lethality with Rad2-defect was expected to be involved in recombination or recombination repair of damaged DNA. Actually *slr3* mutants are sensitive to DNA damage reagents such as MMS, UV, bleomycin and hydroxyurea. Null mutant cell is temperature sensitive for growth, and shows severe growth retardation even at the permissive temperature. The mutant cells arrest at G2 phase with 2C DNA content at the restricted temperature, showing that the bulk DNA synthesis occurred. However, DNA double-strand breaks were found to accumulate in the arrested cells, which was a similar phenotype caused by defect of Okazaki fragment processing in such as *dna2* or *cdc17* mutants. It was further shown that Slr3 interacts not only genetically but also physically with DNA polymerase. These results strongly suggest that Slr3 functions in lagging strand synthesis and Okazaki fragment processing in addition to DNA repair. On the other

hand, *slr3* gene was found allelic to *mcl1* that is required for proper chromosomal segregation. Concomitantly *slr3* mutant cells were sensitive to TBZ. Consequently, highly frequent chromosome loss observed in *mcl1* mutant cells was attributed to defective cohesion of sister chromatids in centromere region(Tsuitsui et al.). Recently, we also found that Slr3 affects heterochromatin structure, which is now under an extensive investigation (Natsume et al. Unpublished results). Thus, Slr3/Mcl1 protein plays multifunctional role to maintain chromosomal stability by connecting DNA replication, recombination, damage repair, cohesion and heterochromatin structure. This work was collaborated with H. Shinagawa's and H. Iwasaki's group in Osaka University and Yokohama-City University, respectively.

PUBLICATIONS

Papers

1. Kotani, T., Nagai, D., Asahi, K., Suzuki, H., Yamao, F., Kataoka, N. and Yagura, T. (2005) Antibacterial Properties of Some cyclic Organobismuth (III) Compounds, **Antimicrob. Agents Chemother.**, *49*, 2729 - 2734

2. Tsutsui, Y., T. Morishita1, T. Natsume, K. Yamashita1, H. Iwasaki, F. Yamao and H. Shinagawa (2005) Genetic and Physical Interactions between Schizosaccharomyces pombe Mcl1 and Rad2, Dna2 and DNA polymerase α: Evidence for a Multifunctional Role of Mcl1 in DNA Replication and Repair., **Curr. Genet.**, *48*, 34 - 43

EDUCATION

1. 山尾文明　生命科学特殊講義(翻訳後修飾とタンパク質機能)　関西学院大学・理工学研究科
2. 山尾文明　分子生物学概論　山口大学・農学部

A-c. Molecular Mechanism Laboratory Hiroshi Mitsuzawa Group

RESEARCH ACTIVITIES

(1) Regulatory roles of the Rpb7 subunit of eukaryotic RNA polymerase II

Hiroshi Mitsuzawa

The Rpb4 and Rpb7 subunits of RNA polymerase II (Pol II) form a dissociable subcomplex. The Rpb4/Rpb7 heterodimer is dispensable for RNA synthesis but is required for promoter-dependent transcription initiation. Rpb4/Rpb7 can thus be viewed as a general transcription factor that associates tightly with Pol II. To elucidate the role of Rpb7 subunit in Pol II transcription, proteins were identified that interact with Rpb7 of the fission yeast *Schizosaccharomyces pombe* (Mitsuzawa *et al.* 2005). Identification of glyceraldehyde-3-phosphate dehydrogenase and actin as Rpb7-interacting proteins has suggested regulatory roles for Rpb7 in both the activation and initiation of Pol II transcription. Interestingly, Rpb4/Rpb7-related subunits have recently been identified in RNA polymerases I (Imazawa *et al.* 2005) and III. Rpb4/Rpb7 and their homologues seem to play diverse regulatory roles in eukaryotic cells.

(2) Identification of ammonium transporter genes in *S. pombe*

Hiroshi Mitsuzawa

Ammonium is an important source of nitrogen for yeast, and its availability has substantial effects on nitrogen metabolism and development of yeast cells. Transport of ammonium across the cell membrane is mediated by members of the Amt/Mep family, proteins with eleven transmembrane domains. Three ammonium transporter genes of *S. pombe*, named *amt1*, *amt2*, and *amt3*, were identified by sequence homology. A series of *amt* deletion strains were constructed and tested for growth on low ammonium, resistance to the toxic ammonium analog methylammonium, and ammonium removal from the growth medium. The results have suggested that the *amt* genes encode functional transporters with different

uptake capacities.

PUBLICATIONS

Papers

1. Imazawa, Y., Hisatake, K., Mitsuzawa, H., Matsumoto, M., Tsukui, T., Nakagawa, K., Nakadai, T., Shimada, M., Ishihama, A., Nogi, Y. (2005) The fission yeast protein Ker1p is an ortholog of RNA polymerase I subunit A14 in *Saccharomyces cerevisiae* and is required for stable association of Rrn3p and RPA21 in RNA polymerase I, **J. Biol. Chem.**, *280 (12)* 11467 - 11474

2. Mitsuzawa, H., Kimura, M., Kanda, E., Ishihama, A. (2005) Glyceraldehyde-3-phosphate dehydrogenase and actin associate with RNA polymerase II and interact with its Rpb7 subunit, **FEBS Lett.**, *579 (1)* 48 - 52

A-c. Molecular Mechanism Laboratory Hiroaki Seino Group

RESEARCH ACTIVITIES

(1) An *in vitro* ubiquitination assay of mitotic cyclin

Hiroaki Seino

Cell cycle events are regulated by sequential activation and inactivation of Cdk kinases. Mitotic exit is accomplished by the inactivation of mitotic Cdk kinase, which is mainly achieved by degradation of cyclins by a ubiquitin-proteasome system.

Previously we reported that two ubiquitin-conjugating enzymes, UbcP1/Ubc4 and UbcP4/Ubc11, were responsible for degradation of mitotic cyclin Cdc13 in fission yeast. Each of these two ubiquitin-conjugating enzymes is essential for cell viability and responsible for degradation of Cdc13. These results suggest that the functions of these two ubiquitin-conjugating enzymes are not redundant and they have distinct functions for ubiquitination of Cdc13. Furthermore, we found that ubiquitin chains of Cdc13 were totally reduced in ubc11 mutant cells, whereas ubiquitin chains were short and not reduced in ubc4 mutant cells. Thus, we proposed a hypothesis that Ubc11 might be involved in initiation of ubiquitination, and Ubc4 might be involved in elongation of ubiquitin chains of Cdc13. However, this hypothesis has not been elucidated yet.

To clarify the functional differences between Ubc4 and Ubc11 for degradation of Cdc13, development of an *in vitro* assay system for ubiquitination for Cdc13 by using fission yeast components is required. Currently, I am attempting to develop this assay system for Cdc13. A ubiquitin-activating enzyme, these two ubiquitin-conjugating enzymes and substrate Cdc13 were expressed as recombinant proteins in bacterial cells and purified. One component of Cdc13-specific ubiquitin ligase anaphase promoting complex/cyclosome (APC/C) was tagged and expressed in fission yeast cells, and APC/C was purified from fission yeast cells. Now I am examining the conditions for reconstitution of a ubiquitination reaction of Cdc13.

(2) Regulation of the DNA damage checkpoint by the ubiquitin pathway

Hiroaki Seino

The mutant strain of *ubc11*$^{+}$ gene showed the defect in mitotic progression. Additionally, this strain showed the cell elongation phenotype. It was shown that this cell elongation phenotype was caused by the delay of G2 phase by cell synchronization experiments. This phenotype is dependent on the DNA damage checkpoint but not the DNA replication checkpoint. When *ubc11*$^{+}$ was inactivated, the checkpoint kinase Chk1 was activated. It is possible that the ubiquitin pathway involving Ubc11 degrades the regulator(s) of the DNA damage checkpoint. It is important to identify the target protein(s) to understand the relationship between this ubiquitin pathway and the DNA damage checkpoint. I am attempting the establishment of the method of screening the target proteins of a specific ubiquitin pathway.

A-d. Division of Nucleic Acid Chemistry Tokuko Haraguchi Group

RESEARCH ACTIVITIES

(1) Dynamics of nuclear structure and function

Tokuko Haraguchi

The cell nucleus is a structure that functions to autonomously decode the genetic information encoded in DNA. Its structure changes in response to biological events such as development, cell growth, differentiation, aging, and the cell's reaction to environmental factors. To study these changes in nuclear structure, we have developed fluorescence microscope systems capable of recording the dynamic behavior of molecular components in living cells. Using these microscope systems, we study the functional and temporal organization of nuclear structures, and attempt to propose a model for the genome operating system which supports fundamental biological activities.

Toward such research goals, we have studied molecular mechanisms of cell division by focusing on the function of centromere proteins. Cdc42 works in metaphase by regulating bi-orient attachment of spindle microtubules to kinetochores. We reported that overexpression of dominant-negative mutants of Ect2 (a Rho GTPase guanine nucleotide exchange factor) and MgcRacGAP (GTPase activating protein), or depletion of Ect2 suppresses the level of GTP-Cdc42 in mitosis, and caused defects in microtubule attachment to kinetochores. In this study, we concluded that Ect2 and MgcRacGAP regulate the activation and function of Cdc42 in mitosis (Oceguera-Yanez et al, 2005). We also studied function of a newly identified kinetochore protein, CENP-50, in mitosis, and found that CENP-50 is a constitutive component of the centromere. To determine the role of CENP-50, we generated a loss-of-function mutant in the chicken DT40 cell line. The CENP-50 knockout was not lethal; however, the time required for CENP-50-deficient cells to complete mitosis was longer than that for wild-type cells. We also observed severe mitotic defects in CENP-50-deficient cells with apparent premature sister chromatid separation when the mitotic checkpoint was activated, indicating that CENP-50 is required for recovery from spindle damage (Minoshima et al, 2005). In fission yeast, centromeres remain clustered at the spindle-pole body (SPB) during mitotic interphase whereas those dissociate from the SPB during meiotic prophase. We examined the behavior of centromere proteins in living meiotic cells of fisson yeast. We show that the Nuf2-Ndc80 complex proteins (Nuf2, Ndc80, Spc24, and Spc25) disappear from the centromere in meiotic prophase when the centromeres are separated from the SPB. We also show that mutation of Nuf2 causes the release of centromeres from the SPB in pat1 mutant cells, suggesting that the Nuf2-Ndc80 complex connects centromeres to the SPB. In this paper, we proposed that the Nuf2-Ndc80 complex may be important in remodeling of the kinetochore for meiotic chromosome segregation (Asakawa et al, 2005).

PUBLICATIONS

Papers

1. Asakawa, H., Hayashi, A., Haraguchi, T., Hiraoka, Y. (2005) Dissociation of the Nuf2-Ndc80 complex releases centromeres from the spindle-pole body during meiotic prophase in fission yeast., **Mol. Biol. Cell**, *16 (5)* 2325 - 2338

2. Oceguera-Yanez, F., Kimura, K., Yasuda, S., Higashida,C., Kitamura,T., Hiraoka, Y., Haraguchi, T. and Narumiya, S. (2005). Ect2 and MgcRacGAP regulate the activation and function of Cdc42 in mitosis. **J. Cell Biol**. *168*, 221 - 232.

3. 平岡泰，原口徳子(2005)タイムラプスイメージングの基本，バイオイメージングがわかる，76 - 82

EDUCATION

会議等主催

1. 原口徳子　第5回細胞生物学ワークショップ　最先端の蛍光顕微鏡技術に関する実機演習　兵庫・(独)情報通信研究機構　関西先端研究センター(現・未来ICT研究センター)

A-d. Division of Nucleic Acid Chemistry
Hiroshi Iwasaki Group

RESEARCH ACTIVITIES

(1) Functional overlap between RecA and MgsA (RarA) in the rescue of stalled replication forks in *Escherichia coli*

Hiroshi Iwasaki (Yokohama City University)

Escherichia coli RecA protein plays a role in DNA homologous recombination, recombination repair, and the rescue of stalled or collapsed replication forks. The *mgsA (rarA)* gene encodes a highly conserved DNA-dependent ATPase, whose yeast orthologue, *MGS1*, plays a role in maintaining genomic stability. In this study, we show a functional relationship between *mgsA* and *recA* during DNA replication. The *mgsA recA* double mutant grows more slowly and has lower viability than a *recA* single mutant, but they are equally sensitive to UV-induced DNA damage. Mutations in *mgsA* and *recA* cause lethality in DNA polymerase I deficient cells, and suppress the temperature-dependent growth defect of *dnaE486* (Pol III alpha-catalytic subunit). Moreover, *recA S25P*, a novel *recA* allele identified in this work, does not complement the slow growth of Δ *mgsA* Δ *recA* cells or the lethality of *polA12* Δ *recA*, but is proficient in DNA repair, homologous recombination, SOS mutagenesis and SOS induction. These results suggest that RecA and MgsA are functionally redundant in rescuing stalled replication forks, and that the DNA repair and homologous recombination functions of RecA are separated from its function to maintain progression of replication fork (Shibata et al., 2005).

(2) Genetic and physical interactions between Schizosaccharomyces pombe Mcl1 and Rad2, Dna2 and DNA polymerase alpha: evidence for a multifunctional role of Mcl1 in DNA replication and repair.

Hiroshi Iwasaki (Yokohama City University)

Schizosaccharomyces pombe rad2 is involved in Okazaki fragments processing during lagging-strand DNA replication. Previous studies identified several *slr* mutants that are co-lethal with *rad2*Δ and sensitive to methyl methanesulfonate as single mutants. One of these mutants, *slr3-1*, is characterized here. Complementation and sequence analyses show that *slr3-1 (mcl1-101)* is allelic to *mcl1(+)*, which is required for chromosome replication, cohesion and segregation. *mcl1-101* is temperature-sensitive for growth and is highly sensitive to DNA damage. mcl1 cells arrest with 2C DNA content and chromosomal DNA double-strand breaks accumulate at the restrictive temperature. Mcl1p, which belongs to the Ctf4p/SepBp family, interacts both genetically and physically with DNA polymerase alpha. Mutations in *rhp51* and *dna2* enhance the growth defect of the *mcl1-101* mutant. These results strongly suggest that Mcl1p is a functional homologue of *Saccharomyces cerevisiae* Ctf4p and plays a role in lagging-strand synthesis and Okazaki fragment processing, in addition to DNA repair (Tsutsui et al., 2005).

(3) Structue-function analysis of the three domains of RuvB motor protein

Hiroshi Iwasaki (Yokohama City University)

RuvB protein forms two hexameric rings that bind to the RuvA tetramer at DNA Holliday junctions. The RuvAB complex utilizes the energy of ATP hydrolysis to promote branch migration of Holliday junctions. The crystal structure of RuvB from *Thermus thermophilus (Tth)* HB8 showed that each RuvB monomer has three domains (N, M, and C). This study is a structure-function analysis of the three domains of RuvB. The results show that domain N is involved in RuvA-RuvB and RuvB-RuvB subunit interactions, domains N and M are required for ATP hydrolysis and ATP binding-induced hexamer formation, and domain C plays an essential role in DNA binding. The side chain of Arg-318 is essential for DNA binding and may directly interact with DNA. The data also provide evidence that coordinated ATP-dependent interactions between domains N, M, and C play an essential role during formation of the RuvAB Holliday junction ternary complex (Ohnishi et al., 2005).

(4) Role of the *Schizosaccharomyces pombe* F-box DNA helicase in processing recombination intermediates

Hiroshi Iwasaki (Yokohama City University)

In an effort to identify novel genes involved in recombination repair, we isolated fission yeast *Schizosaccharomyces pombe* mutants sensitive to methyl methanesulfonate (MMS) and a synthetic lethal with *rad2*. A gene that complements such mutations was isolated from the *S. pombe* genomic library, and subsequent analysis identified it as the fbh1 gene encoding the F-box DNA helicase, which is conserved in mammals but not conserved *in Saccharomyces cerevisiae*. An *fbh1* deletion mutant is moderately sensitive to UV, MMS, and gamma rays. The *rhp51* (RAD51 ortholog) mutation is epistatic to *fbh1*. *fbh1* is essential for viability in stationary-phase cells and in the absence of either Srs2 or Rqh1 DNA helicase. In each case, lethality is suppressed by deletion of the recombination gene *rhp57*. These results suggested that *fbh1* acts downstream of *rhp51* and *rhp57*. Following UV irradiation or entry into the stationary phase, nuclear chromosomal domains of the *fbh1*Δ mutant shrank, and accumulation of some recombination intermediates was suggested by pulsed-field gel electrophoresis. Focus formation of Fbh1 protein was induced by treatment that damages DNA. Thus, the F-box DNA helicase appears to process toxic recombination intermediates, the formation of which is dependent on the function of Rhp51 (Morhisita et al., 2005).

PUBLICATIONS

Papers

1. Functional overlap between RecA and MgsA (RarA) in the rescue of stalled replication forks in *Escherichia coli*. *Shibata* T, Hishida T, Kubota Y, Han YW, Iwasaki H, and Shinagawa H. ***Genes Cells***. (2005) 10:181-191.

2. Genetic and physical interactions between Schizosaccharomyces pombe Mcl1 and Rad2, Dna2 and DNA polymerase alpha: evidence for a multifunctional role of Mcl1 in DNA replication and repair. Tsutsui Y, Morishita T, Natsume T, Yamashita K, Iwasaki H, Yamao F, Shinagawa H. **Curr Genet**. (2005) 48:34-43

3. Role of the *Schizosaccharomyces pombe* F-box DNA helicase in processing recombination intermediates. Morishita T, Furukawa F, Sakaguchi C, Toda T, Carr AM, Iwasaki H and Shinagawa H. ***Mol. Cell. Biol.*** (2005) 25:8074-883.

4. Structue-function analysis of the three domains of RuvB motor protein. Ohnishi T, Hishida T, Harada Y, Iwasaki H, and Shinagawa H. ***J. Biol. Chem.*** (2005) 26:280 (34): 30504-30510.

B. DEPARTMENT OF CELL GENETICS

B-a. Division of Cytogenetics Tamotsu Yoshimori Group

RESEARCH ACTIVITIES

Most of membrane-bound organelles inside eukaryotic cells are linked each other by dynamic membrane trafficking regulated by a set of specific proteins. Membrane traffic is essential not only to survival of each cell but also to various functions organizing the multi-cellular system, e.g., formation of cell polarity and intercellular communication. We aim to unravel molecular mechanisms of membrane traffic and their roles in physiological functions and diseases in animals, which must produce knowledge contributing clinical medicine. We are now focusing on two trafficking routes; autophagy and the endosomal system. Autophagy is membrane traffic delivering cytoplasmic components to lysosomes for bulk degradation. The process is mediated by the formation of the double membrane-bound autophagosomes. Endosomes receive macromolecules taken up by endocytosis from outside. The cargo is then either sorted to lysosomes or recycled back to the plasma membrane.

(1) Intracellular inclusions containing mutant α_1-antitrypsin Z are propagated in the absence of autophagic activity

Takahiro Kamimoto, Shisako Shoji, Tunda Hidvegi[1], Noboru Mizushima[2], Kyohei Umebayashi, David H. Perlmutter[1], and Tamotsu Yoshimori ([1]University of Pittsburgh, [2]Tokyo Metropolitan Institute of Medical Science)

Mutant α_1-antitrypsin Z (α_1-ATZ) protein, which has a tendency to form aggregated polymers as it accumulates within the endoplasmic reticulum of the liver cells, is associated with the development of chronic liver injury and hepatocellular carcinoma in hereditary α_1-antitrypsin (α_1-AT) deficiency. Previous studies have suggested that efficient intracellular degradation of α_1-ATZ is correlated with protection from liver disease in α_1-AT deficiency and that the ubiquitin-proteasome system accounts for a major route, but not the sole route, of α_1-ATZ disposal. Yet another intracellular degradation system, autophagy, has also been implicated in the pathophysiology of α_1-AT deficiency. To provide genetic evidence for autophagy-mediated disposal of α_1-ATZ, here we used cell lines deleted for the *Atg5* gene that is necessary for initiation of autophagy. In the absence of autophagy, the degradation of α_1-ATZ was retarded, and the characteristic cellular inclusions of α_1-ATZ accumulated. In wild-type cells, colocalization of the autophagosomal membrane marker GFP-LC3 and α_1-ATZ was observed, and this colocalization was enhanced when clearance of autophagosomes was prevented by inhibiting fusion between autophagosome and lysosome. By using a transgenic mouse with liver-specific inducible expression of α_1-ATZ mated to the GFP-LC3 mouse, we also found that expression of α_1-ATZ in the liver *in vivo* is sufficient to induce autophagy. These data provide definitive evidence that autopahgy can participate in the quality control/degradative pathway for α_1-ATZ and suggest that autophagic degradation plays a fundamental role in preventing toxic accumulation of α_1-ATZ. (Kamimoto, T., *et al.*, 2005)

(2) LC3 interacts with dynein motor and has an important role in microtubule-dependent autophagosome movement

Shunsuke Kimura and Tamotsu Yoshimori

Time-lapse video microscopy revealed that autophagosomes labeled with GFP-LC3 moved in the cytosol. Nocodazole inhibited the motion, whereas other cytoskeletal inhibitors did not inhibit it. Immunofluorescent microscopic study showed that endogenous LC3 arranged along the tracks of microtubules. These findings indicate that autophagosome moves along microtubules. Then, we examined the motor protein involved in this movement. Overexpression of dynamitin, which is known to disrupt dynein- and dynactin-dependent organelle

movement, inhibited autophagosome movements. In addition, endogenous LC3 colocalized with dynein-dynactin complex. Coimmunoprecipitation and GST pull-down assay showed that LC3 directly interacts with the intermediate chain of cytoplasmic dynein, which is a subunit of dynein motor complex. These results indicate that autophagosomes are linked with the dynein-dynactin complex through the interaction between LC3 and dynein intermediate chain.

To clarify the involvement of LC3 in autophagosome movement, microinjection experiments of anti-LC3 antibodies were performed. While injection of neither anti-GFP nor control IgG had effect on autophagosome movement, injection of anti-LC3 antibodies inhibited the movement. Moreover the anti-LC3 peptide antibodies raised against the N-terminal 1-15 residues, which is known as a microtubule binding region, also inhibited the movement. These results suggest that interaction of microtubule with LC3 is important for the movement. Finally, using FRAP assay, the transport and fusion of autophagosomes to lysosomes were measured. The transport and fusion were significantly inhibited by the injection of anti-LC3 antibody, suggesting that microtubule-dependent movement is necessary for targeting of peripherally-formed autophagosomes to lysosomes localizing to perinuclear region. From these results, we concluded that 1) autophagosome movement is dependent on microtubule and dynein/dynactin motor complex, 2) this movement is necessary for targeting of autophagosomes to lysosomes in mammalian cells, and 3) LC3 plays an important role in assembly of the transport machinery. LC3 may link dynein motor, microtubule and autophagosome. This is the first report elucidating the function of LC3 in autophagy in mammalian cells.

(3) Molecular dissection of internalization of *Porphyromonas gingivalis* by cells using fluorescent beads coated with bacterial membrane vesicle

Kayoko Tsuda, Atsuo Amano[1], Kyohei Umebayashi, Hiroaki Inaba[1], Ichiro Nakagawa[1], Yoshinobu Nakanishi[2], Tamotsu Yoshimori ([1]Osaka University, [2]Kanazawa University)

Porphyromonas gingivalis is one of the causative agents of adult periodontitis, and has been reported to be internalized by nonphagocytic epithelial cells. However, the mechanism for the internalization remains unclear. In the present study, we addressed this issue using fluorescent beads coated with bacterial membrane vesicles (MVs) that retain surface components of *P. gingivalis*. We established an assay system in which we could easily quantify the bead internalization to cells. MVs-coated beads were internalized by HeLa cells in kinetics similar to that of living bacteria. The internalization depended on dynamin but not clathrin. The beads were internalized through the actin-mediated pathway that is controlled by phospatidylinositol (PI) 3-kinase. The dynamics of microtubule assembly and disassembly was also required. Further, the treatement of cells with cholesterol-binding reagents significantly inhibited bead internalization, and the internalized beads were apparently colocalized with ganglioside GM1 and caveolin-1, which suggest the involvement of the lipid raft in the process. These results suggest that *P. gingivalis* accomplishes its internalization utilizing membrane lipid raft and cytoskeletal functions of the target cells. (Tsuda, K., *et al.*, 2005)

(4) Ubiquitin-dependent sorting of the epidermal growth factor receptor in the endocytic pathway

Kyohei Umebayashi and Tamotsu Yoshimori

When plasma membrane proteins are ubiquitinated, they follow the endocytic pathway to lysosomes. In the case of the epidermal growth factor receptor (EGFR), the Cbl E3 ligase is responsible for ligand-dependent ubiquitination. It has been considered that the ubiquitination of the receptor occurs in the plasma membrane. However, we found that Cbl was relocated to early endosomes when cells were stimulated with EGF. Analyses of colocalization as well as coimmunoprecipitation indicated two modes of EGFR-Cbl binding: Cbl was initially targeted to EGFR in the plasma membrane, dissociated after a while, and again bound to the receptor in early endosomes. Notably, Cbl was localized to early endosomal subdomains marked by Hrs, a ubiquitin binding protein required for multivesicular body (MVB) sorting. At longer chase periods after the ligand stimulation, Cbl was not colocalized with EGF, strongly suggesting that Cbl becomes separated from the receptor prior to the MVB sorting. We have obtained evidence that an AAA ATPase SKD1 is required for

the final dissociation of Cbl from EGFR. Expression of the dominant-negative SKD1(E235Q) resulted in prolonged EGFR-Cbl binding and excess ubiquitination of the receptor. Concomitantly, EGFR transport was blocked at aberrant endosomes where the receptor was preferentially colocalized with Hrs. Considering that the functions of SKD1 and its homologues are to disassemble protein complexes, we propose that SKD1 terminates the receptor ubiquitination by dissociating Cbl from EGFR. Termination of ubiquitination and subsequent deubiquitination would be required for proper sorting of cargo proteins into the MVBs.

PUBLICATIONS

Papers

1. Boya, P., Gonzalez-Polo, R.-A., Casares, N., Perfettini, J.-L., Dessen, P., Larochette, N., Metivier, D., Meley, D., Souquere, S., Yoshimori, T., Pierron, G., Codogno, P., Kroemer, G. (2005) Inhibition of macroautophagy triggers apoptosis, **Mol. Cell Biol.**, *25 (3)* 1025 - 1040
2. Hoshino, M., Yoshimori, T., Nakamura, S. (2005) Small GTPase proteins Rin and Rit bind to Par6 GTP-dependently, and regulate cell transformation, J. Biol. Chem., *280 (24)* 22868 - 22874
3. Mizuno, E., Iura, T., Mukai, A., Yoshimori, T., Kitamura, N., Komada, M. (2005) Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes, **Mol. Biol. Cell**, *16 (11)* 5163 - 5174
4. Towns, R., Kabeya, Y., Yoshimori, T., Guo, C., Shangguan, Y., Hong, S., Kaplan, M., Klionsky, D. J., Wiley, J. W. (2005) Sera from patients with type 2 Diabetes and Neuropathy Induce Autophagy and Colocalization with Mitochondria in SY5Y cells, **Autophagy**, *1*, 163 - 170
5. Tsuda, K., Amano, A., Umebayashi, K., Inaba, H., Nakagawa, I., Nakanishi, Y., Yoshimori, T. (2005) Molecular dissection of internalization of Porphyromonas gingivalis by cells using fluorescent beads coated with bacterial membrane vesicle, **Cell Struct. Funct.**, *30 (2)* 81 - 91
6. Yamada, T., Carson, A. R., Caniggia, I., Umebayashi, K., Yoshimori, T., Nakabayashi, K., Scherer, S. W. (2005) Endothelial nitric oxide synthase antisense (NOS3AS) gene encodes an autophagy-related protein (APG9-like2) highly expressed in trophoblast, **J. Biol. Chem.**, *280 (18)* 18283 - 18290

B-b. Division of Microbial Genetics Hiroyuki Araki Group

RESEARCH ACTIVITIES

We have been studying on eukaryotic chromosomal DNA replication and its regulation by the cell cycle. For this purpose, we have employed budding yeast, *Saccharomyces cerevisiae*, as a model system of eukaryotic cells. Using strong genetics of budding yeast, we have identified novel factors involving in chromosomal DNA replication and revealed their functions in chromosomal DNA replication.

(1) The pre-Landing complex of replication proteins formed in a CDK-dependent manner

Sachiko Sakamoto, Kazuyuki Hirai, Yoichiro Kamimura, and Hiroyuki Araki

At replication origins in eukaryotes, the pre-Replicative Complex (pre-RC) forms from late M to G1 phases in the absence of CDK (Cyclin-dependent kinase) activity and other replication proteins including DNA polymerases assemble when CDK activity increases from G1/S boundary. We have reported three yeast complexes, Sld2-Dpb11, Sld3-Cdc45 and GINS, all of which associate with origins in a mutually dependent and the pre-RC dependent manner. Dpb11 has four BRCT repeats and form a complex with the Sld2 protein phosphorylated by CDK. The Sld3-Cdc45 complex is observed throughout the cell cycle. The GINS complex consisting of Sld5, Psf1, Psf2 and Psf3 subunits first associates with origins and then moves with replication forks.

To elucidate how the proteins assemble, we investigated the complex formation between GINS and other proteins by co-immunoprecipitation assay. GINS coprecipitated with Pol ε and with Mcm, a component of the pre-RC, in the S phase. Moreover, when we treated the cells with the cross-linking agent before precipitation, Sld3, Sld2 and Dpb11also coprecipitated with GINS. Without the pre-RC in Cdc6-depleted cells, Sld2-Dpb11 and Pol ε coprecipitated with GINS, suggesting that they form a complex without origin-association. In the same condition, we could precipitate tagged one of Sld2, Dpb11 and Pol ε with remaining

three proteins. Furthermore, there were strong genetic interactions between Sld2, Dpb11, Pol ε and GINS. We therefore propose that Sld2, Dpb11, Pol ε and GINS constitute the pre-Landing Complex (pre-LC), which is formed before associating with origins.

To initiate chromosomal DNA replication, two protein kinases, CDK and DDK/Cdc7 (Dbf4-dependent kinase) are required. The pre-LC formation appeared to depend on CDK but not DDK. Using several mutant cells, we revealed that the Pol ε-GINS and the Sld2-Dpb11 complexes are mainly connected by binding of Pol ε and Sld2 in the presence of CDK activity. The pre-LC seems to associate with the pre-RC via Sld3 since the C-terminal portion of Sld3 interacts with GINS whereas the N-terminal portion interacts with Cdc45.

In parallel with the in vivo analysis, we have purified the proteins involved in the pre-LC to reconstitute it. We expressed all the subunits of GINS in insect cells and purified GINS to be a near homogeneity. We also purified Dpb11, Sld2 and Pol ε from yeast cells. Preliminary result showed that they form a complex in vitro without other proteins. Thus, although we do not know all the components, at least Dpb11, Sld2, Pol ε and GINS are core components of the pre-LC.

(2) Regulatory mechanism of the complex formation between Sld2 and Dpb11 by cyclin-dependent kinase (CDK)

Yon-Soo Tak, Yoshimi Tanaka, Yoichiro Kamimura and Hiroyuki Araki

Phosphorylation often regulates protein-protein interactions to control biological reactions. The Sld2 and Dpb11 proteins of budding yeast form a phosphorylation-dependent complex that is essential for chromosomal DNA replication. The Sld2 protein has a cluster of 11 cyclin-dependent kinase (CDK) phosphorylation-motifs (Ser/Thr-Pro), six of which match the canonical sequences Ser/Thr-Pro-X-Lys/Arg, Lys/Arg-Ser/Thr-Pro and Ser/Thr-Pro-Lys/Arg. Simultaneous alanine-substitution for serine or threonine in all the canonical CDK-phosphorylation motifs severely reduces complex formation between Sld2 and Dpb11, and inhibits DNA replication. We show that phosphorylation of these canonical motifs does not play a direct role in complex formation but rather regulates phosphorylation of another residue, Thr84. This constitutes a non-canonical CDK-phosphorylation motif within a 28-amino acid sequence that is responsible, after phosphorylation, for binding of Sld2 to Dpb11. We further suggest that CDK-catalysed phosphorylation of sites other than Thr84 renders Thr84 accessible to CDK. From these results, we argue that this novel mechanism sets a threshold of CDK activity for formation of the essential Sld2-Dpb11 complex and therefore prevents premature DNA replication.

(3) CDK targets in the initiation of DNA replication

Seiji Tanaka and Hiroyuki Araki

In eukaryotic cells, CDKs play a pivotal role in the regulation of cell cycle progression. Periodic activation and inactivation of stage-specific CDK ensures the cell cycle to progress in one way at the correct timing. Of the cellular processes regulated by CDK, chromosomal DNA replication is one of the most important. Because the failure in chromosomal DNA replication is catastrophic for cells and organisms, cell has the regulatory mechanism that ensures the chromosomal DNA replication to happen exactly once per cell cycle. In this mechanism, CDKs play a central role by regulating two mutually exclusive steps. First, CDKs trigger the initiation of DNA replication, and at the same time, CDKs inhibit formation of the pre-RCs, which is required for the initiation of DNA replication. Because of this inhibitory action of CDK, the pre-RCs are formed at the origins of DNA replication only in late M and G1 in the cell cycle when CDK activity is low. Once CDK is activated, it trigger DNA replication (S phase) by activating the pre-RC. Importantly, re-replication is strictly inhibited at this time because CDK blocks pre-RC re-formation at the fired origins. During following G2-M phase, CDK activity is high and thus keeps to inhibit re-replication. When cells finish M phase and enter into the next G1, CDK activity rapidly decreases and then cells can form the pre-RC again.

To understand how CDK activates pre-RC and how inhibits pre-RC formation has been a major challenge of the research field of DNA replication. Recent studies including ours revealed that CDK inhibits pre-RC formation by downregulating all of known pre-RC components. In contrast, target(s) of

CDK in pre-RC activation had been elusive for a long time. Recently, we have reported that Sld2 is an essential target of CDK for the initiation in budding yeast. We first asked whether Sld2 is a sole target of CDK in the initiation. To test the possibility, we simultaneously substituted aspartic acid for all of serine or threonine residues at CDK phosphorylation motifs in Sld2. This *sld2* mutant *(sld2-11D)* supported cell growth, suggesting that Sld2-11D mimics a phosphorylated active form of Sld2. In this condition, CDK was still required for the initiation of DNA replication. Therefore, we concluded that CDK has essential targets other than Sld2 in the initiation process. To identify unknown target(s) of CDK, we performed a genetic screening and finally isolated a mutant which can initiate DNA replication without CDK activity. The mutant, *JET1-1* (Jumping CDK requirement with Eleven D mutant of SLD-Two), could initiate the CDK-free DNA replication only when Sld2-11D was expressed. Further analyses revealed that Jet1-1 and Sld2-11D can replace the role of CDK in initiation. These results suggest that CDK regulates at least two essential processes in the initiation of DNA replication.

PUBLICATIONS

Papers

1. Ohya, Y., Sese, J., Yukawa, M., Sano, F., Nakatani, Y., Saito, T. L., Saka, A., Fukuda, T., Ishihara, S., Oka, S., Suzuki, G., Watanabe, M., Hirata, A., Ohtani, M., Sawai, H., Fraysse, N., Latge, J. P., Francois, J. M., Aebi, M., Tanaka, S., Muramatsu, S., Araki, H., Sonoike, K., Nogami, S., Morishita, S. (2005) High-dimensional and large-scale phenotyping of yeast mutants, **Proc. Natl. Acad. Sci. USA**, *102 (52)* 19015 - 19020

2. 荒木弘之(2005)真核生物染色体DNAの複製開始領域への複製タンパク質の集合機構, 実験医学, 23 (9) 1334 - 1339

EDUCATION

1. 荒木弘之　遺伝学に関する講義　東京工業大学・生命理工学部
2. 荒木弘之　集中講義　九州大学理学部

B-c. Division of Cytoplasmic Genetics Kazuto Kobayashi Group

RESEARCH ACTIVITIES

(1) Mechanisms of Neural Circuitry Underlying Behavioral Control

Kazuto Kobayashi

Control of behaviors is based on the mechanisms that mediate processing and regulation of information through the complex neural circuitry in the brain. Dysfunction in the neural circuitry is involved in the etiology and pathogenesis of some neural diseases. Our research group is interested in understanding the role of specific neurons or neural pathways that mediate motor control, memory, and emotion as well as the function of the signaling molecules that are involved in these behaviors. In the present year, we analyzed the mechanism by which the subthalamic nucleus, which is a key component of basal ganglia, regulates motor behavior in the neural circuitry. Conditional ablation of the subthalamic neurons by immunotoxin-mediated cell targeting revealed that these neurons act to coordinate motor behavior through differential neural pathways depending on the state of dopamine transmission (Yasoshima et al., 2005). In addition, we developed a new system for physiological studies of identified neurons in cell culture by using transgenic mice expressing green fluorescent protein in tyrosine hydroxylase-positive neurons (Jomphe et al., 2005). This system was used for studying the functional properties of the olfactory bulb dopamine neurons (Pignatelli et al., 2005) and the receptor desensitization/internalization in the locus coeruleus noradrenaline neurons (Arttmangkul et al., 2006). The system was also useful for examining the role of some factors in the development and survival of midbrain dopamine neurons (Rawal et al., 2006; Meurer et al., 2006). Furthermore, we described the mutations of tyrosine hydroxylase gene that are involved in human inherited diseases, including dystonia and movement disorders (Kobayashi and Nagatsu, 2005).

PUBLICATIONS

Papers

1. Arttamangkul, S., Torrecilla, M., Kobayashi, K., Okano, H., and Williams, J.T. (2006) Separation of mu opioid receptor desensitization and internalization: endogenous receptors in primary neuronal cultures. **J. Neurosci.** *in press.*

2. Jomphe, C., Bourque, M.-J., Fortin, G., St-Gelais, F., Okano, H., Kobayashi, K., and Trudeau, L.-E. (2005) Use of TH-GFP transgenic mice as a source of identified dopaminergic neurons for physiological studies in postnatal cell culture, **J. Neurosci. Methods**, *146 (1)* 1 - 12

3. Kobayashi, K. and Nagatsu, T. (2005) Molecular genetics of tyrosine 3-mono- oxygenase and inherited diseases, **Biochem. Biophy. Res. Commun.**, *338 (1)* 267 - 270

4. Meuer, K., Teismann, P., Peters, K., Göricke, B., Lingor, P., Schlachetzki, J., Kobayashi, K., Dietz, G. P.H., Schulz, J.B., Bähr, M., Schneider, A., and Weishaupt, J. H. (2006) Granulocyte-colony stimulating factor is neuroprotective in a model of Parkinson's disease. **J. Neurochem.** *in press.*

5. Pignatelli, A., Kobayashi, K., Okano, H., and Belluzzi, O. (2005) Functional properties of dopaminergic neurones in the mouse olfactory bulb, **J. Physiol.**, *564 (2)* 501 - 514

6. Rawal, N., Castelo-Branco, G., Sousa, K. M., Kobayashi, K., Okano, H., and Arenas, E. (2006) Dynamic temporal and cell type-specific expression of Wnt signaling components in the developing midbrain. **Exp. Cell Res.** *in press.*

7. Yasoshima, Y., Kai, N., Yoshida S., Shiosaka, S., Koyama, K., Kayama, F., and Kobayashi, K. (2005) Subthalamic neurons coordinate basal ganglia function through differential neural pathways, **J. Neurosci.**, *25 (34)* 7743 - 7753

Reviews

8. 小林和人，甲斐信行，小林憲太，小林とも子(2005) 脳研究で実験動物研究がはたしてきた役割：遺伝子改変動物，アニッテクス(Laboratory Animals and Technology) *17*, 13-19.

EDUCATION, SOCIAL CONTRIBUTIONS AND OTHERS:

他大学／研究機関での講義やセミナー

1. 小林和人「カテコールアミン伝達による行動制御の神経回路メカニズム」東京大学医学系研究課機能生物学大学院講義，2005年５月，東京

2. 小林　和人「カテコールアミン伝達と行動制御のメカニズム，遺伝研セミナー」三島，2005年８月

3. 小林　和人「カテコールアミン伝達と行動制御のメカニズム」聖マリアンナ医科大学遺伝子組み換えセミナー，2005年10月，川崎

4. 小林和人「ドーパミン伝達と脳機能」東北大学大学院医学研究科特別講義，2005年11月，仙台

5. 小林和人：ドーパミンによる行動の発達と発現の制御，第３回CREST領域内研究報告会，2006年３月，大阪

客員教授等

大学共同利用機関法人　情報・システム研究機構　国立遺伝学研究所　客員教授

B-c. Division of Cytoplasmic Genetics Masayuki Yamamoto Group

RESEARCH ACTIVITIES

(1) Hrs1p/Mcp6p on the meiotic SPB organises astral microtubule arrays for oscillatory nuclear movement

Masayuki Yamamoto

Microtubules and the motor protein dynein play pivotal roles in the movement and positioning of the nucleus and cytoplasmic organelles in a cell. In fission yeast, oscillatory movement of the nucleus termed horsetail nuclear movement (HNM), has been observed during meiotic prophase. HNM is led by an astral microtubule array emanating from the spindle pole body (SPB), a centrosome equivalent organelle in yeasts, aided by the dynein-dynactin complex, and is proposed to facilitate the alignment of homologous chromosomes necessary for efficient meiotic recombination. We have shown that a meiosis-specific SPB component Hrs1p (also known as Mcp6p) is a key molecule to remodel microtubules into the horsetail-astral array (HAA). Deletion of Hrs1p impaired HAA formation, leading to compromised HNM. Ectopic expression of Hrs1p during the mitotic cell cycle resulted in the formation of a HAA-like astral

microtubule array, which drove an oscillatory nuclear movement in interphase cells. Hrs1p interacted with components of the γ-tubulin ring complex (γ-TuRC) as well as with a meiotic SPB component. Thus, we propose that Hrs1p facilitates formation of the HAA, responsible for the vigorous HNM, by stabilising connection between the SPB and minus ends of microtubules (Tanaka, K., *et al.*, 2005).

(2) The *C. elegans* Homologue of *Deleted in Azoospermia* is Involved in the Sperm/Oocyte Switch

Masayuki Yamamoto

The *Deleted in Azoospermia (DAZ)* gene family encodes putative translational activators that are required for meiosis and other aspects of gametogenesis in animals. The single *C. elegans* homologue of *DAZ, daz-1*, is an essential factor for female meiosis. We have clarified that *daz-1* is important for the switch from spermatogenesis to oogenesis (the sperm/oocyte switch), which is an essential step for the hermaphrodite germline to produce oocytes. RNA interference of the *daz-1* orthologue in a related nemetode *C. briggsae* resulted in a complete loss of the sperm/oocyte switch. The *C. elegans* hermaphrodite deficient in *daz-1* also revealed a failure in the sperm/oocyte switch if the genetic background was conditional Mog (masculinization of germline). DAZ-1 could bind specifically to mRNAs encoding the FBF proteins, which are translational regulators for the sperm/oocyte switch and germ stem cell proliferation. Expression of the FBF proteins seemed to be lowered in the *daz-1* mutant at the stage for the sperm/oocyte switch. Conversely, a mutation in *gld-3*, a gene that functionally counteracts FBF, could partially restore oogenesis in the *daz-1* mutant. Taken together, we propose that *daz-1* plays a role upstream of the pathway for germ cell sex determination (Otori, M., *et al.*, 2006).

(3) *C. elegans* CPB-3 interacts with DAZ-1 and functions in multiple steps of germline development

Masayuki Yamamoto

Cytoplasmic polyadenylation element-binding proteins (CPEBs) are well-conserved RNA-binding proteins, which regulate mRNA translation mainly through control of poly(A) elongation. We have shown that CPB-3, one of the four CPEB homologs in *C. elegans*, positively regulates multiple aspects of oocyte production. CPB-3 protein was highly expressed in early meiotic regions of the hermaphrodite gonad. Worms deficient in *cpb-3* were apparently impaired in germ cell proliferation and differentiation including sperm/oocyte switching and progression of female meiosis. We also show that *cpb-3* is likely to promote the meiotic entry in parallel with *gld-3*, a component of one of the redundant but essential genetic pathways for the entry to and progression through meiosis. Altogether, CPEB appears to have a conserved role in the early phase of meiosis and in the sperm/oocyte specification, in addition to its reported function during meiotic progression. (Hasegawa, E., *et al.*, 2006).

PUBLICATIONS

Papers

1. Izawa, D., Goto, M., Yamashita, A., Yamano, H., Yamamoto, M. (2005) Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p, **Nature**, *434*, 529 - 533
2. Maruyama, R., Endo, S., Sugimoto, A., Yamamoto, M. (2005) C. elegans DAZ-1 is expressed in proliferating germ cells and directs proper nuclear organization and cytoplasmic core formation during oogenesis., **Dev. Biol.**, *277*, 142 - 154
3. Tanaka, K., Kohda, T., Yamashita, A., Nonaka, N., Yamamoto, M. (2005) Hrs1p/Mcp6p on the meiotic SPB organizes astral microtubule arrays for oscillatory nuclear movement, **Curr. Biol.**,*15*,1479 - 1486
4. Yamashita, A., Sato, M., Fujita, A., Yamamoto M., Toda, T. (2005) The roles of fission yeast Ase1 in mitotic cell division, meiotic nuclear oscillation and cytokinesis checkpoint signaling, **Mol. Biol. Cell**, *16*, 1378 - 1395

EDUCATION, SOCIAL CONTRIBUTIONS AND OTHERS

Editor: Genes to Cells; Protein, Nucleic Acid and Enzyme
Editorial Board: Current Genetics; YEAST

C. DEPARTMENT OF DEVELOPMENTAL GENETICS

C-a. Division of Development Genetics Yasushi Hiromi GROUP

RESEARCH ACTIVITIES

(1) Control of ligand trapping for the recognition of spatial cues

Masaki Hiramoto and Yasushi Hiromi

When secreted molecules guide cell migration or navigation of cellular processes, guidance molecules are "trapped" by receptors that are expressed in the motile cells. We have previously shown that trapping of a secreted axon guidance molecule Netrin by its receptor Frazzled can result in the redistribution of Netrin and subsequent presentation to other axons. Here we show that trapping of Netrin by another Netrin receptor, UNC5, has a different consequence; it abolishes Frazzled-mediated Netrin presentation on the cell surface. This activity of UNC5 was achieved by the extracellular domain of UNC5, and was specifically associated with the ability to recognize the direction of navigation, and not with the simple inhibition of the extension. This implies that the control of the ligand trapping is an crucial factor in the recognition of spatial cues.

(2) Intrinsic sub-axonal compartmentalization in *Drosophila* neurons

Takeo Katsuki, Masaki Hiramoto and Yasushi Hiromi

In the developing nervous system of vertebrates and invertebrates, distribution of membrane molecules, especially axon guidance receptors, is often restricted to specific segments of axons. For example, a repulsive axon guidance receptor ROBO and its family members are expressed in distal axon segments, but largely excluded from the proximal regions. It is not known how such sub-axonal distribution patterns are generated and maintained within individual neurons. Using a low-density primary culture system of *Drosophila*, we found that segmental localization of molecules along axons can be generated in the absence of cell-cell contacts, which suggests that the localization is due to an intrinsic property of neurons. Furthermore, fluorescence recovery after photobleaching (FRAP) analysis revealed that the mobility of transmembrane molecules is restricted at the boundary of the localized region, indicative of a barrier mechanism. We propose that axons of *Drosophila* neurons are compartmentalized into sub-axonal regions by a barrier mechanism, and that this compartmentalization is achieved cell-intrinsically.

(3) The relationship between sub-axonal localization of proteins and glycosylation

Tohru Umemura, Takeo Katsuki and Yasushi Hiromi

A majority of cell surface proteins are modified by the addition of carbohydrate moieties, which play important roles in cell-cell interactions and protein sorting. As a part of an effort to understand how membrane proteins are targeted to specific sub-axonal compartments, we examined whether or not sub-axonal localization patterns correlate with modification via a particular sugar moiety. A battery of 20 lectins were screened by staining primary culture of *Drosophila* embryonic neurons. One of the lectins exhibited a striking staining pattern; localization to either the proximal or middle segment of an axon. This pattern was indistinguishable from the staining pattern with a monoclonal antibody BP102, whose epitope is likely a carbohydrate. These results raise a possibility that glycosylation may mark the protein to be delivered to a particular sub-axonal compartment. On Western blots both the lectin and BP102 antibody recognized glycoproteins with molecular weights of about 75kD and 100kD. We are trying to identify the BP102 antigens to investigate how they are targeted to sub-axonal compartments.

(4) Neutralization of repulsive-axon guidance cue on target neurons

Fumikazu Suto, Hajime Fujisawa[1] and Yasushi Hiromi

([1]Nagoya University)

Axon guidance molecules are loosely classified in two categories, attractive and repulsive. While it is easy to see how an attractive guidance molecule may provide a local guidance cue, how repulsive signals pattern axonal trajectories is not well understood. We are using mouse hippocampus, where its dendritic field receives "layer-specific" inputs from multiple neuronal types, to investigate the function and regulation of a repulsive guidance signal Sema6A. One neuronal type that project in a layer-specific fashion is mossy fibers, which mainly connect on the proximal-most part of the apical dendrite of CA3 pyramidal neurons. Although cultured mossy fibers are repelled by Sema6A, Sema6A was strongly localized in the dendritic field of CA3 pyramidal cells, suggesting that there is a mechanism to counteract its repulsive activity to allow mossy fiber projection. We found that Plexin-A2, a receptor for Sema6A, was expressed in CA3 pyramidal cells, and exerted an "attractive" effect on mossy fiber projection; in *plexin-A2* mutant mice, mossy fibers avoided the normal target layer and were redirected to the basal dendrite. This avoidance behavior likely involves Sema6A, because it was suppressed by reducing Sema6A levels. We propose that the target neurons neutralizes the repulsive activity of Sema6A by expressing its receptor on their dendrites, to license layer-specific projections within the dendritic field.

(5) Exploring the factors that regulate temporal gene expression profile in neuronal precursors

Makoto Kanai and Yasushi Hiromi

One of the elements that directs developmental events is the precise temporal regulation of gene expression. Neuroblasts (NBs), neuronal precursors in the *Drosophila* embryonic CNS, are an excellent model to study the temporal regulation of gene expression, because these cells undergo shifts in gene expression profile over time, to generate neuronal diversity of their progeny. A determinant for such temporal specificity is a transcription factor Seven-up (SVP). SVP is expressed in a temporally restricted subsection within the NB's lineage, and this restricted expression directs "switching" of gene expression profile within the NB; in *svp* mutants, NBs maintain gene expression patterns of a younger stage. We are trying to understand how the temporally restricted expression of *svp* is regulated. Preliminary results indicate that cell cycle progression and cytokinesis might affect the expression of *svp*.

(6) Developmental context for Notch-dependent PROS expression in longitudinal glial cells

Yoshihiro Yuasa and Yasushi Hiromi

Notch signaling is utilized in choosing binary cell fates in many aspects of organogenesis. The outcome of Notch activation is context-dependent, resulting in the activation of different target genes in each developmental process. The molecular nature of the developmental context that provides target specificity is poorly understood. One process that Notch activity determines a particular cell type in the nervous system is the subtype specification of longitudinal glia in the *Drosophila* embryonic central nervous system. In each hemisegment, ten longitudinal glial cells are generated from a single precursor and are positioned on the longitudinal axon bundle. These glial cells comprise two subtypes, because six of them activate Notch signaling and express a homeodomain transcription factor Prospero (PROS), whereas the other four are PROS-negative. PROS expression is dependent on Notch activation, and conversely, when Notch is artificially activated all longitudinal glial cells become PROS-positive. To identify the developmental context for Notch-dependent glial subtype specification, we are analyzing the regulation of PROS expression in the longitudinal glia. We found that PROS expression in the longitudinal glia requires three transcriptional factors: homeodomain protein REPO, *ets* transcription factor Pointed and AT-rich binding protein Dead Ringer/Retain (DRI). An enhancer element of the *pros* gene that recapitulates the expression pattern of PROS in the longitudinal glial cells includes the consensus binding sites for all three transcriptional factors. These results suggest that these three transcriptional factors provide a context for Notch-dependent PROS expression in the longitudinal glia.

(7) Stem cell formation in *Drosophila* germline requires gonadal somatic cells

Miho Asaoka, Satoru Kobayashi[1], Ryu Ueda[2] and Yasushi Hiromi ([1]NIBB, [2]Invertebrate Genetics

Laboratory)

Stem cells in the adult tissue are maintained by a special microenvironment called a "niche", which produces signals that maintain stem cell specification and function. However, how stem cell fate is initially established during organogenesis is currently unknown. Stem cell fate may be determined by a cell-autonomous mechanism. Alternatively, it may be induced by a niche-like microenvironment (induction hypothesis). To test these hypotheses, we use *Drosophila* germline stem cells (GSCs) as a model system. Our previous lineage analysis has shown that primordial germ cells (PGCs) contacting somatic cells in the anterior half of embryonic gonad give rise to GSCs in the adult ovary, whereas PGCs in the posterior half of the gonad differentiate directly to cystoblasts. These results suggested that anterior somatic cells in the embryonic gonad may play an inductive role in GSC formation. Here we isolated six genes that are required in the somatic gonad for GSC formation. These genes were identified in our screen involving microarray, *in situ* hybridization, and RNAi-mediated gene knockdown. During embryogenesis all six genes are preferentially expressed in the somatic cells of the gonads, one of them only in the anterior half of the somatic gonad. Gene knock-down in the gonadal somatic cells resulted in the absence or decrease of GSC in adult ovaries. These results suggest that somatic embryonic gonad is required for GSC formation, and support the somatic induction hypothesis.

PUBLICATIONS

Papers

1. Iwanami, M. Hiromi, Y. and Okabe, M. (2005) Cell-type specific utilization of multiple negative feedback loops generates developmental constancy. **Genes Cells**, *10*, 743 - 752.
2. Kanai, M. I., Okabe, M. and Hiromi, Y. (2005) *seven-up* controls switching of transcription factors that specify identities of *Drosophila* neuroblasts. **Developmental Cell**, *8*, 203 - 213.
3. 広海　健(2005)この遺伝子…だれ?? 人物にちなんだ遺伝子の名前，化学，*60*, 28 - 29.

EDUCATION

1. 広海　健　器官構築の原理　連携講義「ライフサイエンスの果実」 静岡産学交流センター「ペガサート」
2. Yasushi Hiromi Intra-axonal patterning: its mechanism and implications. IGBMC, Strasbourg
3. Yasushi Hiromi Intra-axonal patterning: its mechanism and implications. University of Southern California
4. Yasushi Hiromi Intra-axonal patterning: its mechanism and implications. Caltech
5. 広海　健　幹細胞の秘密を遺伝子で解き明かす．遺伝研一般公開
6. 広海　健　遺伝研における男女共同参画－なぜ遺伝研には女性教員が多いのか？－日本生化学会　男女共同参画シンポジウム
7. 浅岡美穂　ショウジョウバエ生殖巣における幹細胞システム～幹細胞形成のための微小環境～，総合研究大学院大学～研究者育成のための研究として～　麻布大学環境保健学部
8. 浅岡美穂　ショウジョウバエ生殖巣における幹細胞システム～幹細胞形成のための微小環境～　徳島大学総合科学部
9. 浅岡美穂　ショウジョウバエ卵巣における幹細胞システム～幹細胞形成のための微小環境～　東北大学加齢医学研究所

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動

広海　健　Dr. Y. Hiromi served as an editor for Development, Growth & Differentiation.
広海　健　Dr. Y. Hiromi served as a member of the council of The Genetics Society of Japan.
広海　健　Dr. Hiromi served as a member of the Gender Equality Committee of The Molecular Biology Society of Japan.

C-a. Division of Development Genetics Toshitaka Fujisawa GROUP

RESEARCH ACTIVITIES

(1) Systematic identification of peptide signaling molecules in *Hydra*

Toshitaka Fujisawa, Chiemi Fujisawa, Eisuke Hayakawa, Yukihiko Noro, Akemi Hayashiuchi,

Hiroshi Shimizu and Toshio Takahashi[1)]
[1)] Suntory Institute for Bioorganic Research, Osaka, Japan

Systematic purification and structural determination of peptide signaling molecules has been carried out by using HPLC, peptide sequencing and mass spectrometry. About 50 new peptides were purified and subjected to structural determination. The peptide sequences were blasted against *Hydra* EST and genome databases. We have also carried out data-mining to identify peptide genes. One of the outcome was the identification of a PW gene. We already identified 4 PW peptides, all of them are short and have a L(I)PW motif at the C-terminus. The encoding gene had been long sought for without success. The EST data showed that the deduced precursor protein contained all 4 PW peptides, one newly purified peptide, LHW and 3 putative PW peptides. Most of the peptides were preceded by a lysine residue and followed by dibasic amino acids, typical processing sites for bioactive peptides. Both immunostaining using anti-AALPW antibody and in situ hybridization showed that PW peptides were localized in ectodermal epithelial cells. PW peptides inhibited neuron differentiation and counteracted by Hym-355, a neuropeptide that enhances neuron differentiation. We have also identified one gene encoding neuropeptide Y like peptides and 3 genes encoding insulin-like peptides.

(2) Aboral end of *Hydra* and oral end of higher organisms share a common ancestral origin

Hiroshi Shimizu, Yasuharu Takaku & Toshitaka Fujisawa

Oral opening of metazoans tends to be formed around the anterior end. Polyps of phylum Cnidaria is exceptional since oral opening is located at the site of *Wnt-3a* homologue expression that usually takes place at the posterior end of developing embryos or adults. Why this odd location of oral opening relative to anterior-posterior axis occurs in this phylum has remained unknown. We provide a solution to this oddness by showing that aboral end of *Hydra* bears structural, genetic and functional resemblance to the oral end of higher organisms. Structural resemblance comes from the fact that there is a pore at the aboral end connecting the interior with the exterior of the animal. Genetic resemblance came from the fact that peduncle tissue of *Hydra* that expresses *CnNk-2*, a hydra homologue of *Nkx-2.5*, shows pumping movement. This is comparable to pharyngeal tissue of nematodes that expresses *ceh-22*, a *Nkx-2.5* homologue, while showing pumping movement. Functional resemblance came from the observation that there is inward and outward fluid transfer through the aboral pore. Taken together, we interpret the resemblance listed above to demonstrate that aboral pore of *Hydra* and oral opening of higher organisms share common ancestral origin although the function of aboral pore is more diverse.

PUBLICATIONS

Papers

1. Takahashi, T., Hatta, M., Yum, S., Koizumi, O., Kobayakawa, Y., Gee, L., Ohtani, M., Fujisawa, T. and Bode, H. R. (2005) Hym-301, a novel peptide, regulates the number of tentacles formed in hydra., **Development**, *132*, 2225 - 2234
2. Takaku, Y., Hariyama, T. and Fujisawa, T. (2005) Motility of endodermal epithelial cells plays a majorole in reorganizing the two epithelial layers in *Hydra.*, **Mechanisms of Development**, *122*, 109 - 122

C-b. Division of Gene Expression Susumu Hirose Group

RESEARCH ACTIVITIES

(1) Role of histone modifications and chromatin remodeling in epigenetic gene expression

Takahiro Nakayama, Kenichi Nishioka, Yi-Xin Dong, Tsukasa Shimojima, Kazuma Hanai, Takashi Yamamoto[1], Koji Akasaka[2] and Susumu Hirose ([1]Graduate Department of Gene Science, Hiroshima University, Higashi-Hiroshima, Japan; [2]Department of Biological Science, University of Tokyo, Tokyo, Japan)

Epigenetic maintenance of the expression state of the genome is important for development. There are two types of epigenetic gene expression in *Drosophila.* One is maintenance of *Hox* gene expression governed by *Polycomb* and *trithorax* group genes. The other is

position effect variegation (PEV). *Trithorax-like (Trl)* encoding GAGA factor is involved in the both types of regulation: *Trl* is a member of *trithorax* group and *Trl* mutation is an enhancer of PEV.

Previous study from this laboratory has demonstrated that the GAGA factor recruits FACT, facilitates chromatin remodeling around its binding site, and contributes to the maintenance of *Hox* gene expression. To further understand the GAGA factor-FACT complex-dependent chromatin remodeling, we searched for remodeling factors that are associated with the GAGA factor, and found PBAP complex as well as NURF. Two types of Brm chromatin remodeling complex are known in *Drosophila*. These are BAP complex containing Osa, and PBAP complex containing Polybromo in addition to shared subunits. Our study showed that the GAGA factor recruits the PBAP but not the BAP complex to the regulatory regions of *Hox*.

We also found that the GAGA factor-FACT complex on a site just downstream of the *white* gene is crucial for PEV. The GAGA factor facilitates chromatin remodeling and replacement of histone H3 by its variant H3.3, and maintains *white* expression under the heterochromatin environment.

RSF (remodeling and spacing factor) is a chromatin remodeling factor consisting of RSF1 and SNF2H (a human counterpart of *Drosophila* ISWI). The factor has a unique activity that can assemble regularly spaced nucleosome arrays in the absence of any histone chaperone. To investigate *in vivo* role of RSF, we carried out genetic analyses on its *Drosophila* counterpart, a heterodimer of dRSF1 and ISWI, and found that it contributes to PEV through facilitating the spreading of silent chromatin and replacement of histone H2A by its variant H2AV.

(2) Chromatin transcription

Mikage Nakajima, Kenichi Nishioka, Toshiharu Komori[1], Tadashi Wada[1], Hiroshi Handa[1], and Susumu Hirose ([1]Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuda, Yokohama, Japan)

Spt6 is an evolutionarily conserved transcription factor. Through biochemical studies on human SPT6, Handa's group has shown it to be a transcriptional elongation factor. To understand *in vivo* role of SPT6 in multicellular organism, we carried out genetic studies on *Drosophila* SPT6. Knockdown of the SPT6 function by heritable RNAi showed some interesting phenotypes that are our target of in depth characterization.

Drosophila Ash1 is a member of *trithorax* group and is known to present as a multi-protein complex. However, molecular nature of the complex remains unknown. We started to isolate the Ash1 complex from a transgenic fly line expressing epitope-tagged Ash1.

(3) Role of DNA topology in the formation of active chromatin

Hirofumi Furuhashi, Kuniharu Matsumoto, Mikage Nakajima, Youhei Ogasawara and Susumu Hirose

Supercoiling factor (SCF) is a protein capable of generating negative supercoils into DNA in conjunction with topoisomerase II. To clarify *in vivo* role of SCF, we carried out genetic studies on *Drosophila* SCF. Knockdown of SCF by heritable RNAi revealed its role in dosage compensation of the X chromosome. SCF co-localizes with the MSL complex along the male X chromosome. Upon overexpression of SCF, the male X chromosome showed a bloated appearance. This phenotype was suppressed by simultaneous overexpression of ISWI. Furthermore, a similar phenotype has been reported for a loss of function mutant of ISWI. These findings suggest that twice activation of the male X chromosome is achieved through a balance between two activities, SCF forming an open and active chromatin and ISWI working toward a compact and silent chromatin.

We have developed a method to visualize negatively supercoiled DNA domains within a cell, which relies on preferencial binding of psoralen to negatively supercoiled DNA. Upon beat shock of *Drosophila* larvae, we observed strong signals of psoralen on heat shock puffs of salivary gland polytene chromosomes. Furthermore, scs and scs' sequences are necessary to detect the strong signals on the *hsp70* gene, suggesting formation of a topologically closed domain through the scs and scs'.

Transcription and supercoiling of the template DNA are closely related each other. DNA supercoiling affects transcription and transcription affects supercoiling of the template DNA. Furthermore, packaging of genomic DNA into chromatin in

eukaryotes raises another type of relation. DNA supercoiling can affect transcription through modulation of the chromatin structure (Hirose, S. and Matsumoto, K., 2005).

(4) Linker histone variants control chromatin dynamics during early embryogenesis

Hideaki Saeki[1], Keita Ohsumi[2], Hitoshi Aihara[2], Takashi Ito[3], Susumu Hirose, Kiyoe Ura[1] and Yasufumi Kaneda[1] ([1]Division of Gene Therapy, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Cell and Developmental Biology, Tokyo Institute of Technology Graduate School of Bioscience and Biotechnology, Nagatsuda, Yokohama, Japan; [3]Department of Biochemistry, Nagasaki University School of Medicine, Sakamoto, Nagasaki, Japan)

Complex transitions in chromatin structure produce changes in genome function during development in metazoa. Linker histones, the last component of nucleosomes to be assembled into chromatin, comprise considerably divergent subtypes as compared with core histones. In all metazoa studied, their composition changes dramatically during early embryogenesis concomitant with zygotic gene activation, leading to distinct functional changes that are still poorly understood. Here, we show that early embryonic linker histone B4, which is maternally expressed, is functionally different from somatic histone H1 in influencing chromatin structure and dynamics. We developed a chromatin assembly system with nucleosome assembly protein-1 as a linker histone chaperone. This assay system revealed that maternal histone B4 allows chromatin to be remodeled by ATP-dependent chromatin remodeling factor, whereas somatic histone H1 prevents this remodeling. Structural analysis shows that histone B4 does not significantly restrict the accessibility of linker DNA. These findings define the functional significance of developmental changes in linker histone variants. We propose a model that holds that maternally expressed linker histones are key molecules specifying nuclear dynamics with respect to embryonic totipotency (Saeki, H. *et al.*, 2005).

(5) Solution structure of the HMG-box domain in the SSRP1 subunit of FACT

Nobuyuki Kasai[1], Yasuo Tsunaka[1], Izuru Ohki[1], Susumu Hirose, Kosuke Morikawa[1] and Shin-ichi Tate[1] ([1]Department of Structural Biology, Biomolecular Engineering Research Instirute, Suita, Osaka, Japan)

The FACT proteins, which are highly conserved in all eukaryotes, form heterodimers consisting of two subunits, structure-specific recognition protein-1 (SSRP1) and SPT16 (also known as CDC68). The smaller SSRP1 subunit contains a High Mobility Group (HMG)-box domain. The FACT HMG-box domain (FACT-HMG) is categorized in the non-sequence-specific HMG-box protein group, which includes HMGB1, HMGB2 and NHP6A. The intact FACT heterodimers also interact with nucleosomes and DNA in a sequence independent manner. The solution structures of free yeast homolog NHP6A and the NHP6A-DNA complex, which shared the FACT function, were already reported. However, the structure of the HMG-box domain belonging to SSRP1 has not been determined yet. Here, we report the solution structure of the HMG-box domain (dFACTHMG) in the SSRP1 subunit (residues 555-624) of FACT from *Drosophila melanogaster* (Kasai, N. *et al.*, 2005).

(6) Roles of the heterotrimeric G proteins in *Drosophila* gastrulation

Naoyuki Fuse, Takuma Kanesaki and Susumu Hirose

In gastrulation of a *Drosophila* embryo, presumptive mesodermal cells undergo sequential cell movements, and invaginate into the ventral furrow. It has been shown that the *concertina*, encoding a G α subunit of the heterotrimeric G protein (G protein), is involved in an initial step of the cell movements. However, mechanisms that govern various cell movements during gastrulation are largely unknown. To address this issue, we investigated roles of the G proteins in gastrulation.

We utilized Moesin-GFP to characterize cell movements in living embryos and classified three steps for the mesoderm invagination. First, mid-ventral cells among presumptive mesodermal cells coordinately constrict their apical cell surfaces. Secondly, remaining mesodermal cells on the side extend filopodia-like

protrusions toward the mid-ventral and actively migrate into furrow. Thirdly, cells, which migrate from each side and meet at the mid-ventral, immediately adhere and cease movement. The *concertina* mutant perturbed the first and the second steps, however, mutant for another G α subunit, G α i, compromised the second and the third steps. These observations suggest that differential G protein signaling organize various cell movements during gastrulation.

(7) Anterior epidermis-specific expression of the cuticle gene *EDG84A* is controlled by many *cis*-regulatory elements in *Drosophila melanogaster*

Yasunori Kayashima, Susumu Hirose and Hitoshi Ueda[1] ([1]The Graduate School of Natural Science and Technology, Okayama University, Tsushima-naka, Okayama, Japan)

During insect metamorphosis, a pulse of ecdysteroids induces many different morphological changes depending on different parts of the body. In *Drosophila*, although a number of transcription factors are expressed in a stage-specific manner in response to an ecdysteroid pulse, little is known on the regulatory mechanism for space-specific gene expression during metamorphosis. The *EDG84A* gene encoding pupal cuticle protein is one of the targets of ecdysteroid-inducible transcription factor βFTZ-F1 and is expressed only in anterior epidermis of the body during mid- to late prepupal period, whereas βFTZ-F1 is expressed in almost all tissues. To address the regulatory mechanism of the tissue-specific expression of the *EDG84A* gene, we established transgenic fly lines which carry various upstream regions of the gene fused to the *LacZ* gene and examined the expression pattern of the reporter gene. Results of the transgenic fly reporter assays showed that the space-specific expression is controlled by at least four positive and two negative elements within a 263-bp region near the transcription start site, and at least three of them showed space-specific effects to the anterior body trunk. These results suggest that both high expression level and differential expression are achieved through many cis-regulatory elements (Kayashima, Y. *et al.*, 2005).

(8) Molecular mechanisms of Sonic hedgehog mutant effects in holoprosencephaly

Tapan Maity[1], Naoyuki Fuse and Philip A. Beachy[1] ([1]Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA)

Holoprosencephaly (HPE), a human developmental brain defect, usually is also associated with varying degrees of midline facial dysmorphism. Heterozygous mutations in the *Sonic hedgehog (SHH)* gene are the most common genetic lesions associated with HPE, and loss of *Shh* function in the mouse produces cyclopia and alobar forebrain development. The N-terminal domain (ShhNp) of Sonic hedgehog protein, generated by cholesterol-dependent autoprocessing and modification at the C terminus and by palmitate addition at the N terminus, is the active ligand in the Shh signal transduction pathway. Here, we analyze seven reported missense mutations (G31R, D88V, Q100H, N115K, W117G, W117R, and E188Q) that alter the N-terminal signaling domain of Shh protein, and show that two of these mutations (Q100H and E188Q), which are questionably linked to HPE, produce no detectable effects on function. The remaining five alterations affect normal processing, Ptc binding, and signaling to varying degrees. These effects include introduction of a recognition site for furin-like proteases by the G31R alteration, resulting in cleavage of 11 amino acid residues from the N terminus of ShhNp and consequent reduced signaling potency. Two other alterations, W117G and W117R, cause temperature-dependent misfolding and retention in the sterol-poor endoplasmic reticulum, thus disrupting cholesterol-dependent autoprocessing (Maity, T. *et al.*, 2005).

PUBLICATIONS

Papers

1. KASAI, N., TSUNAKA, Y., OHKI, I., HIROSE, S., MORIKAWA, K. and TATE, S. (2005) Solution Structure of the HMG-box domain in the SSRP1 subunit of FACT, **J. Biomol. NMR**, *32*, 83 - 88

2. KAWASAKI, H., HIROSE, S. and UEDA, H. (2005) A simple and quick method to isolate nuclear extracts from pupae of *Drosophila melanogaster*, **Cytotechnology**,

49, 67 - 70
3. KAYASHIMA, Y., HIROSE, S. and UEDA, H. (2005) Anterior epidermis-specific expression of the cuticle gene *EDG84A* is controlled by many cis-regulatory elements in *Drosophila melanogaster*, **Dev. Genes Evol.**, *215*, 545 - 552
4. MAITY, T., FUSE, N. and BEACHY, P. A. (2005) Molecular mechanisms of sonic hedgehog mutant effects in holoprosencephaly, **Proc. Natl. Acad. Sci. USA**, *102*, 17026 - 17031
5. SAEKI, H., OHSUMI, K., AIHARA, H., ITO, T., HIROSE, S., URA, K., and KANEDA, Y. (2005) Linker histone variants control chromatin dynamics during early embryogenesis, **Proc. Natl. Acad. Sci.** USA, *102*, 5697 - 5702
6. 広瀬　進　(2005)細胞記憶を支えるクロマチン，生命誌ジャーナル，*46*
7. 広瀬　進　(2005)酸化ストレス応答を支配するコアクチベーターMBF1，**蛋白質・核酸・酵素**，*50*, 136 - 140
8. 広瀬　進　(2005)長寿に関わるタンパク質，生化学，*77*, 1417 - 1419

Books

9. HIROSE, S. and MATSUMOTO, K. (2005) Possible roles of DNA supercoiling in transcription, DNA Conformation and Transcription, (OHYAMA, T.), Landes Bioscience・Texas, USA, 138 - 143

EDUCATION

1. 広瀬　進　クロマチン構造と転写調節　山口大学
2. 広瀬　進　クロマチン構造と転写調節　岡山大学

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

広瀬　進，清水光弘　国立遺伝学研究所研究会　クロマチンダイナミクスとゲノム機能制御　三島

HIROSE, S. and SHINKAI, Y. The 28th Annual Meeting of the Molecular Biology Society of Japan, Symposium Chromatin dynamics FUKUOKA

特許

特願2005-091475，負の超らせんDNAの検出法(3)，広瀬　進，松本国治，大学共同利用機関法人情報・システム研究機構

C-c. Division of Molecular and Developmental Biology Koichi Kawakami Group

RESEARCH ACTIVITIES

(1) Transposon tools and methods in zebrafish

Koichi Kawakami

Zebrafish is an excellent model animal to study vertebrate development by genetic approaches. Hundreds of mutations affecting various processes of development have been isolated by chemical mutagenesis and insertional mutagenesis using a pseudotyped retrovirus. However, useful transposon tools and methods had not been available in zebrafish. This is mainly because no active transposable element has been found from the zebrafish genome. Recently, we have developed efficient transgenesis, gene trap and enhancer trap methods in zebrafish by using the *Tol2* transposon systems. These methods should increase the usefulness of zebrafish as a model vertebrate and facilitate the study of developmental biology, genetics and genomics (Kawakami. K., 2005).

(2) Expression pattern-driven insertional mutagenesis in zebrafish

Saori Nagayoshi, Eriko Hayashi[1], Kazuhide Asakawa, Akihiro Urasaki, Kazuki Horikawa[1], Hiroyuki Takeda[1] and Koichi Kawakami ([1]University of Tokyo)

Although gene and enhancer trap methods using transposons and a retrovirus have been recently described in zebrafish, no insertional mutation has been created by these methods and it is not clear whether those are applicable to insertional mutagenesis. We report here the development of an efficient enhancer trap method in zebrafish using the *Tol2* transposable element and the identification of a developmental mutant created by this method. We constructed a transposon vector containing the zebrafish *hsp70* promoter upstream of the GFP gene. Transgenic fish carrying chromosomal insertions of the construct expressed GFP in various tissues and organs at normal temperatures, indicating that locus-specific enhancers activated the *hsp70* promoter.

More than half of the insertions gave rise to unique GFP expression patterns, suggesting that the *hsp70* promoter is highly capable of responding to chromosomal enhancers. 16 enhancer trap lines with a single insertion were established and characterized. These insertions were frequently found within protein-coding genes; i.e., 13 out of 16 were inserted within a gene. Furthermore, three were located within an exon. One of them expressed GFP strongly in the median fin folds and the pectoral fin. The insertion disrupted the first exon of the *tcf7* gene, which encoded a transcription factor involved in the Wnt signaling pathway. Homozygous embryos exhibited anomalies in the median fin folds and the pectoral fin, where GFP expression was detected. Thus, we demonstrated that our enhancer trap method can generate an insertion mutation in a developmentally important gene, which is identifiable by observing GFP expression patterns in living vertebrate embryos.

(3) Development of the Gal4-enhancer trap system using the *Tol2* transposable element

Kazuhide Asakawa, Akihiro Urasaki, Tomoya Kotani, Saori Nagayoshi, Yasuyuki Kishimoto, Masahiko Hibi[1] and Koichi Kawakami ([1]RIKEN CDB)

First, by using the *Tol2* transposable element, we constructed an enhancer trap vector *hsp-gal4*, which contains the yeast transcription factor *GAL4* gene placed under the heat shock promoter, and reporter vectors, UAS-EGFP and UAS-DsRED. Then, we established transgenic zebrafish lines carrying these transposon vectors by *Tol2*-mediated transgenesis. We found that, upon heat shock, embryos obtained from crosses between the *hsp-gal4* fish and these reporter lines could express EGFP and DsRed ubiquitously, suggesting that Gal4 expressed from *hsp-gal4* can induce expression of a gene placed under UAS. Next, to determine whether Gal4-enhancer trapping is feasible, we created random insertions of the *hsp-gal4* vector in the genome. In a pilot screen, we identified 30 lines that showed unique Gal4 expression patterns during embryonic stages by crossing the *hsp-gal4* fish with the reporter lines. Inverse PCR and the following database search allowed us to map rapidly the insertion sites of *hsp-gal4* on the genome and to identify candidate genes regulated by the trapped enhancer. These Gal4 lines should be used to express a gene of interest in the specific organs and tissues.

(4) *misty somites*, a maternally expressed gene identified by the transposon-mediated gene trap method in zebrafish, is required for somite boundary formation

Tomoya Kotani, and Koichi Kawakami

Maternal factors, which are transmitted from mother to offspring through eggs, play important roles during early development. Although chemical mutagenesis screens to isolate maternal effect mutants have been performed, it is difficult to identify genes responsible for maternal effect mutants by positional cloning. We have been developing the gene trap method in zebrafish using the *Tol2* transposon. The insertions of the *Tol2*-based gene trap vector cause GFP-expression in unique patterns during development. We hypothesized that when the insertions were integrated in genes expressed maternally and captured their transcripts, GFP should be expressed in eggs. In fact, we isolated transgenic fish expressing GFP in one-cell stage embryos. Then, we generated females homozygous for the insertions and analyzed embryos from these females for morphological defects during embryonic development. By screening 16 homozygous females, we found that embryos from one homozygous female exhibited defects in somite boundary formation at 13 hpf (the 6-somite stage). The maternal insertional mutation was named *misty somites (mys)* and further analyzed. In the *mys* mutant, the gene trap construct was integrated within an intron of a novel gene, and captured its transcript. The *in situ* hybridization analysis using a probe for the *mys* gene revealed that the *mys* mRNA was indeed accumulated in one-cell stage embryos. To prohibit expression of the Mys protein completely, we injected an antisense morpholino oligonucleotide (MO) that blocks the translation initiation site of the *mys* gene to the *mys* homozygous embryos. The MO injection resulted in a phenotype severer than that of the *mys* mutant and in embryonic lethality, indicating that the *mys* mutant phenotype is hypomorph. In the morphants, the genes expressed during somitogenesis, such as *fgf8, deltaC, her1, tbx24* and *mespb*, were expressed and somite boundaries were observable at 16 hpf (the 14-somite stage). At 20 hpf (the 20-somite stage), however, the boundaries were disappeared in the MO-injected embryos. Thus, the *mys* gene encodes

a maternal factor required for the maintenance of the somite boundaries during vertebrate development.

(5) Remobilization of integrated transposons: the jump starter system in zebrafish

Akihiro Urasaki, Kazuhide Asakawa and Koichi Kawakami

We have been developing the gene and enhancer trap methods using the *Tol2* transposon, and creating transgenic zebrafish expressing GFP in specific tissues and organs. Currently we are making transposon insertions in the genome by microinjection of both a transposon-donor plasmid and the transposase mRNA into fertilized eggs. Creating a number of zebrafish lines with different transposon insertions would be easier if the jump-starter system, which enables creation of new transposon insertions without microinjection, was developed. Toward this goal, first we tested whether integrated transposons can be remobilized. We injected transposase mRNA into embryos homozygous for a single transposon insertion in the *hoxc3a* and the *pax6b* gene. The integrated transposons were excised from these two loci, and inserted into different loci efficiently. 11 out of 13 reintegration loci mapped on the genome were located on different chromosomes, and two were located on the same chromosome, only 100-kb and 550-kb from the original locus. Thus, integrated transposons can be mobilized by supplying the transposase activity, and, upon remobilization, both genome-wide global hopping and local hopping, predominantly global hopping, were observed. Then, we constructed transgenic fish carrying the transposase cDNA under the control of the zebrafish *hsp70* promoter. Double transgenic fish carrying the transposase cDNA and an insertion of the *Tol2* enhancer trap construct, causing specific GFP expression, were heat-shocked repeatedly, and their offspring were screened for new patterns during embryonic development. We found that the germ cells of the heat-shocked fish became highly mosaic, and a pair of the male and the female could generate more than 11 new patterns at the frequency of approximately one pattern per 20 embryos. Southern blot analysis and inverse PCR revealed that these new GFP expression patterns indeed resulted from transposon insertions at new loci. In these cases, global hopping was also predominant. These results indicate that the jump starter system we established here can be used to create transposon insertions in new loci efficiently in the germ cells.

(6) The transposon-mediated Gal4 gene trapping identified genes expressing in unique patterns in the adult zebrafish

Kanta Mizusawa, Kazuhide Asakawa, Akihiro Urasaki, Tomoya Kotani, Saori Nagayoshi, Yasuyuki Kishimoto, Shigeru Kondo and Koichi Kawakami

Gene-trap and enhancer trap methods have recently been developed in zebrafish. The previous studies have shown that these methods are powerful to study the function of developmental genes. However, these methods have not been applied to the study of genes functioning in the adult stages. To identify and characterize genes that are expressed in spatially restricted patterns in the adult and play important roles to maintain the adult system, we have developed the Gal4-gene trap system in zebrafish and performed a screen for unique Gal4 expression patterns in the adult. First, 247 fish injected with the Gal4 gene trap transposon construct-donor plasmid and transposase mRNA were mated with the UAS-GFP reporter fish, and the offspring were screened for unique GFP expression during embryonic development. 154 unique GFP expression patterns were identified, and the F1 fish were raised. Then, 1252 F1 fish with these 154 embryonic patterns were analyzed for unique GFP expression at the adult stages. We observed unique GFP expression in various organs; i.e., eye, nose, jaw, fin, skin, gill, muscle, skeleton and part of the brain, and, in total, 121 unique GFP expression patterns were identified. To determine whether the prescreen at the embryo stages enriched fish with unique GFP expression patterns at the adult stage, we performed the adult screen without the embryonic prescreen. A total of 1984 F1 fish from 99 injected fish were analyzed at the adult stage, and 79 unique GFP expression patterns were identified. Thus, the adult screen with the embryonic prescreen (79%:121/154) was comparable with that without the prescreen (80%:79/99), suggesting that the adult and the embryonic patterns may be irrelevant. We are now focusing on fish lines exhibiting unique Gal4 expression in different parts of the adult brain (telencephalon, diencephalon, and cerebellum) and in the pigment cells on the skin (the inter-stripe

and the stripe boundary regions). Further studies of these lines should disclose genes functioning these organs. Also, these Gal4 lines will be useful to modify the activities of those cells in the adult zebrafish.

(7) The zebrafish *bobtail* mutation identifies the *MOCS1* (the molybdenum cofactor synthesis step-1) gene that is required for the posterior body formation as an essential element of the Fgf/ERK signaling

Yasuyuki Kishimoto, Sumito Koshida[1], Makoto Furutani-Seiki[2], Atsushi Kawakami[3], J. Reiss[4], G. Schwarz[5], Yuji Kohara, Hisato Kondoh[2] and Koichi Kawakami ([1]National Institute of Natural Science, [2]ERATO/SORST JST, [3]Tokyo Institute of Technology, [4]Goettingen Univ., [5]Univ. of Cologne)

The formation of vertebrate posterior body and tail requires coordinated growth, differentiation and movement of multipotent progenitor cells located in the tailbud, and is dependent upon the Fgf signaling. Here we describe the zebrafish *bobtail (btl)* mutation that exhibits a recessive maternal effect, causing a strong reduction of the tail region and abnormal somite patterning. Through positional cloning, we show that the gene mutated in the *btl* mutant is a zebrafish homologue of the molybdenum cofactor synthesis step-1 *(MOCS1)* gene. In humans, mutations in *MOCS1* have been described for a lethal genetic disorder, molybdenum cofactor deficiency. *MOCS1* catalyzes conversion of GTP to cyclic pyranopterin monophosphate (cPMP), a biosynthetic intermediate for molybdenum cofactor. Injection of cPMP at the one-cell stage completely rescued the *btl* mutant phenotype, demonstrating that the defects in the *btl* mutant are caused by the lack of cPMP biosynthesis. Furthermore, we found that phosphorylation of the ERK protein in the tailbud region, which has been shown to be regulated through the Fgf signaling, strongly decreased during gastrulation and segmentation stages in the *btl* mutant embryos. Implantation of Fgf-soaked beads could not induce ectopic ERK phosphorylation in the btl mutant, indicating that MOCS1 is required for Fgf-dependent ERK activation. Our results suggest a molecular link between molybdenum cofactor biosynthesis and Fgf/ERK signaling during the development of the vertebrate posterior structures.

(8) The Zebrafish *pob* Gene Encodes a Novel Protein Required for Survival of Red Cone Photoreceptor Cells

Michael R. Taylor[1], Satoshi Kikkawa[1], Vishvanathan Ramamurthy[1], Koichi Kawakami, and Susan E Brockerhoff[1] ([1]University of Washington)

The zebrafish mutant, *partial optokinetic response b (pob)*, was isolated using an n-ethyl n-nitrosourea (ENU)-based screening strategy designed to identify larvae with defective optokinetic responses in red but not white light. Previous studies showed that red-light blindness in *pob* was due to the specific loss of long-wavelength photoreceptor cells via an apoptotic mechanism. Here, we used a positional cloning strategy to identify the mutated *pob* gene. We find that *pob* encodes a highly conserved 30kDa protein of unknown function. Pob contains putative transmembrane regions and protein sorting signals, and is localized to intracellular compartments in COS-7 cells and the inner segment and synapse in photoreceptor cells. To demonstrate that the correct gene was isolated, we used the *Tol2* transposon system to generate transgenic animals and rescue the mutant phenotype. We also show that the degeneration of red cone photoreceptors in the mutants is independent of light. Based on these findings, we propose that Pob is not involved in phototransduction but rather plays an essential role in protein sorting and/or trafficking (Taylor, M. *et al.*, 2005).

PUBLICATIONS

Papers

1. Kawakami, K. (2005) Transposon tools and methods in zebrafish, **Developmental Dynamics**, *234 (2)* 244 - 254

2. Miskey, C., Izsvak, Z., Kawakami, K., Ivics, Z. (2005) DNA transposons in vertebrate functional genomics, **Cellular and Molecular Life Sciences**, *62*, 629 - 641

3. Taylor, M. R., Kikkawa, S., Diez-Juan, A., Ramamurthy, V., Kawakami, K., Carmeliet, P., Brockerhoff, S. E. (2005) The zebrafish pob gene encodes a novel protein required for survival of red cone photoreceptor cells, **Genetics**, *170*, 263 - 273

EDUCATION

1. Kawakami, K. Transposon-mediated gene

trapping and enhancer trapping in zebrafish. Johns Hopkins School of Medicine

2. Kawakami, K. Transposon-mediated gene trapping and enhancer trapping in zebrafish. Peking University

SOCIAL CONTRIBUTIONS AND OTHERS

1. K.Kawakami was served as a Chair of the 3rd Annual International Conference on Transposition and Animal Biotechnology at Minneapolis, U.S.A. on June, 2005.

2. K. Kawakami was a lecturer in EMBO practical course: enhancer detection, gene trapping, and TILLING in zebrafish held at Bergen, Norway on August, 2005.

3. K. Kawakami had a seminar at Johns Hopkins School of Medicine, Baltimore, U.S.A. on September, 2005.

4. K. Kawakami had a seminar at Peking University, China on November, 2005.

5. K. Kawakami organized a workshop entitled "Functional genomics using transposable elements" in the 28th annual meeting of the molecular biology society of Japan at Fukuoka on December, 2005.

会議等主催

Mead, P., Hackett, P., McIvor, S., Kawakami, K., Fahrenkru, S. The 3rd Annual International Conference on Transposition and Animal Biotechnology Germline Mutagenesis. Minneapolis, Minnesota

Kawakami, K., Becker, T., Burgess, S., Cuppen, E. EMBO Practical Course: Enhancer Detection, Gene Trapping, and TILLING in Zebrafish. Bergen, Norway

川上浩一，竹田潤二　第28回日本分子生物学会年会ワークショップ　転移因子によるゲノムワイド遺伝子機能研究　福岡

C-d. Division of Physiological Genetics Fumitoshi Ishino Group

RESEARCH ACTIVITIES

哺乳類の個体発生のエピジェネティック的理解／石野史敏

EPIGENETIC VIEW OF MAMMALIAN DEVELOPMENT/FUMITOSHI ISHINO

概要

1) Genomic imprinting in an Australian marsupial species, tammar wallaby

We demonstrated that *IGF2* and *PEG1/MEST* in the tammar wallaby showed paternal expressions as well as humans and mice (eutherians). However, there found no DMR (differentially methylated regions) in the wallaby *PEG1/MEST* (Suzuki, S., *et al.* 2005). It was previously reported that *IGF2R* in a South American marsupial species, grey-tail opossum, was imprinted without apparent DMR. As DMR is the central regulator of genomic imprinting in the eutherians, these results suggest the differential regulatory mechanisms between the marsupials and eutherians although existence of their common imprinted genes (regions).

2) Abnormal gene expression profiles in somatic cloned mice

Comprehensive gene expression analysis of somatic cloned mice clearly demonstrated that they were epigenetically heterologous creatures in spite of their genetic identity. Although we analyzed several normal-looking somatic cloned mice, up to 40 % of genes showed over two fold changes compared with normal mice produced from sexual mating. Interestingly, some gene clusters sensitive to somatic cloning process were identified (Kohda, T. *et al.* 2005).

3) A retrotransposon-derived gene essential for mammalian development

We previously identified *Peg10* (paternally expressed 10) as a Sushi-ichi retrotransposon derived gene. Recently, it turned out that *Peg10* was a mammal-specific gene that was conserved in all eutherian mammals registered in GenBank. Gene targeting experiment showed that *Peg10* KO embryos were early embryonic lethal due to severe placental defects. This is the first demonstration that an acquired gene from a retrotransposon has essential function in mammalian development (Ono, R. *et al.* Nat Genet 2005 online).

PUBLICATIONS

Papers

1. Isotani, A., Nakanishi, T., Kobayashi, S., Lee, J., Chuma, S., Nakatsuji, N., Ishino, F. and Okabe, M. (2005) Genomic imprinting of XX spermatogonia and XX oocytes recovered from XX - XY chimeric testis, **Proc. Natl. Acad. Sci. U. S. A**, *102 (11)* 4039 - 4044

2. Kagami, M., Nishimura, G., Okuyama, T., Hayashidani, M., Takeuchi, T., Tanaka, S., Ishino, F., Kurosawa, K., Ogata, T. (2005) Segmental and full paternal isodisomy for chromosome 14 in three patients: narrowing the critical region and implication for the clinical features., **Am. J. Med. Genet.**, *138 (A)* 127 - 132

3. Kanatsu-Shinohara, M., Ogonuki, N., Iwano, T., Lee, J., Kazuki, Y., Inoue, K., Miki, H., Takehashi, M., Toyokuni, S., Shinkai, Y., Oshimura, M., Ishino, F., Ogura, A. and Shinohara, T. (2005) Genetic and epigenetic properties of mouse male germline stem cells during long-term culture., **Development**, *132 (18)* 4155 - 4163

4. Kohda, T., Inoue, K., Ogonuki, N., Miki, H., Naruse, M., Kaneko-Ishino, T., Ogura, A., and Ishino, F. (2005) Variation in gene expression and aberrantly regulated chromosome regions in cloned mice., **Biol. Reprod.**, *73 (6)* 1302 - 1311

5. Miki, H, Inoue, K., Kohda, T., Honda, A., Ogonuki, N., Yuzuriha, M., Mise, N., Matsui, Y., Baba, T., Abe, K., Ishino, F. and Ogura, A (2005) Birth of Mice Produced by Germ Cell Nuclear Transfer., **Genesis**, *41 (2)* 81 - 86

6. Ono, R., Nakamura, K., Inoue, K., Naruse, M., Usami, T., Wakisaka-Saito, N., Hino, T., Suzuki-Migishima, Ogonuki, N., Miki, H, Kohda, T., Ogura, A., Yokoyama, M., Kaneko-Ishino, T., and Ishino, F. (2005) Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality., **Nature Genetics**, *online, 11* Dec

7. Shiura, H., Miyoshi, N., Konishi, A., Wakisaka-Saito, N., Suzuki, R., Muguruma, K., Kohda, T., Wakana, S., Yokoyama, M., Ishino, F. and Kaneko-Ishino, T. (2005) Meg1/Grb10 overexpression causes postnatal growth retardation and insulin resistance via negative modulation of the IGF1R and IR cascades., **Biochem. Biophys. Res. Commun.**, *329 (3)* 909 - 916

8. Suzuki, S., Renfree, M. B., Pask, A., Shaw, G., Kobayashi, S., Kohda, T., Kaneko-Ishino, T. and Ishino, F. (2005) Genomic imprinting of IGF2, P57KIP2 and PEG1/MEST in a marsupial, the tammar wallaby., **Mechanisms of Development**, *122 (2)* 213 - 222

9. 石野史敏，金児－石野 知子(2005)ゲノムインプリンティングのリプログラミングとその生物学的意義，**実験医学　増刊**，*23 (18)* 33 - 39

10. 金児－石野 知子，石野史敏(2005)ゲノムインプリンティングの機構と進化，**Molecular Medicine**, *42 (2)* 181 - 188

11. 金児－石野 知子，石野史敏(2005)個体発生とエピジェネティクス，**Hormone Frontier in Gynecology**，*12 (4)* 54 - 60

12. 金児－石野 知子，石野史敏(2005)Peg/Meg ～インプリンティングの役者たち～私の名付けた遺伝子，**実験医学**，*23 (12)* 1952 - 1954

Books

13. 石野史敏，金児－石野 知子(2005)ゲノムインプリンティング　転写研究集中マスター　バイオ研究マスターシリーズ　(半田宏，和田忠士，山口雄輝)，羊土社・東京，134 - 143

C-d. Division of Physiological Genetics Yukiko Gotoh Group

RESEARCH ACTIVITIES

(1) The Wnt-ß-catenin pathway directs neuronal differentiation of cortical neural precursor cells

Yusuke Hirabayashi, Yasuhiro Itoh, Norihisa Masuyama and Yukiko Gotoh

Neural precursor cells (NPCs) have the ability to self-renew and to give rise to neuronal and glial lineages. The fate decision of NPCs between proliferation and differentiation determines the number of differentiated cells and the size of each region of the brain. However, the signals that regulate the timing of neuronal differentiation remain unclear. Here we show that the Wnt signaling inhibits self-renewal capacity of mouse cortical NPCs, and instructively promotes their neuronal differentiation. Overexpression of Wnt7a or of a stabilized form of B-catenin in mouse cortical NPC culture induced neuronal differentiation even in the presence of FGF2, a self-renewal-promoting factor in this system. Moreover, blockade of the Wnt signaling led to

inhibition of neuronal differentiation of cortical NPCs in the developing mouse neocortex. Furthermore, the B-catenin/TCF complex appears to directly regulate the promoter of neurogenin1, a gene implicated in cortical neuronal differentiation. Importantly, stabilized B-catenin did not induce neuronal differentiation of cortical NPCs at earlier developmental stages, consistent with previous reports, suggesting stage-specific functions of the Wnt signaling. Collectively, these results reveal pivotal physiological roles for the Wnt signaling in neuronal differentiation.

(2) Hes binding to STAT3 mediates crosstalk betweenNotch and JAK-STAT signaling

Sachiko Kamakura, Koji Oishi, Takeshi Yoshimatsu, Norihisa Masuyama, Yukiko Gotoh

Although the Notch and JAK-STAT signaling pathways fulfill overlapping roles in growth and differentiation regulation, no coordination mechanism has been proposed to explain their relationship. Here we show that STAT3 is activated in the presence of active Notch as well as the Notch effectors Hes1 and Hes5. Hes proteins associate with JAK2 and STAT3, and facilitate complex formation between JAK2 and STAT3, thus promoting STAT3 phosphorylation and activation. Furthermore, suppression of endogenous Hes1 expression reduces growth factor induction of STAT3 phosphorylation. STAT3 appears to be essential for maintenance of radial glial cells and differentiation of astrocytes by Notch in the developing central nervous system. These results imply that direct protein-protein interactions coordinate cross-talk between the Notch-Hes and JAK-STAT pathways.

(3) Notch promotes survival of neural precursor cells via mechanisms distinct from those regulating neurogenesis

Koji Oishi, Sachiko Kamakura, Yuko Isazawa, Takeshi Yoshimatsu, Keisuke Kuida, Norihisa Masuyama and Yukiko Gotoh

During development of the mammalian brain, many neural precursor cells (NPCs) undergo apoptosis. The regulation of such cell death, however, is poorly understood. We now show that the survival of mouse embryonic NPCs in vitro was increased by culture at a high cell density and that this effect was attributable to activation of Notch signaling. Expression of an active form of Notch1 thus markedly promoted NPC survival. Hes proteins, key effectors of Notch signaling in inhibition of neurogenesis, were not sufficient for the survival-promoting effect of Notch1. This effect of Notch1 required a region of the protein containing the RAM domain and was accompanied by up-regulation of the anti-apoptotic proteins Bcl-2 and Mcl-1. Moreover, knockdown of these proteins by RNA interference resulted in blockade of the Notch1-induced survival. These results reveal a new function of Notch, the promotion of NPC survival.

(4) JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3

Jun Sunayama, Fuminori Tsuruta, Yasunori Mori, Norihisa Masuyama and Yukiko Gotoh

Life and death decisions are made by integrating a variety of apoptotic signals and survival signals in mammalian cells. Therefore, there is likely to be a common mechanism that integrates multiple signals adjudicating between the alternatives. We propose in this study that 14-3-3 represents such an integration point. A number of proapoptotic proteins commonly become associated with 14-3-3 upon phosphorylation by survival-mediating kinases such as Akt. We reported previously that cellular stresses induce JNK-mediated 14-3-3ζ phosphorylation at Ser184. Here we show that phosphorylation of 14-3-3 by JNK releases the proapoptotic proteins Bad and FOXO3a from 14-3-3, and antagonizes the effects of Akt signaling. As a result of dissociation, Bad is dephosphorylated, and translocates to the mitochondria, where it associates with Bcl-2/Bcl-x^L. Since Bad and FOXO3a share the 14-3-3 binding motif with other proapoptotic proteins, we propose that this JNK-mediated phosphorylation of 14-3-3 regulates these proapoptotitc proteins in concert and makes cells more susceptible to apoptotic signals.

PUBLICATIONS

Papers

1. Hirabayashi, Y. and Gotoh, Y. (2005)
Stage-dependent fate determination of neural precursor cells in mouse forebrain.

Neurosci. Res. *51*, 331-336.

2. Sunayama, J.,Tsuruta, F., Masuyama, N. and Gotoh, Y. (2005)
JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3.
J. Cell. Biol. *170*, 295-304.

3. Takada, T., Suzuki, H., Gotoh, Y. and Sugiyama, Y. (2005)
Regulation of the cell surface expression of human BCRP/ABCG2 by the phosphorylation state of Akt in polarized cells.
Drug Metab. Dispos. *33*, 905-909.

3. Yoshimatsu, T., Kawaguchi, D., Oishi, K., Takeda, K., Akira, S., Masuyama, N and Gotoh, Y. (2006)
Non-cell-autonomous action of STAT3 in maintenance of neural precursor cells in the mouse neocortex.
Development, 133, 2553-2563. (2006)

4. 伊藤靖浩, 後藤由季子(2005)神経系前駆細胞と脳がん幹細胞, **Medical Science Digest** *31*, 353-357

5. 砂山潤, 鶴田文憲, 後藤由季子(2005)細胞生存シグナルと死シグナルのバランスによる生死決定機構, 実験医学, *23*, 2006-2011

6. 樋口麻衣子, 後藤由季子(2005)Aktの活性化因子と哺乳類における機能, 実験医学, 増刊, *23*, 1679-1685

SOCIAL CONTRIBUTIONS AND OTHERS

Oncogene (Editor)
Journal of Biochemistry (Associate Editor)
Cell Structure and Function (Associate Editor)
Journal of Histochemistry and Cytochemistry (Editorial Board Member)

学会活動
日本分子生物学会　評議員
日本生化学会　常務理事, 会計幹事, 学術集会企画委員, 男女共同参画推進委員
日本学術会議　連携会員

2006年(平成17年度)　JBSバイオフロンティアシンポジウム"タンパク質修飾と遺伝子制御：組織分化／幹細胞における役割", を主催
2006年3月1日－3日, グリーンプラザ軽井沢(群馬県)

D. DEPARTMENT OF POPULATION GENETICS

D-a. Division of Population Genetics Naruya Saitou Group

(1) Ape Genome Project Silver

Saitou Naruya, Sumiyama Kenta

In search of genetic changes responsible for human uniqueness, we are determining genomic sequences of chimpanzee and gorilla that are phylogenetically close to human, and do molecular evolutionary analyses. In 2005, we have three publications on this subject.
(i) We examined evolutionary conservation of 5' upstream sequence of nine genes between human and great apes (Kitano, T., et.al., 2005).
(ii) We compared nucleotide sequences of chromosome rearrangement on human chromosome 12 and corresponding ape chromosomes (Shimada, M., et al., 2005).
(iii) We reviewed on evolution of hominoids and search for genetic basis for creating humanness (Saitou, N., 2005).

(2) Molecular evolution of developmental regulation

Sumiyama Kenta, Kim Hyung-Cheol, Saitou Naruya

We are studying cis-control elements of the developmental genes by sequence analysis and gene transfer experiments of large scale genomic clones, in order to elucidate relationship between evolution of cis-elements and body plan.

(3) Analysis of evolution of closely related populations

Saitou Naruya, Ishibashi Minaka

We are mainly interested in evolutionary relationship of various human populations. In 2005, we hade two publications on this subject.
(i) Phylogenetic relationship of the populations within and around Japan was examined by using 105 short tandem repeat polymorphic loci (Li, S. L., et al., 2005).
(ii) During ancient DNA investigation in China, we discovered bronze weapon based on an embedded fragment in a 3000-year-old skull (Kurosaki, K., et al., 2005).

(4) Evolution of blood group genes

Saitou Naruya

Blood group antigens are expressed on cell surface, and have a higher chance of being affected by bacteria or virus. Therefore, their genes may have undergone positive selection. We are studying evolution of ABO and Rh blood group genes for humans and non-human primates. Several papers are now in preparation.

(5) Large scale evolutionary analysis of many gene sequences

Saitou Naruya, Ezawa Kiyoshi

We do genome-wide comparison of many protein coding and non-protein coding nucleotide sequences among various organisms to elucidate evolutionary pattern. In 2005, we conducted systematic analysis of mouse and rat genome on gene conversion. Paper was accepted and is published in 2006 (Ezawa, K., et al., Mol Biol Evol)

(6) Development of new methods for the study of gene evolution

Saitou Naruya Kirill Kryukov

We developed new algorithm for multiple alignment, named MISHIMA (Method to Infer Sequence History In terms of Multiple Alignment), and successfully applied this method to 100 mammalian whole mitochondorial genome sequences and 4 closely related bacterial genome sequences. Paper is now in preparation.

PUBLICATIONS

Papers

1. Kitano, T. and Saitou, N. (2005) Evolutionary conservation of 5' upstream sequence of nine genes between human and great apes, **Genes and Genetics Systems**, *80 (3)* 225 - 232
2. Kurosaki, K., Wang, L., Tang, J., Wang, W., Saitou, N., Endo, T., and Ueda, S. (2005) Identification of a bronze weapon based on an embedded fragment in a 3000-year-old skull, **Forensic Science International**, *151 (1)* 105 - 108
3. Li, S. L., Yamamoto, T., Yoshimoto, T., Uchihi, R., Mizutani, M., Kurimoto, Y., Tokunaga, K., Jin, F., Katsumata, Y., and Saitou, N. (2005) Phylogenetic relationship of the populations within and around Japan using 105 short tandem repeat polymorphic loci, **Human Genetics**, *118 (6)* 695 - 707
4. Nakajima, T., Wooding, S., Satta, Y., Jinnai, N., Goto, S., Hayasaka, I., Saitou, N., Guan-Jun, J., Tokunaga, K., Jorde, L. B., Emi, M., and Inoue, I. (2005) Evidence for natural selection in the HAVCR1 gene: high degree of amino-acid variability in the mucin domain of human HAVCR1 protein, **Genes Immunology**, *6 (5)* 398 - 406
5. Saitou, N. (2005) Evolution of hominoids and the search for a genetic basis for creating humanness, **Cytogenetic and Genome Research**, *108 (1-3)* 16 - 21
6. Shimada, M., Kim, C.-G, Kitano, T., Ferell, R. E., Kohara, Y., and Saitou, N. (2005) Nucleotide sequence comparison of a chromosome rearrangement on the human chromosome 12 and corresponding ape chromosomes, **Cytogenetic and Genome Research**, *108*, 83 - 90
7. Tateno, Y., Saitou, N., Okubo, K., Sugawara, H., and Gojobori, T. (2005) DDBJ in collaboration with mass-sequencing teams on annotation, **Nucleic Acids Research**,*33*,25 - 28
8. 北野誉，斎藤成也(2005)ヒトと類人猿のゲノム比較からわかること，分子精神医学，*5 (4)* 381 - 385
9. 斎藤成也(2005)塩基配列以外のゲノム進化解析，数理科学，*43 (12)* 70 - 76
10. 斎藤成也(2005)中立進化するゲノム，数理科学，*43 (9)* 70 - 76
11. 斎藤成也(2005)ゲノムの進化学的解析，数理科学，*43 (7)* 64 - 70
12. 斎藤成也(2005)ゲノム解析の諸法，数理科学，*43 (6)* 77 - 83
13. 斎藤成也(2005)ゲノムとは，数理科学，*43 (4)* 68 - 75
14. 斎藤成也(2005)系統ネットワークを用いた配列間関係の表現，数理科学，*43 (3)* 60 - 66
15. 斎藤成也(2005)形質状態データから系統樹を作成する方法，数理科学，*43 (1)* 64 - 72
16. 隅山健太，斎藤成也(2005)脊椎動物の比較ゲノム：遺伝子間領域の比較解析，細胞工学，*24 (10)* 1108 - 1111
17. 颯田葉子，太田聡史，斎藤成也(2005)ゲノムから見た霊長類としてのヒト，蛋白質核酸酵素，*50 (16)* 2078 - 2082

Books

18. 北野誉，斎藤成也(2005)DDBJのホームページを用いた配列解析，バイオデータベース利用法，(金久実，小川温子，西原祥子編)，学進出版，158 - 29
19. 斎藤成也(2005)DNAから見た日本人，筑摩書房
20. 斎藤成也(2005)遺伝子から探る，ネアンデルタール人の正体，(赤澤威編)，朝日新聞社，141 - 164
21. 斎藤成也(2005)人種よさらば，人種概念の普遍性を問う，(竹沢泰子編)，人文書院，468 - 486

EDUCATION

1. 斎藤成也　分子系統進化学　集中講義　関西学院大学大学院理工学部
2. 斎藤成也　分子進化学　講義　東京大学理学部
3. 斎藤成也　言語能力の遺伝的基礎　21世紀COEテーマ講義「心とことば」　東京大学教養学部
4. 斎藤成也　ヒトゲノムと類人猿ゲノムの比較解析　講義　東京大学大学院獣医学部
5. 斎藤成也　遺伝子から見た人類の進化　ヒトゲノムの進化　特別講義　福井大学医学部
6. 斎藤成也　遺伝子の進化　講義　山形大学医学部
7. 斎藤成也　遺伝子から見た人間の進化　講義　山形大学大学院医学部

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動

斎藤成也　日本学術会議第20期会員
斎藤成也　日本遺伝学会会計幹事

その他

斎藤成也　海洋研究開発機構にて「哺乳類を中心とした比較ゲノム解析によるヒトへの進化過程の研究」について講演
斎藤成也　山梨県立甲府南高等学校のサイエンスフロンティアフォーラムにて「ゲノムからたどる人間の進化」について講演
斎藤成也　IBIC遺伝子多様性モデル解析事業部門会議にて「チンパンジーとヒトのSNP比較」について講演

D-a. Division of Population Genetics
Toshiyuki Takano Group

RESEARCH ACTIVITIES

(1) Detecting single-generation selection acting on inversion polymorphism in Drosophila

Toshiyuki Takano-Shimizu, Nobuyuki Inomata, Masanobu Itoh, Rumi Kondo, Miki Ohshima and Yutaka Inoue

Signatures of past natural selection have been found in DNA sequences. However, detecting ongoing selection has been difficult so far. Such selection are particularly relevant to the future status of populations. Furthermore, there is very little evidence for strong natural selection. Here, we developed a new method to detect viability selection acting on inversion polymorphisms in extant natural populations. The central issue of the method is a difference in the amount and pattern of molecular variation between two samples of inversion-free (standard) chromosomes: one from standard-chromosome homokaryotype and the other from standard and inversion heterokaryotype. Under no selective differential among genotypes, we expect no difference in the scale of variation between these two samples. Alternatively, under models with selection, some differences are expected. We applied this method to *In(2L)t* inversion polymorphism in a natural population of *D. melanogaster*. The number of haplotypes on standard chromosomes from the heterokaryotype was significantly smaller than that from the homokaryotype. This result suggests that strong selection currently acting on inversion polymorphism in Drosophila. Our method is applicable to a wide variety of sequence data, especially for humans, which are accumulating very fast. Our findings also imply the evolutionary significance of multi-locus interactions. Strong selection may be more common than we have believed.

(2) A high frequency null mutant of an odorant-binding protein, *Obp57e*, in *Drosophila melanogaster*

Aya Takahashi and Toshiyuki Takano-Shimizu

We found a null mutant of an odorant-binding protein, *Obp57e*, in *Drosophila melanogaster*. This frameshift mutation, which is a 10 bp deletion in the coding region, was at a high frequency in the Kyoto population, and was also present in Taiwan and Africa. We analyzed an about 1.5kb DNA sequence including the tandemly duplicated gene, *Obp57d*, from 16 inbred lines sampled in Kyoto, Japan. The analyses suggested that this presence/absence polymorphism is under balancing selection (Takahashi and Takano-Shimizu, 2005).

In order to assess if there is overdominance selection affecting this locus, we genotyped 211 wild-caught individuals from the same population using RFLP (Restriction Fragment Length Polymorphism), which can distinguish wild-type and deletion type alleles. These data did not indicate significant deviation from the genotype frequencies expected under Hardy-Weinberg equilibrium. Therefore, the locus is probably under other type of balancing selection such as frequency dependent selection or selection caused by environmental heterogeneity.

(3) Analyses of assortative mating and morphological differentiations in populations of *Drosophila melanogaster*

Aya Takahashi and Toshiyuki Takano-Shimizu

We surveyed multiple strains of *D. melanogaster* and *D. simulans* for differences in heterospecific copulation frequencies, and identified two *D. melanogaster* inbred lines between which mating frequencies of males against *D. simulans* females differ by a large extent. One line was originated in West Africa (Mel6) and the other was collected in Taiwan (Twn). We also performed an intraspecific mating experiment and found that these two lines show assortative mating. Especially, males from these two lines have different mating preference against conspecific females of different lines. These two lines have been subjected to over 20 generations of sib-mating, and are suitable materials for genetic mapping. Our genetic analyses showed that the third chromosome has a major effect on these behavioral differences of males. We have constructed 70 third chromosome recombinant lines between the two inbred lines and have been testing their mating behavior.

We noticed that these two lines differ in thorax pigmentation pattern as well. The thorax trident is sharply pigmented in Twn individuals whereas it is not obvious in Mel6. We mapped this phenotypic differences using the recombinant lines, and narrowed down the genetic region responsible for this phenotype to 75kb. We have been analyzing a candidate gene in this region.

(4) Spontaneous mutation spectrum in *Drosophila melanogaster*

Yutaka Watanabe, Aya Takahashi and Toshiyuki Takano-Shimizu

Mutation is among the most fundamental of all evolutionary biology. Its rate and pattern often vary greatly among different individuals, species, and even among different chromosomal regions in a single genome; however, so little is known about the mechanisms of such surprising variation. Mutation rate cannot be estimated from a comparison of nucleotide sequences from different species since many factors such as selection, population structure, and random genetic drift would change mutant frequency and then the information obtained provides only a partial picture of the range of spontaneous mutations. On the other hand, direct studies on spontaneous mutation have been limited to a few model organisms. The fruit fly (*D. melanogaster*) is one of them. However, the issue of spontaneous mutation rate is still far from resolved partly because many internal and external factors affecting the rate are not controlled adequately to estimate rates in natural conditions and partly because there are large variation in rate among individuals. We have started large mutation screen experiments aimed at understanding the nature of spontaneous mutation and obtaining the picture of the standard mutation spectrum in natural populations of *D. melanogaster*. We have designed an experimental scheme to screen mutations that occur in germ cells of the first generation offspring of wild-caught inseminated-females. The offspring were produced from crosses occurred in nature and therefore carried genetic compositions close to that of flies in nature. Male and female F1 offspring were individually crossed to mutant stocks carrying three or four visible recessive mutations, and the F2 offspring were screened for mutations. From more than 800,000 F2 flies, we obtained eight independent mutants and are currently investigating the nature of these mutations at molecular level.

(5) Nucleotide variation and linkage disequilibrium in 150-kb region around the *Scr* gene in *D. melanogaster* and *D. simulans*

Yutaka Watanabe, Haruki Tatsuta and Toshiyuki Takano-Shimizu

The sex comb is a specific row of enlarged bristles on the foreleg of male flies and a recently derived trait that is found almost exclusively in the *melanogaster* and *obscura* species groups in the subgenus *Sophophora*. Its structure varies greatly among species with respect to the numbers of sex combs and their teeth, and orientation of the sex comb. We performed QTL analyses of sex-comb tooth-number variation within *Drosophila simulans*, and identified, in total, seven QTL. One of candidate regions is the *Antennapedia Complex (ANTC)*, which consists of the *Hox* genes including the *Sex comb reduced (Scr)* gene. The *Hox* genes are well known as selector genes, but may also be involved more directly in the formation of morphological structures. Despite many functional and comparative studies, the gene regulation mechanisms of the *HOX* genes have remained elusive. As a first step in identifying QTN for the sex-comb tooth number variation, we surveyed nucleotide variation in about 150kb region surrounding the *Scr* gene in both *D melanogaster* ($n = 10$) and *D. simulans* ($n = 10$). The amount of nucleotide variation within species (nucleotide diversity = π) was lower than the typical values in both species. This may reflect low recombination rate at *ANTC*, but the degree of linkage disequilibrium was not so strong in both species. Further analysis is currently in progress.

(6) Fluctuation in linkage disequilibrium scale in *Drosophila melanogaster* genome

Toshiyuki Takano-Shimizu, Masanobu Itoh, Rumi Kondo, Nobuyuki Inomata, Noriko Nanba, Masako Hasegawa, Miki Ohshima and Yutaka Inoue

Linkage disequilibrium (LD) is a complex measure and influenced by many factors, of which relative effects are largely unknown. Nevertheless, it is not only

useful to infer population history but also necessary to describe the genetic structure of populations in multi-locus context. By using two fly samples from the same collection site, we have analyzed linkage disequilibria between polymorphisms at about 100 Drosophila chemoreceptor genes, finding larger LD in a spring sample than in an autumn sample and a significant excess of associations between one frequent and one less common allele in the replacement polymorphisms. To gain a finer picture of LD in Drosophila genome, we extended our analysis to further samples and genes. Despite the seasonal fluctuation in the scale of LD between the chemoreceptor genes, the amount of LD between randomly chosen genes did not differ so much among samples of different collection sites and seasons. Seasonal change in the amount of LD may generally be smaller than we may have expected from the census number. By using a spring sample of a different collection year, we confirmed a significant excess of haplotypes composed of one frequent and one rarer allele in replacement polymorphisms. At the same time, we found a significant bias in the opposite direction in silent polymorphisms. Further surveys on LD are under way for a quantitative assessment of natural selection in shaping patterns of LD.

PUBLICATIONS

PAPERS

1. Kobayashi, S., Noro, Y., Nagano, H., Yoshida, K. T., Takano-Shimizu, T., Kishima, Y. Sano, Y. (2005) Evidence for an evolutionary force that prevents epigenetic silencing between tail-to-tail rice genes with a short spacer., **Gene**, *346*, 231 - 240

2. Takahashi, A., Takano-Shimizu, T. (2005) A high frequency null mutant of an odorant-binding protein gene, *Obp57e*, in *Drosophila melanogaster*., **Genetics**, *170*, 709 - 718

EDUCATION

1. Dr. Takano gave a two-day training course for Mishima Kita Junior High School students, October, 2004.

SOCIAL CONTRIBUTIONS AND OTHERS

1. Dr. Takano served as a member of the steering committee of National Bio-Resource Project "DROSOPHILA."

D-c. Division of Theoretical Genetics Sumio Sugano Group

RESEARCH ACTIVITIES

(1) Functional Genomics Based on the full-length cDNA collection

Sumio Sugano

We have been constructing full-length cDNA libraries from various tissues and cells using oligo-capping method. From these libraries, we have been isolating a number of clones and determining their 5' end sequences[15]. Furthermore, we collaborated with Shin-Ichi Hashimoto and are isolating 5' sequence tags of mRNAs[1]. Using these 5' end sequence data, we made and are maintaining database of transcriptional start sites, DBTSS, the We also gathered an international consortium, H-invitational, for the annotation of human full-length cDNAs with Takashi Gojobori and Nomura Nobuo and made H-invitational database[12]. Using this database, we compared human and cynomolgus monkey 5' UTR region[8] and devised and evaluated tissue specific expression and promoter motifs[6]. Our data also contributed to make dbQSNP, that provides sequence and allele frequency information for single-nucleotide polymorphisms located in the promoter regions of human genes[11].

We also collaborated with various groups to make full-length cDNA libraries and to collect full-length cDNA clones. This includes, Dictyostelium discoideum[4], red cynomolgus monkey[6], and malaria[10]. We also helped isolating various full-length cDNA clones and provided various clones for analysis [2) 3) 5) 7) 9) 13) 16)].

Finally, we performed mass spectrometry analysis of surface proteins of ES cells and found 59 clusters of differentiation-related molecules and more than 80 components of multiple cell signaling pathways that are characteristic of a number of different cell lineages.

PUBLICATIONS

Papers

1. Eichinger L, Pachebat JA, Glockner G, Rajandream MA, Sucgang R, Berriman M, Song J, Olsen R, Szafranski K, Xu Q, Tunggal B, Kummerfeld S, Madera M, Konfortov BA, Rivero F, Bankier AT, Lehmann R, Hamlin N, Davies R, Gaudet P, Fey P, Pilcher K, Chen G, Saunders D, Sodergren E, Davis P, Kerhornou A, Nie X, Hall N, Anjard C, Hemphill L, Bason N, Farbrother P, Desany B, Just E, Morio T, Rost R, Churcher C, Cooper J, Haydock S, van Driessche N, Cronin A, Goodhead I, Muzny D, Mourier T, Pain A, Lu M, Harper D, Lindsay R, Hauser H, James K, Quiles M, Madan Babu M, Saito T, Buchrieser C, Wardroper A, Felder M, Thangavelu M, Johnson D, Knights A, Loulseged H, Mungall K, Oliver K, Price C, Quail MA, Urushihara H, Hernandez J, Rabbinowitsch E, Steffen D, Sanders M, Ma J, Kohara Y, Sharp S, Simmonds M, Spiegler S, Tivey A, Sugano S, White B, Walker D, Woodward J, Winckler T, Tanaka Y, Shaulsky G, Schleicher M, Weinstock G, Rosenthal A, Cox EC, Chisholm RL, Gibbs R, Loomis WF, Platzer M, Kay RR, Williams J, Dear PH, Noegel AA, Barrell B, Kuspa A. The genome of the social amoeba Dictyostelium discoideum. **Nature**. *435*: 43-57, 2005.

2. Hayashi H, Matsuzaki O, Muramatsu S, Tsuchiya Y, Harada T, Suzuki Y, Sugano S, Matsuda A, Nishida E. Centaurin-alpha 1 is a PI3K dependent activator of ERK1 / 2 map kinases. **J Biol Chem**. *281*: 1332-1337, 2005.

3. Kamei Y, Aoyama Y, Fujimoto T, Kenmotsu N, Kishi C, Koushi M, Sugano S, Morohashi K, Kamiyama R, Asakai R. A steroidogenic cell line with differentiation potential from mouse granulosa cells, transfected with Ad4BP and SV40 large T antigen genes. **J Endocrinol**. *185*: 187-195, 2005.

4. Kasai Y, Hashimoto SI, Yamada T, Sese J, Sugano S, Matsushima K, Morishita S. 5'SAGE: 5'-end Serial Analysis of Gene Expression database. **Nucleic Acids Res**. *33*: D550-D552, 2005.

5. Lan NT, Yamaguchi R, Uchida K, Sugano S, Tateyama S. Growth Profiles of Recent Canine Distemper Isolates on Vero Cells Expressing Canine Signalling Lymphocyte Activation Molecule (SLAM). **J Comp Pathol**. *133*: 77-81, 2005.

6. Miyajima N, Watanabe M, Ohashi E, Ohmori K, Mochizuki M, Nishimura R, Ogawa H, Sugano S, Sasaki N. Identification and characterization of a canine highly similar to retinoic acid receptor alpha. **DNA Seq**. *16*: 7-15, 2005.

7. Nakagawa T, Watanabe M, Ohashi E, Uyama R, Takauji S, Mochizuki M, Nishimura R, Ogawa H, Sugano S, Sasaki N. Cyclopedic protein expression analysis of cultured canine mammary gland adenocarcinoma cells from six tumours. **Res Vet Sci**. *80*: 317-323 (2006) 2005 Sep 19;[Epub ahead of print]

8. Nunomura K, Nagano K, Itagaki C, Taoka M, Okamura N, Yamauchi Y, Sugano S, Takahashi N, Izumi T, Isobe T. Cell surface labeling and mass spectrometry reveal diversity of cell-surface markers and signaling molecules expressed in undifferentiated mouse embryonic stem cells. **Mol Cell Proteomics**. *4*: 1968-1976 (2005) 2005 Sep 20;[Epub ahead of print]

9. Osada N, Hirata M, Tanuma R, Kusuda J, Hida M, Suzuki Y, Sugano S, Gojobori T, Shen CK, Wu CI, Hashimoto K. Substitution Rate and Structural Divergence of 5'UTR Evolution: Comparative Analysis Between Human and Cynomolgus Monkey cDNAs. **Mol Biol Evol**. *22*: 1976-1982, 2005. 2005 Jun 8;[Epub ahead of print]

10. Otsuki T, Ota T, Nishikawa T, Hayashi K, Suzuki Y, Yamamoto J, Wakamatsu A, Kimura K, Sakamoto K, Hatano N, Kawai Y, Ishii S, Saito K, Kojima S, Sugiyama T, Ono T, Okano K, Yoshikawa Y, Aotsuka S, Sasaki N, Hattori A, Okumura K, Nagai K, Sugano S, Isogai T. Signal Sequence and Keyword Trap in silico for Selection of Full-Length Human cDNAs Encoding Secretion or Membrane Proteins from Oligo-Capped cDNA Libraries. **DNA Res**. *12*: 117-126, 2005.

11. Scott A, Pantoja-Uceda D, Koshiba S, Inoue M, Kigawa T, Terada T, Shirouzu M, Tanaka A, Sugano S, Yokoyama S, Guntert P. Solution structure of the Src homology 2 domain from the human feline sarcoma oncogene Fes. **J Biomol NMR**. *31*: 357-361, 2005.

12. Shibui A, Shiibashi T, Nogami S, Sugano S, Watanabe J. A novel method for development of malaria vaccines using full-length cDNA libraries. **Vaccine**. *23*: 4359-4366, 2005.

13. Tahira T, Baba S, Higasa K, Kukita Y, Suzuki Y, Sugano S, Hayashi K. dbQSNP: a database of SNPs in human promoter regions with allele frequency information determined by single-strand conformation polymorphism-based methods. **Hum Mutat**. *26*: 69-77, 2005.

14. Yamaguchi R, Kojimoto A, Sakai H, Uchida K, Sugano S, Tateyama S. Growth characteristics of canine distemper virus in a new cell line CCT cells

originated from canine malignant histiocytosis. **J Vet Med Sci**. *67*: 203-206, 2005.
15. Yamasaki C, Koyanagi KO, Fujii Y, Itoh T, Barrero R, Tamura T, Yamaguchi-Kabata Y, Tanino M, Takeda JI, Fukuchi S, Miyazaki S, Nomura N, Sugano S, Imanishi T, Gojobori T. Investigation of protein functions through data-mining on integrated human transcriptome database, H-Invitational database (H-InvDB). **Gene**. *364*: 99-107 (2005). 2005 Sep 23;[Epub ahead of print]
16. Yamashita R, Suzuki Y, Sugano S, Nakai K. Genome-wide analysis reveals strong correlation between CpG islands with nearby transcription start sites of genes and their tissue specificity. **Gene**. *350*: 129-136, 2005.

Databases
http://dbtss.hgc.jp/
https://www.jbirc.aist.go.jp/hinv/index.jsp

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動
HUGO: Council
HUGO Pacific: Co-Chair
遺伝子治療学会：評議員

E. DEPARTMENT OF INTEGRATED GENETICS

E-a. Division of Human Genetics Hiroyuki Sasaki Group

RESEARCH ACTIVITIES

(1) Establishment and maintenance of DNA methylation imprints in the germline and early mouse embryos

Hiroyuki Sasaki, Yuzuru Kato, Ryutaro Hirasawa, Kenji Kumaki, Hiroyasu Furuumi, Masahiro Kaneda[1], Masaki Okano[2], En LI[3], Mizue Hisano[4], Masami Nozaki[4], Tomohiro Suzuki[5], Shigeharu Wakana[5], and Toshihiko Shiroishi[5] ([1]Gurdon Inst. Cancer Dev. Biol.; [2]CDB, RIKEN; [3]Novartis; [4]Osaka Univ.; [5]GSC, RIKEN)

Genomic imprinting, an epigenetic gene-marking phenomenon in the germline, causes parent-of-origin-specific monoallelic expression of a subset of mammalian genes in offspring. DNA methylation serves as an important mechanism to mark the parental alleles of the imprinted genes. Evidence suggests that the imprinted genes are regulated by nearby differentially methylated regions (DMRs). We previously showed by conditional gene targeting that the de *novo* DNA methyltransferase gene *Dnmt3a*, but not *Dnmt3b*, is essential for the establishment of the methylation imprints in both male and female germlines. We are now studying in detail how the methylation imprints are affected in germ cells of the conditional mutants. Another question is how the methylation imprints are maintained in cleavage stage mouse embryos, in which the rest of the genome is extensively demethylated. We are therefore studying cleavage stage embryos lacking either *Dnmt1*, *Dnmt3a*, *Dnmt3b*, or combinations of these to see which methyltransferase(s) maintains the imprints. Lastly, we have also set out to screen ENU-treated mutant mouse stocks for new mutants that have a defect in establishment of the imprints.

(2) Comparative analyses of the distal imprinted domain on mouse chromnosome 7 and the orthologous domain on chicken chromosome 5

Hiroyuki Sasaki, Takaaki Yokomine, Yuzuru Kato, Wahyu Purbowasito, Hisao Shirohzu, Hisakazu Iwama, Kazuho Ikeo, Tetsuya Hori, Masaoki Tsuzuki[1], Shigeki Mizuno[2], Yo-ichi Matsuda[3], Atsushi Toyoda[4], Masahira Hattori[4], and Yoshiyuki Sakaki[4] ([1]Hiroshima Univ.; [2]Nihon Univ.; [3]Hokkaido Univ.; [4]GSC, RIKEN)

Imprinted genes tend to form clusters in the genome (imprinted domains), which may be related to the mechanism of imprinting or to the evolution of imprinting. We have cloned and sequenced a 1-Mb imprinted domain in mouse chromosome 7F4/F5 and its orthologous domain in chicken chromosome 5 (0.5 Mb). Using one of the mouse YAC clones, we showed that this domain is in fact composed of two autonomous subdomains (Cerrato *et al.*, 2005). We then found that the genes of the chicken domain are not imprinted and, furthermore, that the chicken domain lacks the unique tandem repeat cluster of 0.2 Mb, the *H19* gene, and the imprinting control elements, all of which are present in the mouse. The results indicate that the mammalian imprinted genes were already clustered in the common ancestors of mammals and birds and that the imprinting mechanism, which affects multiple genes in the cluster, came in later during mammalian evolution (Yokomine *et al.*, 2005). We also published a review on the interactions between the DMRs within this domain by chromatin looping (Kato and Sasaki, 2005).

(3) Computer-assisted search for the sequence features common to the imprinted DMRs

Hiroyuki Sasaki, Hisato Kobayashi, Takashi Abe, Yuji Kohara and Toshimichi Ikemura[1] ([1]SOKENDAI)

Although the imprinted DMRs, which show differential methylation depending on parental origin, often play crucial roles in imprinting, features common to the DMRs have not been identified. We therefore set out to look for the sequence features common to the DMRs by computer-assisted programs. We first determined the extent of each mouse DMR by bisulphite sequencing in 12.5-day embryos. We then located the DMR sequences on self-organizing maps

(SOMs) produced from the mouse genome sequences for di-, tri- and tetra-nucleotides. We found that most DMRs are located in the periphery of the SOMs: they are more CpG-rich than most of the genome but less CpG-rich than the CpG islands. Furthermore, the paternally methylated DMRs contain less CpGs than the maternally methylated DMRs (Kobayashi et al. in press). These findings provide a basis for the further characterization of the DMRs.

(4) Antisense regulation at the *Xist* locus by *Tsix*

Takashi Sado, Yuko Hoki, Hiroyuki Sasaki

X-inactivation is controlled by *Xist* and its antisense gene, *Tsix*, neither of which encodes a protein. *Xist* is essential for X-inactivation to occur in cis and its differential expression is a key event in the initiation of X-inactivation. *Xist* and *Tsix* are imprinted in the extraembryonic tissues of mouse embryos, so that they are expressed from the paternal and maternal X, respectively, resulting in the preferential inactivation of the paternal X. Targeted disruption of *Tsix* causes ectopic expression of *Xist*, suggesting that *Tsix* negatively regulates *Xist* in *cis*. However, the molecular mechanism of this antisense regulation remains unknown. We have demonstrated that *Tsix* transcriptionally silences *Xist* in both embryonic and extraembryonic tissues of mouse embryos. Moreover, we have shown that disruption of *Tsix* impairs establishment of repressive epigenetic modifications and chromatin structure at the *Xist* locus. We propose that *Tsix* silences *Xist* through modification of the chromatin structure (Sado *et al.*, 2005).

(5) *Tsix*-independent mechanism for *Xist* silencing

Takashi Sado, Tatsuya Ohhata, Yuko Hoki, Hiroyuki Sasaki

Differential induction of the X-linked non-coding *Xist* gene is a key event in the process of X-inactivation occurring in female mammalian embryos. *Xist* is negatively regulated in *cis* by its antisense gene *Tsix* through modification of the chromatin structure. The maternal *Xist* allele, which is normally silent in the extraembryonic lineages, is ectopically activated when *Tsix* on the same chromosome is disrupted, and subsequently the maternal X chromosome undergoes inactivation in the extraembryonic lineages even in males. However, it is still unknown whether the single *Tsix*-deficient X chromosome ($X^{\Delta Tsix}$) in males is also inactivated in the embryonic lineage. Here, we show that both male and female embryos carrying a maternally derived $X^{\Delta Tsix}$ could survive if the extraembryonic tissues were complemented by wild-type tetraploid cells. In addition, *Xist* on the $X^{\Delta Tsix}$ was properly silenced and methylated at CpG sites in adult male somatic cells. These results indicate that the embryonic lethality caused by the maternal $X^{\Delta Tsix}$ is solely attributable to the defects in the extraembryonic lineages. $X^{\Delta Tsix}$ does not seem to undergo inactivation in the embryonic lineage in males, suggesting the presence of a *Tsix*-independent silencing mechanism for *Xist* in the embryonic lineage (Ohhata *et al.*, in press).

(6) Role of Dnmt3L in gametogenesis

Kenichiro Hata, Maki Kusumi, Takaaki Yokomine, En LI[1] and Hiroyuki Sasaki ([1]Novartis)

The *Dnmt3L* (DNA cytosine-5-methyltransferase 3-Like) gene encodes a protein of 421 amino acid residues and harbors a putative zing finger domain that shares a high degree of homology with the PHD-like domains of Dnmt3a and Dnmt3b. The C-terminal part of Dnmt3L is related to Dnmts, but it does not possess critical motifs for methyltransferase activity. We have generated *Dnmt3L*-deficient mice by gene targeting. While $Dnmt3L^{-/-}$ mice grew normally, both males and females are infertile. All embryos from pregnant $Dnmt3L^{-/-}$ mothers died around E10.5. The maternally methylated imprinted genes, e.g. *Igf2r* and *Peg1*, were hypomethylated in embryos derived from $Dnmt3L^{-/-}$ females x $Dnmt3L^{+/+}$ males, but paternally methylated DMRs were unaffected. $Dnmt3L^{-/-}$ male mice showed azoospermia. These abnormal germ cells have methylation defects in paternally imprinted DMRs and IAP retrotransposon sequences (Hata *et al.*, in press). We speculate that Dnmt3L functions via interactions with Dnmt3a and/or Dnmt3b to control de *novo* methylation of the imprinted DMRs and other sequences including retrotransposons in male germ cells.

(7) Role of Dnmt3L in placentation

Kenichiro Hata, Takahiro Arima[1], Maki Kusumi[2], Satoru Tanaka[2], Takaaki Yokomine, Kunio Shiota[2], Norio Wake[1] , Masaoki Tsudzuki[3] and Hiroyuki Sasaki ([1]Kyushu Univ., [2]Tokyo Univ., [3]Hiroshima Univ.)

Although the Dnmt3L protein lacks DNA methyltransferase activity, it probably cooperates with Dnmts to establish methylation imprints. Oogenesis apparently proceeds normally in female *Dnmt3L*$^{-/-}$ mice but their heterozygous offspring (*Dnmt3L*$^{mat-/-}$) die before midgestation probably due to a lack of imprints. The *Dnmt3L*$^{mat-/-}$ embryos also showed defective formation of the labyrinth, reduced formation of the spongiotrophoblast layer, excess trophoblast giant cells and insufficient attachment between the chorion layer and ectoplacental cone. Cells of the extraembryonic tissues were arrested but not apoptotic. *Dnmt3L*$^{mat-/-}$ trophoblastic stem cells showed a disturbed cell fate *in vitro*. It was also suggested that a maternal methylation imprint is required for *Mash2* imprinting. (Arima *et al.*, submitted). Intriguingly, our comparative studies showed that the presence of the *Dnmt3L* gene is restricted to the species that have imprinting. The acquisition of Dnmt3L by a common ancestor of eutherians and marsupials might have been closely related to the evolution of imprinting (Yokomine *et al.*, in press). These findings indicate that the maternal imprints established by de *novo* methylation are required for placentation.

PUBLICATIONS

Papers

1. Cerrato, F., Sparago, A., Zou, X., Dean, W., Bruggemann, M., Sasaki, H., Reik, W. & Riccio, A. (2005) The two domain hypothesis in Beckwith-Wiedemann syndrome: autonomous imprinting of the telomeric domain of the distal chromosome 7 cluster., **Hum. Mol. Genet.**, *14 (4)* 503 - 511

2. Kato, Y. and Sasaki, H. (2005) Imprinting and looping: epigenetic marks control interactions between regulatory elements., **BioEssays**, *27 (1)* 1 - 4

3. Sado, T., Hoki, Y. and Sasaki, H. (2005) Tsix silences Xist through modification of chromatin structure., **Dev. Cell**, *9 (1)* 159 - 165

4. Sado, T. and Ferguson-Smith, A. C. (2005) Imprinted X inactivation in the preimplantation mouse embryos., **Hum. Mol. Genet.**, *14 (Spec.1)* 59 - 64

5. Yokomine, T., Shirohzu, H., Purbowasito, W., Toyoda, A., Iwama, H., Ikeo, K., Hori, T., Mizuno, S., Tsudzuki, M., Matsuda, Y., Hattori, M., Sakaki, Y. and Sasaki, H. (2005) Structural and functional analysis of a 0.5-Mb chicken region orthologous to the imprinted mammalian Ascl2/Mash2-Igf2-H19 region., **Genome Res.**, *15 (1)* 154 - 165

6. 熊木健治，佐々木裕之(2005)幹細胞とゲノムインプリンティング，バイオインダストリー，*22 (2)* 29 - 34

7. 佐々木裕之(2005)ゲノムインプリンティング：新しい潮流をもたらしたスーパーモデル(Overview)，**Molecular Medicine**，*429*，146 - 148

8. 佐々木裕之(2005)エピジェネティクスと疾患ーはじめに，医学のあゆみ，*215 (105)*

9. 佐渡敬，大畑樹也(2005)マウス胚体外組織におけるインプリント型X染色体不活性化，**Molecular Medicine**，*42*，173 - 180

10. 秦健一郎，佐々木裕之(2005)生殖系列におけるインプリント成立機構，**Molecular Medicine**，42

Books

11. Sasaki, H. (2005) DNA methylation in epigenetics, development and imprinting. Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics (Dunn, M., Jorde, L., Little, P. & Subramaniam, S.) John Wiley & Sons, Chichester, 285 - 291

12. 佐々木裕之(2005)エピジェネティクス入門ー三毛猫の模様はどう決まるのか，佐々木裕之，岩波書店

EDUCATION

1. 佐々木裕之　医学部講義　山梨大学医学部講義

2. 佐々木裕之　エピジェネティクスとは何か　総合研究大学院大学葉山高等研究センター

3. 佐々木裕之　ゲノムインプリンティングの分子遺伝学　遺伝医学セミナー(人類遺伝学会臨床遺伝学認定医制度研修会・臨床遺伝専門医制度研究会)

4. 佐々木裕之　連携講義　静岡大学，浜松医科大学，静岡県立大学，国立遺伝学研究所

5. 佐々木裕之　理学部講義　北里大学理学部

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動

佐々木裕之　財)遺伝学普及会評議員

佐々木裕之　日本生殖医療エンジニアリング研究会世話人

その他
佐々木裕之　パパ似？ママ似？遺伝大研究. プレモ(主婦の友社)
佐々木裕之　静岡県立静岡がんセンター研究所遺伝子組換え実験安全委員会委員
佐々木裕之　浜松医科大学遺伝子組換え実験安全委員会委員
佐々木裕之　三島社会保険病院倫理委員会委員
佐々木裕之　エピジェネティクス－遺伝子だけで決定されない. Bang!(NTT-BJグループ広報誌)

E-b. Division of Agricultural Genetics Tetsuji Kakutani Group

RESEARCH ACTIVITIES

(1) Developmental abnormalities induced by DNA methylation mutation of Arabidopsis

Tetsuji Kakutani, Tetsu Kinoshita, Asuka Miura, Yuki Kinoshita, Masaomi Kato, Miyuki Nakamura, Hodetoshi Saze

Arabidopsis provides a genetically tractable system to learn role of DNA methylation, since viable mutants with reduced genomic DNA methylation are available. Arabidopsis MET1 (METHYLTRANSFERASE 1, ortholog of mammalian DNA methyltransferase Dnmt1) is necessary for maintaining genomic cytosine methylation at 5'-CG-3' sites. Arabidopsis additionally methylates non-CG sites using CHROMOMETHYLASE3 (CMT3). A third Arabidopsis gene necessary for DNA methylation is *DDM1 (DECREASE IN DNA METHYLATION)*, which encodes a chromatin remodeling factor (Jeddeloh et al., 1999, Nat. Genet 22, 94-). The *ddm1* mutation affects both CG and non-CG methylation. A striking feature of *ddm1* mutation is that it induces a variety of developmental abnormalities by causing heritable change in other loci (Kakutani et al., 1996, PNAS 93, 12406-). The molecular basis has been clarified in two of the loci directly causing the developmental abnormalities (see below). In addition, we are starting genetic characterization of other types of developmental abnormalities (Kakutani et al., 2004, Cold Spring Harbor Symp Quant Biol 69, 139-).

(2) Epigenetic behavior of *CACTA* transposon

Asuka Miura, Masaomi Kato, Miyuki Nakamura, Kazuya Takashima, Yuki Kinoshita, Tetsuji Kakutani

Through genetic characterization of one of the *ddm1*-induced developmental abnormalities, we identified a novel endogenous Arabidopsis transposon, named *CACTA1*. This transposon transposes and increases in the copy number in DNA hypomethylation background in *ddm1* mutant (Miura et al., 2001, Nature 411, 212-). Loss of DNA methylation seems to be sufficient for mobilization of *CACTA1*, because it was mobilized in mutants of DNA methyltransferase genes, *MET1* and *CMT3*. High frequency transposition of *CACTA* elements was detected in *cmt3-met1* double mutants. Single mutants in either *met1* or *cmt3* were much less effective in mobilization. Thus CG and non-CG methylation systems redundantly function for immobilization of transposons (Kato et al., 2003 Curr Biol 13, 421-). *CMT3* gene and non-CG methylation in plants may have evolved as an additional epigenetic tag dedicated to transposon control.

CACTA1 activated by the *ddm1* mutation remained mobile in the presence of the wild type *DDM1* gene, suggesting that de novo silencing is not efficient for the defense of the genome against *CACTA* movement. The defense depends on maintenance of transposon silencing over generations (Kato et al., 2004, Genetics 168, 961-).

Differentiation of gene-rich and transposon-rich (gene-poor) regions is a common feature in plant genomes. That may be due to preferential integration of transposons to gene-poor regions or may be due to purifying selection against transposon insertion to gene-rich regions in natural populations. To evaluate the possible contribution of natural selection to the formation of transposon distribution pattern, we examined the distribution of the *CACTA* transposons in genomes of 19 natural variants (ecotypes) and compared that to integration induced in laboratory in the *ddm1* mutants. Sequences similar to mobile *CACTA1* copy distribute among the ecotypes and show high polymorphism in genomic localization. Despite the high polymorphism, the copy number was low in all the examined ecotypes and they localized preferentially in pericentromeric and transposon-rich regions (Miura et al, 2004, MGG 270, 524-). This contrasts to transposition induced in laboratory, in

which the integration sites are less biased and the copy number frequently increases. In addition, transposition induced in *DDM1* wild type background also showed unbiased integration specificity (Kato et al., 2004, Genetics 168, 961-). The differences in the integration sites may be due to natural selection against deleterious insertion into chromosomal arm regions.

(3) Inheritance of epigenetic developmental abnormality.

Yuki Kinoshita, Asuka Miura, Tetsu Kinoshita, Tetsuji Kakutani

Another developmental abnormality, late flowering trait, was induced by ectopic expression of *FWA* gene associated with hypomethylation of tandem repeat upstream of the coding region (Soppe et al., 2000, Mol Cell 6, 791-). Interestingly, this region is similar to SINE retroelement in the sequences (Lippman et al., Nature 430, 471-). We examined if DNA methylation of this SINE-related region is really critical for the silencing of FWA using RNA-directed DNA methylation (RdDM) strategy (Mette et al., EMBO J. 19, 5194-) and the *ddm1*-induced gain-of-function epigenetic *FWA* allele. Re-silencing of the epigenetic *FWA* allele was induced when the SINE-related region was methylated de novo. The silent state is heritable and associated with methylation at CG sites (manuscript submitted).

(4) Epigenetic control of *FWA* gene expression in endosperm

Tetsu Kinoshita, Asuka Miura, Yuki Kinoshita, Tetsuji Kakutani

Although *FWA* is ectopically expressed in the epigenetic alleles stated above, the role of *FWA* gene product in normal development remained unknown. To understand why the *FWA* gene is epigenetically controlled, we further examined expression of this gene during normal development in wild type. Results of GFP reporter system and direct detection of the transcript both suggest that *FWA* is expressed specifically in the endosperm. Endosperm is a plant tissue analogous to mammalian placenta; it serves as nutritional support to the embryo. Furthermore, the *FWA* gene was expressed in parent-of-origin-specific manner; only maternal gene is expressed. The *FWA* imprint depends on the maintenance DNA methyltransferase MET1, as is the case in mammals. Unlike mammals, however, the *FWA* imprint is not established by allele-specific de *novo* methylation. It is established by maternal gametophyte-specific gene activation, which depends on a DNA glycosylase gene, *DEMETER*. Since endosperm does not contribute to the next generation, the activated *FWA* gene need not be silenced again. Double fertilization enables plants to use such 'one-way' control of imprinting and DNA methylation in endosperm (Kinoshita et al, 2004 Science 303, 521-).

In order to identify factors involved in the establishment of imprinted *FWA* expression, we screened mutant defective in this process. A transgenic line expressing *FWA-GFP* was mutagenized and the mutants with defective *FWA* expression were selected by monitoring the GFP signal. Among 1200 EMS-mutagenized M_1 plants, we obtained several mutant candidates. Further genetic and molecular characterization of them is in progress.

PUBLICATIONS

Papers

1. Ono T, Kaya H, Takeda S, Abe M, Ogawa Y, Kato M, Kakutani T, Mittelsten Scheid O, Araki T, and Shibahara K (2006). Chromatin assembly factor 1 insures the stable maintenance of silent chromatin states in Arabidopsis. Genes Cells 11, 153-162.
2. Jullien P, Kinoshita T, Ohad N, Berger F (2006) Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. Plant Cell *in press.*
3. Takamiyama T, Hosobuchi S, Asai K, Nakamura E, Tomioka K, Kawase M, Kakutani T, Paterson A, Murakami Y, and Okuizumi H (2006) Restriction landmark genome scanning method using isoschizomers (MspI/HpaII) for DNA methylation analysis. Electrophoresis *in press.*
4. 加藤政臣，角谷徹仁(2005)RNAiとDNAメチル化によるゲノムの多様な制御，**化学と生物**，*43*，542 - 547

EDUCATION

1. 角谷徹仁　集中講義　東京大学理学部

SOCIAL CONTRIBUTIONS AND OTHERS

各種受賞

木下哲：日本遺伝学会奨励賞授賞

木下由紀，三浦明日香，木下哲，角谷徹仁：日本遺伝学会第77回大会Best Paper賞

E-b. Division of Agricultural Genetics Kei-ichi Shibahara Group

RESEARCH ACTIVITIES

(1) Mechanism of nucleosome assembly during DNA replication

Tatsuya Ono, Yasunari Takami[1], Fumiyuki Sanematsu, Tatsuo Nakayama[1], and Kei-ichi Shibahara
[1]Department of Biochemistry, Miyazaki Medical College, University of Miyazaki

A newly replicated DNA is assembled into nucleosome soon after the passage of replication fork. This rapid formation of nucleosome is functionally linked to DNA replication machineries and plays a critical role for the maintenance of genome integrity in proliferating cells. Two histone binding proteins, CAF-1 (Chromatin Assembly Factor-1) and ASF1 (Anti-Silencing Function1), are involved in some process of this nucleosome assembly reaction.

In collaboration with Dr. Nakayama in University of Miyazaki, using CAF-1 and ASF1-deficint chicken DT40 cell lines, we recently showed that without CAF-1 and/or ASF1 function, S-phase progression was delayed and a rapid nucleosome assembly during DNA replication was disturbed *in vivo*. (Sanematsu, F, *et al., J.Biol.Chem.* in press, 2006). This is the first direct evidence for the involvement of CAF-1 and ASF1 in a rapid nucleosome assembly during DNA replication *in vivo*. In addition, we obtained some interesting evidence for that CAF-1 is involved in Chk1-dependent checkpoint pathway after the treatment with HU (Takami, Y., *et al.*, submitted).

(2) Physiological implications of CAF-1 and CAF-1-dependent nucleosome assembly in higher eukaryotes

Tatsuya Ono, Hidetaka Kaya[1], Shin Takeda[2], Tetsuji Kakutani, Takashi Araki[1], Kei-ichi Shibahara
[1]Department of Botany, Graduate School of Science, Kyoto University
[2] Department of Plant Biology, University of Geneva, Switzerland

The genetic approach of *Arabidopsis* is a powerful tool to see physiological implications of CAF-1 and CAF-1dependent nucleosome assembly in higher eukaryotes. We have analyzed loss-of-function mutants of *caf-1 (fasciata : fas)* in *Arabidopsis* and have shown that the *fas* mutants displayed severely disturbed cellular and functional organization of both meristems (Kaya *et al., Cell*, 2001; Takeda *et al., Genes Dev.*, 2004). We recently showed that transcriptional gene silencing (TGS) of endogenous *CACTA* transposons was released infrequently in a stochastic manner in *fas*, without decreasing DNA methylation. Other endogenous silent genes at different chromosomal sites were also transcriptionally activated non-concomitantly with each other. Furthermore, TGS of the silent transgene *β-glucuronidase (GUS)* was also de-repressed randomly in *fas* mutant plants, irrespectively of developmental abnormalities, and the activated state of *GUS* was maintained during growth to produce clusters of cells expressing *GUS* (Ono et al., *Genes Cells*, 2006). Taken together, we strongly suggest that CAF-1 ensures stable inheritance of epigenetic states through multiple rounds of cell divisions and that defects in CAF-1 functions explain the stochastic occurrence of pleiotropic phenotypes in the *fas* mutants.

(3) Histone macroH2A-mediated formation of transcriptionally repressed states of chromatin

Yuya Ogawa[1], Hitoshi Nishijima, and Kei-ichi Shibahara
[1] Department of Genetics, Harvard Medical School, USA

The unusual histone variant macroH2A (macroH2A) is predicted to be functionally associated with transcriptional repression as it appears to be enriched in the inactive X chromosome by immunostaining. However, molecular function of macroH2A in modulating chromatin structures remains unknown. To reveal the roles of macroH2A, we purified macroH2A-containing nucleosome by affinity purification with anti-epitope tag antibodies, and eventually, we found mono-ubiquitinated form of maroH2A and determined the ubiquitinated sites in

macroH2A (Ogawa, Y., *et al.*, 2005). We are currently clarifying the function of these modifications and trying to isolate the molecules enriched in and associate with macroH2A-containing nucleosomes.

(4) Analysis of *S. pombe* RanGAP SpRna1 protein in the heterochromatin assembly via histone H3.

Hitoshi Nishijima, Ayumi Kusano[1], Tomoyuki Ohba[1], Hideo Nishitani[1], Kei-ichi Shibahara, Takeharu Nishimoto[1]
1: Department of Molecular Biology, Graduate School of Medical Science, Kyushu University

We have isolated temperature-sensitive mutants of the RanGAP homologue, *Sprna1*, in *S. pombe*. *Spran1*^{ts} strains display a strong defect in mitotic chromosome segregation (Kusano, A., *et al., Mol.Biol. Cell*, 2004). This phenotype is suppressed by the overexpression of Clr4, which methylates Lys 9 of histone H3 (H3K9). We found that histone H3 controls the RanGAP activity of *Sp*Rna1 to prevent disruption of the RanGTP/GDP gradient and that *Sp*Rna1 enhances the activity of the Clr4-dependent methylation of histone H3K9 (Nishijima, H., *et al., Mol.Biol.Cell*, in press). In addition, we had isolated another suppressor, Snf2SR, which is likely to be a novel member of the SNF2 family involved in the Ran GTPase cycle (Ohba, T., *et al.*, in preparation).

PUBLICATIONS

Papers

Ogawa, Y., Ono, T., Wakata, Y., Okawa, H., Tagami, H. and Shibahara, K-i. (2005) Histone variant macroH2A1.2 is mono-ubiquitinated at its histone domain., **Biochem. Biophys. Res. Commun.**, *336*, 204 - 209

EDUCATION

柴原慶一　次世代志向境界領域講義：エピジェネティック論　大学院講義　国立遺伝学研究所・セミナー室

E-c. Division of Brain Function Tatsumi Hirata Group

RESEARCH ACTIVITIES

(1) Regulation of ventral tangential migration of guidepost neurons in the lateral olfactory tract

Takahiko Kawasaki, Keisuke Ito and Tatsumi Hirata

In the developing nervous system, many neurons migrate for a long distance from their birthplace to the final destination. We found a ventral tangential migration stream of early-generated neurons in the mouse telencephalon (Tomioka et al., 2000). Although this migration mode had been overlooked for a long time, this is the most common migration mode in the early telencephalon when the neurogenesis has just started, and therefore has immeasurable impact on all the following developmental processes. One of the important function of this migration is to convey a specific type of neocortex-born neurons called lot cells to the future pathway of the lateral olfactory tract (LOT), where the cells construct a tight cellular array and guide the following projection of LOT axons.

We developed an organotypic culture system to investigate the regulation of this cell migration in the developing telencephalon (Kawasaki et al., 2006). Using the system, we found that two types of signals govern the ventral tangential migration. One is the signal contained in the neocortex, which directs the neurons ventrally. The other is the repulsive signal in the ganglionic eminence, a ventral structure of the telencephalon, which excludes neocortex-born neurons from invading this ventral area. These two types of signals appear to cooperatively function to arrange the lot cells in a packed cellular array at the boundary between the neocortex and the ganglionic eminence.

To investigate molecular mechanisms that control the ventral tangential migration, we examined effects of several signaling molecules on the migration lot cells in the organotipic culture and found that netrin 1, an axon guidance factor, attracts these cells (Kawasaki et al., 2006). Furthermore, we demonstrated that mutations in the genes encoding netrin 1 and its functional receptor Dcc lead to inappropriate distribution of lot cells and subsequent partial disruption of LOT projection. These results suggest

that netrin 1 regulates the migration of lot cells and LOT projections, possibly by ensuring the correct distribution of these guidepost neurons.

(2) Identification of the Antigens Recognized by Monoclonal Antibodies Raised against the Developing Lateral Olfactory Tract

Takahiko Kawasaki, Yoshiko Takagi, Hitoshi Yamatani, and Tatsumi Hirata

During development, olfactory bulb axons navigate the complex microenvironment composed of myriads of molecules in the telencephalon to construct a bundle called the lateral olfactory tract. The axons, themselves, also express thousands of different molecules during the navigation. Previously, we have produced many monoclonal antibodies that label the lateral olfactory tract and the surroundings in a unique pattern. The labeling profiles suggested that the antigen molecules recognized by each antibody are heterogeneously distributed around the developing lateral olfactory tract. We developed an efficient screening method to identify the antigen molecules by combining expression of a cDNA library in COS-7 cells and the subsequent immunohistochemical staining of the cells (Kawasaki et al., 2005). The systematic screening successfully identified the specific cDNA clones for many of the monoclonal antibodies, which highly probably code for the antigen molecules, and therefore unveiled the molecular nature of local components that embrace the developing lateral olfactory tract.

PUBLICATIONS

Papers

1. Kawasaki, T., Takagi, Y., Yamatani, H. and Hirata, T. (2005) Systematic screening and identification of the antigens recognized by monoclonal antibodies raised against the developing lateral olfactory tract, **J. Neurobiol.**, *62*, 330 - 340

2. Koma, Y., Ito, A., Watabe, K., Hirata, T., Mizuki, M., Kitamura, T., Kanakura, Y. and Kitamura, Y. (2005) Distinct role for c-kit receptor tyrosine kinase and SgIGSF adhesion molecule in attachment of mast cells to fibroblasts., **Lab. Invest.**, *85*, 426 - 435

3. 平田たつみ(2005)脳の領域特異化と軸索束形成，**神経研究の進歩**，*49*，77 - 83

EDUCATION

1. 平田たつみ　神経細胞の軸索伸長とガイダンス機構　広島大学・工学部

2. 平田たつみ　細胞生物学　特別講義　東北大学・医学部

E-d. Division of Applied Genetics Kunio Shiota Group

RESEARCH ACTIVITIES

(1) Stage-by-stage change in DNA methylation status of *Dnmt1* locus during mouse early development

Yeoung-Gyu Ko, Koichiro Nishino, Naoko Hattori, Yoshikazu Arai, Satoshi Tanaka and Kunio Shiota (The University of Tokyo)

Methylation of DNA is involved in tissue-specific gene control, and establishment of DNA methylation pattern in the genome is thought to be essential for embryonic development. Three isoforms of *Dnmt1* (DNA methyltransferase 1) transcripts, Dnmt1s, Dnmt1o, and Dnmt1p, are produced by alternative usage of multiple first exons. Dnmt1s is expressed in somatic cells. Dnmt1p is found only in pachytene spermatocytes, whereas Dnmt1o is specific to oocytes and preimplantation embryos. Here we determined that there is a tissue-dependent differentially methylated region (T-DMR) in the 5' region of *Dnmt1o* but not in that of the *Dnmt1s/1p*. The methylation status of the Dnmt1o T-DMR was distinctively different in the oocyte from that in the sperm and adult somatic tissues and changed at each stage from fertilization to blastocyst stage, suggesting that active methylation and demethylation occur during preimplantation development. The T-DMR was highly methylated in somatic cells and embryonic stem cells. Analysis using Dnmt-deficient embryonic stem cell lines revealed that Dnmt1, Dnmt3a, and Dnmt3b are each partially responsible for maintenance of methylation of *Dnmt1o* T-DMR. In particular, there are compensatory and cooperative roles between Dnmt3a and Dnmt3b. Thus, the regulatory region of *Dnmt1o*, but not of *Dnmt1s/1p*, appeared to be a target of DNA methylation. The present study also suggested that the DNA methylation status of the gene region dynamically

changes during embryogenesis independently of the change in the bulk DNA methylation status. (Ko, *et al.*, 2005).

(2) DNA methylation-dependent epigenetic regulation of *dimethylarginine dimethylaminohydrolase 2* gene in trophoblast cell lineage

Junko Tomikawa, Kazumi Fukatsu, Satoshi Tanaka and Kunio Shiota
(The University of Tokyo)

Trophoblast cell lineage is established through the first cellular differentiation in mammalian embryogenesis, and its developmental potential is restricted to the extraembryonic tissues contributing solely to the placenta. Several lines of evidence suggest a relative lack of importance of DNA methylation in gene regulation in the extraembryonic tissues compared to embryonic ones. Here we analyzed the dynamics of epigenetic status in the upstream region of mouse *Ddah2* gene, which was found to be specifically repressed in a stem cell population of trophoblast cell lineage. We found a tissue-dependent differentially methylated region in the regulatory region of *Ddah2* gene. This region was hypermethylated in trophoblast stem cells and was hypomethylated in differentiated cells both in vivo and in vitro. This change was well correlated with *Ddah2* expression. In addition, in vitro methylation confined to the differentially methylated region was sufficient to repress promoter activity in the reporter assay. Furthermore, a repressive pattern of histone modifications was formed around the differentially methylated region in undifferentiated TS cells with repressed *Ddah2*. Our data suggest that DNA methylation-mediated chromatin remodeling is involved in the regulation of *Ddah2* gene expression, and thus is important even in trophoblast cell lineage (Tomokawa, *et al.*, 2006).

(3) DNA methylation profiles of donor nuclei cells and tissues of cloned bovine fetuses

Kremenskoy Maksym[1], Kremenska Yuliya[1], Masako Suzuki[1], Kei Imai[2], Seiya Takahashi[3], Kazuyoshi Hashizume[4], Shintaro Yagi[1], Kunio Shiota[1]
([1]The University of Tokyo, [2]National Livestock Breeding Center, [3]National Institute of Livestock and Grassland Science, [4]Iwate University)

Methylation of DNA in CpG islands plays an important role during fetal development and differentiation, because in mammalian genomic DNA, CpG islands are preferentially located at upstream regions including the transcription start site of housekeeping genes, as well as associated with tissue-specific genes. Somatic nuclear transfer (NT) technology has been used to generate live clones in numerous mammalian species, but only a low percentage of nuclear transferred animals develop to term. Abnormal epigenetic changes in CpG islands of donor nuclei after nuclear transfer could contribute to a high rate of abortion during early gestation and increase perinatal death; these changes have yet to be explored. Thus, we investigated genome-wide DNA methylation profiles of CpG islands in nuclei donor cells and NT animals. Using Restriction Landmark Genomic Scanning (RLGS), we showed for the first time, the epigenetic profile formation of tissues from NT bovine fetuses produced from cumulus cells. From approximately 2600 unmethylated *Not*I sites visualized on the RLGS profile, at least 35 *Not*I sites showed different methylation status. Moreover, we proved that fetal and placental tissues from artificially inseminated and cloned cattle have tissue-specific differences in genome-wide methylation profiles of CpG islands. We also found that possible abnormalities occurred in fetal brain and placental tissues of cloned animals (Maksym, *et. al.*, 2006a).

(4) Epigenetic characterization of CpG islands of bovine *leptin* and *POU5F1* genes in cloned bovine fetuses

Kremenskoy Maksym[1], Kremenska Yuliya[1], Masako Suzuki[1], Kei Imai[2], Seiya Takahashi[3], Kazuyoshi Hashizume[4], Shintaro Yagi[1], Kunio Shiota[1]. ([1]The University of Tokyo, [2] National Livestock Breeding Center, [3]National Institute of Livestock and Grassland Science, [4] Iwate University)

Production of nuclear transferred animals concerns with number of abnormal development and fetal loss during postimplantation period. Abnormal DNA methylation is one of the reasons of poor survival of cloned animals. In mammalian genome DNA, CpG islands are preferentially located at the start of transcription of housekeeping genes and they are also

associated with tissue-specific genes. The correct and consecutive mechanisms of DNA methylation in CpG islands are necessary for selective gene expressions that determine the properties of individual cells, tissues or organs. In this study we investigated CpG islands methylation status in fetal and placental tissues of bovine *Leptin* and *POU5F1* genes in fetuses produced by artificial insemination (AI) and nuclear transfer (NT) at days 48 and 59 of pregnancy. Altered DNA methylation was observed in the normal and cloned fetal, placental and endometrial tissues using bisulfite sequencing and pyrosequensing. Tissue-specific differently methylated region in bovine *Leptin* gene and *POU5F1* gene shows variable methylation status in NT fetuses compare to AI control (Maksym, *et. al.*, 2006b).

(5) Proliferation related acidic leucine rich protein PAL31 functions as a caspase-3 inhibitor

Weiyong Sun[1,2], Hiromichi Kimura[2], Naka Hattori[2], Satoshi Tanaka[2], Shigemi Matsuyama [b], and Kunio Shiota[1,*] ([a] The University of Tokyo, [b] Case Western Reserve University)

Proliferation related acidic leucine rich protein PAL31 (PAL31) is expressed in proliferating cells and consists of 272 amino acids with a tandem structure of leucine-rich repeats in the N-terminus and a highly acidic region with a putative nuclear localization signal in the C-terminus. We previously reported that PAL31 is required for cell cycle progression. In the present study, we found that the antisense oligonucleotide of PAL31 induced apoptosis to the transfected Nb2 cells. Stable transfectants, in which PAL31 was regulated by an inducible promoter, were generated to gain further insight into the signaling role of PAL31 in the regulation of apoptosis. Expression of PAL31 resulted in the marked rescue of Rat1 cells from etoposide and UV radiation-induced apoptosis and the cytoprotection was correlated with the levels of PAL31 protein. Thus, cytoprotection from apoptosis is a physiological function of PAL31. PAL31 can suppress caspase-3 activity but not cytochrome c release in vitro, indicating that PAL31 is a direct caspase-3 inhibitor. In conclusion, PAL31 is a multifunctional protein working as a cell cycle progression factor as well as a cell survival factor (Sun, et al., 2006).

(6) Dimethyl Sulfoxide(DMSO) Increases Expression of Dnmt3as and Affects Genome-wide DNA Methylation Profiles in Mouse Embryoid Body

Misa Iwatani, Kohta Ikegami, Yuliya Kremenska, Naka Hattori, Satoshi Tanaka, Shintaro Yagi and Kunio Shiota (The University of Tokyo, Tokyo, Japan)

Dimethyl sulfoxide (DMSO), an amphipathic molecule, is widely used not only as a solvent for water-insoluble substances, but also as a cryopreservant for various types of cells. Exposure to DMSO sometimes causes unexpected changes in cell fates. Because mammalian development and cellular differentiation are controlled epigenetically by DNA methylation and histone modifications, DMSO likely affects the epigenetic system. The effects of DMSO on transcription of three major DNA methyltransferases (Dnmts) and five well studied histone modification enzymes were examined in mouse embryonic stem cells (ES cells) and embryoid bodies (EBs) by RT-PCR. Addition of DMSO (0.02-1.0%) to EBs in culture induced an increase in Dnmt3a mRNA level with increasing dosage. Increased expression of two subtypes of Dnmt3a in protein level was confirmed by Western blotting. Southern blot analysis revealed that DMSO caused hypermethylation of two kinds of repetitive sequences in EBs. Furthermore, Restriction Landmark Genomic Scanning (RLGS), by which DNA methylation status can be analyzed on thousands of loci in genic regions, revealed that DMSO affected DNA methylation status at multiple loci, inducing hypomethylation as well as hypermethylation depending on the genomic loci. In conclusion, DMSO has an impact on the epigenetic profile: upregulation of Dnmt3as' expression and alteration of genome-wide DNA methylation profiles with phenotypic changes in EBs.

PUBLICATIONS

Papers

1. Ko, YG, Nishino, K, Hattori, N, Arai, Y, Tanaka, S, Shiota, K (2005) Stage-by-stage change in DNA methylation status of Dnmt1 locus during mouse early development., **J. Biol. Chem.**, *280 (10)* 9627 - 9634

2. 岩谷美沙，大鐘潤，塩田邦郎(2005)発生・分化とエピジェネティクス，ゲノム医学，*5*，25 - 30

3. 塩田邦郎(2005)概論－生命科学の新たなパラダイム：エピジェネティスク，実験医学，*23*，2096 - 2099

4. 田中智, 塩田邦郎(2005)ＤＮＡメチル化情報からみた哺乳類胚発生と細胞分化, 実験医学, *23*, 2100 - 2106

E-d. Division of Applied Genetics Takashi Araki Group

RESEARCH ACTIVITIES

1-(1). Physiological roles of chromatin assembly factor-1 (CAF-1) in epigenetic control of meristem activity during post-embryonic development in plants

Takashi Araki[1,2], Mitsutomo Abe[1,3], Hidetaka Kaya[4], Yuya Ogawa, Masaomi, Kato, Tetsuji Kakutani, Kei-ichi Shibahara ([1]Graduate School of Science, Kyoto University, [2]Japan Science and Technology Agency, [3]PROBRAIN, [4] Genome & Drug Research Center, Tokyo University of Science)

Loss-of-function mutants of chromatin assembly factor-1 (CAF-1) in *Arabidopsis, fasciata (fas)*, show a wide variety of morphological abnormalities and unique defects in the expression of *WUSCHEL* and *SCARECROW* genes in meristems (Kaya *et al.*, Cell 104, 131-142. 2001). We found that transcriptional gene silencing (TGS) of endogenous *CACTA* transposons was released in a stochastic manner in *fas*, without decreasing global levels of DNA methylation. Other endogenous silent genes at different chromosomal sites, a DNA transposon *AtMu1* and a hypothetical gene *T5L23.26* at a heterochromatin knob, were also transcriptionally activated. Activation of the three different silent loci occurred non-concomitantly to each other. Furthermore, TGS of a silent ß-glucuronidase (GUS) transgene was also released randomly in *fas* plants, without apparent correlation with developmental abnormalities. Activated state of GUS was maintained during growth to produce clusters of cells expressing GUS. Analysis of patterns in cotyledons suggested that release from TGS may sometimes occur during endoreduplication and/or post-mitoptically. Based on these observations, we proposed a model that CAF-1 contributes to the stable inheritance of epigenetic states through multiple rounds of cell divisions. Defect of CAF-1 function explains the stochastic occurrence of pleiotropic morphological phenotypes observed in *fas* mutants (Ono *et al., Genes to Cells* 11, 153-162. 2006; issue cover).

1-(2). Physiological roles of anti-silencing function 1 (ASF1) in embryonic and post-embryonic development in plants

Takashi Araki[1,2], Ken-ichi Kurotani, Mitsutomo Abe[1,3], Hidetaka Kaya[4], Tetsuji Kakutani, Kei-ichi Shibahara ([1]Graduate School of Science, Kyoto University, [2]Japan Science and Technology Agency, [3]PROBRAIN, [4] Genome & Drug Research Center, Tokyo University of Science)

Arabidopsis has two genes for a histone chaperone, anti-silencing function 1 (ASF1), ASF1a and ASF1b. We are interested in physiological role of ASF1 in plant, especially functional overlap and differentiation between ASF1 and CAF-1. To address these questions, loss-of-function mutations of ASF1a, ASF1b, and CAF-1 (represented by *fas2* lacking the second largest subunit) are being analyzed in various combinations. Biochemical work of ASF1 is also being performed.

asf1a; asf1b double mutants were viable as are CAF-1 mutants, but with much more subtle abnormality in gross morphology. *asf1a; fas2* and *asf1b; fas2* had more severe morphological defect than *fas2* single mutant indicating the functional redundancy. Pollen formation was arrested at the tetrad stage causing complete male sterility in the double mutants suggesting that either meiosis or pollen maturation is severely perturbed. *asf1a; asf1b; fas2*/+ plants gave rise to about a quarter aborted seeds which are likely *asf1a; asf1b; fas2* triple homozygotes. Embryos in these seeds were arrested at the late globular stage. Therefore, in plants histone chaperon activity represented by CAF-1 or ASF1 is indispensable for completion of embryonic development.

1-(3). Genes involved in the integration of various genetic pathways for regulation of the floral transition

Takashi Araki[1,2], Mitsutomo Abe[1,3], Sumiko Yamamoto[1,2], Yasufumi Daimon[1], Ayako Yamaguchi[1], Yoko Ikeda[1], Michitaka Notaguchi[1], Masaki Kobayashi[1] ([1]Graduate School of Science, Kyoto University, [2]Japan Science and Technology Agency, [3]PROBRAIN)

Floral transition in *Arabidopsis* is regulated by several pathways which converge on the transcriptional regulation of floral pathway integrators

including *FT*. *FT* is a direct target of CO and encodes a protein with similarity to mammalian proteins (PEBP/RKIP) involved in cellular signaling. Function of *FT* as a potent promoter of flowering is conserved among various plant species (Kojima *et al., Plant Cell Physiol.* 43, 1096-1105. 2002; Endo, T. *et al.* 2005).

FT transcription was immediately induced in cotyledon and leaf vascular tissues upon transfer from short-day to inductive long-day photoperiods. We found that the promotion of flowering by *FT* requires the activity of another flowering-time gene *FD* which encodes a bZIP transcription factor preferentially expressed in the shoot apex. FT-FD protein interaction was observed in yeast cells, *in vitro*, and *in planta*. FD is involved in transcriptional activation of the floral meristem identity genes *AP1* and *CAL* redundantly with LFY. *ft; lfy* and *fd; lfy* double mutants were very similar in severe reduction of *AP1* mRNA levels and strong defects in floral specification. Loss of *FT* function expression suppressed ectopic up-regulation of *AP1* in seedlings by *FD* over-expression. Mutant forms of FD which lack a potential phosphorylation site at the C-terminus did not interact with *FT* in yeast cells and failed to complement *fd* late-flowering phenotype even by over-expression. These and other evidences suggest that *FT* and *FD* are inter-dependent in promotion of floral transition and activation of *AP1* expression. Since the activity of FD, which is preferentially expressed shoot apex, seems to require protein/protein interaction with FT, shoot apex is likely the site of action of FT protein. Consistent with this, restoration of *FT* function in the whole region or the outermost layer (L1) of the shoot apex rescued late-flowering phenotype of *ft*. Based on these observations we proposed a hypothesis that the *FT* gene products (protein and/or mRNA) represent a long-distance signal generated in photoperiodically-induced leaves (mainly in vascular tissues) and act at the shoot apex to initiate floral development (Abe *et al.*, 2005).

We also found the involvement of short-distance signal(s) in regulation of *FT* by phytochrome B (phyB) which senses light quality. Cell type-specific restoration experiments of phyB function demonstrated that phyB in mesophyll cells is essential for down-regulation of *FT* in phloem tissue. These suggest that phyB in mesophyll cell generate signal(s) which are transported to the phloem companion and parenchyma cells which is the site of *FT* expression (Endo, M. *et al.*, 2005).

Arabidopsis genome has a homolog of *FT*, called *TWIN SISTER OF FT (TSF)*. We demonstrated that *TSF* acts redundantly with *FT* as a floral pathway integrator. Interestingly, regions of expression of *FT* and *TSF* did not overlap (*FT* was expressed in cotyledons and leaves, whereas *TSF* was expressed in hypocotyls), although they shared the same tissue-specificity in phloem. Based on these and other observations we proposed a model in which various regulatory pathways of flowering are integrated in the phloem tissue to activate transcription of *FT* and *TSF*, products of which in turn act as the long-distance signals (Yamaguchi *et al.*, 2005).

1-(4). Molecular basis of late-flowering phenotype in epialleles of *FWA* in *Arabidopsis*

Takashi Araki[1,2], Yoko Ikeda[1], Mitsutomo Abe[1,3]
([1]Graduate School of Science, Kyoto University, [2]Japan Science and Technology Agency, [3]PROBRAIN)

Dominant late-flowering mutantation *fwa* is an "epigenetic mutation" that causes ectopic expression of *FWA* due to hypomethylation of direct repeats in the promoter (Soppe *et al., Mol. Cell* 6, 791-802, 2000). We have shown that *FWA* is not expressed in seedlings and that ectopically-expressed *FWA* inhibits floral transition by interfering with the *FT* function through protein interaction. Since FWA protein is a HD-ZIP protein which is likely a transcription factor, we further tested the possibility that FWA inhibits flowering through the transcriptional mis-regulation of its target genes. We performed microarray analysis using two *fwa* epialleles (*fwa-2* and *fwa-101D*) and 35S::*FWA*. The transcriptional profiles of these plants suggest that late-flowering phenotype of *fwa* is unlikely due to the mis-regulation of transcription. We also investigated the site of action of FT protein using FWA protein as a specific inhibitor of the *FT* function. *FWA* expressed in shoot apex by the *FD* promoter delayed flowering, whereas *FWA* expression in vascular tissues did not. These support the notion that shoot apex is the site of action of FT protein (Ikeda *et al.*, in preparation).

PUBLICATIONS

Papers

1. Abe, M., Kobayashi, Y., Yamamoto, S., Daimon,

Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K., Araki, T. (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex., **Science**, *309 (5737)* 1052 - 1059

2. Endo, M., Nakamura, S., Araki, T., Mochizuki, N., Nagatani, A. (2005) Phytochrome B in the mesophyll delays flowering by suppressing FLOWERING LOCUS T expression in Arabidopsis vascular bundles., **Plant Cell**, *17 (7)* 1941 - 1952

3. Endo, T., Shimada, T., Fujii, H., Kobayashi, Y., Araki, T., Omura, M. (2005)Ectopic expression of an FT homolog from Citrus confers an early flowering phenotype on trifoliate orange (Poncirus trifoliata L. Raf.)., **Transgenic Res.**, *14 (5)* 703 - 712

4. Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., Araki, T. (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT., **Plant Cell Physiol.**, *46 (8)* 1175 - 1179

Books

5. 大門靖史，阿部光知，荒木　崇，(2005)減圧浸潤法および花序浸し法によるシロイヌナズナの形質転換. 改訂3版，モデル植物の実験プロトコール　イネ・シロイヌナズナ編(植物細胞工学シリーズ　21)，(島本　功，岡田清孝，田畑哲之)，秀潤社，149 - 154

EDUCATION

1. 荒木　崇　生物科学特別講義Ⅳ　北海道大学・理学部
2. 荒木　崇　農学特別講義Ⅰ　近畿大学・農学部
3. 荒木　崇　第7回屋久島フィールドワーク講座　京都大学21COE・上屋久町
4. 荒木　崇　長距離花成シグナルの実体解明に向けて　北海道大学・理学部
5. 荒木　崇　シロイヌナズナのＦＴ遺伝子と長距離花成シグナル　近畿大学・農学部

SOCIAL CONTRIBUTIONS AND OTHERS

その他

荒木　崇　Abe et al. (2005) Science 309, 1052-1056.がScience (AAAS)の"Breakthroughs of the Year"の第3位(Blooming Marvelous)に選出.

F. GENETIC STRAINS RESEARCH CENTER

F-a. Mammalian Genetics Laboratory Toshihiko Shiroishi Group

RESEARCH ACTIVITIES

(1) A novel long-range enhancer to specify the Sonic hedgehog *(Shh)* expression in the oral cavity morphogenesis

Tomoko Sagai, Takanori Amano, Yoichi Mizushina, Hiromi Yamamoto, Noriko Sakurai-Yamatani, Ayaka Okagaki, Masaru Tamura and Toshihiko Shiroishi

Shh is a secreted glycoprotein that is essential for the fundamental morphogenesis in vertebrate embryogenesis. In developing embryos, *Shh* expresses in various organogenesis processes including head, lung, heart and limbs formation. Mutant mice in which normal *Shh* expression is affected in the organogenesis exhibit severe defects in the relevant organs. The *Shh* expression in a particular organogenesis is controlled by specific *cis*-acting regulatory elements. Recently, such several long-range enhancers have been identified by cross species comparison of genome sequences and exploration of conserved sequences among evolutionary remote species. A noncoding sequence, MFCS1, localized in the 1Mb upstream of the *Shh* coding sequence is one of such *cis*-acting elements, which includes limb bud-specific *Shh* enhancer (Sagai et al., 2005).

Here we report another novel long-range enhancer involving in morphogenesis of mouse oral cavity. MFCS4 is a phylogenetically conserved noncoding sequence residing in the 800kb upstream region of the *Shh* coding. In order to test whether the sequence acts as *cis*-acting regulatory element, first we carried out transgenic assay with a *LacZ*-reporter gene flanked by the MFCS4 fragment. In the transgenic mice, we found that MFCS4 induces expression of the reporter gene specifically in the oral epithelium of palate, epiglottis and arytenoid. Next, we conducted ES targeting to generate MFCS4 knockout (KO) mice. The mice heterozygous for the MFCS4 KO did not show any defect. By contrast, the KO homozygotes caused hypoplastic defects in the palate and epiglottis surrounding the naso-and oropharyngeal opening. Because of this defect, airway of the homozygotes was disturbed, and eventually it resulted in neonatal lethality.

In the oral cavity of wild-type embryos, endogenous *Shh* expression was detected in palatal and tongue epithelium and partially overlapped the expression domain of the reporter gene in the transgenic mice. Then, we examined the *Shh* expression in the MFCS4 KO homozygotes. Section *in situ* hybridization with a *Shh* riboprobe showed that in the MFCS4 KO homozygotes, *Shh* did not express in the oral epithelium where the signal of transgenic reporter gene was detected. Finally, in order to confirm that MFCS4 is a *cis*-acting element, we carried out a test cross of the *Shh*-coding KO and the MFCS4 KO mice. From the above cross, we generated compound heterozygotes of the both KO alleles. We observed that all compound heterozygotes exhibited the phenotype almost identical to that of the single MFCS4 KO homozygotes. Thus, all data demonstrated that MFCS4 is a *cis*-acting element including naso-and oropharyngeal specific long-range enhancer of *Shh*.

(2) Chromosomal dynamics of a long-range *cis*-regulator for limb bud-specific *Shh* expression

Takanori Amano, Tomoko Sagai, Hideyuki Tanabe[1] and Toshihiko Shiroishi ([1]The Graduate University for Advanced Studies)

MFCS1 (mammal-fish conserved sequence) is located at the intron 5 of *Lmbr1* locus, which is 1 Mb apart from the *Shh* coding sequence. We have reported that elimination of MFCS1 causes a defect of *Shh* expression and distal truncation of skeletal elements of mouse limb. It is suggested that MFCS1 is a limb bud-specific *cis*-acting regulatory element of *Shh* (Sagai et al., 2005). This fact raises a question of how MFCS1 controls a transcription of *Shh* beyond such a long distance. To address this question, we paid attention on higher-order chromosomal structure surrounding the *Shh* locus.

To examine topological relationship of the *Shh* coding region and MFCS1, we performed 3D-FISH analysis using hapten-labeled probes that hybridize with these two genomic regions. We dissected anterior, intermediate and posterior portions of developing limb buds at E10.5 embryos. The posterior portion of limb bud is referred to as zone of polarizing activity (ZPA) and it expresses *Shh* exclusively in wild-type mice. We immediately prepared single cell suspension from the each dissected specimen, and used for the FISH analysis. After capturing 3D images of the two fluorescent signals, physical distance between the *Shh* coding region and MFCS1 was computationally calculated. This result clearly indicated that the mean distance in the posterior limb bud cells was significantly shorter than that in the intermediate limb bud cells, suggesting that MFCS1 tends to approach the *Shh* locus in the *Shh*-expressing cells. Notably, the mean *Shh*-MFCS1 distance in the anterior limb bud cells was somewhat smaller than that in the posterior limb bud cells, but the value was close to that in the posterior limb bud cells. It is known that many mouse mutants exhibit ectopic *Shh* expression at the anterior margin of limb buds, and it leads to preaxial polydactyly. It is, therefore, conceivable that the anterior limb bud cell potentially has a competence to express *Shh*. Thus, adjoining of physically distant two regions, MFCS1 and the *Shh* coding, implies that the cells have a competence for expressing *Shh*.

To further investigate the chromatin dynamism surrounding the *Shh* coding and MFCS1 regions, we adopted a method of chromosome conformation capture (3C). Limb buds from E10.5 embryos, which express *Shh* at very high level, were used for the analysis. We used limb buds from E12.5 embryo, in which *Shh* expression already ceased, as a control. As a result, we captured an expected positive signal only in the limb buds from E10.5 embryos, suggesting that MFCS1 physically interacts with the *Shh* coding region in *Shh*-expressing cells.

(3) Molecular mechanisms of epithelium formation of skin and gastrointestinal tract in mice

Masaru Tamura, Shigekazu Tanaka, Tomoaki Fujii, Hiromitsu Komiyama[1] and Toshihiko Shiroishi
([1]Graduate School of Medicine, Juntendo University)

Epithelial cells are tightly connected to one another in sheets and tubes, and form epidermis and mucosal epithelium of gastrointestinal tracts. Homeostasis of the epithelial linings is controlled by the balance between proliferation of the stem cells and apoptosis following terminal differentiation. Disruption of this balance may cause abnormal cell proliferation and several diseases like as cancers. However, our knowledge on the molecular basis underlying the epidermis development and homeostasis is still limited. We have overcome this problem using the forward and reverse genetics approaches. We performed three fundamental studies: (i) Screening and phenotype analysis of mutant mice, which exhibit abnormal epidermal development; (ii) identification of causative gene of these mutant mice; (iii) analysis of these gene functions using the transgenic and gene knockout technique.

We identify five novel genes during the analysis of just one mutant mouse. Four of five these genes are clustering in the mouse chromosome 15, and these genes have several conserved sequences and novel functional motifs. Each of these genes is expressed in the epidermis and gastrointestinal tract (GI) in a tissue specific manner, and expression patterns and levels were depended heavily on proximal-distal axis in the GI tract. We are now examining the function of these genes using the reverse genetics approach.

(4) Transgenesis with a modified-BAC DNA harboring the *Rim3*-type *GsdmA3* mutation recaptures the mutant skin phenotype

Shigekazu Tanaka, Masaru Tamura, Aya Aoki[1], Tomoaki Fujii, Hiromitsu Komiyama[2], Toshihiko Shiroishi ([1]Department of Neurosurgery, Tokyo Metropolitan Hiroo Hospital [2]Graduate School of Medicine, Juntendo University)

In our previous study, we showed that a spontaneous dominant skin mutant, *Rim3*, has a point mutation in Gasdermin A3 *(GsdmA3)*. The *Rim3* mutation has a single base substitution, 1124 G to A, which results in a missense change, alanine 348 threonine. To confirm that the genetic alteration found in the *GsdmA3* gene causes the mutant phenotype, in this study we carried out transgenesis using a modified-BAC DNA containing the *Rim3*-type *GsdmA3* mutation (alanine 348 threonine). A BAC clone RP23-438D7 contains 160 kb genomic region that

includes *GsdmA* cluster except for *GsdmA1* and most likely contains the promoter and the *cis*-regulatory elements of *GsdmA3* gene within its 115 kb upstream fragment of *GsdmA3*. We introduced the *Rim3*-type mutation into the exon 9 of *GsdmA3* in the RP23-438D7 clone by means of BAC modification technology. The modified BAC construct was transferred into genome of wild-type (DBA/2J x C57BL/6J)F_1 mice. We selected transgenic mice by genotyping for the vector sequence, and established three transgenic founder lines carrying the *Rim3*-type *GsdmA3* mutation. We found that these all three lines exhibit skin phenotype similar to that of *Rim3*. Histological analysis revealed that the transgenic mice have degenerated hair follicles at 10 months of age as was observed in *Rim3* mutant mice. In contrast, transgenic mice that express wild-type *GsdmA3* did not exhibited any phenotype in the skin. The result clearly demonstrated that *GsdmA3* is the causative gene for *Rim3*.

(5) Disruption of genetic interaction between two autosomal regions and the X chromosome causes hybrid breakdown in a mouse intersubspecific cross

Ayako Oka, Toshihiro Aoto[1], Yoshikazu Totsuka[1], Riichi Takahashi[1], Masatsugu Ueda[1], Akihiko Mita, Noriko Sakurai-Yamatani, Hiromi Yamamoto Nobuo Takagi[2], Kazuo Moriwaki[3] and Toshihiko Shiroishi
([1]The YS Institute, Inc., [2]Hokusei Gakuen University, [3]RIKEN BioResource Center)

Reproductive isolation that initiates speciation is likely caused by incompatibility between multiple loci in organisms belonging to genetically diverging populations. Laboratory C57BL/6J mice, which predominantly originated from *Mus musculus domesticus*, and a MSM/Ms strain derived from Japanese wild mice (*M. m. molossinus*) are reproductively isolated. These two strains exhibit hybrid breakdown: F_1 hybrids are fertile, but succeeding intercrosses result in male sterility. A consomic strain, C57BL/6J-ChrX^{MSM}, which carries the X chromosome of MSM/Ms in the C57BL/6J background, shows male sterility, suggesting genetic incompatibility of the MSM/Ms X chromosome and other C57BL/6J chromosome(s). In this study, genome-wide linkage analysis and subsequent QTL mapping successfully detected significant QTLs on chromosomes 1 and 11 that interact with the X chromosome and are responsible for the hybrid breakdown. This was confirmed by the observation that introduction of the MSM/Ms chromosomes 1 and 11 into the C57BL/6J-ChrX^{MSM} background partially restored fertility. Thus, genetic interactions between the two autosomal regions and the X chromosome have a crucial role in proper sperm differentiation, and their disruption causes hybrid breakdown. Intracytoplasmic sperm injection and zona-free *in vitro* fertilization showed that the C57BL/6J-ChrX^{MSM} spermatozoa have a defect in penetration of the extracellular zona pellucida. Moreover, two-dimensional electrophoretic proteome study of whole proteins from sperms provided considerable candidates responsible for the sterility of C57BL/6J-ChrX^{MSM} males.

(6) QTL analysis of hereditary predisposition of obesity based on inter-subspecific cross of C57BL/6J and MSM/Ms strains

Akiteru Maeno, Hideko Watanabe, Akihiko Mita, Ayako Oka and Toshihiko Shiroishi

Obesity is a genetically complex trait, and more than hundreds of potential loci have been identified so far. In order to elucidate the genetic determinants of this disease, we are taking approach to use mouse models for heritable obesity. In this study, we conducted genetic analysis of obesity-related quantitative traits based on the cross between a standard laboratory mouse strain C57BL/6J (B6) and a Japanese wild mouse-derived strain MSM/Ms (MSM). The quantitative traits that we analyzed in this study are followings: body weight, body weight that excludes total fat pads weights (lean body weight), percentage of the weight of each of fat pad surrounding three visceral tissues (gonad, retroperitoneum and mesenterium) to lean body weight, percentage of the total weight of the above three visceral fad pads to lean body weight, percentage of total subcutaneous fad pads weight to lean body weight and total fat pads weights to lean body weight (adiposity index: A.I.).

MSM has higher A.I. value than that of B6 at ten weeks of age, and this tendency became more prominent as age proceeded. This suggests that MSM shows higher predisposition to obesity as compared with B6. For the quantitative trait loci (QTL) analysis, we crossed B6 and MSM mice and generated F_1 hybirds, and subsequently intercrossed the F_1 mice and

obtained totally 272 F_2 progeny. Then, we carried out QTL analysis with the F_2 progeny at 10 weeks of age to identify loci responsible for eight obesity-related traits described above. First, single-locus genome scans analysis identified four QTLs on Chr 2, 6, 9 and 13, all of which control A.I. value. All these QTLs showed sex-dependent effect. For the three QTLs on Chr 6, 9 and 13, MSM alleles increase A.I. value, while B6 allele increases the A.I. value for the QTL on Chr 2. Because all four QTLs explain relatively small proportion of the variance observed in the F_2 progeny (only 9 to 11% of the total variance), many other minor QTLs and epistatic interaction of two independent loci were expected to be contributed to the remaining genetic variance. Therefore, we implemented pairwise genome scan analysis for the same data set of the 272 F_2 progeny. As a result, we identified a significant epistatic interaction between two loci on Chr 9 and 13.

QTL analyses with Multiple Interval Mapping (MIM) and Bayesian Interval Mapping (BIM) are thought to be more suitable for the cases in which multiple QTLs are responsible for complex traits. We need further studies using the above analyses to reveal hidden QTLs that were not detected in this study.

(7) Genetic dissection of obesity using mouse consomic strains

Toyoyuki Takada, Akiteru Maeno and Toshihiko Shiroishi

Obesity is a common disease and a major risk to develop type 2 diabetes mellitus and metabolic syndrome. Genetic obesity is generally thought to be multifactorial disease, which is caused by multiple genetic determinants and influence of environmental factors. To understand pathology of obesity, genetic analysis of responsible genes for this complex trait has been prominent issue as in many common human diseases. We are taking approach to use mouse models for obesity. C57BL/6J-Chr^{MSM} consomic strains were constructed by transferring individual chromosomes from MSM/Ms strain, derived from Japanese wild mouse, *Mus musuculs molossinus*, into the genetic background of a standard laboratory strain C57BL/6J, which was derived from West European wild mouse, *M. m. domesticus*. These consomic strains are strikingly useful for chromosome mapping of various phenotypes, because above two mouse strains showed extremely large extent of phenotypic variations based on vast amount of genomic differences (~ 1% SNPs). Our preliminary phenotyping of the consomic strains indicated a variety of range in susceptibility or resistance to obesity or obesity-related phenotypes among the strains. Based upon the strain difference, we intend to explore genes and genetic pathways underlying the polygenic obesity. At the first step, now we are conducting more detailed characterization of the phenotype of each consomic strain using following tests: 1) measurement of consumption of foods (normal chaw and high fat diet, etc.) and body weight, 2) detection of metabolism-related parameters (activity, body fat content, blood and hepatic lipid content, plasma insulin and leptin levels, etc), 3) radiographical measurement of adipose tissue mass using computer tomography (CT) scanning, 4) transcriptome analysis of metabolism-related tissue of all consomic strains.

PUBLICATIONS

Papers

1. Ogasawara, M., Imanishi, T., Moriwaki, K., Gaudieri, S., Tsuda, H., Hashimoto, H., Shiroishi, T., Gojobori, T., Koide, T.(2005)Length variation of CAG/CAA triplet repeats in 50 genes among 16 inbred mouse strains, **Gene**, *349*, 109 - 117

2. Sagai, T., Hosoya, M., Mizushina, Y., Tamura, M., Shiroishi, T. (2005) Elimination of a long-range cis-regulatory module causes complete loss of limb-specific Shh expression and truncation of the mouse limb, **Development**, *132 (4)* 797 - 803

3. Sakai, T., Kikkawa, Y., Miura, I., Inoue, T., Moriwaki, K., Shiroishi, T., Satta, Y., Yonekawa, H. (2005) Origins of mouse inbred strains deduced from whole-genome scanning by polymorphic microsatellite loci, Mammal. **Genome**, *16 (1)* 11 - 19

4. Taguchi, Y., Koide, T., Shiroishi, T., Yagi, T. (2005) Molecular evolution of cadherin-related neuronal receptor/protocadherin (alpha) (CNR/Pcdh (alpha)) gene cluster in Mus musculus subspecies, **Mol Biol Evol**., *22 (6)* 1433 - 1443

5. 城石俊彦(2005)高次形質の遺伝基盤　マウスのゲノム多型から体質関連遺伝子を探る, 蛋白質・核酸・酵素, *50 (16)* 2184 - 2190

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動
城石俊彦　遺伝学会企画集会幹事
城石俊彦　実験動物学会評議委員
城石俊彦　Editorial boad of Mammalian Genome
城石俊彦　Editor of Gene & Genetic Systems
城石俊彦　"The Scientific World" Associate editor for Genetics

その他
城石俊彦　文部科学省科学技術政策研究所科学技術動向センター専門調査員
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城石俊彦　日本学術振興会　ゲノムテクノロジー第164委員会委員
城石俊彦　日米科学技術協力事業　組み換えDNA研究計画委員会委員
城石俊彦　(財)順天堂災害医学研究所　理事

F-b. Mammalian Development Laboratory Yumiko Saga Group

RESEARCH ACTIVITIES

(1) Molecular mechanism of somite segmentation

Mitsuru Morimoto, Makoto Kiso and Yumiko Saga

The somite is the first morphologically distinct segmental unit formed in a vertebrate embryo and give rise to metameric structures such as vertebrae, ribs and skeletal muscles. A 'clock and wavefront' model has been proposed to explain the underlying mechanism, in which the periodicity is generated by a 'clock' in the posterior PSM and this temporal periodicity is then translated into the segmental units in the 'wavefront'. The wavefront is thought to exist in the anterior PSM and progress backwards at a constant rate. Mesp2 plays a crucial role in translation of the temporal oscillation of Notch activity into the regularly-spaced somite (Morimoto et al. 2005).

Somitogenesis is not only an attractive example of metameric pattern formation but is also a good model system for studies of morphogenesis, particularly epithelial-mesenchymal interconversion in vertebrate embryos. Mesp1 and Mesp2 are homologous bHLH transcription factors that are co-expressed in the anterior presomitic mesoderm (PSM) just prior to somite formation. Analysis of possible functional redundancy of Mesp1 and Mesp2 has been prevented by the early developmental arrest of Mesp1/Mesp2 double-null embryos. We performed chimera analysis using either Mesp2-null cells or Mesp1/Mesp2 double-null cells, to clarify (1) possible functional redundancy and the relative contributions of both Mesp1 and Mesp2 in somitogenesis and (2) the cell autonomy of Mesp functions in several aspects of somitogenesis. Both Mesp2-null and Mesp1/Mesp2 double-null cells fail to form initial segment borders and to acquire rostral properties, confirming that the contribution of Mesp1 is trivial in these aspects. In contrast, Mesp1/Mesp2 double-null cells contribute to neither epithelial somite nor dermomyotome formation while Mesp2-null cells partially contribute to incomplete somites and dermomyotome. This indicates that Mesp1 has a significant role in the epithelialization of somitic mesoderm (Takahashi et al. 2005).

(2) Regulation of *Mesp1* and *Mesp2* expression

Masayuki Oginuma, and Yumiko Saga

Mesp1 and *Mesp2* encoding bHLH-type transcription factors Mesp1 and Mesp2, locate close to each other on chromosome 7. The both genes are co-expressed in the anterior presomitic mesoderm (PSM) just prior to somite formation. In our previous study, we identified the enhancer for the PSM in the 185bp upstream region of Mesp2, and called it the P2PSME (Mesp2 PSM enhancer). To identify global transcriptional regulation of both *Mesp1* and *Mesp2*, we conducted enhancer analyses using BAC transgenic system. Using BAC recombination system, we first constructed a *BAC-P1AP-P2Z* transgenic vector that has inserted *Alp* gene in *Mesp1* locus and inserted *nlacZ* gene in *Mesp2* locus. *BAC-P1AP-P2Z* transgenic mouse showed similar expression pattern to that of endogenous *Mesp1* and *Mesp2*. Next, we constructed the *BAC-P1AP-P2ZΔ (P2PSME)* vector which lacked *P2PSME* from the *BAC-P1AP-P2Z* vector. Transgenic (TG) embryos that have *BAC-P1AP-P2ZΔ (P2PSME)* did not express *Mesp2* in the PSM but the *Mesp1* expression was intact. We also identified the enhancer for the PSM in the 4kb upstream region of *Mesp1*, and

called it the *P1PSME* (*Mesp1* PSM enhancer). To know the enhancer specificity, we constructed a *BAC-P1AP-P2ZΔ (P1PSME)* which lacked *P1PSME* from the *BAC-P1AP-P2Z* vector. TG embryos that have *BAC-P1APP2ZΔ (P1PSME)* did not express *Mesp1* in the PSM but the *Mesp2* expression was intact. These results indicate that *Mesp1* and *Mesp2* use different enhancers for the expression in the PSM. By the comparative analysis of these enhancer sequences, we identified conserved motives for several transcription factors.

(3) Search for target genes of Mesp2 transcription factor

Yoshiro Nakajima and Yumiko Saga

Somites provide the basic body plan for metameric axial structures in vertebrates and establish the segmental features thorough the sequential gene expression in the presomitic mesoderm (PSM). A critical gene for segment border formation is the bHLH transcription factor Mesp2, the expression of which is restricted to the anterior PSM. A gene candidate that is activated by Mesp2 is *EphA4*, since its expression pattern resembles *Mesp2* and is absent in Mesp2-null embryos. We have analyzed the enhancer region of *EphA4*, which is responsible for its expression in the anterior PSM, and identified an E-box containing region. Subsequent transgenic and transient luciferase analyses successfully determined that the presence of repeated E-box sequences is a minimum essential requirement for faithful *EphA4* expression. We also show that Mesp2 directly binds the enhancer sequence of *EphA4*. Furthermore, the forced expression of Mesp2 in somitic cells results in the activation of *EphA4* and repression of the caudal gene *Uncx4.1*, which may trigger the events leading to the formation of abnormal somites and rostralized vertebra. In addition, ectopic *Mesp2* expression induces abnormally epithelialized structures, which support to the idea that Mesp2 induces the formation of segmental borders by activating genes, which play roles in cellular epithelialization.

(4) Functional analyses of Hesr1 and Hesr2 in the cardiovascular development

Hiroki Kokubo, Raul VizcardoSakoda, and Yumiko Saga

Establishment of atrial and ventricular chamber specificity is initial requirement for complex cardiac morphogenesis and function of the heart. We have shown the *hesr1* and *hesr2* genes, which encode the *Hairy and enhance of split (Hes)*-related bHLH transcription repressors, are required for the normal cardiovascular development (Kokubo et al. 2005). They are expressed specifically in the atria or the ventricle, implicating to function in the chamber specification. We found that the forced expression of *Hesr1* or *Hesr2* in the entire cardiac lineage resulted in the reduction or loss of the atrioventricular (AV) canal. In the *Hesr1*-activated embryos, gene expressions demarcating boundaries between chambers and AV canal appeared to be vague on either side of atria and ventricle, and the expression of specific marker for AV myocardium, *Bmp2* or *Tbx2*, was weakened or undetected. Similarly in the *Hesr2*-activated embryos, gene expression revealed as like that the atrium and ventricle seems to be directly connected, and neither expression of *Bmp2* nor *Tbx2* was detected in the AV canal region. These data suggest that *Hesr1* and *Hesr2* are involved in the AV boundary formation.

(5) Analyses toward understanding the function of Protein O-fucosyltransferase1 in the Notch signaling

Yoshiaki Okamura, and Yumiko Saga

Notch signaling is an evolutionarily conserved signaling pathway, which plays key roles in regulating cell fate decisions and morphogenesis. The glycosylation of Notch receptor, especially O-fucosylation by Protein O-fucosyltransferase1 *(Pofut1)*, is indispensable for signal activation. However, it remains unclear how the Pofut1 acts on Notch signaling in mouse embryogenesis. Using conditional strategy in the mouse, we could delete *Pofut1* alleles specifically in cardiovascular system, which showed the defects in the cardiogenesis including valve formation. Moreover, we have already shown that the forced expression of the activated Notch1 (actN1) in cardiovascular system causes the myocardial defect. Taking advantage of this system, we have examined the epistasis between Pofut1 and actN1 on Notch signaling. Both in the control and Pofut1 mutant embryos with forced expression of actN1, the myocardial defect was observed. This result suggests that the Pofut1 acts upstream of the actN1.

(6) Analyses of nanso2 expression and function during mouse germ cell development

Atushi Suzuki, Kaoru Mitsui, and Yumiko Saga

Mouse nanos proteins, nanos2 and nanos3 are required for germ cell development and both proteins share highly conserved zinc finger domain. However, the expression pattern during development is different each other. nanos3 expression starts in the primordial germ cell (PGC)s just after their formation and the lack of this protein results in germ cell-less phenotype in both sexes. However, nanos2 expression starts in the male PGC only after entering to the genital ridge and the lack of nanos2 results in the lack of male germ cells irrespective of the co-expression of nanos3. These results indicate that these proteins have distinct function depending on the time and place of their expression. We addressed the question by producing transgenic mouse lines which express nanos2 under the control of Oct4 ΔPE promoter and examined the nanos2 function in the nanos3-null genetic background. The ectopically produced nanos2 protein rescued the lack of nanos3 and germ cells developed in both sexes. This result indicates that the nanos2 can substitute nanos3 function in the early PGC development.

(7) Functional analysis of nanos3

Hitomi Suzuki, Makoto Kiso and Yumiko Saga

nanos3 is one of *nanos* genes essential for germ cell development. *nanos3* expression starts in PGCs (primordial germ cells) just after their formation and continues during migration until reaching the gonads. In the *nanos3*-null mice, the number of PGCs rapidly decreased during their migration, indicating that *nanos3* is responsible for the maintenance of PGCs rather than their generation. There are two possibilities for the loss of PGCs, apoptotic cell death or abnormal differentiation to somatic cells. By the immunostaining, we detected apoptosis in migrating PGCs of *nanos3*$^{-/-}$ mice, which prompted us to rescue the loss of PGC by preventing apoptosis in PGCs. We have then generated double knockout mice of nanos3 and a pro-apoptotic factor Bax that is known to be involved in the germ cell development. Although adult *nanos3*$^{-/-}$*bax*$^{-/-}$male mice were sterile and showed the similar phenotype to *bax*$^{-/-}$male mice, they had germ cells that were never seen in *nanos3*$^{-/-}$male mice. The adult *nanos3*$^{-/-}$*bax*$^{-/-}$female mice had ovaries which contained morphologically normal oocytes although the number was considerably low. In the *nanos3*$^{-/-}$*bax*$^{-/-}$embryo, a few PGCs reached gonads and were maintained until adulthood. These observations suggest that one reason for PGC loss in *nanos3*$^{-/-}$mice is BAX-dependent apoptosis but nanos3 is responsible for the other pathway to maintain germ cells.

PUBLICATIONS

Papers

1. Kokubo H, Miyagawa-Tomita S, Saga Y, Johnson RL. (2005) Mouse hesr1 and hesr2 genes are redundantly required to mediate Notch signaling in the developing cardiovascular system, **Dev. Biol.**, *278*, 301 - 309
2. Morimoto M, Takahashi Y, Endo M, Saga Y (2005) The transcription factor Mesp2 establishes segmental borders by suppressing Notch activity., **Nature**, *435*, 354 - 359
3. Takahashi, Y., Kitajima S, Inoue T, Kanno, J., Saga Y (2005) Differential contributions of Mesp1 and Mesp2 to the epithelialization and rostrocaudal patterning of somites., **Development**, *132*, 787 - 796

Books

4. 相賀裕美子 (2005) Notchシグナルの多様性, 実験医学(増刊), 「発生・分化・再生研究2005」, *23*, 64 - 72

F-c. Mouse Genomics Resource Laboratory (MGRL) Tsuyoshi Koide Group

RESEARCH ACTIVITIES

(1) A systematic analysis of genetic factors associated with the spontaneous activity in mice

Akinori Nishi, Toshihiko Shiroishi[1] and Tsuyoshi Koide ([1] Mammalian Genetics Laboratory)

A great variation of behavioral pattern is observed in a variety of animals. A number of studies indicated that the behavioral diversity is greatly influenced by genetic factors. The aim of our study is to identify the genetic factors associate with the diversity of behavioral traits.

In order to study genetic mechanism associate with the behavioral diversity, we are using mouse strains, C57BL/6, which is mostly derived from *Mus musculus domesticus* and MSM, which belongs to *M. m. molossinus*, and the consomic strains. The consomic strains were established by replacing each chromosome of C57BL/6 by corresponding chromosome of MSM. These strains are expected as powerful tools for investigating complex traits, which are influenced by multiple genetic factors. In the result of systematic test battery on the consomic strains, we found that B6-6CMSM consomic strain, which carries MSM-derived chromosomal segment from D6Mit166 to D6Mit12 on chromosome six, exhibited significantly lower activity in the home cage test and the novel home cage test, and indicated higher anxiety related behavior in the light/dark box test comparing to C57BL/6.

To identify the quantitative trait loci (QTLs) associated with the behavioral differences between C57BL/6 and B6-6CMSM, I performed the QTL analysis by use of the F2 population established between these two strains. In the result of QTL analysis, I detected the several QTLs. The QTL located on centromere side mainly associated with general activity and emotionality related traits and the QTL located on telomere side associated with general activity.

(2) Genetic analyses of psychological traits using consomic mouse strains of C57BL/6 and MSM

Aki Takahashi, Akinori Nishi, Toshihiko Shiroishi[1], Tsuyoshi Koide ([1]Mammalian Genetics Laboratory)

It is thought that genetic variation that underlies emotionality is complex and is regulated by loci that have quantitative effect on the phenotype. We have been examining to reveal those genetic mechanisms by using B6-MSM consomic mouse strains. It is known that the laboratory strain C57BL/6 (B6) and the wild-derived strain MSM have a wide genetic diversity and behavioral differences. B6-MSM consomic strains have same genetic background as B6 except for one chromosome from MSM. By examining bunch of consomic strains, it was found that over a half of consomic strains changed anxiety-related behavior from B6. Detailed observations of behavior in the open-field, assumed as anxiety-evoked situation, revealed that some of consomic strains have specific characters. Such as, B6-3MSM and B6-9MSM showed higher locomotion in the open-field than B6, and B6-3MSM exhibited jumping behavior and characterized by vertical movement while B6-9MSM never showed jumping and tend to avoid central part of the open-field. This result suggested that many genetic components that have variety effect associated to anxiety-related behavior. Also, we performed social interaction test in those consomic mouse strains, and found some chromosomes associated with social or aggressive behavior.

One strain B6-17MSM, which have MSM derived chromosome 17, showed reduced locomotion in the novel situation, increased fear responses, and risk-assessment behavior, and thus it is thought that there is a locus/loci related to the emotionality. To identify the gene/genes on the chromosome 17, we are currently making subconsomic strains that have narrower part of chromosome 17 from MSM. By analyzing some of those subconsomic strains, it has been suggested that a locus/loci is in the telomeric region.

(3) Genetic factors responsible for difference in spontaneous home-cage activity between KJR and C57BL/6J

Juzouh Umemori and Tsuyoshi Koide

It has been previously reported that mice move spontaneously in the habituated home-cage according to a light/dark rhythm, and that most mice are active during the dark period. This kind of activity is called as spontaneous home-cage activity. In a variety of strains, mice of C57BL/6J were relatively hypoactive, in contrast to mice of KJR those were especially hyperactive in the habituated home-cage. We studied the spontaneous home-cage activity in terms of the ethological components and biological functions.

F2 progeny (BKF2) were made between C57BL/6J and KJR strains to be analyzed for the ethological and genetic studies. Ethological studies showed that total home-cage activity (THA) could be divided to two independent components, active time (AT) that indicated temporal elements of spontaneous home-cage activity, and average activity (AA) that indicated quantitative elements. A little correlation between AT and AA suggested that AA and AT are independent measurement.

In order to study the genetic bases responsible for the differences in spontaneous locomotive activity between KJR and C57BL/6J, we conducted quantitative trait loci (QTL) analysis using BKF2 progeny. QTL analysis showed three significant QTLs associated with the spontaneous home-cage activity. These QTLs were designated as *hyperlocomotive activity related QTL1 (Hylaq1), Hylaq2 and Hylaq3.* All *Hylaq* loci controlled THA that was defined by summing up effects of the AT and the AA. *Hylaq1* was located in a middle region of chr2 and mainly controlled activity time. *Hylaq2* was located on the distal side of *Hylaq1* on chr2 and controlled both of AT and AA. *Hyaq3* was located near the telomeric region on chr10, and controlled mainly AA. In order to analyze the single gene effect more precisely, we are currently making congenic strains that carry KJR allele at Hylaq loci in BLG2 background. They will be used to conduct further fine mapping and phenotypic analyses. We have currently made N5 generation of the congenic strains.

It has been reported that dopamine (DA) system in basal ganglia in brain is associated with movement and spontaneous locomotive activity. To search for candidate genes in the above QTL region, and to understand the biological mechanism that is associated with the spontaneous home-cage activity, strain-comparative analyses on the DA system have been conducted with pharmacological and biochemical methods. The pharmacological study suggested that the function of D1-like DA pathway might be reduced in KJR while that of D2-like pathway might be highly suppressive for the activity only in C57BL/6J. Expression analyses and microdialysis analyses indicated that there was no significant difference in DA synthesis, release and uptake in presynaptic dopaminergic neuron in striatum. These results suggested that C57BL/6J and KJR have difference in the downstream pathways following activation of the DA receptors.

PUBLICATIONS

Papers

1. Esumi, S., Kakazu, N., Taguchi, Y., Hirayama, T., Sasaki, A., Hirabayashi, T., Koide, T., Kitsukawa, T., Hamada, S., Yagi, T. (2005) Monoallelic yet combinatorial expression of variable exons of the protocadherin-a gene cluster in single neurons., **Nature Genetics**, *37*, 171 - 176

2. Ogasawara, M., Imanishi, T., Moriwaki, K., Gaudieri, S., Tsuda, H., Hashimoto, H., Shiroishi, T., Gojobori, T., Koide, T. (2005) Length variation of CAG/CAA triplet repeats in 50 genes among 16 inbred mouse strains., **Gene**, *349*, 107 - 119

3. Taguchi, Y., Koide, T., Shiroishi, T., Yagi, T. (2005) Molecular evolution of Cadherin-related neuronal receptor / protocadherin α (CNR/Pcdhα) gene cluster in Mus musculus subspecies., **Molecular Biology and Evolution**, *22*, 1433 - 1443

EDUCATION

1. 小出剛　野生マウスを用いた行動遺伝学　岡山理科大学
2. Koide, T.　System genetics: Toward identification of genetic factors for behavioral diversity in mice University of Strasbourg, France
3. Koide, T.　System genetics: Toward identification of genetic factors for behavioral diversity in mice University of Zurich, Swiss
4. 小出剛　野生由来マウス系統の遺伝的多様性を活用した行動の遺伝学的解析　長岡科学技術大学
5. 小出剛　マウス行動多様性をもたらす遺伝的基盤の解明に向けて　国立遺伝学研究所公開講演会　京都

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

小出剛，山元大輔　第77回日本遺伝学会大会ミニシンポジウム　行動遺伝学の最前線　東京

森裕司，小出剛　国立遺伝学研究所研究会「動物行動の遺伝学」　三島

特許

特願2005-312366，髄鞘形成異常モデルマウス，小出剛・湯浅茂樹・梅森十三，大学共同利用機関法人情報・システム研究機構

特願2005-310000，温痛感覚を調節する蛋白EH4，鈴木誠・小出剛，学校法人自治医科大学

F-d. Model Fish Genomics Resource
Noriyoshi Sakai Group

RESEARCH ACTIVITIES

(1) Gene targeting system with RNA interference (RNAi) in zebrafish

Minori Shinya, Kimiko Saka, Kayo Kobayashi and Noriyoshi Sakai

Gene silencing via small interfering RNAs (siRNAs) has proved to be a useful tool in studying gene function in plants, invertebrates and mammalian systems. To date, gene silencing effects of siRNAs have confirmed in the zebrafish, which is an emerging model for developmental and diseases analysis. However, the effects were temporal (only in early developmental stages) and sometimes mosaic in an embryo because of the method injecting siRNAs into the one-cell stage of embryos. We recently succeeded in the production of transgenic zebrafish from *in vitro*-cultured sperm. The advantage of this technique is that the mosaicism inherent in other conventional transgenic methods is avoided. Our aim in the present study is the establishment of a rapid system with cultured sperm to generate transgenic zebrafish for gene silencing by siRNA.

As targeted genes, *sonic hedgehog (shh), signal transducers and activators of transcription 3 (stat3)* and *green fluorescent protein (gfp)* were selected because of their clear silenced phenotypes. To determine the best sequence of siRNA for the specific suppression of each gene, we planned two kinds of assays: one is the injection of siRNA into one-cell stage embryos and the other is the transfection of siRNA to the zebrafish cultured cells. In the first assay, we tested four sequences of siRNA for *shh*, two for *stat3* and one for *gfp*. Northern blot analyses of the siRNA-injected embryos revealed that one siRNA for *shh* (sishh) and one for *gfp* (sigfp) suppressed each transcript efficiently. However, sishh-injected embryos showed not only the *shh* null mutant phenotype but also the malformed development and cell death. Further analyses e.g. precise dosage test, are required. In contrast, sigfp-injected embryos were developed normally. In addition, sigfp injection gave us an insight in the effective time of siRNA in zebrafish embryos. Northern blot analysis of sigfp-injected embryos in every two hours revealed that sigfp suppressed the transcript only for 2-4 hours. The result suggests that stable expression of a short hairpin RNA(shRNA) would be required for efficient and constant gene silencing in zebrafish. We are currently working on the construction of a plasmid DNA encoding a shRNA which is expected to generate sigfp *in vivo* through processing by Dicer, double strand RNA-specific endonuclease. Using retroviral system and the shRNA construct, we will produce the cultured sperm with foreign DNA encoding the shRNA.

The second assay, the transfection of siRNA to the zebrafish cultured cells, is easier and faster than the injection to embryos. Thus, the establishment of this assay will be a great help to build a high-throughput system for genome-wide loss-of-function studies. For the assay, we established the zebrafish cultured cells expressing *shh* and *stat3*, and are trying to isolate the cell line expressing GFP stably. As there was no method for the efficient transfection to the zebrafish cultured cells (less than 1% transfection efficiency), several known transfection methods were tested in various condition. So far, we found to achieve 30-40% introduction of foreign DNA into the cells by some electroporation protocols.

(2) Analysis of functionally distinctive testicular cell lines of zebrafish to support male germ cell development

Aki Masuda and Noriyoshi Sakai

Sertoli cells are important to germ cells in everything from male sex-determination to spermatogenesis. In spermatogenesis, Sertoli cells interact directly with germ cells in the testis to induce the complex process required for the production of functional sperm. These cells mediate the production of many molecules as well as cell junctions and adhesion. The function of many of these molecules and the regulation of gene expression remain unclear. We recently established two testicular cell lines of zebrafish with distinct functions to support the development of male germ cells.

The lines, ZtA6-2 and ZtA6-12, showed almost the same characteristics as Sertoli cells, but exhibited distinctive features when male germ cells were co-cultured with each line as feeders: the function of the ZtA6-2 cells was directed to stimulate the

proliferation of spermatogonia, and ZtA6-12 to stimulate the differentiation into sperm.

These cell lines facilitate investigation of Sertoli cell molecules that contribute to the proliferation and differentiation of spermatogonia. The microarray analysis for approximately 20,000 genes showed that 45 genes were expressed 3 times higher in ZtA6-2 cells than ZtA6-12 cells, and that 156 genes in ZtA6-12 cells than ZtA6-2 cells. Interestingly, several steroid-related genes were found in up-regulated genes of ZtA6-12 cells. In a zebrafish testis, Sertoli cells constitute a cyst structure in which single A-type spermatogonium develops and differentiates synchronously. This characteristic makes it easy to determine the stages of Sertoli cells corresponding to the stages of germ cells. To investigate the stage specific expression of these genes in Sertoli cells, we developed a protocol for frozen sections of a zebrafish testis. The section kept the testicular structure enough to determine each developmental stage of germ cells. *In situ* hybridization of the profiled cDNAs to the frozen sections is under investigation.

(3) Culture condition for zebrafish spermatogonial stem cells

Kenji Saito and Noriyoshi Sakai

In zebrafish, a culture system using primary cultures of male germ cells on a Sertoli cell line, in which the differentiation from spermatogonia to functional sperms can occur *in vitro*, allows us to generate transgenic zebrafish lines rapidly through a simple *in vitro* fertilization using transfected sperm. However, the proliferation of spermatogonial stem cells (SSCs) was not observed under the culture conditions. Here, we studied the enrichment procedure of SSCs in zebrafish testis by *in vivo* treatment with busulfan reagent, and a culture condition to proliferate SSCs with newly isolated Sertoli cell line as feeder cells.

When zebrafish were soaked in busulfan solution for 24 hr, we observed decline in the amount of differentiating germ cells, such as spermatocytes and spermatids, and increase of SSCs on day 4 after treatment. Germ cells in the testis consisted of only SSCs and sperm on day 7. After 14 days, type B spermatogonia appeared, and after 1 month the testis became normal and the fish became fertile. When we used enzymatically dissociated cells of busulfan-treated testes on day 7, the germ cells proliferated on newly isolated Sertoli cell line, ZtA6-6. Morphology of the germ cells resembles to the SSC of paraffin section, and did not change during the culture. Furthermore, the cells expressed a germ cell marker gene *vasa*, and un-synchronous proliferation of the germ cells was observed by BrdU incorporation experiments. These results indicate that SSCs of zebrafish proliferate under these culture conditions. Growth of the SSCs did not seem to recover the amount of lost cells at re-plating every 7 days, however, we were not able to maintain the SSCs more than 1 month. Towards better culture conditions, we are now investigating the effect of growth factors on the proliferation of zebrafish SSCs, such as glial cell line-derived neurotrophic factor (GDNF) which is an essential growth factor for mouse SSCs, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1).

(4) Analysis of the ability of cultured embryonic cells derived from different developmental stages to induce the anterior-posterior axis.

Megumi Hashiguchi, Minori Shinya and Noriyoshi Sakai

One of the most fascinating phenomena in primary embryonic induction is the regional specificity of the neural structures that are produced. Primary embryonic induction can be divided into three major components: head specific, trunk specific and tail specific induction.

We established the culture condition for zebrafish embryonic cells that continuously proliferate without any artificial immortalization treatment. When cultured cells were implanted into a blastula embryo (3.5 hours post fertilization, hpf), the cells from different developmental stages showed different abilities to induce specific second axes. Cells derived from the early gastrula stage (6 hpf) induced complete anterior structure. Cells from the pharyngula stage (embryos segmented, 24 hpf) also induced anterior structures with cyclopia or two eyes. However, cells from later stages (protruding mouth larva, 72 hpf, and swimming larva, 120 hpf) induced posterior structure with otic vesicles and a heart. Interestingly, cells from later stages had low induction efficiency, but the efficiency increased when proliferation of the cells was arrested

by mitomycin C. Whole-mount *in situ* hybridization for *emx-1* (telencephalon marker), *krox 20* (rhombomere marker), and *shh* (notochord marker) indicated appropriate gene expression in the observed specific structure of induction. Cultured cells derived from embryos at various developmental stages, therefore, change their properties to induce the second axis from an anterior to a posterior orientation according to their developmental stage. In addition, cells may secrete more inducer(s) when proliferation is stopped, particularly in later stages.

Following four reasons indicate that these induction of the secondary axis results from induction of organizer. (1) A organizer marker, *goosecoid* was ectopically expressed around the cultured cells at the early gastrula stage. (2) Cultured cells formed clumps without differentiation, while notochord was induced. (3) Growth-arrested cells by mitomycin C still have the induction activity. (4) When cultured cells were implanted into an early gastrula embryo, we did not observe the secondary axis. Implanting cultured cells into maternal-zygotic *one-eyed pinhead* mutants that defect Nodal co-receptor revealed that secondary axis was induced without Nodal signaling. These cultured cells would be used to find new cellular factors involved in induction of organizer.

PUBLICATIONS

Papers

1. Kimura, T., Yoshida, K., Shimada, A., Jindo, T., Sakaizumi, M., Mitani, H., Naruse, K., Takeda, H., Inoko, H., Tamiya, G., Shinya, M. (2005) Genetic linkage map of medaka with polymerase chain reaction length polymorphisms. **Gene** *363*, 24 - 31.

2. Tamiya, G., Shinya, M., Imanishi, T., Ikuta, T., Makino, S., Okamoto, K., Furugaki, K., Matsumoto, T., Mano, S., Ando, S., Nozaki, Y., Yukawa, W., Nakashige, R., Yamaguchi, D., Ishibashi, H., Yonekura, M., Nakami, Y., Takayama, S., Endo, T., Saruwatari, T., Yagura, M., Yoshikawa, Y., Fujimoto, K., Oka, A., Chiku, S., Linsen, S. E., Giphart, M. J., Kulski, J. K., Fukazawa, T., Hashimoto, H., Kimura, M., Hoshina, Y., Suzuki, Y., Hotta, T., Mochida, J., Minezaki, T., Komai, K., Shiozawa, S., Taniguchi, A., Yamanaka, H., Kamatani, N., Gojobori, T., Bahram, S., Inoko, H. (2005) Whole genome association study of rheumatoid arthritis using 27 039 microsatellites. **Hum. Mol. Genet.** *14 (16)*, 2305 - 2321

EDUCATION

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F-e. Plant Genetics Laboratory
Nori Kurata Group

RESEARCH ACTIVITIES

(1) Cytogenetic and molecular analysis of rice meiotic mutants

Ken-ichi Nonomura, Mutsuko Nakano, Akane Morohoshi, Mitsugu Eiguchi, Akio Miyao*, Hirohiko Hirochika* and Nori Kurata (*Natl. Inst. Agrobiol. Sci.)

This study aims to dissect genetic machinery controlling meiosis and sporogenesis of rice (*Oryza sativa* L.). We have identified four meiotic genes and their insertional mutants to date; *PAIR1 (HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS 1), PAIR2, PAIR3* and *MEAL (MEIOSIS ARRESTED AT LEPTOTENE).* The *PAIR1* and *PAIR2* genes were found to encode a putative DNA-binding protein and a HORMA-domain protein, respectively. Identification of *PAIR3* and *MEAL* genes is in progress.

The PAIR2 is an important protein to complete synaptonemal complex (SC) formation by associating transiently with chromosomal axial elements (AEs) and recruiting transverse filaments and central elements between homologous AE pairs. Most of PAIR2 proteins dissociate from AEs at pachytene, but small amounts of them continue to associate with centromeric regions until nuclear envelope breakdown. We found that non-disjunction of chromosomes was occurred in meiocytes of *pair2* mutant, but absence of PAIR2 proteins from the centromere did not affect any centromere functions such as sister-chromatid cohesion and kinetochore assembly in rice meiosis (Nonomura et al., J. Cell Sci. (in press)).

The *meal* mutant showed an interesting phenotype and its candidate gene was expressed in a specific pattern that have not been reported yet. In male and female meiocytes of the mutant, meiosis was almost arrested at early stages around at leptotene and zygotene. In addition, the size and shape of meiocytes

became irregular, indicating that the *meal* mutation affected not only meiosis progression but also pre-meiotic mitosis of germ cells. *In situ* hybridization revealed that expression of the *meal* candidate mRNA is only observed in germ cells in a specific pattern. Analyses of the *MEAL* gene will shed light on the germ-cell lineage development of higher plants.

(2) Structural and functional analysis of rice *OsHAP3* genes

Yukihiro Ito, Thiruvengadam Thirumurugan and Nori Kurata

We identified 11 genes *(OsHAP3A to OsHAP3K)* which encode a HAP3/NF-YB subunit of CCAAT-box binding complex in rice by cDNA screening and a database search. We showed that three genes, *OsHAP3A, OsHAP3B* and *OsHAP3C*, are involved in chloroplast biogenesis (Miyoshi, et al. 2003), and *OsHAP3E* might be involved in brassinosteroid-related responses and panicle development. We also revealed that several specific members of OsHAP2 and OsHAP5 interacted with OsHAP3A and OsHAP3E, indicating specific combinations of OsHAP3, OsHAP2 and OsHAP5 in the formation of heterotrimeric CCAAT-box binding complex.

This year we examined expression patterns of *OsHAP2* and *OsHAP5* genes by RT-PCR. Among ten HAP2 genes, five (*OsHAP2A* to *OsHAP2E*) showed ubiquitous expression, while remaining five showed various or gene-specific expression patterns. *OsHAP5* genes also showed ubiquitous expression (*OsHAP5A, B, D,* and *E*) or organ-specific expression (three genes). For example, *OsHAP2G* and *OsHAP2H* were mainly expressed in shoot apex and callus, respectively, and *OsHAP5F* and *OsHAP5G* were expressed in callus and root, respectively. These results suggest that subunit interaction of a HAP complex could be controlled by specificities of gene expression as well as the combination of protein-protein interaction.

(3) Regulation of expression of *KNOX* family class 1 homeobox genes of rice

Yukihiro Ito, Katsutoshi Tsuda and Nori Kurata

KNOX family class1 homeobox genes, which show SAM-specific expression, play a vital function for shoot apical meristem (SAM) formation and maintenance. We found that cytokinin, which is necessary for shoot regeneration, induced the expression of *OSH1*, a member of the *KNOX* family, in callus. Overexpression of homologues of cytokinin signalling genes such as a histidine kinase gene *OHK3*, histidine-containing phosphotransmitter gene *OHP2* and response regulator gene *ORR1* enhanced the cytokinin-induced *OSH1* expression, and conversely, expression of a dominant negative of *OHK3* reduced it. *In situ* hybridization showed overlapped expression of these three genes in regenerating callus. Yeast two-hybrid analysis showed interaction of OHK3 and OHP2, and OHP2 and ORR1. These results indicate that these genes mediate cytokinin-induced expression of *OSH1* in regenerating callus.

Analyses of dominant mutants of *KNOX* genes in maize and barley, and our transgenic analyses of rice *KNOX* genes showed that introns and exons have cis-regulatory elements of their expression. To identify trans-factors that bind to the cis-elements and control the *OSH1* expression, we started yeast one-hybrid screening. We also searched and identified a recessive mutant showing ectopic expression of *OSH1* in leaf and resembling overexpressors of *OSH1*. The mutant shoot was very small, and no leaf was formed after germination. The mutant was seedling-lethal probably due to a failure of the SAM maintenance. These results indicate that this gene is required for the SAM maintenance and the suppression of the *OSH1* expression in leaf. Detail analyses of the mutant and the one-hybrid screening are underway.

(4) Analysis of a genetic program in embryosac/ovary formation

Shinichiro Yamaki, Yasuo Nagato* and Nori Kurata (*Univ. Tokyo, Agr. Sci)

Ovule is a female reproductive organ where megasporogenesis and megagametogenesis take place. To elucidate the mechanism of rice ovule development, the *gypsy embryo (gym)* mutant was found and analysed. The mutant showed an interesting phenotype of apically displaced embryos. Observation of the mutant indicated that the apically displaced embryo came from the apically displaced egg cells caused by incomplete ovule curvature. Apically displaced egg cells resulted in low fertility compared

to the normally positioned egg cells. This suggests that the normal ovule needs to be curved for allocating an egg cell to the position advantageous for fertilization. *In situ* hybridization analysis revealed that *OSH1* expression, a marker of indeterminate cells, was ectopically remained in ovule primordium. In contrast, *OsMADS13* expression, a marker of ovule, was restricted to a smaller region than that of wild type. These results indicated that the primary function of *GYM* gene is establishment of ovule identity by suppressing an indeterminate state of primordial cells. This suggestion was supported by the result of double mutant analysis that combined *gym* with other related mutations (Yamaki et al. 2005). We have mapped the *GYM* gene within 30 kb region on the short arm of chromosome 2. Cloning and identification of the *GYM* gene is now in progress.

(5) Establishment of rice TILLING system and mutant pools

Tadzunu Suzuki, Mitsugu Eiguchi, Toshihiro Kumamaru*, Hikaru Satoh* and Nori Kurata (*Kyushu Univ., Faculty of Agr.)

Many resources originated from rice insertion mutagenesis have been stacked for functional genomic studies in the world. These insertion lines, however, are difficult to cover knockout mutants for all genes because of the integration of DNA or transposons in the preferential target sites. In contrast, chemically mutagenized population is thought to cover all mutations for every gene. We collected MNU-treated mutant M2 population for screening mutations.

To establish a system for rice mutant screening, we applied a "Targeting Induced Local Lesions IN Genomes (TILLING)" method with a few modifications (Suzuki, et al., 2005). The modified TILLNG method, in which non-labeled primers for PCR and rapid capillary gel electrophoresis are used in place of fluorescence primers and polyacrylamide gel electrophoresis, was applied to screen mutations in the MNU-induced mutant population of T65 rice. More than 700 M2 lines were used to search base substitutions in two genes of known function, and many mutations were successfully detected by the modified TILLING system. The result suggested that around ten different mutant lines would be expected in 1,000 lines for 1 kb gene region. Thus, the MNU-induced mutant pool could be a useful resource for the reverse genetics approach and to compensate other mutant populations that cannot cover all genes.

(6) Positional cloning and statistical analysis of segregation distortion genes between Japonica and Indica rice

Yoshiaki Harushima, Satoshi Kuriki*, Youko Mizuta, Hironori Fujisawa*, and Nori Kurata (*The Institute of Statistical Mathematics)

Reproductive barrier is a genetic mechanism to isolate "species". The aim of this study is to understand molecular mechanisms of reproductive barriers by mapping genes, isolating genes, and observing phenotypes relating to its isolation mechanisms. Asian cultivated rice *O. sativa* can be divided into two ecotype groups, Japonica and Indica, and many reproductive barriers had been identified in their crosses (Harushima et al. 2002). Reproductive isolation in the crosses is known to be resulted from gene interactions. In 2005, we have been developing a statistical method to detect interactive locus/gene pairs of reproductive isolation in an F_2 population. In the development of the method, a gene pair of male gametophytic reproductive barrier was detected on chromosome 1 and 6 using an F_2 population of Nipponbare x Kasalath, and was confirmed reciprocal backcross populations. Positional cloning of the gene pair has been started.

(7) Comparative and evolutional studies for cultivated and wild rice species

Ken-ichi Nonomura, Yukie Sano, Akane Morohoshi and Nori Kurata

The genus *Oryza* is composed of 23 species, of which two species of *O. sativa* and *O. glaberrima* are cultivated and the rest are wild relatives. An F1 hybrid can be produced in almost all cross-combinations among *Oryza* species. However, F2 seeds showed complete sterility in many hybrids. This F1 sterility is often accompanied by aberrant meiosis and gametogenesis caused by reproductive isolation mechanisms. Elucidation of genetic diversity among *Oryza* species especially in genes related to meiosis/gametogenesis events could help to reveal a

reproductive isolation mechanism that will then be used for overcoming reproductive barriers.

The *PAIR1* gene plays an important role in homologous chromosome recognition and condensation in rice meiosis. It is possible that the *PAIR1* might be one of the candidate genes working in the reproductive isolation process. The *PAIR1* mRNA was expressed in young flowers from several wild relatives. In tetraploid species, two types of *PAIR1* mRNAs were detected. Sequencing of genomic DNA and cDNA from wild relatives revealed that the *PAIR1* locus possesses high diversity in several important domains. To examine relationship between F1 sterility and *PAIR1* sequence diversity among species, a complementation test of *pair1* mutation of cultivated species with *PAIR1* genes of wild relatives is in progress.

We also performed comparison of ESTs (expression sequence tags) from shoot and panicle between a cultivated species and wild relatives (BB and CC genome species) as described in the report of Experimental Farm. A total of 2300 cDNAs from BB and CC genomes analyzed so far showed about 3% base substitutions with AA genomes and scores of highly diverged genes and several wild specific genes have been identified. A detailed characterization of these divergent genes is now underway. This approach will bring us useful information to have knowledge about global molecular diversity in genus *Oryza*.

(8) Detection of genetic/expression variation among rice species

Yoshiaki Harushima, Youko Horiuchi, Masanori Kawakita*, Shinto Eguch*, and Nori Kurata (*The Institute of Statistical Mathematics)

In the preliminary microarray comparative analysis between wild and cultivated rice, we saw abnormally higher expression of a set of cultivated rice genes. The higher expression was thought to come from sequence diversity between two rice species. The aim of this study is discriminate genetic and expression variation among rice species using Affymetrix Rice Genome array that contains 631,066 25mer-probes to target 49,824 transcripts mainly from Japonica rice. To distinguish genetic diversity from expression variation, we are going to develop a combinational analytical method. The method is composed of a comparison between experimental hybridization and in silico hybridization of the probe sequences of Nipponbare (Japonica) and 93-11 (Indica), whose sequences are available and averaged differences were 3.0 SNP/kb in coding region and 4.5 SNP/kb in 3'UTR. The method would become indispensable in the expression analysis of genetically diverged wild rice genes on the array of cultivated rice genes.

(9) Rice genetic resource project in NBRP and Oryzabase

Nori Kurata, Toshie Miyabayashi, Shinichiro Yamaki and Ken-ichi Nonomura

The National Bioresource Project (NBRP) works to conserve and distribute genetic and biological resources for scientific communities. In the NIG, we are dealing 2,000 wild rice accessions covering 10 genomes and 23 species collected all over the world for over 50 years. Four rice sub-centers in other institutions deal with different kinds of genetic materials and work together in the project. Resource materials and their information can be accessed at;
http: / / www.shigen.nig.ac.jp / rice / oryzabase / nbrpStrains/aboutNbrp.jsp

To evaluate the NIG *Oryza* collection in a molecular level, we measured DNA contents of 39 accessions from 20 species by flow cytometric analysis. The karyotype analysis is also performed to confirm the ploidy level. Flow cytometric analysis revealed that DNA contents of the accessions involved in the *officinalis* complex (CC genome species) are various. In addition, *O. granulata* and *O. meyeriana*, assigned as GG genome species, possessed two-fold or more DNA contents than that of *O. sativa*. Karyotype analysis revealed that the accession W1525 that belongs to the species of *officinalis* complex and previously assigned as a diploid species of *O. eichingeri*, possessed a tetraploid chromosome set, indicating contamination in the process of maintenance. Identification of each accession with species specific DNA markers is also in progress.

In addition, we observed morphological features of vegetative and reproductive architectures of 44 Rankl core accessions of wild rice. These observations revealed some relationships of each feature. First, the accessions with many tillers per plant tended to differentiate few spikelets per panicle and vice versa.

Second, seed weight was also in inverse proportion to the number of spikelets per panicle. Third, the number of panicles per plant had positive correlation with seed fertility. Because it is supposed that seed fertility is involved in pereniality, the number of panicles per plant may also be involved in pereniality. The intimate analyses of these relationships are underway.

The Oryzabase is a comprehensive rice biological database composed of 15 sections of information. Major data characteristic to the Oryzabase are; mutants and their trait genes collection, wild rice collection and development/anatomy of rice, together with genetic maps, physical maps, basic biological data and so on. In 2005, we finalized an important section of rice developmental stages that is served as one of the bases of plant ontology activities in cereal plants. NBRP data have also been added. The rice genetic resources committee centered in the Plant Genetics Laboratory is responsible for collecting and managing the contents of information. Construction and maintenance of the DB have been carried out by the Genetic Informatics Laboratory. The DB is accessible at;
http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp

PUBLICATIONS

Papers

1. Ito, Y., Takaya, K. and Kurata, N. (2005) Expression of SERK family receptor-like protein kinase genes in rice, **Biochim. Biophys. Acta**, *1730*, 253 - 258
2. Ito, Y. and Kurata, N.(2005) Identification of enhancer trap lines with tissue-specific GUS activities in rice, **Rice Genet. Newslet.**, *22*, 32 - 33
3. Itoh, J. I., Nonomura, K. I., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H. and Nagato, Y. (2005) Rice plant development: from zygote to spikelet, **Plant Cell Physiol.**, *46*, 23 - 47
4. Kurata, N., Miyoshi, K., Nonomura, K. I., Yamazaki, Y. and Ito, Y. (2005) Rice mutants and genes related to organ development, morphogenesis and physiological traits, **Plant Cell Physiol.**, *46*, 48 - 62
5. Moriguchi, K., Suzuki, T., Ito, Y., Yamazaki, Y., Niwa, Y. and Kurata, N. (2005) Functional isolation of novel nuclear proteins showing a variety of subnuclear localizations, **Plant Cell**, *17*, 389 - 403
6. Suzuki, T., Eiguchi, M., Satoh, H., Kumamaru, T. and Kurata, N. (2005) A modified TILLING system for rice mutant screening, **Rice Genet. Newslet.**, *22*, 89 - 90
7. Xu, J-H., Kurata, N., Akimoto, M., Ohtsubo, H. and Ohtsubo, E. (2005) Identification and characterization of Australian wild rice strains of *Oryza meridionalis* and *Oryza rufipogon* by SINE insertion polymorphism, **Genes Genet. Syst.**, *80*, 129 - 134
8. Yamaki, S., Satoh, H. and Nagato, Y. (2005) *Gypsy embryo* specifies ovule curvature by regulating ovule / integument development in rice, **Planta**, *222*, 408 - 417

Books

9. 伊藤幸博(2005)エンハンサー，エンハンサートラップ，アクティベーションタギング　植物育種学辞典　(日本育種学会)　培風館

EDUCATION

1. 野々村賢一　イネの減数分裂における染色体認識と動原体　東京大学大学院理学系研究科
2. 野々村賢一　減数分裂遺伝子の解析とイネ属の種分化　名古屋大学大学院
3. 倉田のり　ゲノム生物学の基礎と意味－植物ゲノム研究　京都大学大学院・農学研究科

SOCIAL CONTRIBUTIONS

1. 倉田のり：日本学術会議育種学連携委員
2. 倉田のり：日本育種学会幹事
3. 倉田のり：日本育種学会学会賞選考委員
4. 倉田のり：生物遺伝資源イネ小委員会委員長
5. 倉田のり：Rice Genetics Newsletter Editor
6. 倉田のり：NSF project advisory committee member

F-f. Microbial Genetics Laboratory Hironori Niki Group

RESEARCH ACTIVITIES

(1) Guidance of Actively Partitioning Plasmid by a Helical Structure of an ATPase, SopA

Toshiyuki Hatano and Hironori Niki

In a bacterial cell, replicated chromosomal segments including the replication origin and the F plasmid actively move from midcell toward cell poles and are located at cell quarter positions. The Sop ABC partitioning system of F plasmid has been characterized genetically and biochemically to elucidate

molecular mechanism of DNA partitioning. The SopABC system consists of two proteins, SopA ATPase and SopB, which binds to centromere site, *sopC*. The SopA protein is a hypothetical motor protein because of its walker-type ATPase activity. We have previously constructed an active SopA-YFP and observed oscillation of YFP fluorescence from one cell pole to the other with a helical structure extending along the long axis of a cell. Simultaneously, migration of the plasmid DNA was observed fluorescent labeling technique with lacO array and LacI-CFP. The analyses of movement of both the fluorescence suggest that SopA could be a motor protein to govern "direction" of movement of the plasmid DNA.

We further analyzed dynamics of movement of SopA-YFP. SopA-YFP oscillated between the "nucleoid borders" in a cell. The result indicates that SopA may recognize and cluster at the positions of nucleoid borders during oscillation. To test this possibility, we observed localization of SopA-YFP in anucleated cells (DNA less cells). Surprisingly, SopA-YFP showed discrete foci and oscillated in anucleated cells. Statistical analysis revealed that the positions of foci were corresponding to the potential positions of the nucleoid borders. Furthermore, we observed helical structure of SopA-YFP in anucleated cells. These results indicate that SopA can assemble at the specific subcellular positions where overlap with nucleoid boader.

Furthermore, we found the helical structure of SopA-YFP kept constant length in a cell during oscillation. In E. coli, a bacterial action like filamentous protein maintains cell morphology. The cytoskeletal protein, MreB makes a helical structure along the long axis of a cell To test the possibility that the bacterial cytoskeleton protein might be required for the helical structure of SopA, we observed the localization of SopA-YFP in a cell lacking MreB. SopA-YFP formed filamentous structure in the deletion mutant cells, and the foci were oscillated. In addtiton, we found that F plasmids are stably maintained in *ΔmreBCD* mutant cells. These results indicate that the bacterial actin, MreB is not required for F plasmid partitioning itself and movement of SopA. SopA may govern a partitioning system independent from nucleoid structure and cytoskeleton.

(2) Analysis of Dynamics of ATPase, P1 ParA in Partitioning of P1 Plasmid

Toshiyki Hatano and Hironori Niki

ParA/SopA family proteins are conserved and involved in partitioning of a variety of plasmids and bacterial chromosomes. P1 ParA protein is a member of ParA/SopA family and essential for stable partitioning of P1 phage plasmids. The P1 Par system consists of two proteins, ParA ATPase and ParB, which binds to centromere site, *parS*. Because P1 ParA protein also contains a walker type ATPase motif, P1 ParA may have function as motor for migration of P1 plasmids like the SopA protein.

We have been constructed a P1 ParA-YFP fusion protein that sustained biological function for the plasmid partitioning. Interestingly, in spite of similarity with SopA as walker ATPase, the P1 ParA-YFP fusion protein did not show oscillation in a cell, but showed localization spread on nucleoid with weak foci at cell quarter positions. Comparison of the dynamics between SopA and ParA in a living cell may elucidate conserved or diverse functions of the ATPase motor involved in plasmid partitioning. Especially, difference of dynamics between them may explain difference of incompatibility of partitioning between F and P1 plasmids.

(3) Characterization of proteins that are interact with a bacterial centromere site, migS

Yashuyuki Ogata, Toshiyuki Hatano and Hironori Niki

The replicated Ori domains migrates towards opposite cell poles. This indicates that a putative cis-acting site for bipolar migration of the *oriC* segment is located within the domain. A new method to split a bacterial chromosome into two sub-chromosomes has enabled to identify the cis-acting site. In the end, we found that a 25 bp sequence, *migS*, is responsible for bipolar migration of the *oriC* segment . Although rapid bipolar migration of the *oriC* segments is inhibited, the *migS* is not a defect in chromosome segregation. This suggests that a redundant mechanism to compensate for the *migS* pathway might be involved in bacterial chromosome partitioning. To examine this idea, synthetic lethal mutations with the *migS* deletion were screened in a library with insertion

mutations of a transposon. We are investigating isolated mutations at the present time.

We would like to focus on study of the molecular mechanism for bipolar migration of DNA in a bacterial cell. Although actin-like proteins have been found in prokaryotes, a motor function to partition chromosomes into daughter cells has not yet been identified on bipolar migration of the chromsome. Presumably, a motor protein works on the process to generate a driving force. Investigation of the molecular mechanism of *migS* dependent migration is providing vital new clues as how the putative motor protein contributes to chromosome partitioning. Proteomics approaches have already been identified two proteins as *migS* binding proteins. Further investigation of the genes that encode the *migS* binding proteins have already been carried out, and the results indicates the genes would be involved in bipolar migration of *migS*. We investigate on property of the proteins for bipolar migration of *migS*.

EDUCATION

1. 仁木　宏典　発生細胞化学特論　東京大学大学院薬学系研究科

会議等主催

2. 仁木　宏典　21世紀大腸菌研究会－2005－　モデル生物大腸菌の統合的理解にむけて　志摩
3. 仁木　宏典　第28回日本分子生物学会年会　ワークショップ　染色体の高次機能領域　福岡

F-g. Invertebrate Genetics Laboratory Ryu Ueda Group

RESEARCH ACTIVITIES

(1) RNAi mutant fly bank for comprehensive analyses of gene function in *Drosophila*

Ryu Ueda, Yukiko Sado[1], Kazuko Fujitani, and Kuniaki Takahashi ([1]JST)

Genome sequencing projects have revealed the number of genes for several model organisms for genetics. The small worm *Caenolabditis elegans*, which is composed of only 959 cells, has 19,000 genes in its genome. On the other hand, *Drosophila melanogaster*, which has a long and sophisticated alimentary canal, a tubular heart that circulates hemolymph, and a large brain composed of over 10^4 cells, harbors only 14,066 protein-coding genes. Considering there is such a small number of fly genes, each one of them may have an essential function in fly development and behavior. In other words, it may be easy to detect and analyze gene function in the fly by reverse genetics because the abnormal phenotype will frequently appear when knocking down a target gene whose function is unknown. We are planning to investigate the function of fly genes comprehensively as a suitable model for studying the functional genomics of multicellular organisms.

How does one investigate the function of all 14,066 genes in the fly? We use RNA interference (RNAi) to knock down the activity of the target gene. RNAi is one of the emerging technologies with which to investigate gene function in multicellular organisms. When introduced into the cell, double stranded RNA (dsRNA) works as a specific mutagen for each gene. That is, dsRNA recognizes host mRNA and digests it in a sequence-specific manner, and consequently brings a loss-of-function mutation phenotype to the host cell. The detailed mechanism of this RNAi phenomenon has not yet been elucidated, but it works efficiently in many multicellular organisms, including humans.

We coupled the RNAi with the GAL4-UAS gene expression system to induce a conditional loss-of-function mutation in the fly. The GAL4-UAS system is a binary system for inducing transgene expression, in which two fly lines are used. One is the GAL4 driver fly line, which expresses yeast transcription factor, GAL4, in a specific cell/tissue or at a specific developmental stage in favor of the GAL4 transgene. The other fly line harbors a transgene on the chromosome, in which an appropriate gene to be expressed is fused to the UAS promoter, the GAL4 target. When these two fly lines are crossed with each other, we can observe in the fly progeny that the GAL4 protein induces target transgene expression in a driver-specific conditional fashion. In this GAL4-UAS system, when we use a UAS-transgene having an inverted repeat (IR) sequence, the transcribed RNA may form a dsRNA in the cell and induce a loss-of-function mutation by the RNAi mechanism. Such inducible RNAi caused by the transcription of an IR sequence was first successfully adopted to gene

function analysis in *C. elegans*. It was then also found to be effective in fly genetics. By making a UAS-transformation vector containing an IR sequence of the gene predicted by the fly genome project, and by introducing it into a fly line (IR fly), a mutant phenotype of the gene can be easily observed in any cell or at any developmental stage of the progeny, whenever the IR fly is crossed to an appropriate GAL4 driver fly.

We are expanding this inducible RNAi to the whole genome of the fly. This process involves two major procedures.

1) *in vitro* construction of transformation vectors containing an IR sequence from each of the 14,066 predicted genes.

2) Transformation of IR vectors by injecting them into fly eggs and establishment of IR fly lines by traditional genetic methods.

As of the end of 2005, over 8,200 transformation vectors had been constructed, 7,300 of which have been successfully introduced into the fly to establish 12,000 IR fly lines. We will be continuing this work to the end of the next year, and may be able to add up to 9,000 vectors and 14,000 IR fly lines to our fly bank.

Along with the establishment of IR fly lines, basic characterization of these fly lines is conducted. All of the IR fly lines are crossed to the Act5C-GAL4 fly. The Act5C-GAL4 induces the UAS-transgene in all cells at all developmental stages. Thus, if the gene targeted by RNAi has functions that are indispensable for fly development, the progeny of IR and GAL4 flies should die before the adult flies emerge. Among the 1,954 genes (3,908 IR fly lines) tested, 51.1% of the fly lines showed lethality. This value is rather high compared to that obtained by classical genetics (25%), while the fact that many of the genes tested here were considered to have important functions in various aspects of fly development by our collaborators may bring about such a high score. Detailed analyses on known genes and greater accumulation of data are necessary.

We are currently collaborating with over 100 research groups in the world with our IR flies. The usefulness of inducible RNAi for investigating gene function in *Drosophila* is being revealed in many biological phenomena, such as morphogenesis (Adachi-Yamada, T., *et al.*, 2005; Hirota, Y., *et al.*, 2005), immunity (Kleino, A., *et al.*, 2005), behavior (Ishimoto, H., *et al.*, 2005; Usui-Aoki, K., *et al.*, 2005), circadian rhythm, brain development, cell proliferation, DNA repair, glycobiology, neurodegenerative disease, sex determination, *etc.*

Through feedback from these collaborations, we convinced the efficacy of our fly bank. Thus, a part of the bank has been opened to the community (http://www.shigen.nig.ac.jp/fly/nigfly/) with a support of National Bio-Resource Project in Japan. Significant part of the bank will be opened in the next year after re-inspection and integration of many associated data with these flies.

This work was supported in part by financial assistance from the Mitsubishi Kagaku Institute of Life Sciences (MITILS) to Dr. Ueda.

PUBLICATIONS

Papers

1. Adachi-Yamada, T., Harumoto, T., Sakurai, K., Ueda, R., Saigo, K., O'Connor, M. B., and Nakato, H. (2005) Wing-to-leg homeosis by Spineless causes an apoptosis regulated by Fish-lips, a novel Leucine-Rich Repeats transmembrane protein., **Mol. Cell. Biol.**, *25 (8)* 3140 - 3150

2. Hirota, Y., Sawamoto, K., Takahashi, K., Ueda, R., and Okano, H. (2005) The transmembrane protein, Tincar, is involved in the development of the compound eye in Drosophila melanogaster., **Dev. Genes Evol.**, *215*, 90 - 96

3. Ishimoto, H., Takahashi, K., Ueda, R., and Tanimura, T. (2005) G-protein gamma subunit 1 is required for sugar reception in *Drosophila.*, **The EMBO J.**, *24*, 3259 - 3265

4. Kleino, A., Valanne, S., Ulvila, J., Kallio, J., Myllymaki, H., Enwald, H., Stoven, S., Poidevin, M., Ueda, R., Hultmark, D., Lemaitre, B., and Ramet, M. (2005) Inhibitor of apoptosis 2 and TAK1-binding protein are components of the Drosophila Imd pathway, **The EMBO J.**, *24*, 3423 - 3434

5. Usui-Aoki, k., Matsumoto, K., Koganezawa, M., Kohatsu, S., Isono, K., Matsubayashi, H., Yamamoto, M., Ueda, R., Takahashi, K., Saigo, K., Mikoshiba, K., and Yamamoto, D. (2005) TARGETED EXPRESSION OF IP3 SPONGE AND IP3DSRNA IMPAIRES SUGAR TASTE SENSATION IN DROSOPHILA, **J. Neurogenet.**, *19*, 123 - 141

6. 相垣敏郎，上田龍(2005)ショウジョウバエゲノムの体系的機能解明---発生・分化の遺伝子システム解明に向け

て，蛋白質核酸酵素　別冊「ゲノムから生命システムへ」，*50 (16)* 2146 - 2152

EDUCATION

1. 上田龍　遺伝情報特別講義　都立大学・理学部
2. 上田龍　ゲノム時代にショウジョウバエを利用して遺伝子の働きを研究する　遺伝学フォーラム京都2005　京都市・ぱ・る・るプラザ京都

SOCIAL CONTRIBUTIONS AND OTHERS

その他
上田龍　独立行政法人・農業生物資源研究所・評価助言委員

F-h. Laboratory for Frontier Research Takako Isshiki Group

RESEARCH ACTIVITIES

(1) Analysis of temporal specification within late phases of *Drosophila* neuroblast lineage

Takako Isshiki

During development, neural progenitors often generate diverse cell types in an invariant order, changing their property over time. Although substantial progresses have been made in understanding the molecular mechanisms of how different cell fates are generated in order, many parts of the mechanisms still remain unknown. We utilize the *Drosophila* central nervous system (CNS) as an excellent model system to study temporal specification of cell fates within a lineage. We previously showed that *Drosophila* neuroblasts sequentially express the transcription factors Hunchback, Krüppel, Pdm and Castor over time. However, most neuroblasts divide additional 10 times on average after they start expressing Castor. Thus, there must be subsequent mechanisms for temporal specification.

We have found, to date, several transcription factors expressed later than Castor in most neuroblasts, and elucidated the precise order of their expression. This year we investigated functions of these factors by genetic analyses. These analyses revealed that, among these factors, Castor plays a pivotal role in orchestrating temporal change of neuroblasts at the late stages of embryonic development.

(2) Investigation of molecular functions of Castor in Development of the CNS

Horiuchi Takayuki

Our genetic study indicated that Castor is one of crucial transcription factors for temporal change of neuroblasts, although it remains mostly unknown what molecular mechanisms enable Castor to perform function. Aiming to elucidate the molecular mechanism, we searched the proteins that directly bind to Castor by using Yeast 2 Hybrid screening. So far, we could obtain 4 good candidates that might bind to Castor *in vivo*. We are now investigating whether each caudidate has any effect on Castor by genetic analyses.

(3) Temporal change, quiescence, and reactivation of neuroblasts

Katsutomo Okamura, Takako Isshiki

At the end of embryogenesis, most, not all, abdominal neuroblasts cease cell division and undergo apoptosis eventually. In contrast, thoracic and brain neuroblasts go into the quiescent state, and then get reactivated at the larval stages. Upon the reactivation, neuroblasts change their property dramatically. For example, larval neuroblasts require glial niche and continue cell growth, which enables them to generate 50-100 neurons per a single neuroblasts during a long larval period. However the mechanisms of the quiescence, the reactivation and the transformation of neuroblasts are poorly understood. We have started addressing the questions by observing the expression pattern of the temporal transcription factors just before entering the quiescence and during the larval and pupal stages.

G. CENTER FOR GENETIC RESOURCE INFORMATION

G-a. Genetic Informatics Laboratory Yukiko Yamazaki Group

RESEARCH ACTIVITIES

(1) Wheat mitochondrial genome analysis

Ogihara, Y., Yamazaki, Y., Murai, K., Kanno, A., Terachi, T., Shiina, T., Miyashita, N., Nasuda, S., Nakamura, C., Mori, N., Takumi, S., Murata, M., Futo, S. and Tsunewaki, K..

The application of a new gene-based strategy for sequencing the wheat mitochondrial genome shows its structure to be a 452528 bp circular molecule, and provides nucleotide-level evidence of intra-molecular recombination. Single, reciprocal and double recombinant products, and the nucleotide sequences of the repeats that mediate their formation have been identified. The genome has 55 genes with exons, including 35 protein-coding, 3 rRNA and 17 tRNA genes. Nucleotide sequences of seven wheat genes have been determined here for the first time. Nine genes have an exon-intron structure. Gene amplification responsible for the production of multicopy mitochondrial genes, in general, is species-specific, suggesting the recent origin of these genes. About 16, 17, 15, 3.0 and 0.2% of wheat mitochondrial DNA (mtDNA) may be of genic (including introns), open reading frame, repetitive sequence, chloroplast and retro-element origin, respectively. The gene order of the wheat mitochondrial gene map shows little synteny to the rice and maize maps, indicative that thorough gene shuffling occurred during speciation. Almost all unique mtDNA sequences of wheat, as compared with rice and maize mtDNAs, are redundant DNA. Features of the gene-based strategy are discussed, and a mechanistic model of mitochondrial gene amplification is proposed. (Ogihara Y. et al. 2005)

(2) CARD R-BASE

Takehiro Yamakawa, Hideki Kato, Naomi Nakagata, Kenichi Yamamura and Yukiko Yamazaki

CARD R-BASE is a database of transgenic mouse strains established and deposited by individual researchers. All resources are stored as frozen embryos and are available on request. The database mainly contains genetic backgrounds, destroyed / transferred genes, relevant human diseases and journal articles for each strain. In this year, we developed an on-line application system. This system not only supports depositors to submit data of their mice but also allows mouse maintainers, data reviewer and database manager to carry out their own tasks on-line while taking an overview of whole processes.

CARD R-BASE became a member of FIMRe (Federation of International Mouse Resources) and has been periodically sending data files to the IMSR, a database of FIMRe. The FIMRe established in 2005 is a collaborating group of Mouse Repository and Resource Centers worldwide whose collective goal is to archive and provide strains of mice as cryopreserved embryos and gametes, ES cell lines, and live breeding stock to the research community. CARD group also started collaboration with Asian mouse archiving centers.

(3) Guide dog database

Kazuhiro Oogushi, Yukiko Yamazaki, Yoshinori Suwa and Hiroshi Suzuki

There are 900 guide dogs in Japan but the number of people requiring guide dogs exceeds this number by five times and there are 10 times more people who wish to own a guide dog. In addition, only 30% of the dogs which undergo strict training manage to pass the aptitude test necessary for them to become guide dogs. Whether or not the inborn characteristics of a dog meet the conditions required for a guide dog is also an important factor. Since the dog's training process only begins after its contraception or castration, a guide dog cannot reproduce even if it is found to be of a superior breed. Therefore, a dog can only be either a guide dog or a breeding dog, disabling a guide dog to be used as a breeding dog at a later stage. If the number of

breeding dogs decreases, the offspring will become genetically closer, and this may cause various problems in the future. At present, it is also difficult to import guide dogs from abroad.

In order to overcome the shortage of guide dogs, the project entitled "Reproduction engineering research on breeding of superior guide dogs" has been started. This project aims to establish "K9 BioBank" wherein the genetic resources (sperms, embryos, ovaries, etc.) of superior guide dogs are frozen and stored, and the reproduction of superior guide dogs can be realized, if needed.

The database construction of the K9 BioBank is one of the pillars of this project. We have constructed a trial database of individual dogs information (blood line, case history, existence of etiologic gene, character evaluation result) this year and plan to construct a system that facilitates the sharing of information between related organizations and consequently fulfill requests from users.

The Asia GuideDogs Breeding Network (AGBN) was established in 2002 with eight facilities in Japan, one in South Korea, and two in Taiwan. The construction of this database is expected to lead to further cooperation among these facilities.

(4) Rice

Koji Watanabe, Nori Kurata, Atsushi Yoshimura, Yasuo Nagato and Yukiko Yamazaki

Oryzabase is a comprehensive rice database integrating biological data derived from various studies on morphology, physiology and ecology with molecular genomic information. The data in Oryzabase are divided into 15 sections: development / anatomy, mutants, trait genes, linkage maps, physical maps, comparative maps, references, basic biological data, DNA sequence, BLAST search, chloroplast and mitochondrion, tools and protocols, strains, stock centers and wild rice.

The biological data incorporated in Oryzabase include morphological and gene expression characteristics of rice at different developmental stages and in various mutants. These data are classified into four sections, namely, development / anatomy, trait genes, mutants, and basic biological information. The development / anatomy is the most distinguishing feature of Oryzabase. The database contains information on 1,698 trait genes and 136 unclassified genes that has been identified from a mutant or natural variant Each entry (trait gene) is provided with a gene symbol, gene name, chromosome (location if identified), mutant class name, GO and / or trait ontology number.

The Oryzabase is also a repository for about 20,000 rice strains including wild rice accessions, cultivars, mutant lines, chromosome substitution lines, recombinant inbred lines and marker gene lines in conjunction with the National Bioresource Project (NBRP) in Japan. The wild rice strains include 23 species from the AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ genomes. A core collection of 289 accessions from wild species was chosen and ranked for convenience in accessing. The crossed lines include RILs of four *japonica* x *indica* crosses, chromosome substitution lines with *japonica* backgrounds crossed with other AA genome species. A collection of more than 6,000 mutants induced by N-methyl-N-nitrosourea (MNU) and which has been classified into 12 classes of visible phenotypes is also available.

As to data on rice genomics, Oryzabase provides access to genome maps, sequences and comparative mapping resources. The four basic linkage maps of rice, namely, the classical linkage map with 571 phenotypic genes, the integrated linkage map with 83 RFLP markers and 40 phenotypic markers, the recombinant inbred (RI) map with 375 RFLP markers, and the high-density Nipponbare / Kasalath linkage map with 2,275 DNA markers were integrated using commonly mapped markers. A genome viewer showing the physical maps of the 12 rice chromosomes with windows ranging from 250 kb to 1 Mb and 10 to 100 kb has been added recently. The genome sequences represent the latest submissions in DDBJ / EMBL / GenBank. The comparative maps include barley clones and wheat ESTs mapped in the rice genome.

Oryzabase also aims to establish a comprehensive ontology for all morphological features and trait genes in rice. All trait genes are manually annotated and assigned with gene ontology (GO) IDs in agreement with the central GO database at Gramene (http://www.gramene.org/). A major priority is to develop, curate and share controlled vocabularies describing various morphological structures of the rice plant at different stages of growth and development in line with the Plant Ontology Consortium (http://www.plantontology.org/) to establish a widely accepted plant ontology (PO) and plant and trait ontology

(PATO) framework for rice. An integrated viewer, O3, will soon be incorporated in the database to correlate the concept of ontology with various biological data. Therefore Oryzabase can be a source of both information and resources that will provide the foundation for rice functional genomics.

(5) SHIGEN project

Takahiro Yamakawa, Koji Watanabe, Rie Tsuchiya, Sumiko Yano, Kazuhiro Oogushi, Shingo Sakaniwa, Mutsumi Saito, Sharoh Yip, Toru Watanabe, Miharu Yoshioka and Yukiko Yamazaki

In 2002 the National Bioresource Project was started in Japan to reinforce the system for collecting, maintaining, and providing research resources and their information effectively. Twenty-four resource centers, each of which is representative organization of a species and one information center were established.

Our missions as the information center are collection of resource information and provision of the databases in hopes of promoting effective use of the resources. Twenty-three individual databases are now available for use through the internet and both intra-species integration such as genetic resources and genomic resources integration, and inter-species integration using ontology have been proceeding. We are developing a system, the BioResource World, by which resource maintainer could upload and provide their information through the online database without having to maintain a database. This system also allows resource users to search several different resource databases simultaneously.

According to the access logs, nearly 40000 visits per month and over 100 feedbacks from users were recorded each month in 2005.

Featured contents of each database in 2005

5-1: Mouse Polymorphism Database

Mouse Polymorphism Database provides SSLPs among different mouse strains, cSNPs between B6 and MSM mice based on their full length cDNA sequence information and gSNPs between B6 / MSM mice based on BAC end sequences. We applied the Ensembl, which is provided for free by EBI, as a genome browser. The original data of MSM BAC ends, MSM / B6 cSNPs and microsatellite polymorphism was added to the browser as DAS (Distributed annotation source). We also developed an original browser, the SHIGEN genomic browser, to overcome several functional limitations of Ensembl. Sequence alignment and SNP positions as well as genomic position can be displayed by using the SHIGEN browser.

5-2: Medaka Atlas

The on-line medaka atlas was developed on the bases of an original atlas published in an article. The content of the first release was the adult brain of a wild-type inbred strain (HNI). This year, new content of the blood vessel was added. The system was implemented using flash technology and presents a detailed characterization of the vascular anatomy of a developing medaka embryo from the stage 24 through stage30 with dorsal-, dorsolateral- and ventrolateral views.

5-3: Drosophila

Information of over 2000 RNAi-mutant flies was added to the NIG-Fly database. Each stock has basic information (STOCK ID, CG No, Synonyms, Accession No. Inserted chromosome number, phenotype and references) and sequences including primers, fragment sequences, assemble data, and off-target gene candidates. FLYSTOCK database is a one-stop-shop of Drosophila resources and compiles all available drosophila resources maintained by four organizations, Kyoto DGRC, NIG, KYORIN and Ehime.

5-4: Wheat

Wheat database, KOMUGI, provided an online journal submission and editorial system and started distribution of the electronic newsletter for wheat researchers "eWIS" this year. This activity was carried out by the Japanese wheat geneticist community. KOMUGI collected EST end sequences from various tissues and stress conditions. Sequence analysis was performed by the NBRP project and all clones are available through the on-line order system. Wheat gene database was updated by using MacGene2003, 2004-, 2005-supplement data. We applied the CMAP tool to display markers on maps instead of incorporating

them into the gene database as we used to.

5-5: Legume

NBRP-LegumeBase is a resource ordering site shared by Lotus japonica resource database and Glycine max. resource database. Lotus japonica database provides wild accessions, root culture, Recombinant Inbred Lines (RILs), BAC / TAC clones, and cDNA clones as resources. Some strains were characterized by phenotype when growing in northern areas and southern areas. Glycine max database contains wild species, Glycine soja, RILs and mutant strains. Legume follows rice (monocot model plant) and arabidopsis (dicot model plant) as the third model plant.

5-6: E.coli

Sixty-four hundreds transposon insertion disruptants were added to the E. coli strain database after the analysis of insertion position on E. coli genome. A collection of cloning vectors of E. coli was incorporated into this E. coli strain database. Essentiality of genes based on different criteria as well as huge amount of resource collections would become very useful for E. coli researchers and accelerate functional genomics studies.

PUBLICATIONS

Papers

1. Hashimoto, M., Ichimura, T., Mozoguchi, H., Tanaka, K., Fujimitsu, K., Keyamura, K., Ote, T., Yamakawa, T., Yamazaki, Y., Mori, H., Katayama, T., Kato, J. (2005) Cell size and nucleoid organization of engineered Escherichia coli cells with a reduced genome., **Mol. Microbiol.**, *55 (1)* 137
2. Kawaura, K., Mochida, K., Yamazaki, Y., Ogihara, Y. (2005) Transcriptome analysis of salinity stress responses in common wheat using a 22k oligo-DNA microarray., **Funct Integr Genomics**, 132 - 142
3. Kurata, N., Miyoshi, K., Nonomura, K., Yamazaki, Y., Ito, Y. (2005) Rice mutants and genes related to organ development, morphogenesis and physiological traits., **Plant and Cell Physiology**, *46 (1)* 48 - 62
4. Moriguchi, K., Suzuki, Y., Ito, Y., Yamazaki, Y., Niwa, Y., Kurata, N. (2005) Functional Isolation of Novel Nuclear Proteins Showing a Variety of Subnuclear Localizations., **Plant Cell**, *17 (2)* 389 - 403
5. Ogihara, Y., Yamazaki, Y., Murai, K., Kanno, A., Terachi, T., Shiina, T., Miyashita, N., Nasuda, S., Nakamura, C., Mori, N., Takumi, S., Murata, M., Futo, S., Tsunewaki, K. (2005) Structural dynamics of cereal mitochondrial genomes as revealed by complete nucleotide sequenceing of the wheat mitochondrial genome., **Nucleic Acid Res.**, *33 (19)* 6235
6. Yamazaki, Y. and Jaiswal, P. (2005) Biological Ontologies in Rice Databases., **Plant and Cell Physiology**, *46 (1)* 63 - 68

Books

7. 山崎由紀子 (2005) Gene Ontology (GO) データベース，細胞工学，1207

Database

8. Mouse Genetic Resources (July, 2005) http://shigen.lab.nig.ac.jp/mouse/strain/
9. Mouse Polymorphism DB (2005) http://shigen.lab.nig.ac.jp/mouse/polymorphism/top/top.jsp
10. CARD R-BASE 2005 http://cardb.cc.kumamoto-u.ac.jp/transgenic/
11. Japan Mouse Strain Resources (JMSR 2005) http://shigen.lab.nig.ac.jp/mouse/jmsr/
12. National BioResource Project MEDAKA 2005 http://shigen.lab.nig.ac.jp/medaka/
13. National BioResource Project XENOPUS 2005 http://shigen.lab.nig.ac.jp/xenopus/top.jsp
14. National BioResource Project ZEBRAFISH 2005 http://shigen.lab.nig.ac.jp/zebra/
15. NIG-FLY 2005 http://shigen.lab.nig.ac.jp/fly/nigfly/
16. Flystock 2005 http://218.44.182.89/%7Eflystock/html/index_j.html
17. C. elegans MUTANTS 2005 http://shigen.lab.nig.ac.jp/c.elegans/mutants/
18. SilkwormBase 205 http://shigen.lab.nig.ac.jp/silkwormbase/
19. KOMUGI 2005 http://shigen.lab.nig.ac.jp/wheat/komugi/
20. Oryzabase 2005 http://shigen.lab.nig.ac.jp/rice/oryzabase/
21. BARLEY DB 2005 http://shigen.lab.nig.ac.jp/barley/
22. NBRP Chrysanthemum 2005 http://shigen.lab.nig.ac.jp/chrysanthemum/
23. NBRP LegumeBase 2005 http://shigen.lab.nig.ac.jp/legume/legumebase/

24. 藻類資源DBシステム2005 http://shigen.lab.nig.ac.jp/algae/
25. NBRP E.coli Strain 2005 http://shigen.lab.nig.ac.jp/ecoli/strain/
26. Profiling of E.coli Chromosome (PEC) 2005 http://shigen.lab.nig.ac.jp/ecoli/pec/
27. National BioResource Project YEAST 2005 http://yeast.lab.nig.ac.jp/nig/
28. National BioResource Project病原微生物2005 http://wdcm.nig.ac.jp/byogen/

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

Bruskiewich, R., Constanzo, M., Dolinski, K., Gojobori, T., Hide, W., Howe, D., May, S., Millburn, G., Misra, S., Schaeffer, M., Rhee, S., Twigger, Simon, Yamazaki, Y. 1st International Biocurator Meeting Asilomar

G-b. Genome Biology Laboratory Yuji Kohara Group

RESEARCH ACTIVITIES

Genome Biology Laboratory, Center for Genetic Resource Information

(1) Extension of the nematode expression pattern map database (NEXTDB) towards comparative genomics.

Tadasu Shin-i, Hiroshi Kagoshima, Kaori Ikeda, Ikuko Muramatsu-Sugiura, Masumi Obara, Wakako Shimizu, Aya Hamakawa, Sachiko Takahashi, Takayo Hamanaka, Yasuko Sugiyama, Yumiko Ueta, Hiroko Ochi, Chihiro Hijikata, Yuji Kohara

We are updating the WWW-based database "NEXTDB" to integrate all the information of ESTs, gene expression patterns and gene functions of C.elegans and related nematodes which are being produced and analyzed in this laboratory.

Based on 14,000 unique C.elegans cDNA species classified from 130,000 clones, whole mount in-situ hybridization of about 11,000 genes were carried out and their images were loaded to the database. Then they were annotated by their developmental stages and expression patterns. The images and their description of RNAi phenotypes of 900 genes were also stored in the database. All the images and annotations are arranged based on the corresponding cDNA clones. All the data are integrated with the genome map based on WWW, and the information of maps and their relations are displayed visually. Based on the results, we have carried out various collaborations (Kunitomo, H. et al. 2005, Lazakovitch, E. et al., 2005). The latest version is opened through the following URL. ⟨http://nematode.lab.nig.ac.jp⟩

We have started EST/genome analysis of another nematode Diploscapter sp. in collaboration with Dr. Einhard Schierenberg at Univ. of Koeln. We are interested to compare gene expression and function between different developmental systems, and this nematode is one of the case, which has a different cell cleavage and arrangement pattern until gastrulation stage. EST sequences got from about 70.000 cDNA clones were classified and about 10,000 clusters were identified. About 5,800 clusters of them were found to have homologs or orthologs to C.elegans. To identify homologues systematically, we also did whole-genome shotgun sequencing of Diploscapter sp. and got reads of x1.0 amount of the genome size. They were assembled preliminarily and the contigs were compared to C.elegans peptides by use of WormPep. We found additional 1,000 homologs in the contigs. All the results were also incorporated to NEXTDB.

(2) The C. elegans RUNX transcription factor MAB-2/RNT-1 is required for asymmetrical cell division of the T blast cell.

Hiroshi Kagoshima, Hitoshi Sawa[1], Shohei Mitani[2], Thomas R. Burglin[3], Katsuya Shigesada[4], Yuji Kohara

1) RIKEN Center for Developmental Biology
2) School of Medicine, Tokyo Women's Medical University
3) Department of Biosciences, Karolinska Institutet, Sweden
4) Institute for Virus Research, Kyoto University

The RUNX genes encode conserved transcription factors, which play vital roles in the development of various animals and human diseases. Drosophila runt is a secondary pair-rule gene, which regulates embryo segmentation. Human RUNX1, previously known as AML1, is essential for hematopoiesis. C. elegans rnt-1 is co-orthologous to the human RUNX genes. We found

that RNT-1::GFP is expressed in the H0-2, V1-6, and T blast cells in the embryo, and predominantly in the seam cells during larval to adult stages. rnt-1 mutants exhibit a loss of polarity in the asymmetrical T cell division in hermaphrodites and abnormal ray morphology in the male tail. Genetic and molecular analysis revealed that rnt-1 is allelic to mab-2. Mutant analysis suggested that mab-2/rnt-1 is involved in regulating T blast cell polarity in cooperation with the Wnt signaling pathway. Expression studies of GFP::POP-1 and TLP-1::GFP reporters in mab-2/rnt-1 mutants indicated that this gene functions upstream of tlp-1 and downstream, or in parallel to, pop-1 in the genetic cascade that controls asymmetry of the T cell division. All our data suggest that MAB-2/RNT-1 functions with POP-1 to control the asymmetry of the T cell division (Kagoshima, H. et al., 2005).

(3) POLO like kinase, PLK-1,is required for the establishment and the maintenance of anterior-posterior cell polarity in C. elegans zygotes

Eisuke Sumiyoshi and Yuji Kohara

Embryonic cell polarity is important to produce diverse cell lineages from a single cell oocyte in C. elegans. Establishment of embryonic cell polarity requires PAR proteins and MEX-5. The asymmetric localization of these proteins is important for diversification of cell lineage. We demonstrate that PLK-1, a C. elegans POLO-like kinase, is required for the asymmetric localization of PAR-1 and the maintenance of the asymmetric distribution of MEX-5 and PIE-1 in C. elegans zygotes. POLO-like kinase is a widely conserved ser/thr protein kinase and is involved in the cell cycle progression in many organisms from yeast, fly, worm to vertebrate. However, the role of POLO-like kinase in development is largely unknown. RNA interference of plk-1 caused the aberrant distribution of P granules to the entire cytoplasm and the expansion of the distribution of MEX-5 and PIE-1 to the posterior and the anterior cytoplasm, respectively, after the completion of pseudocleavage. RNAi of plk-1 also caused the localization of MEX-5 and PIE-1 to P granules both in the anterior and the posterior cytoplasm after the pseudocleavage furrow regressed. Furthermore, PAR-1, which is localized to the posterior cortex in wild type, is not localized to the posterior cortex in plk-1 (RNAi) embryos. Therefore, plk-1 seems to be involved in both the establishment and the maintenance of cell polarity. PLK-1 is localized to the anterior cytoplasm in 1-cell embryos, and to the cytoplasm of the somatic blastomeres and the P granules in the germline blastomeres in the later stage. The proper localization of PLK-1 to the anterior cytoplasm at 1-cell stage requires MEX-5 activity. Therefore, PLK-1 and MEX-5 may compose a feedback loop that maintains the polarity in the cytoplasm of C. elegans embryos.

(4) Studies on localization mechanisms of pos-1 mRNA in C.elegans embryos.

Kouki Noguchi, Yuji Kohara

Maternal pos-1 mRNA localizes to posterior half of embryo at the 1st cleavage and localized to germ lineage in early embryogenesis. We are trying to elucidate this localization mechanisms.

For this purpose, first, we injected various reporter RNA into the gonad of adult hermaphrodite and examined the RNA distribution in embryos by in situ hybridization when the reporter RNA reached to the embryos (4 hours after the injection). When we used a RNA construct (Cap - pos-1 5'-UTR - lacZ - pos-1 3'-UTR - polyA, using pJK370 vector kindly provided by T. Evans), although the localization of the RNA was not observed, the RNA signals disappeared after 1st cleavage, probably due to degradation. Interestingly, the dagaradation depended on the presence of pos-1 3'-UTR. This suggest that a balance between degradation and protection might produce the localized distribution of pos-1 mRNA. However, this phenomenon may be caused by the nature of the reporter RNA that are supplied artificially into cytoplasm, therefore, secondly, we are trying to examine the distribution of reporter RNA that are transcribed from the genome in nuclei. For this purpose, by the genomic co-injection method, we obtained the transformant lines that expressed reporter RNAs (expression vector are constructed from pJH4.52 vector kindly provided by G. Seydoux) maternally. Although it took time to device to detect the low level expression of the reporter construct, currently we are analyzing these strains.

(5) Effects of poly (A) elongation on the translational activation of maternally transcribed mRNA in *C. elegans*

Yoshiki Andachi, Shuichi Onami[1,2], Yuji Kohara ([1]Keio Univ., [2]JST, BIRD)

Many proteins required for early embryogenesis are produced by the translation of mRNAs that are transcribed during oogenesis. Maternal gene *glp-1*, a *C. elegans* Notch homolog, determines the fate of cells derived from AB that is the anterior cell at the two-cell stage. Whereas *glp-1* mRNA is distributed in both cells of two-cell stage embryos, the mRNA is translated only in the AB cell. The translational regulation is directed by 3' untranslated region (3'UTR) of the mRNA, where two sites are present, Temporal Control Region responsible for translational repression from gonad to one-cell stage, and Spatial Control Region responsible for translational repression in the posterior cell P1 at the two-cell stage. The 3'UTR interacts with three protein factors, GLD-1, POS-1 and SPN-4, which work for translational repression throughout the stages, for translational repression in the P1 cell, and for translational activation in the AB cell, respectively. Although the roles of poly(A) in translational regulation have been studied in several model organisms, ambivalent data are preventing to make clear conclusion. We previously reported correlation between translational activation and poly(A) length of *glp-1* mRNA, that is, the poly(A) length of *glp-1* mRNA was shortened to 40 bases in oocytes, and was slightly elongated to 70 bases in the P1 cell and was highly elongated to 160 bases in the AB cell.

To further confirm the correlation, we measured poly(A) length of *glp-1* mRNA in mutants where *glp-1* translation is affected. poly(A) length of *glp-1* mRNA was 150 bases in *pos-1* mutant at the two-cell stage, whereas the length was less than 30 bases in *spn-4* mutant at the two-cell stage, indicating correlation between translational activation and poly(A) length of *glp-1* mRNA in these mutants. Next, we prepared synthetic mRNA consisting of *lacZ* reporter sequence, *glp-1* 3'UTR sequence and poly(A) chain, and injected it into gonad of wild type worms. Translation of synthetic mRNA whose poly(A) length was 30 bases was regulated in the same manner as the endogenous *glp-1* mRNA. On the other hand, synthetic mRNA whose poly(A) length was 150 bases was actively translated in gonad, though translation of the mRNA was restricted in AB lineage cells of the embryo. Since poly(A) length of the injected mRNA turned out to be shortened by the onset of embryogenesis, we were not able to evaluate the effects of long poly(A) chain on the activation of translation in embryo. Nevertheless, the results suggest that long poly(A) chain can cancel the translational repression of *glp-1* mRNA in gonad.

(6) A physical model for C. elegans embryo: towards computer simulation of gastrulation.

Atsushi Kajita, Masayuki Yamamura[1], Yuji Kohara
[1] Tokyo Institute of Technology

Cellular arrangements are important for cell-fate determination in development. In C. elegans embryos, the arrangement of cells is largely restricted by physical conditions: the existence of a hard egg shell; the properties of membrane and cytoskeleton; the specificity of cell adhesion; and the force and direction of cell division. In order to understand the mechanisms underlying the dynamic cellular arrangements, we have studied computer simulations of C. elegans early embryos. We previously constructed a physical model of cells for C. elegans embryo using deformable and dividable polyhedrons, in which we modeled cell division as the main force generator. This modeling was based solely on mechanics of cells in order to make the model as simple as possible. We found that the model successfully simulated the cellular arrangements in wild type embryos up to the 8-cell stage, in embryos whose eggshell were removed, and in laser-ablated embryos.

To take the next step, we applied our model with some extensions to mutant embryos and the embryos at gastrulation. For this purpose, we added two structures to the model: microtubules(MT) and cytoplasm. Here we report computer simulation results by the extended models.

First, we modeled MTs by thin spring rods which compose a spindle. We found that the model could generate the same cellular arrangement and cleavage pattern at the 1-cell stage as those of real embryos in wild type and zen-4 mutant. In zen-4 mutant, the center connection of a spindle is not bridged between two centrosomes, thus two centrosomes separate faster than those in wild type. This phenotype was simulated by the model.

Second, we added cytoplasm as an elastic body,

and implemented direct interactions between cells: the spindle elongation force is directly transferred from a cell to the neighboring cells, and the friction force is directly exerted within a cell to the spindle that contacts eggshell. Although the orientation of the two daughter cells of E cell were still unsatisfactory, our model simulated essentially the cellular arrangements of embryos up to the 26-cell stage.

(7) Semi-automatic system for the creation of cell shape models in C. elegans embryogenesis

Hideaki Hiraki, Yuji Kohara.

Cell to cell interactions play critical roles in early embryogenesis, therefore, it is very important to have information about the arrangements of cells, cell shapes and the contact among them. Early embryogenesis of C. elegans can be analyzed utilizing time-lapse recording of multiple optical sections with a Nomarski DIC microscope. Though cell shape models of early embryo can be reconstructed from DIC images, delineating cell boundaries becomes harder as the cell number increases. Conversely, cell shapes can be directly visualized by confocal fluorescent microscopy with vital stain of plasma membranes. We have been developing a computer system to create cell shape models from a time series of confocal images of plasma membranes.

In this system, cell shapes are automatically calculated by a seeded region growing algorithm from a 3D image and a set of seed point coordinates. Manual editing of the seed coordinates is assisted by a graphical user interface. The region growing algorithm segments the image volume in such a way that one region contains one seed. A cell boundary is detected as ridges of high fluorescent signal between two seed points. We tested the system on the "dub" data set in WORM 4D CDs kindly provided by Bill Mohler, and obtained cell shape models successfully for the most part. However, we encountered a problem that some contours of cells weren't delineated correctly where the signal to noise ratio was low.

This time, we reinforced the system with the following two methods to fix the problem. One is to merge some regions after the region growing step. By this method, a cell could be represented by a few regions and the noise-derived false region boundary became minimized. Another one is a correction method based on the comparisons of the segmentation results among neighboring timepoints. Even if the cell shape is delineated incorrectly, the images of its neighboring timepoints can be sometimes processed correctly. However, because cells deform during development, comparing cell shapes among the neighbor timepoints is not straightforward. Therefore, we devised a program to calculate a smooth deformation field between two timepoints assuming that cells deformed continuously. Some errors of the segmentation could be corrected by voting among the candidate shapes that were generated by deformation of the segmentation results from the neighbor timepoints.

We are improving the system more, and plan to apply this system to compare the cellular arrangements and the cell-to-cell contacts among mutant embryos and the embryos from other species closely related to C. elegans.

(8) Towards comparative genomics: Genome/EST sequencing

Takanori Narita, Kazuko Oishi, Fumiko Ohta, Tomomi Morishita, Tomoko Endo, Keiko Nogata, Akiko Hase, Hisayo Nomoto, Noriko Hasegawa, Masumi Mizukoshi, Etsuko Yokoyama, Nanayo Ishihara, Junko Miyamoto, Shigeko Iiyama, Tadasu Shin-i, Toshinobu Ebata, Yohei Minakuchi, Kumiko Kawaguchi, Naoko Sakamoto, Yasuko Sugiyama, Yuji Kohara

As a core facility of the group grant "Genome" (consisted of about 170 labs) and the National BioResource Project, we are running a DNA sequencing center. Currently our capacity is 12 million reads per year. This year, we have carried out the sequencing of the followings;

(a) EST: Ascidian *Ciona savignyo* (Nori Satoh, Kyoto U.), Ascidian *Halocynthia roretzi* (Kazuhiro Makabe, Tokushima U.), related species to Drosophila melanogaster (Masatoshi Yamamoto, Kyoto Inst. Tech), starfish (Takeo Kishimoto, Tokyo Inst. Tech.), Yeast *Schizosaccharomyces pombe* (Chikashi Shimoda, Osaka City U.), tomato *Lycopersicon esculentum* (Hiroshi Ezura, Tsukuba U.), wheat *Triticim aestivum cv. Chinese Spring* (Yasunari Ogiwara, Kyoto Pref. U.). High quality sequences were obtained with 70-90% of the reads (depending on the samples) and deposited in DDBJ. The analyzed cDNA clones are also

distributed from this laboratory. (Suzuki, T. et al., 2005; Eichinger, L. et al., 2005; Kijimoto, T. et al., 2005; Shoguchi, E. et al., 2005)
(b) SAGE: Medaka fish *Oryzias latipes* (Hiroyuki Takeda, U. Tokyo),
(c) Genome (BAC, fosmid clones): silkworm (Tohru Shimada, Tokyo U.), platypus *Orinihorhynchus anatinus* (Fumitoshi Ishino, Tokyo MDU), Acidian *Ciona intestinalis* (Nori Satoh, Kyoto U.), medaka fish *Oryzias latipes* (Hiroyuki Takeda, U. Tokyo).(Harumi Terasaki, H. et al., 2005)
(d) Genome (whole genome): Medaka fish *Oryzias latipes* (800Mb) (Hiroyuki Takeda, Shin-ichi Morishita, U. Tokyo), freshwater choanoflagellate *Monosiga ovata* (Naoyuki Iwabe, Kyoto U., Asao Fujiyama, NII), mouse MSM strain (Toshihiko Shiroishi, NIG)

PUBLICATIONS

Papers

1. Elena Lazakovitch, John M Kalb, Reiko Matsumoto, Keiko Hirono, Yuji Kohara, Richard M Gronostajski (2005) nfi-1 affects behavior and life-span in C. elegans but is not essential for DNA replication or survival, **BMC Developmental Biology**, *5*, 24 - 24

2. Hirofumi Kunitomo, Hiroko Uesugi, Yuji Kohara, and Yuichi Iino (2005) Identification of ciliated sensory neuron-expressed genes in Caenorhabditis elegans using targeted pull-down of poly (A) tails, **Genome Biology**, *6 (2 R)* 17 - 17

3. Hiroshi KAGOSHIMA, Hitoshi SAWA, Shohei MITANI, Thomas R. BÜRGLIN, Katsuya SHIGESADA and Yuji KOHARA (2005) The C. elegans RUNX transcription factor MAB-2/RNT-1 is required for asymmetrical cell division of the T blast cell, **Developmental Biology**, *287*, 262 - 273

4. L. Eichinger, J. A. Pachebat, G. Glöckner, M.-A. Rajandream, R. Sucgang, M. Berriman, J. Song, R. Olsen, K. Szafranski, Q. Xu, B. Tunggal, S. Kummerfeld, M. Madera, B. A. Konfortov, F. Rivero, A. T. Bankier, R. Lehmann, N. Hamlin, R. Davies, P. Gaudet, P. Fey , K. Pilcher, G. Chen, D. Saunders, E. Sodergren, P. Davis, A. Kerhornou, X. Nie, N. Hall, C. Anjard, L. Hemphill, N. Bason, P. Farbrother, B. Desany, E. Just, T. Morio, R. Rost, C. Churcher, J. Cooper, S. Haydock, N. van Driessche, A. Cronin, I. Goodhead, D. Muzny, T. Mourier, A. Pain, M. Lu, D. Harper, R. Lindsay, H. Hauser, K. James, M. Quiles, M. Madan Babu, T. Saito, C. Buchrieser, A. Wardroper, M. Felder, M. Thangavelu, D. Johnson, A. Knights, H. Loulseged, K. Mungal, K. Oliver, C. Price, M. A. Quai, H. Urushihara, J. Hernandez, E. Rabbinowitsch, D. Steffen, M. Sanders, J. Ma, Y. Kohara, S. Sharp, M. Simmonds, S. Spiegler, A. Tivey, S. Sugano, B. White, D. Walker, J. Woodward, T. Winckler, Y. Tanaka, G. Shaulsky, M. Schleicher, G. Weinstock, A. Rosentha, E. C. Cox, R. L. Chisholm, R. Gibbs, W. F. Loomis, M. Platzer, R. R. Kay, J. Williams, P. H. Dear, A. A. Noegel, B. Barrell and A. Kuspa (2005) The genome of the social amoeba Dictyostelium discoideum, **Nature**, *435 (7038)* 43 - 57

5. Takashi Suzuki, Tadasu Shin-I, Asao Fujiyama,, Yuji Kohara and Masanori Kasahara (2005) Hagfish Leukocytes Express a Paired Receptor Family with a Variable Domain Resembling Those of Antigen Receptors., **J. Immunology**, *174*, 2885 - 2891

6. Teiya Kijimoto, Masakatsu Watanabe, Koji Fujimura, Masumi Nakazawa, Yasunori Murakami, Shigeru Kuratani, Yuji Kohara, Takashi Gojobori and Norihiro Okada (2005) cimp1, A Novel Astacin Family Metalloproteinase Gene from East African Cichlids, Is Differentially Expressed Between Species During Growth, **Mol. Biol. Evol**, *22 (8)* 1649 - 1660

H. STRUCTURAL BIOLOGY CENTER

H-a. Biological Macromolecules Laboratory Makio Tokunaga Group

(1) Single Molecule Imaging, Quantitative Analysis and Model Simulation of Nuclear Transport in Cells

Makio Tokunaga and Naoko Imamoto[1] ([1]Cellular Dynamics Laboratory, Riken)

What is a key to enter the nuclei through nuclear pores? This question has been answered by visualizing single molecules inside cells. Little is known about the specific molecular interactions between the assembled nuclear pore complex (NPC) and molecules in nucleocytoplasmic transport. Using novel microscopy we were able to clearly visualize single fluorescent molecules during transport on the nuclear envelope in permeabilized cells and in living cells. Kinetic parameters of the interactions between translocating molecules and NPCs were obtained. Two types of binding were found: weaker binding site gathers up to ~100 molecules/NPC, concentrating substrates locally; stronger binding site, where up to ~8 molecules/NPC are bound, changes its affinity drastically upon nuclear accumulation ability. In the presence of RanGTP, the stronger binding was not found and the active site emerges. Retention times by single molecule analysis and translocation rates by single nuclear analysis showed a correlation with a coefficient of ~8 molecules/NPC, which exhibits the stoichiometry of the active site. Computer simulation was carried out based on parameters obtained by single molecule analysis. Simulation based on a model with two types of multi-binding sites using these parameters well explained the molecular kinetic features in cells.

(2) Identification and Characterization of Associated Proteins with RNG105, An RNA-Binding Protein Responsible for Neuronal Local Translation

Nobuyuki Shiina, Kazumi Shinkura and Makio Tokunaga

We have identified and characterized RNG105-associated proteins: Caprin-2, G3BP and RACK1. Caprin-2 is a homolog of RNG105, and has the coiled-coil domain in the N-terminal and the RGG-box in the C-terminal as well as RNG105. Caprin-2 showed RNA-binding and translation-inhibition activities, and the N-terminal coiled-coil domain was required for the activities. These characteristics of Caprin-2 were very similar to those of RNG105. Caprin-2 was expressed highly in brain, and localized in granular structures in neurons. Caprin-2 was colocalized with RNG105 in some of the granules in the dendrites, but was not colocalized in other granules, especially in the cell body.

We have identified G3BP (RasGAP SH3-binding protein) as an RNG105-associated protein by immunoprecipitation, and some papers also reported that G3BP is associated with RNG105. G3BP is reported to be an RNA-binding protein and localized in stress granules in cultured cells, which are similar to the RNA granules in neurons. We found that G3BP was bound directly to RNG105 in vitro, and that G3BP regulated the RNA-binding specificity of RNG105 in vitro. G3BP was highly expressed in brain. Its localization in neurons was mainly in the cell body, but some of G3BP was localized with RNG105 in the dendritic RNA granules.

RACK1 (receptor for activated protein kinase C 1) is reported to bind to ribosomal 40S subunit and regulate translation by controlling 40S-60S docking. RACK1 is also reported to be associated with G3BP and RNG105 in polysome fractions. We found that although RACK1 was not bound directly to RNG105, it was co-immunoprecipitated with RNG105 from rat brain extracts. We transfected cultured cells with RACK1-GFP fusion protein along with RNG105-RFP fusion protein, and found that the localization of RNG105 in the cytoplasmic granules was canceled by the co-expression of RACK1. In rat hippocampal neurons, RACK1 was localized diffusely in the cell body and the proximal dendrite, where RNG105-positive RNA granules were not detected although they were located in the distal dendrites.

These results suggest that RACK1 may facilitate RNG105 release from the RNA granules or RNA granule disassembly.

(3) Single Hydrogen Bonds of DNA Base Pairs Detected in Unzipping Force by Intermolecular Force Microscopy

Michio Hiroshima[1] and Makio Tokunaga ([1]Single Molecule Immunoimaging, Research Center for Allergy and Immunology, RIKEN)

Single hydrogen bonds of DNA base pairs have been measured by unzipping double-stranded DNA oligomers with an intermolecular force microscope (IMF). IFM has achieved the force resolution of subpico-newton using ultrasensitive cantilevers. The probe position is controlled with nanometer accuracy using a feedback system, which uses laser radiation pressure to reduce thermal fluctuation of the cantilever. Previous methods of mechanical unfolding often yield "stick-slip" processes, reducing the resolution. The stick-slip processes are drops in the force caused by a sudden increase in the extension of the unfolding molecule. The feedback system eliminates the stick-slip process by controlling the probe tip position by laser radiation pressure.

DNA is bridged between a substrate and a bead bound to the tip of the cantilever. The duplex in the stem region was unzipped by pulling up the DNA end bound to the cantilever tip. Force vs. extension curves showed repeated force peaks of 10-15pN. Auto- or cross-correlation analysis and averaging of force curves were carried out to reduce noises in the force curve. The previous studies showed that the force for separating poly(G-C) DNA was 1.5 to 2 times stronger than that for poly(A-T). However, no difference was found in the force between individual G-C and A-T base pairs. The force curve of individual G-C and A-T pairs showed three and two peaks, respectively, which are assigned to single hydrogen bonds. The force is variable but the work is constant. The work to break single hydrogen bonds is 1.3 kB · T, in other words, about 1.3-fold of the thermal energy. This is the first report to detect the force of single hydrogen bonds in biological macromolecules.

(4) Single Molecule Detection of Mechanical Unfolding of Proteins

Akihiro Fukagawa, Michio Hiroshima[1] and Makio Tokunaga ([1]Single Molecule Immunoimaging, Research Center for Allergy and Immunology, RIKEN)

Single molecule measurement of the mechanical unfolding of staphylococcal nuclease (SNase) has been carried out using the intermolecular force microscope (IFM). Cysteinyl residues at both the N- and C-termini of SNase was attached to gold-coated surfaces of an IFM probe and a glass substrate. Previous methods of mechanical unfolding often yield "stick-slip" processes, reducing the resolution. The stick-slip process was eliminated by the feedback system with laser radiation pressure. Therefore, force-extension curves by IMF showed a clear breakdown of the force due to structural changes. It has yielded more than 10 times better resolution in the force-displacement detection than previous methods.

Clear force-extention curves corresponding to single molecules were obtained. The extension distance of approximately 55 nm showed accordance with the calculated distance of the single-molecule extension, 54 nm (0.36 nm/residues by 149 residues). The force-extension curves of single molecules showed some peaks, which corresponding to unfolding of subdomain structures. A few types of peak patterns were found, which indicates some pathways in the unfolding. We are demonstrating whether the fine peaks in the force-extention curves are attributed to single alpha-helices, single beta-sheets and single turns.

(5) Visualization of initiation of T cell activation

Kumiko Sakata-Sogawa[1], Michio Hiroshima[1], Tadashi Yokosuka[2], Sho Yamasaki[2], Takashi Saito[2], and Makio Tokunaga ([1]Single Molecule Immunoimaging, [2]Cell Signaling, Research Center for Allergy and Immunology, RIKEN)

Immunological synapse (IS) has been thought to mediate antigen recognition and activation of T cells. Although recent studies suggest that the activation signals precede IS formation and sustain for hours, the sites for initiating and sustaining TCR signals remain elusive. We have developed a "time 0 method" and clearly visualized initiation of T cell activation from

the moment of the activation. We reported that T cell activation is initiated and sustained in TCR-containing microclusters (MC) generated at the initial contact areas and at the periphery of the mature IS. MCs containing TCR, ZAP-70 and SLP-76 are continuously generated in the periphery. MCs containing TCR, ZAP-70 and SLP-76 migrate toward the cSMAC, but SLP-76 disappeared completely and ZAP-70 was lost mostly before the MCs join the TCR rich cSMAC. Tyrosine phosphorylation and Ca2+ influx was induced as MCs formed in the initial contact sites. These results suggest that TCR MCs initiate and sustain TCR signals.

(6) Three-dimensional Multi-color Visualization and Quantitative Analysis of Nulcear Fine Structures

Michio Hiroshima[1], Kumiko Sakata-Sogawa[1], Kazumi Shinkura, Kyoichi Isono[2], Haruhiko Koseki[2], and Makio Tokunaga ([1]Single Molecule Immunoimaging, [2] Developmental Genetics, Research Center for Allergy and Immunology, RIKEN)

Using multi-color molecular imaging, fine structures of distributions of DNA and nuclear proteins were visualized. Three kinds of fluorescent dyes were simultaneously examined. Using DNA-labeling dyes and fluorescently labeled antibodies, distributions of nuclear proteins were visualized along the string and beads structures of DNA in the nucleus.

Three-dimensional visualization and molecular quantification was carried out using GFP-fusion Polycomb Group proteins by HILO spectroscopy technique in living cells. Three-dimensional images were reconstructed from a series of z-scanned images. Defocus was eliminated using deconvolution technique. The number of molecular clusters in single nuclei and, furthermore, the number of molecules in single clusters are evaluated on the three-dimensional images. The present technique opens a new way for quantifications of three-dimensional distribution, dynamics and the numbers of multi-molecules in living cells.

(7) Asymmetric nucleocytoplasmic transport revealed by a novel assay system using planner reconstituted nuclear envelope

Atsuhito Okonogi, Michio Hiroshima[1], Nobuyuki Shiina, Shingo Kose[2], Naoko Imamoto[2] and Makio Tokunaga ([1]Single Molecule Immunoimaging, RCAI, Riken, [2]Cellular Dynamics Laboratory, Riken)

Using a novel in vitro assay system of nucleocytoplasmic transport, we found asymmetric molecular interactions with the nuclear pore complexes between import and export. Import and export of importin alpha was examined in the absence of RanGTP and other soluble factors using the present cell-free in vitro assay system. Asymmetry between import and export was discovered in the interaction of importin alpha and the NPC in the absence of Ran-GTP and other soluble factors. In the apparent equilibrium, import of importin alpha showed a difference in the concentration between at the cytoplasmic side and at the nucleoplasmic side, whereas export of importin alpha showed no difference. The concentration difference of importin alpha is found to correlate with the amount of binding of importin alpha with the NPC. The asymmetry between import and export was also found in the transport of importin alpha in the presence of NLS cargo molecules. In the presence of NLS-protein, importin alpha showed no biding with the NPC. The control molecules showed no asymmetry.

Re-addition of importin alpha to the apparently steady distribution of importin alpha at different concentrations between "inside" and "outside" of nuclear envelope showed again the same asymmetric distribution of importin alpha between "inside" and "outside". The present finding shows that the NPC has an asymmetric feature in the interactions with cargo molecules and transport factors between import and export.

(8) Molecular Imaging of Local Translation in Neuronal Dendrites by LTP Electrical Stimulation

Hiraku Miyagi, Kumiko Sakata-Sogawa[1], Michio Hiroshima[1] and Makio Tokunaga ([1]Single Molecule Immunoimaging, RCAI, Riken)

Local protein synthesis in neuronal dendrites is required for synaptic plasticity, which is associated with long-term memory storage. This protein synthesis is reported to be induced at activated postsynaptic sites. In order to investigate when and where the translation is initiated locally in the dendrites, we visualized local translation in dendrites of rat

hippocampal neurons after LTP electrical stimulation. Local translation was visualized by the fluorescence of GFP translated from EGFP-CaMKIIa 3'UTR mRNA. Only in the vicinity of the tip of electrode, fluorescence increase, i.e. local translation was observed. It was occurred at the root of spines. Shape change of spines and sprouting of filopodia-like protrusion was also found in the vicinity of the electrode tip after simulation.

(9) Development and Optimization of Molecular Microscopy and Software

Makio Tokunaga, Michio Hiroshima[1]and Kumiko Sakata-Sogawa[1] ([1]Single Molecule Immunoimaging, RCAI, Riken)

We are presently developing and optimizing the Highly Inclined and Laminated Optical sheet (HILO) microscopy technique for multi-color imaging. Stable observation during long time was achieved by a novel autofocusing system using total internal reflection with near-red sensing light, whose resolution of the height measurement of the specimen was higher than 0.1 micrometer. The resolution of images has been refined especially by development of a high-sensitive camera and an objective lens. Control system of the microscope with a personal computer has been developed in both hardware and software. We are also developing a software system of single molecule imaging for image analysis and quantification of molecular interactions and kinetics.

PUBLICATIONS

Papers

1. Kitamura K., Tokunaga M., Esaki, S., Iwane A. H. & Yanagida T (2005) Mechanism of muscle contraction based on stochastic properties of single actomyosin motors observed in vitro, **BIOPHYSICS**, *1*, 1 - 19

2. Shiina N., Shinkura K., Tokunaga M (2005) A novel RNA-binding protein in neuronal RNA granules: Regulatory machinery for local translation, **J. Neuroscience**, *25 (17)* 4420 - 4434

3. Yokosuka T., Sakata-Sogawa K., Kobayashi, W., Hiroshima, M., Hashimoto-Tane, A., Tokunaga M., Dustin, M. L., Saito, T, (2005) Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76, **Nature Immunol**, *6 (12)* 1253 - 1262

Books

4. Tokunaga M (2005) 「分子モーター」 物理学大辞典 (鈴木増雄・荒船次郎・和達三樹編) 朝倉書店 766 - 775

EDUCATION

他大学／研究機関での講義やセミナー

1. Dr. M. Tokunaga gave a Seminar at Institute of Molecular and Cellular Biosciences, the University of Tokyo, February, 2005 (in Japanese).
2. Dr. M. Tokunaga gave a Seminar at Yukawa Institute, Kyoto University, December, 2005 (in Japanese).

客員教授等

Tokunaga M 科学を育む光学顕微鏡 Olympus伊那工場

SOCIAL CONTRIBUTIONS AND OTHERS

特許出願

各種委員

1. 徳永万喜洋 バイオテクノロジー開発技術研究組合「細胞内ネットワークのダイナミズム解析技術開発」研究開発委員
2. 徳永万喜洋 科学技術振興調整費研究評価部会「細胞・生体システム研究評価WG」科学技術・学術審議会専門委員

集会／シンポジウムの主宰

H-b. Molecular Biomechanism Laboratory Nobuo Shimamoto Group

RESEARCH ACTIVITIES

(1) Regulatory Mechanism of transcription initiation by switching conformations of RNA polymerase: a homogeneous protein works inhomogeneously.

Motoki Susa[1], Takashi Miyamoto[1], and Nobuo Shimamoto[1] ([1]Structural Biology Center, National Institute of Genetics and and Department of Genetics,

School of Life Science, The Graduate University for Advanced Studies)

For several decades, all RNA polymerase once initiating formation of phosphodiester bonds at a promoter (the initiation complex) has been supposed to synthesize mature transcript. Accordingly, the mechanism of transcription initiation has been assumed to be a sequence of three essential steps: formation of a complex of RNA polymerase and a promoter (closed complex), isomerization into a complex with a DNA bubbled duplex, (open complex), and escape of RNA polymerase from the promoter associated with elongation of RNA (promoter clearance). All RNA polymerases so far examined in vitro are known to catalyze not only the synthesis of full-length RNA but also abortive synthesis, which is an iterative synthesis and release of larger amounts of oligo-RNA molecules, typically 2 to 15 nucleotides in length. In the conventional sequential mechanism, abortive synthesis is often excluded from the mechanism or supposed to be stochastic failure by the homogeneous initiation complex. This sequential model is based on the idea that a homogeneous fraction of a protein should work homogeneously, which is a basic hypothesis used in studies on various biological systems.

However, we found that this idea is wrong at least for transcription in *E. coli*. We identified a new form of initiation complex, named moribund complex, which exclusively synthesize abortive transcript. Since 1996, we show it is

1. generated from homogeneous RNA polymerase [JMB 256, 449 ('96), Genes Cells 6, 389 ('01)]
2. the major source of abortive products on a λP_R promoter and mitigated by GreA/B [the same refs in 1]
3. arrested at the promoter with a different structure from productive form [JBC 275, 10899 ('00)]
4. detected as persistent abortive synthesis after completion of full-length synthesis [JBC 277, 15407 ('02), the same refs in 1]
5. induced by force and could work as a safety valve for misincorporation [*NAR* 25, 2640-47 ('97)]

In 2005, we finally proved that moribund complex contributes transcriptional regulation in *E. coli* cells at least for three genes *(atp, cspA, rpsA)* [Ref. 1 (2005)]

To examine the existence and significance of the branched pathway *in vivo*, we selected GreA and GreB for clues. At the λP_RAL promoter these factors enhance conversion of the moribund complex into the productive one, in the presence of high concentrations of initiating nucleoside triphosphate *in vitro*. If the branched mechanism exists *in vivo*, absence of the Gre factors should result in reduction of productive transcription from promoters at which the moribund complex is susceptible to these factors. We constructed a double-disruptant of *E.coli*, $\Delta greA \Delta greB$, and then arbitrarily selected 10 genes from among those whose levels of transcripts in the mutant strain were found to be lower than those in the parental $greA^+ greB^+$ strain. Finally, the promoter for three of these genes, *atpC (uncC), cspA*, and *rpsA*, passed a further conventional test which confirmed that they displayed a branched initiation pathway in a reconstituted transcription system composed of purified components. The results obtained prove that the branched initiation pathway exists *in vivo* and is utilized in regulation of transcription initiation from some promoters, through modulation of the fraction of polymerase-promoter complexes entering each branch of the pathway.

In this year, we examined various physiological conditions that affect on the levels of the Gre factors. The determination of the level of GreB was observed to be constitutive. The level of GreA remained the same through the growth phase, and did not respond much to the richness of the culture media. However, it decreased into half in aerobic conditions, indicating that some genes are regulated by GreA. Therefore, the regulatory circuit responding to the levels of proteins involving GreA, namely the branched pathway mechanism, is working in cells.

(2) Applicability of thermodynamics to equilibria in biology.

Nobuo Shimamoto[1] and Jyun-ichi Tomizawa[1],
([1]Structural Biology Center, National Institute of Genetics and Department of Genetics, School of Life Science, The Graduate University for Advanced Studies)

Most DNA-binding proteins are biologically functional as a specific complex, one containing a special short DNA segment. Such a complex is usually assumed as a state tenable for thermodynamic analysis of binding equilibrium. Thus, forward and backward reactions should balance at equilibrium in every

pathway, and the affinity should be independent of the length of DNA. However, we have found that the balance at equilibrium is broken for some proteins by their sliding along DNA during association but not dissociation and that their affinities for their specific sites dependent on the length of DNA harboring the sites. This seeming disagreement is explained by an indeliberate use of the state of specific complex in thermodynamics. In the presence of sliding, the state does not satisfy the second law (the ergodic condition) and thus is disqualified for thermodynamic analysis. A general treatment of binding equilibrium, while maintaining the specific complex as a distinct state, is proposed on the base of the master equation or chemical kinetics.

(3) Systematic search for promoters encoded in the genomic DNA sequences of *E. coli*

Nobuo Shimamoto[1], Hideki Nakayama[1] and Hironori Aromaki[2] ([1]Structural Biology Center, National Institute of Genetics and Department of Genetics, School of Life Science, The Graduate University for Advanced Studies. [2]Daiichi College of Pharmaceutical Sciences)

Irrespective of a pile of compiled sequences identified as promoters for E. coli vegetative RNA polymerase holoenzyme, σ^{70} holoenzyme, there is no successful methods to predict strength of a promoter. To construct such prediction method, we started to design a functional SELEX to select promoter sequences. The complexity of random oligo-DNA available in a lab scale, $4^{12\sim14}$ is too small to cover the all-possible promoters. Therefore we limited the candidate sequences to those involved in the genomic sequence. In order to construct and select a library, we transferred and amplified parts of the genomic sequence with PCR with a single primer. As a drawback of the use of single primer, the constructed library contains DNA fragments generated from in vitro recombination. A theoretical method to diminish the effect of such recombinants has been developed.

PUBLICATIONS

Papers

1. 十川久美子，嶋本伸雄(2005)ナノ回転計が明かしたＤＮＡ結合タンパク質の挙動，Bionics，2，62 - 63

Books

2. 嶋本伸雄, Ed. (2005). ナノバイオ入門. 1 vols. Tokyo：サイエンス社.

3. 嶋本伸雄(2005)．ナノバイオテクノロジー産業化推進調査(ナノバイオロードマップ)報告書　NEDO

講演，学会報告

International

4. Miyamoto T.,Susa,M. and Shimamoto N. Coupling between transcription initiation and DNA damage, "The 9th Asian-Pacific Conference on Transcription", Miaoli, Taiwan, 2005

5. Nakayama H., Shimamoto N., A system for single-molecule dynamics of translation "The 9th Asian-Pacific Conference on Transcription", Miaoli, Taiwan, 2005

6. Shimamoto N., Chemical and Biological consequences of one-dimensional diffusion of proteins along DNA "International Congress of Biorheology and International Conference on Clinical Hemorheolog", Chongqing, 2005, 5/30

7. Shimamoto N., Chemical processes involved in control switches of transcription initiation, FASEB Meeting "Prokaryotic Transcription Initiation", Saxton Rever, VT., 2005, 6/21

8. Shimamoto N., Control switches of transcription initiation (and big questions), "The 9th Asian-Pacific Conference on Transcription", Miaoli, Taiwan, 2005 12/15

9. Shimamoto N., Ceremonial speech for the president Chen-Wen Wu, "The 9th Asian-Pacific Conference on Transcription", Miaoli, Taiwan, 2005, 12/15

10. Suganthan Rajan Babu, Sato Y.,Yagi S., Gyedu Ampaabeng and Shimamoto N. Unexpected cleavage occurred in modified sigma70 subunit of active *E. coli* RNA polymerase, "The 9th Asian-Pacific Conference on Transcription", Miaoli, Taiwan, 2005

国内

11. Suganthan Rajan Babu, Sato, Y., Yagi, S., Gyedu Ampaabeng and Shimamoto N., Unexpected cleavage occurred in modified sigma70 subunit of active E. coli RNA polymerase　第43回日本生物物理学会，札幌市，2005，11／23

12. 中山秀喜，嶋本伸雄　翻訳の１分子ダイナミックスの測定系の構築, RNA情報網第３回サテライトミーティング，伊賀，2005

13. 中山秀喜，嶋本伸雄，伊藤耕　翻訳の１分子ダイナ

ミックスの測定系の構築，第７回RNAミーティング，弘前，2005
14. 中山秀喜，嶋本伸雄，伊藤耕一，翻訳の１分子ダイナミックスの測定系の構築，第43回日本生物物理学会，札幌市，2005，11／23
15. 宮本貴史，須佐太樹，嶋本伸雄　転写開始とDNA損傷との共役点，21世紀大腸菌研究会，志摩，2005
16. 宮本貴史，須佐太樹，嶋本伸雄，転写開始とDNA損傷との共役点，第７回RNAミーティング，弘前，2005
17. 宮本貴史，須佐太樹，嶋本伸雄，DNAのUV損傷による転写スイッチ，第43回日本生物物理学会，札幌市，2005，11／23

EDUCATION

1. Dean of School of Life Science, Graduate School of Advansed Studies
2. 嶋本伸雄，富澤純一　反応平衡における熱揺動の生理学的役割　基研研究会　ナノバイオダイナミクス　京大基礎物理学研究所
3. 嶋本伸雄　基礎から学ぶ分子細胞生物学コースー機能分子としてのタンパク質　神奈川科学技術アカデミー　東大医科研
4. 嶋本伸雄　生体分子のナノバイオ的利用　ナノバイオ産業化セミナー　大阪商工会議所
5. 嶋本伸雄　計測ワーキンググループ報告　ナノバイオロードマップと産業化を考えるシンポジウム　大阪商工会議所国際会議ホール

SOCIAL CONTRIBUTIONS AND OTHERS

各種委員

1. 嶋本伸雄，文部科学省科学技術・学術審議会専門委員
2. 嶋本伸雄，NEDO「ナノバイオテクノロジー産業化推進調査」委員，WG

学会活動等

1. ember of international Committee of Asian Conference of Transcription (ACT), the delegate of Japan. (Organizing members of ACT8, Bangkok, November, 2004)
2. 嶋本伸雄，財団法人未踏科学技術協会「生命を測る」組織幹事

H-c. Multicellular Organization Laboratory
Isao Katsura Group

RSEARCH ACTIVITIES

(1) Enhancement of chemotaxis by pre-exposure to odorants and food in the nematode *C. elegans*

Ichiro Torayama, Hiroshi Ichijo, Kotaro Kimura, Takeshi Ishihara[1] and Isao Katsura ([1]Kyushu University)

The nematode *C. elegans* provides a good system for the analysis of behavioral plasticity at the levels from molecules to organisms. We have found that pre-exposure to butanone and food enhances chemotaxis to butanone but not to benzaldehyde, although butanone and benzaldehyde are sensed by the same olfactory neurons called AWC. This odor-specific plasticity, which we call butanone enhancement, is different from the already known plasticity in which food inhibits olfactory adaptation. In the latter case, the chemotaxis index never exceeds that of naive animals, and food can be replaced by serotonin, which is not true for butanone enhancement. Butanone enhancement is beneficial to animals for their survival and proliferation, because it helps them find food at higher probability. It resembles classical conditioning in this aspect, but differs in the following aspects. (a) Animals are attracted by the conditioned stimulus (butanone) even without conditioning, while conditioning only enhances the degree of attraction. (b) The conditioned stimulus must be presented at the same time as and not before the unconditioned stimulus (food). To elucidate the molecular mechanism, we isolated ten mutants that showed abnormality in this plasticity. Of those mutants, *olrn-1(ut305)* and *olrn-2(ut306)*, which showed the strongest abnormality, were studied in detail. *olrn-1* gene encoded a novel protein containing predicted transmembrane domains and showing limited homology to the *Drosophila* Raw protein. Expression of *olrn-1* in AWC sensory neurons was sufficient for normal butanone enhancement. The *olrn-1(ut305)* mutant showed abnormality in the neuronal asymmetry of AWC neurons: the wild-type animal expresses *str-2* gene in only one of the two AWC neurons, but the *olrn-1(ut305)* mutant expresses *str-2* in neither of the AWC neurons. We also cloned

olrn-2 gene, which was allelic to *bbs-8* gene, one of the Bardet-Biedl syndrome genes. The *olrn-2(ut306)* mutant showed the wild-type phenotype in the AWC asymmetry.

In 2005, we obtained the following results. (1) Since the *olrn-1* mutant does not express *str-2*, we hypothesized that the AWC^{ON} neuron, which expresses *str-2*, is required for butanone enhancement. This hypothesis was proved by various experiments, including the butanone enhancement assay of AWC^{ON}-killed wild type animals. (2) The *olrn-2(ut306)* mutant showed structural defects in sensory cilia like known Bardet-Biedl syndrome mutants. We therefore tested many cilium structure mutants for butanone enhancement. The results revealed that all the Bardet-Biedl syndrome mutants tested, *olrn-2/ bbs-8*, *bbs-1*, and *osm-12/ bbs-7*, were abnormal, while other cilium structure mutants, including *osm-3*, *dyf-1*, *daf-10*, *che-11*, *osm-1* and *che-2* mutants, were normal in butanone enhancement. (3) We found that pre-exposure to isoamyl alcohol and food enhances chemotaxis to isoamyl alcohol, and isolated mutants defective in this behavioral plasticity.

(2) Genetic analysis of plasticity of avoidance behaviors in *C. elegans*

Kotaro Kimura and Isao Katsura

Although *C. elegans* exhibits several types of behavioral plasticity, the plasticity of its avoidance behaviors has not been studied enough. We have discovered a novel type of plasticity in the avoidance response of *C. elegans*. The avoidance response of the animal to repellent odors was enhanced, rather than being reduced, after preceded exposure to the odor. It was somewhat surprising because almost all of the previously known stimuli (including other repellent stimuli) to the animals cause adaptation, i.e., the reduction of the sensory response after preceded exposure. In general, adaptation has been extensively studied using various experimental systems. However, the enhancement of the sensory response has been far less studied, particularly at the molecular level, with an exception of mammalian pain sensation and gill-withdrawal reflex in *Aplysia*. Therefore, analyzing the enhancement of the avoidance response of *C. elegans* may lead to the identification of a novel regulatory mechanism of the sensory response enhancement in general.

To gain insight into the molecular mechanism of the enhancement of 2-nonanone avoidance behavior, we have analyzed the phenotype of ~60 mutants of genes that may play roles in neuronal function, such as ion channels, neurotransmitter receptors, and signaling proteins. We found that several mutants showed specific defects in the enhancement of avoidance behavior, but not in naive avoidance behavior itself. We are currently analyzing detail of the phenotype.

(3) Class 1 *flr* mutants of the nematode *Caenorhabditis elegans*

Yuri Kobayashi, Kotaro Kimura and Isao Katsura

Class 1 *flr* mutants of *C. elegans*, which map in *flr-1*, *flr-3* and *flr-4*, were isolated originally by resistance to 0.4 mg/ml NaF (Katsura, I. *et al.*: Genetics **136**, 145-154, 1994). They also show many other phenotypes including slow growth, short defecation cycle periods (Dec-s), frequent skip of the expulsion step of defecation, synthetic abnormality in dauer larva formation, weak tendency to stay on food, and hypersensitivity to serotonin. The *flr-1* gene encodes a cation channel of the DEG/ENaC superfamily, while *flr-4* and *flr-3* code for a novel Ser/Thr protein kinase and a kinase-like molecule, respectively, both having a hydrophobic domain at the carboxyl terminus. They are expressed essentially in the intestinal cells, except that *flr-4* is expressed also in the isthmus of the pharynx and in a pair of head neurons called AUA. We consider that class 1 *flr* genes constitute a regulatory system that acts in differentiated intestinal cells.

In 2005, we found that animals expressing the FLR-1 ion channel without the C-terminal cytoplasmic domain (FLR-1DC) show long defecation cycle periods (Dec-l phenotype). By carrying out RNAi of many genes that are expressed in the intestine, we identified genes, the RNAi of which suppresses the Dec-l phenotype of FLR-1DC and the Dec-s phenotype of the *flr-1(ut11)* mutant, respectively. We think these genes act in the regulation of defecation cycle periods, and plan to analyze them in detail.

(4) Class 2 *flr* mutants of the nematode *Caenorhabditis elegans*

Akane OISHI, Kotaro Kimura, Takeshi Ishihara[1] and Isao Katsura ([1]Kyushu University)

Class 2 *flr* mutations were isolated first as a weak fluoride-resistant mutation and then as suppressors of the slow growth or serotonin-hypersensitivity of class 1 *flr* mutations. Besides these phenotypes, they also suppress the dauer larva formation abnormality and weak tendency to stay on food, but not the defecation abnormalities or strong fluoride-resistance. By themselves, class 2 *flr* mutations show the phenotypes of weak resistance to NaF and short average longevity as compared with wild-type animals. The phenotypes suggest two possibilities on the relationship between class 1 and class 2 *flr* genes. (a) Class 2 *flr* genes may act downstream of the class 1 regulatory pathway. At the downstream, the regulatory pathway bifurcates into two branches, the growth/dauer branch and the defecation branch, while class 2 genes act in the former branch and not the latter. (b) Class 2 *flr* genes may act antagonistically to class 1 genes, while the threshold of the phenotypes is different between the growth/dauer phenotypes and the defecation phenotypes.

Class 2 mutations map in four genes, *flr-2, flr-5, flr-6* and *flr-7*, of which only *flr-2* has been cloned. *flr-2* encodes a secretory protein belonging to the gremlin/DAN/cerberus family. A functional *flr-2*::GFP fusion gene was expressed in some neurons in the head and the tail as well as many pharyngeal neurons. We also found that FLR-2 interacts in vitro with a secretory protein encoded by ZK20.1 gene, and isolated a deletion mutant in this gene.

In 2005, we obtained the following results. First, we found that the *ZK20.1* mutation increased defecation cycle periods under certain genetic backgrounds but not under the wild-type background. For instance, the *ZK20.1; flr-2(ut5)* double mutant showed longer defecation cycle periods than the wild-type or the *flr-2(ut5)* mutant. Furthermore, the *ZK20.1; flr-2(ut5)* double mutation suppressed the short defecation cycle periods and the expulsion defect of *various flr-4* mutants *(ut7, sa201, n2259)*. Second, although we tried to clone *flr-5* gene by the positional cloning method, we could not succeed because of technical difficulties. Therefore, we started to clone *flr-6* and *flr-7* genes.

(5) Analysis of synthetic dauer-constitutive mutants in the nematode *Caenorhabditis elegans*

Tomoko Yabe, Norio Suzuki[1], Tatsuo Furukawa[2], Takeshi Ishihara[3] and Isao Katsura ([1]Riken CDB, [2]Kagoshima University, [3]Kyushu University)

If newly hatched larvae of *C. elegans* are in a crowded state and given a limited food supply, they sense the environmental signal of pheromone and food with a head sensory organ called amphid, and deviate from the normal life cycle, ending in non-feeding larvae called dauer larvae. Although mutations showing abnormality in this phenomenon were isolated to saturation, the regulation of dauer larva formation has not been fully elucidated. We, therefore, are investigating synthetic dauer-constitutive (synDaf) mutants, i.e., mutants that form dauer larva in a certain mutant background even in the presence of plenty food and at low population density. We found that mutations in more than 50 known neural genes show synDaf phenotypes. The synthetic nature of the phenotype, we think, is based on the pathway of sensory signals. Namely, the signals are transmitted through parallel routes, and therefore two mutations are required to block them. We are determining the combinations of mutations for the synDaf phenotype and the pattern of suppression of the synDaf phenotype by various suppressor mutations. In this way we hope to elucidate the detailed regulatory pathway for dauer larva formation.

Furthermore, to identify new genes for the regulation of dauer larva formation, we have isolated and mapped 44 mutations that show the synDaf phenotype in the *unc-31(e169)* background, where *unc-31* gene encodes CAPS protein, which acts in secretion from dense core vesicles. These mutations mapped at least to 17 genes, of which *sdf-9, sdf-13*, and *sdf-14* have been cloned. *sdf-9* encoded a protein tyrosine phosphatase-like molecule, was expressed in a pair of neuron-associated cells called XXXL/R, and regulated dauer larva formation in the steroid hormone signaling pathway. *sdf-13* encoded a homologue of the transcription factors Tbx2 andTbx3, was expressed in AWB, AWC, and ASJ sensory neurons as well as many pharyngeal neurons, and controlled olfactory adaptation in AWC and dauer larva formation in cells

other than AWC (possibly ASJ). *sdf-14* was allelic to *mrp-1*, which was formerly identified by its homology to multidrug resistance-associated protein genes in mammals. A functional *sdf-14*::GFP fusion gene was expressed in many tissues including neurons, pharyngeal muscles and intestinal cells, and expression in multiple tissues was required for the wild-type phenotype. Interestingly, human MRP1 could substitute for *C. elegans* MRP-1 in dauer larva regulation, and an inhibitor of the MRP1 export activity impaired this function, showing that the export activity is required for normal dauer larva regulation. sdf-14 mutations strongly enhance the dauer-constitutive phenotype of the *daf-2(e1370)* mutation, which confers resistance to many environmental stresses. Furthermore, sodium arsenite, which is a substrate of human MRP1, enhanced dauer larva formation in the background of the *unc-31(e169)* mutation, which partially blocks the *daf-2* insulin signaling pathway. Thus, although we could not show direct evidence, *sdf-14* gene may be involved in stress responses.

In 2005, we wrote a paper on *sdf-14* and published in the journal Development (Yabe et al., 2005). We are also continuing epistasis studies to reveal the regulatory pathway.

PUBLICATIONS

Papers

1. Take-uchi, M., Kobayashi, Y., Kimura, K. D., Ishihara, T., Katsura, I.(2005) FLR-4, a novel Serine / Threonine protein kinase, regulates defecation rhythm in *Caenorhabditis elegans.*, **Mol. Biol. Cell**, *16*, 1355 - 1365

2. Yabe, T., Suzuki, N., Furukawa, T., Ishihara, T., Katsura, I. (2005) Multidrug resistance-associated protein MRP-1 regulates dauer diapause by its export activity in *Caenorhabditis elegans.*, **Development**, *132*, 3197 - 3207

3. 桂　勲(2005)線虫*C. elegans*の発生・分化研究とゲノム情報，実験医学増刊，*23 (1)* 157 - 163

EDUCATION

1. 桂　勲　生命理工学部特別講義Ⅰ　東京工業大学・生命理工学部

2. 桂　勲　線虫*C. elegans*の行動の分子遺伝学的解析－行動の中心的制御機構の探索－東京工業大学・生命理工学部

SOCIAL CONTRIBUTIONS AND OTHERS

学会活動

桂　勲　Genes to Cells, Associate Edditor
桂　勲　日本分子生物学会，広報幹事
桂　勲　日本生物物理学会，委員

H-d. Biomolecular Structure Laboratory Yasuo Shirakihara Group

RESEARCH ACTIVITIES

(1) Crystallographic Study of ATP synthase

Yasuo Shirakihara and Aya Shiratori

ATP synthase is responsible for ATP production in living cells, and is a membrane protein located in the energy conversion membranes. ATP synthase consists of a channel Fo portion (about 100,000 dalton, subunit composition of $ab_2c_{8\text{-}12}$) and a large soluble catalytic F1 portion (380,000 dalton, $\alpha_3\beta_3\gamma\delta\varepsilon$). The unique rotational catalysis mechanism of F1 includes rotation of the rod-like γ subunit, which is thought to control the conformations of the three catalytic β-subunits in a cyclic manner by its rotation. Starting from elucidation of the $\alpha_3\beta_3$ sub-assembly structure of the thermophilic F1, we have moved up to the higher sub-assemblies. After the $\alpha_3\beta_3\gamma$ and $\alpha_3\beta_3\gamma\varepsilon$ sub assemblies, we have been working on the holo-enzyme, ATP synthase.

ATP synthase is still a challenging target for a structural study, in view of relatively few solved structures of the membrane proteins so far. Though last year we were able to record the first diffraction patterns from ATP synthase crystals to about 7 Å, we obviously had to improve quality of the crystals and this was done this year.

Firstly, we have continued intensive examination of the protein preparation by changing detergents species, PS3 culture batches, kinds of nucleotide present in the extraction step and various purification parameters. Quality of each preparation was evaluated by its crystallization capability and by diffraction capability of the resultant crystals, if crystallized. This procedure established the following. Decyl-maltoside and dodecyl-maltoside, both of which had been useful for crystallization but indistinguishable between the

two, were shown to have their own features. Though dodecyl-maltoside was much better than decy-maltoside in a number of respects, we have encountered a problem that crystals from dodecyl-maltoside were produced less frequently. The problem was cured partially by finding better PS3 cell stocks, examining dodecyl-maltoside from different manufacturers and knowing properties of the used chromatographic materials better.

Secondly, we did a laser experiment in a hope to get better crystals, because it was shown that a well defined application of laser beam to crystallization setups is useful for inducing crystallization and sometimes for getting better ordered crystals. Although the laser beam clearly induced crystallization in the experiment, they were small compared with crystals grown without the laser beam application. We could not record protein diffraction pattern from either of the crystals. Further efforts to optimize the target crystallization conditions may be necessary for a successful laser experiment.

Thirdly, we made further efforts to analyze the diffraction patterns obtained last year. The analysis had been hampered by incorrect beam position parameters supplied and a high mosaicity of crystals. Data were recollected in a way that should allow diffraction analysis, and an analysis is going with our refined procedures.

The preparation and crystallization study was done in collaboration with Satoshi MURAKAMI at Institute of scientific and industrial research, Osaka University, Toshiharu SUZUKI and Masasuke YOSHIDA, at Research Laboratory of Resource Utilization, Tokyo Institute of technology. The laser experiment was done with Satoshi MURAKAMI and his colleagues in Osaka University and in Sosho.

(2) Comprehensive Crystallographic Study of Transcription factors and Genome-partitioning Factors from *E.coli*

Yasuo Shirakihara and Aya Shiratori

In *E.coli*, more than 160 transcription factors control transcription of their target gene(s) by binding to both their specific DNA sequence and the transcription apparatus. Sixty-five such transcription factors had been purified in Ishihama laboratory. In Niki laboratory, a number of novel proteins, that are judged to play roles in the genome and plasmid partitioning, have been prepared. Setting these transcription and genome partitioning factors as targets for a structural study, we have been doing comprehensive structure determination in the Protein 3000 project (the sub-field of 'transcription and translation'). In the previous first three years, we examined 65 proteins for their crystals but identified only three proteins that gave crystals of diffraction quality. This year, we have concentrated to refine the crystals obtained so far.

YmcB, a protein believed to be involved in the genome and plasmid partitioning, was found last year to form extremely good crystals. The SeMet derivative of YmcB was expressed, successfully purified owing to knowledge of purification procedures accumulated over the last year, and was crystallized under the conditions similar to those for the native protein. Most of the crystals were trigonal ($a=69.2$Å, $b=69.2$Å, $c=172.8$Å) with a resolution limit of 2.6 Å, but a minor proportion was tetragonal ($a=b=68.5$Å, $c=99.2$Å) with a resolution limit of 2.0 Å. MAD data sets were collected from a number of SeMet derivative crystals, because initial data sets could not be analyzed successfully possibly due to deteriorative effects of inappropriate crystal freezing. A data set obtained at a late stage of study was processed in a satisfactory way. SAD analysis of the data set with SOLVE looked promising, and a further analysis is going on.

This work has been done in collaboration with Akira ISHIHAMA, Emi KANDA (Nippon Institute for Biological Science), Hironori NIKI, Rie INABA, Katsynori YATA, Yasushi OGATA (isotope center).

(3) Structure-based functional studies on transcription factors

Hiroshi Itou and Yasuo Shirakihara

Genomic-DNA information on number of organisms is now available. Using the information, comprehensive structure analysis of transcription factors for their structure-based functional understanding is in progress. We had already succeeded in structure analysis of five of crystals; two from an archaeon *P.horikoshii* and three from a bacterium *C.glutamicum*. As a functional approach, target DNA sequences for these proteins were determined using SELEX (Systematic Evolution of Ligand by EXponential enrichment) method, and this

led to a tentative identification of the target genes for these transcription factors. Based on the results from these two approaches, further experimental strategies toward detailed functional understanding of the proteins were set out. Through the combination of structure and functional analysis, we successively determined function of the CGL2612 protein, one of the hypothetical transcription factors from *C.glutamicum*, as a transcriptional repressor that responsible for the antimicrobial resistance system in the bacterium. The strategy used in this study is one of the most convenient and powerful methods to analyze functionally unknown transcription factors newly identified by genomic analysis.

This work has been done in collaboration with Ui OKADA, Yonggui Gao and Isao TANAKA at Division of Biological Sciences, Graduate School of Science, Hokkaido University, and Hiroaki Suzuki and Masaaki Wachi at Department of Bioengineering, Tokyo Institute of Technology.

(4) Expression and crystallization Study of Eukaryotic Transcription Factors

Hiroshi Itou and Yasuo Shirakihara

Eight eukaryotic transcription factors (four from *H.sapience*, two from *O.lapites*, one from *M.musculus*, and one from *O.sativa*) have been selected as our research target. To prepare sufficient amount of extremely purified protein samples required for protein crystallization experiment, optimization of protein preparation strategies for these targets have been in progress. Despite of the complexity of protein folding inherent in eukaryotic proteins, we've be getting good results in the sample preparation step for three of proteins, under the condition of co-expressed them with several chaperon proteins using an *E.coli* over-expression system.

This work has been done in collaboration with Isao TANAKA at Division of Biological Sciences, Graduate School of Science, Hokkaido University (*H.sapeience* and *O.lapites*), and Sumio SUGANO at Division of Biosciences, Graduate School of Frontier Sciences, Tokyo University *(H. sapience)*, Minoru TANAKA at Laboratory of molecular genetics for reproduction, National Institute for Basic Biology *(O.lapites)*, Mitsuru MORIMOTO and Yumiko SAGA at Mammalian Development Laboratory, Genetic Strains Research Center, National Institute of Genetics *(M.musculus)*, and Ken-ichi NONOMURA at Experimental Farm, National Institute of Genetics *(O.sativa)*.

(5) Expression and crystallization Study of Transcription factors from *Pseudomonas aeruginosa*

Yasuo Shirakihara and Aya Shiratori

Pseudomonas aeruginosa is a well-known opportunistic bacterial pathogen, and a number of transcription factors responsible for the pathogenicity have been identified. After structural studies of PtxR, PtxS, PhzR, and PA3547 done over the last two years, we have included 12 of such transcription factors this year. Those were AlgP, AlgQ, Anr, FleN,Dnr, PA0275,PA2591,RhlR, Vfr, AlgR, FleQ, FleR, and FleS.

The genes for the proteins were cloned into pET21b so that the expressed protein has a His tag at the C-terminus, and their protein expression was examined by a locally developed system using *E.coli* hosts. Among those twelve proteins, 6 of those (AlgQ, FleN, Dnr, PA2591, Vfr, FleQ) were obtained in large amounts. However, annoyingly, in cases of Anr and AlgR, proteins that had molecular weight around 32,000 and were presumably due to a cloning vector, were strongly expressed irrespective of host cells and expression conditions. The rest were not expressed. The proteins were purified with a Ni-NTA column and, when possible, an ion exchange column, and have been subjected to crystallization studies.

This work has been done in collaboration with Hironori ARAMAKI (Daiichi Pharmaceutical College).

(6) Crystallization of Kid, a chromosome mover

Yasuo Shirakihara and Aya Shiratori

Kid is involved in spindle formation and chromosome movements in mitosis/meiosis. It consists of three domains: an N-terminal kinesin-like motor domain (35-370), a C-terminal chromosome-binding domain (594-647) and a connecting domain (371-593) including a coiled-coil region. So far, we have tried to crystallize fragments of the molecule: the N-terminal motor domain, the C-terminal chromosome-binding domains and a longer N-terminal fragment (1-462). This year, we examined properties of the hopeful crystals

of the longer N-terminal fragment, obtained with jeffamine or isopropanol in the presence of MgADP. However, the observed diffraction patterns suggested that the crystals were not genuine N-terminal fragment crystals. Then we started a crystallization experiment of the full-length Kid, expressed in *E.coli* as a GST fusion protein and subsequently the GST portion was cleaved-off. From a preliminary crystallization study, we think that we have to increase the available amount of the protein, improve quality pf the prep, and devise a successful strategy for the molecule with predicted disordered regions covering a third of the molecule.

The study was done in collaboration with Noriko TOKAI, Miho OHSUGI and Jun-ichiro INOUE at the Institute of medical science, the university of Tokyo.

(7) Structural analysis of glutaminase from *Micrococcus luteus K-3*

Yasuo Shirakihara and Aya Shiratori

Glutaminase (EC 3.5.1.2) catalyzes the hydrolytic degradation of L-glutamine to L-glutamic acid. The salt-tolerant glutaminase from marine *Micrococcus luteus* K-3 has an unusual property. Its maximum activity is observed at about 2M salt, but is also active at low salt concentration. This is in contrast to the halophilic proteins which are active at high salt conditions but loose activity at low salt conditions. Previously the structure of the truncated form of the enzyme (1~306) had been solved in our hands. We have tried to solve the structure of the intact enzyme with its MAD data and its native data (2.4 Å resolution).

In the initial structure analysis of the intact form, the refinement was stuck with R-factor of 41%. It was then found that the crystal was merohedrally twinned with a twinning factor of 0.43, close to the worst. A refinement dealing with twinning by CNS gave an R-factor 26% and Rfree 33%, however, the resultant maps were not good enough to trace the rest of the molecule (307~456).

The structural study was done in collaboration with Kazuaki Yoshimune and Mitsuaki Moriguchi at Oita University, and Mamoru Wakayama at Ritsumeikan University.

PUBLICATIONS

Papers

1. Itou, H., Okada, U., Suzuki, H., Yao, M., Wachi, M., Watanabe, N., Tanaka, I. (2005) The CGL2612 protein from *Corynebacterium glutamicum* is a drug resitance-related transcriptional repressor, **J. Biol. Chem.**, *280 (46)* 38711 - 38719

2. Maenaka, K., Fukushi, K, Aramaki, H., Shirakihara, Y. (2005) Expression, crystallization and preliminary diffraction studies of the Pseudomonas putida cytochrome P-450cam operon repressor CamR, **Acta Crystallogr**, *F61*, 796 - 798

3. Motohashi, T., Shimojima, T., Fukagawa, T., Maenaka, K., Park, EY (2005) Efficient large-scale protein production of larvae and pupae of silkworm by Bombyx mori nuclear polyhedrosis virus bacmid system., **BioChem Biophys Res Commun.**, *326 (3)* 564 - 569

EDUCATION

Shirakihara, Y. A Crystal Structure of Thermophilic F1-ATPase and Crystals of Thermophilic ATPsynthase MRC Dunn Human Nutrition Unit, Cambridge, UK

H-e. Gene Network Laboratory Emiko Suzuki Group

RESEARCH ACTIVITIES

(1) Searching for cell-surface and secreted proteins controlling motor axon targeting and synaptogenesis

Mitsuhiko Kurusu, Kai Zinn[1] and Emiko Suzuki ([1]Division of Biology, California Institute of Technology)

The aim of this study is to understand two aspects concerning the development of neural wiring; how motor axons recognize specific muscle fibers as targets for synapse formation and how their synapses expand and change as the muscles grow. Despite the advances in characterizing molecules that regulate motor axon pathfinding in both invertebrate and vertebrate systems, we still understand little about the questions noted above. In particular, difficulty in identifying

molecules controlling target recognition was due to the lack of single loss-of function (LOF) mutations that block recognition of a specific muscle target whereas many mutations affect pathfinding decisions, leading to aberrant wiring of the neuromuscular system. This result is most easily explained by genetic redundancy in target labeling. If each muscle fiber were defined by a combination of several cell surface labels, removing one of the labels might not have a major effect on targeting of axons to that fiber.

To overcome the redundancy problem, we employed gain-of-function (GOF) screen in which candidate molecules are ectopically expressed on all muscle fibers. If these proteins are functional labels, their misexpression might produce alterations in target recognition. Furthermore, this GOF screen is available for an identification of genes involved in the synaptogenesis. This is assessed on the aberrant synapse shape caused by an effect of overexpression of candidate molecules.

To conduct this GOF screen, we first created a database of all cell-surface and secreted (CSS) proteins in *Drosophila* that are likely to be involved in specific cell-cell interactions. The database was generated by defining all fly genes encoding proteins that contain domains known to be present in CSS proteins in other eukaryotes. It currently contains 994 genes. To drive expression of these genes in muscles, we used the "EP" system, in which a P element containing a block of UAS sequences that are responsive to the yeast transcription factor GAL4 is jumped around the genome. Like other P elements, EPs usually land upstream of genes. If a line bearing an EP upstream of a gene is crossed to a 'driver' line expressing GAL4 in all muscle fibers, the gene will now be expressed at high levels in muscles in the resulting progenies. To find EP-like elements upstream of the CSS genes, we searched through about 40,000 different insertions, including the original EP set generated by Pernille Rorth, the EY insertions lines generated in the Bellen lab, the GS lines developed in Japan, and the GE lines developed by GenExel, Inc. We were able to identify insertions that can confer expression of about 300 of the 1005 CSS genes in our database. To screen for genes encoding potential targeting molecules, we are crossing each of these insertions to a muscle GAL4 driver and visualizing motor axons and neuromuscular junction synapses in the resulting F1 progeny larvae by immunostaining. We have already identified a number of genes that cause specific mistargeting phenotypes and/or aberrant synaptic boutons when they are expressed on muscles.

(2) High-resolution *in vivo* analysis of synaptogenesis in *Drosophila* neuromuscular system

Franklin Carrero-Martinez[1,2], Akira Chiba[1] and Emiko Suzuki ([1]Department of Structural Biology and Neuroscience, University of Illinois, [2]Summer student of JSPS program)

The embryonic *Drosophila* body-wall neuromuscular system serves as an ideal model system for studying the early events during synaptogenesis. *In vivo* synaptogenesis can be visualized using cell-specific GFP marker that genetically labels single muscle cells or neurons. Our previous studies using confocal microscopy and electron microscopy in this system have revealed that neuronal growth cones and target muscle cells interact mutually with highly dynamic actin-based filopodia when they meet each other (Ritzenthaler et al., 2000). Several other investigations on both vertebrates and invertebrates have also reported such filopodial interactions, suggesting that these processes are common at early stages of synaptogenesis. However, their significance has not been established yet. To address this problem, we genetically inhibited the function of certain components that is likely to be involved in the filopodial interaction, and analysed the results carefully to correlate this process and synaptogenesis. We focused on a particular synapse between an RP5 motor neuron and an M12 muscle cell, for it is the easiest synapse to observe with high optical and time resolution. Using whole-mount high-resolution time-lapse in vivo confocal imaging, we could describe the sequence of filopodial interaction processes with up to 2-minutes temporal resolution (Carrero-Martinez et al., submitted). Once filopodia from RP5 neuron and those from M12 muscle cell make contact, they gradually cluster at the definite site on M12. This is stereotypic in each segment. As soon as filopodial clusters were formed, a synaptic scaffold protein DLG, the Drosophila orthologue of PSD95, accumulated at these sites. As PSD95 in vertebrates is known to accumulate at the initial site of synaptogenesis, our results strongly suggest that the filopodial clustering initiates synaptogenesis. To test this possibility, we

designed an experiment to express a truncated form of human actin-interacting protein Ezrin (Ezrin^{DN}) in M12 to disturb filopodial cluster formation. Over-expression of Ezrin^{DN} did not eliminate filopodia in muscles, but the filopodia clustering was completely abolished. RP5 growth cone still reached M12 under this condition. However, it failed to form a normal terminal and, instead, extended abnormally thin and long processes that spread diffusely over the muscle surface. The results indicate that filopodial cluster provides a cellular platform on which to assemble synaptic molecules (Carrero-Martinez et al., 2006, submitted).

Next, we asked whether post-synaptic Dlg in filopodial cluster is essential for the synapse formation. We over-expressed DLG^{DN} protein that lacks both PDZ1 and PDZ2 domains, the domains for the interaction with synaptic essential proteins. DLG^{DN} molecule dimerizes with endogenous DLG and acts dominant negatively. When DLG^{DN} was expressed in M12 of wild-type, the filopodial cluster was normally formed. However, RP5 axon subsequently retracted and did not make a synapse with M12. Sometimes RP5 neuron extended again and exhibited a wide range of miswiring (Carrero-Martinez et al., 2006, submitted). These results indicate that DLG is not required for filopodial clustering, rather it is required for the subsequent processes. How filopodial clustering recruits DLG remains to be uncovered in the future project.

(3) Formation of topological axon layer in *Drosophila* mushroom body

Mitsuhiko Kurusu, Kai Zinn[1] and Emiko Suzuki
([1]Division of Biology, California Institute of Technology)

Topographically organized neural circuits in the brain are essential for information processing. Although their functional significance has been defined, the genetic mechanisms underlying the construction of topologically organized circuits are still largely unknown. *Drosophila* mushroom body (MB) functioning as a learning center is a good model system to study the molecular mechanisms that control the sequential generation of neurons and their topological projections into layers. Structurally, axonal projections of *Drosophila* larval MB are topologically organized into concentric layers. Developmental analyses have shown that four independent small axon bundles from newborn neurons originating from the quadruple cell clusters intermingle with each other and sort out into the core of peduncle, and they then migrate outwards more peripheral region as the neurons differentiate. To define the cellular and molecular mechanisms underlying the construction of the topologically organized concentric axonal projections, we are trying to understand how four independent axon bundles from newly born neurons of each cell cluster sort out into the core within the single bundle of the peduncle.

We began these studies with the hypothesis that cell surface molecule expressed on the axons of newly born neurons should represent attractive cue for the axons of follower neurons that choose the same pathways. To identify candidate cue molecules, we screened for known axon guidance molecules by careful immunostaining of the MB with antibodies against these proteins. We identified a cell adhesion protein, N-Cadherin (N-Cad) specifically expressed on the axons of younger neurons that are localized within the core fibers of the peduncle. To understand the functional significance of the gene for core fiber development, we conducted systematic analyses of their loss-of-function (LOF) and gain-of-function (GOF) MB phenotypes. Our results demonstrate that N-Cad is essential for the four bundles of axons originating from the quadruple cell clusters to converge into a single tract in the proximal part of the peduncle and coalesce into a unified core. It has been reported that another cell adhesion protein, Fasciclin II (Fas II), is expressed in the outer layer of axons from mature neurons but is absent from the core, and that FasII is required for the clonal integrity of mature neurons (Kurusu et al., 2002). These results, combined with our newer findings, suggest a model in which N-Cad mediated adhesion between younger axons and Fas II-mediated adhesion between mature axons control the formation of concentric axonal layers. This model implies that precise temporal regulation of expression of these cell adhesion proteins is essential for the proper development of the topologically organized axonal layers of the MB.

Having identified differential temporal expression of cell adhesion molecules as a critical factor in distinguishing the axonal projections of younger neurons vs. mature neurons, we then wondered what mechanisms regulate transitions in gene expression

profiles in the course of differentiation of post-mitotic neurons. One possibility is that the acquisition of electrical activity by young neurons may facilitate the transition to the 'mature' state of gene expression. To test this idea, we examined MB development under the condition in which electrical activity was inhibited using dominant expression of EKO and dORK, modified potassium channels, which shunt depolarizing by inducing hyperpolarization. Preliminary tests showed contradictory effects of the two different electric knockout systems on the core structure and FasII distribution in the MB axon bundles. Whereas no defect in MB morphology or altered expression patterns of N-Cad and Fas II in EKO-expressing MBs, dORK expression caused severe morphological abnormality in the core structure composed of younger axon bundles and down-regulation of Fas II distribution in the mature neurons. Considering that the effect of electrical silencing is stronger in dORK than EKO expressing cells, we conclude that the phenotype induced by dORK expression is plausible, implying the layer development of MB depending on the electrical activity.

(4) Molecular studies on the diacylglycerol kinase function in *Drosophila* photoreceptor neurons

Takuma Yamada and Emiko Suzuki

Phototransduction in *Drosophila* photoreceptor neurons is a G-protein coupled phosphoinositide (PI)-signaling cascade. It is the most rapid process among G-protein coupled cascades known to date (receptor potential is generated within ~20 milliseconds after light stimulation), and the responsiveness is dynamically regulated. We are studying how such elaborated signaling cascade is accomplished, from the viewpoint of intracellular organization of the molecules involved in this system. Our previous studies by combination of functional analysis and electron-microscopic immunocytochemistry have shown that close association of the core components in phototransduction cascade in photoreceptive cell organella, rhabdomeric microvilli, is essential for generation of light responses (Collaboration with Prof.C.Zuker at U.C. San Diego). We also revealed that the PI regeneration cycle is located close to rhabdomeric microvilli. At the bases of rhabdomeric microvilli, there is a network of smooth endoplasmic reticulum that we call subrhabdomeric cisternae (SRC). We found that most of the enzymes involved in PI regeneration and a phosphatidylinositol transfer protein are localized to SRC membranes or in the cytoplasm around it. Mutations of these components not only affect light response but also destroy the network of SRC, leading to retinal degeneration at the end. Among the genetic mutations of PI regeneration cycle, *retinal degeneration A (rdgA)*, the mutation of eye-specific diacylglycerol kinase (DGK) that mediates phosphorylation of diacylglycerol (DG) to produce phosphatidic acid (PA), causes severest retinal degeneration. In *rdgA* mutants, PA in the photoreceptor cell layer is dramatically reduced, indicating that RDGA is a key player for the PA homeostasis in photoreceptors. As PA molecules are thought to be required in membrane regions with large curvatures, as is the case for SRC, and breakdown of SRC networks is the initial process of photoreceptor degeneration in *rdgA* mutants, we hypothesize that the homeostasis of PA content in SRC maintained by RDGA is essential for the structural integrity of SRC. To address this hypothesis we planned to study the molecular mechanism how DGK activity of RDGA is localized to SRC. RDGA protein molecule has two cysteine-rich domains (CRDs) arranged in tandem in N-terminal region. By the analogy with CRDs in potein kinase C, it has been suggested that CRDs in DGK function as binding sites for DG in plasma membranes. However, this has not been conclusively demonstrated. To test this possibility, we designed experiments of expression of modified RDGA proteins with deletion or mutation within CRD regions. Our previous experiments showed that expression of RDGA protein that lacks both CRDs does not rescue the degeneration phenotype of *rdgA* mutants, suggeting that these domains are essential for its function. By that experiment however, we could not precisely examine the subcellular localization of expressed protein because of cell degeneration. This year, we tried to study the localization of modified DGK by use of myc-tag. Expression of myc-tagged RDGA in *rdgA* mutant compound eyes rescued the degeneration phenotype, and the expressed protein localized normally in photoreceptors. With this construct as an entry point, we are now carrying out further experiments with modified RDGA proteins.

PUBLICATIONS

Papers

1. Orihara-Ono, M., Suzuki, E., Saito, M., Yoneda, Y., Aigaki, T., Hama, C. (2005) The slender lobes gene, identified by retarded mushroom body development, is required for proper nucleolar organization in *Drosophila.*, **Developmental Biology**, *281*, 121 - 133

2. Tokai-Nishizumi N,. Ohsugi, M., Suzuki, E., Yamamoto, T. (2005) The Chromokinesin Kid Is Required for Maintenance of Proper Metaphase Spindle Size, **Mol. Biol. Cell.**, *16*, 5455 - 5463

EDUCATION

鈴木えみ子　細胞超微形態学特論　横浜市立大学・国際総合科学部

I. CENTER FOR INFORMATION BIOLOGY AND DNA DATA BANK OF JAPAN

I-a. Laboratory for DNA Data Analysis Takashi Gojobori Group

RESEARCH ACTIVITIES

(1) Various adaptations for the perpetual darkness in the cave-dwelling population of Mexican tetra, *Astyanax mexicanus*, by cDNA microarrays

Nobuhiko Tanaka, Shozo Yokoyama, Kazuho Ikeo and Takashi Gojobori

Mexican tetra *(Astyanax mexicanus)*, a single species with eyed surface (surface fish) and eyeless cave (cavefish) dwelling forms, gives unique opportunities for studying the genetics of speciation processes because the direction of evolutionary processes is known with certainty: cavefish are derived from surface-dwelling ancestors. In this study, to find genes that can differentiate evolutionary changes between the surface fish and the cavefish, we conducted cDNA microarray analyses. Each of the microarrays has 3,070 non-redundant genes from an entire body of an adult surface fish. Target genes for the microarrays were made from an entire body of each surface fish and cavefish. On the basis of the results of comparative microarray analysis for 716 functionally known genes, relatively highly expressed genes in the surface fish and in the cavefish were listed up, respectively. As a result, a lot of functional information on relatively highly expressed genes in the surface fish and in the cavefish was obtained and at suggested various adaptations for the dark cave environment. These adaptations covered behavioral, physiological, and morphological changes between the surface fish and the cavefish. In conclusion, at the gene expression level, various adaptations for the perpetual darkness were raised in the diversification process from the surface fish to the cavefish. This is the first report that differences of gene expression between the surface fish and the cavefish were extensively examined throughout a whole body.

(2) Compensatory change of interacting amino acids in the coevolution of transcriptional coactivator MBF1 and TATA-box binding protein TBP

Qing-Xin Liu, Naomi Nakashima-Kamimura, Kazuho Ikeo, Susumu Hirose and Takashi Gojobori

To elucidate the transcriptional regulation in eukaryotic genome network, it is important to understand coevolution of transcription factors, transcriptional coactivators and TATA-box binding protein TBP. In this study, coevolution of transcriptional coactivator MBF1 and its interacting target TBP was first deduced from comparison of their phylogenetic trees. The proposed coevolution was, then, evaluated experimentally by examining if compensatory amino acid changes took place at interacting sites of both proteins. The experiments were conducted by identifying interaction sites and comparing the amino acids at these sites among different organisms. Here we provide direct evidence for compensatory changes of transcription coactivator and its interacting target, presenting the first report that transcription coactivator has undergone coevolution with TBP.

(3) Targets analysis of the *Drosophila tdf* by using oligonucleotide microarrays

Qing-Xin Liu, Kazuho Ikeo, Yasushi Hiromi, Susumu Hirose and Takashi Gojobori

In *Drosophila*, the *tracheas defective (tdf)* gene encodes a bZIP protein that required for the development of trachea, heart, head and neural system. The targets of *tdf* responsible for these responses have not been identified. To identify *tdf* downstream genes in a comprehensive manner, we used genome-wide oligonucleotide arrays and analyzed differential gene expression in wild-type embryos versus *tdf* mutant embryos. Upon knockout of *tdf* function, expression of 340 genes was decreased and 338 genes were increased. Many of these genes can be assigned to specific aspects of the tracheal and neural system development. We also discovered *tdf* target genes

which are likely to play specific roles in eye morphogenesis. Moreover, we identified lim3 to be a putative direct target of *tdf*, carrying a TDF binding sequence.

(4) Functional analysis of *Drosophila* Midline during the neuronal development

Qing-Xin Liu, Masaki Hiramoto, Hitoshi Ueda, Takashi Gojobori, Yasushi Hiromi and Susumu Hirose

Formation of the neural network requires coordinated pathfinding behavior of many neurons. Each neuron must express axon guidance receptors in their growth cone, and also act as a secretion source of guidance molecules. How neurons orchestrate the expression of multiple guidance genes is poorly understood. Here we show that *Drosophila midline* encoding a T-box protein controls expression multiple axon guidance molecules: Frazzled, ROBO, and Slit. In *midline* mutant expression of all these molecules are reduced, resulting in severe axon guidance defects, whereas misexpression of Midline induces their expression. Midline is present on the promoter of these genes, indicating that it directly controls transcription. Our results provide evidence that Midline controls the switch to axon growth.

(5) A possible regulatory role of *cis* sense-antisense mRNAs in gene expression

Naoki Osato, Yoshiyuki Suzuki, Kazuho Ikeo and Takashi Gojobori

Cis sense-antisense mRNAs (CSA) are composed of a pair of mRNAs with complementary regions, and are located on each strand at the same genomic locus. A pair of CSA changes its expression level and regulates the activation of the mRNAs. Although the number of known CSA was less than 100, more than 2,000 of human and mouse CSA pairs have been predicted from the genome and cDNA sequences. Some of the predicted CSA pairs are expected to regulate their expression, acting as CSA. However, it is unclear what properties of CSA affect their expression. In this study, we examined whether overlapping arrangements of predicted CSA affect their expression. From human predicted CSA pairs, we found that when the genomic length of their overlapping regions increased, highly expressed CSA pairs decreased. Moreover, depending on overlapping patterns of the predicted CSA pairs in the human genome, the distribution of their expression levels changed. the predicted CSA pairs of the mouse showed the same tendency of expression as that of human. These findings suggest that overlapping arrangements of CSA pairs significantly affect their expression.

(6) The Rice Annotation Project Database (RAP-DB): hub for *Oryza sativa* ssp. *japonica* genome information

Hajime Ohyanagi, Yasumasa Shigemoto, Kazuho Ikeo and Takashi Gojobori

Rice is considered a model cereal plant because of its small genome size and a high degree of chromosomal co-linearity with other major cereal crops such as maize, wheat, barley and sorghum. The International Rice Genome Sequencing Project (IRGSP), a consortium of publicly-funded laboratories from 10 countries, initiated the sequencing of *Oryza sativa* ssp. *japonica* cultivar Nipponbare in 1998 using the clone-by-clone sequencing strategy. In 2004, the finished-quality sequence of the entire genome was completed and is now available in the public domain. The annotation of the sequence is indispensable in understanding the overall structure and function of the rice genome. However, most of the annotations of the rice genome sequences were obtained by automated methods. Although this provides an overview of the composition of the genes that comprise the genome, the limitations in prediction programs often result in probable errors and artifacts among predicted genes. Therefore, in concordance with the completion of the rice genome sequence, the Rice Annotation Project (RAP) was organized in 2004 with the aim of providing standardized and highly accurate annotations of the rice genome. To facilitate efficient management of the results of annotation and to establish a platform for integrating the data with other rice resources, an annotation database called the Rice Annotation Project Database (RAP-DB) was developed (Ohyanagi, *et al.*, 2006). The RAP-DB integrates the IRGSP genome sequence and the RAP annotations with other data on rice researches, and makes them available to the public through HTTP access. All of the resources can be accessed through http://rapdb.lab.nig.ac.jp/.

(7) Intracellular transporters evolve slowly

Hajime Ohyanagi and Takashi Gojobori

On the contrary to prokaryotes, eukaryotes have membranous organelles in cells. As a result, some eukaryotic proteins might be under a different evolutionary pressure. Therefore, the gain of the membrane system could affect the evolution of particular proteins, such as intracellular transporters. With the motivation of giving insight to the evolutionary studies of the membrane system, we are conducting the following analyses. First, we computed the evolutionary rates of proteins involved in the large molecule transport in cells. Second, we classified proteomes into two groups; transporters and non-transporters, and compared the evolutionary rates between of the two groups. The results suggest that intracellular transporters, especially transporters for large molecules are slowly evolving now.

(8) An analysis of gene expression for sexual dimorphism in a mouse brain

Kazuya Yuge, Kazuho Ikeo and Takashi Gojobori

Sexually differentiated animals show distinctive sex differences of phenotypic traits such as reproductive organs and behavioral patterns. In particular, sex differences of behavioral patterns may be due to sexual dimorphism of a brain. It is generally known that in spite of remarkable differences in behavioral patterns between the sexes, there are only a few differences in anatomical, biochemical, and molecular characteristics in the brain between the sexes. In particular, little is known about sex differences in the brain at the gene expression level.

To investigate whether there are significant differences in a gene expression pattern of adult mouse brains between the sexes, we compared the gene expression patterns between the sexes. In practice, we isolated a preoptic area, a hypothalamus, and a pituitary from male and female mouse brains, which are known to show sex differences at the anatomical and biochemical levels. we then analysed a pattern of gene expression using a 16.3 K oligo microarray, which contains 93% of mouse genes. As a result, we found that there were 14 genes in preoptic area, 16 genes in hypothalamus, 133 genes in pituitary that were differentially expressed between the sexes. It is noted that two categories of genes, peptide hormone genes and sex chromosome genes, have a remarkably differential pattern of gene expression between the sexes. we also validated the microarray results of preoptic area using real-time RT-PCR by quantifying the expression level of genes for growth hormone and glycoprotein hormones, which are the peptide hormone encoding genes, and Eif2s3y, which is the sex chromosome gene. As a result, these genes were expressed more highly in male than in female. These findings suggest that peptide hormone genes and sex chromosome genes are important for determining sex differences in mouse adult brains.

(9) On the origin of mechanosensory system

Jung Shan Hwang, Shiho Hayakawa, Kazuho Ikeo, Toshitaka Fujisawa and Takashi Gojobori

Nematocyte or cnidocyte is well being used to characterize all members of the phylum Cnidaria and they act as a mechanosensory cell in response to the environmental stimuli. Similar structures can be identified as extrusive organelles in many protists. Not only the nematocyst and extrusome share the morphological similarity, they both are readily discharged when encountered stimuli and some extrusomes could even produce toxins. Therefore, a hypothesis of protist as the origin of nematocyst is generally proposed. More than 60 nematocyte related genes were isolated from hydra by using cDNA microarray. All of them have been ensured with their expression patterns specific to the nematocyte lineage. Searching the sequence homology against the protein data set of Genbank revealed that 25 have the significant sequence match to the currently known proteins, and 5 out of 25 are unique to the phylum of Protoctista. These genes mainly express at the later stage of nematocyte differentiation, possibly they are essential for the assembly of mature nematocyst. Furthermore, phylogenetic analysis revealed that one of the protein disulfide isomerase of hydra was clustered with the ones from protists rather than metazoans. The findings suggested that nematocyst must have originated from the protist. Ultrastructures of two heterotrophic dinoflagellates, *Polykrikos* and *Nematodinium*, have demonstrated that the species contain nematocysts. We plan to isolate and

characterize genes that encode the nematocyst in either *Polykrikos* or *Nematodinium*. Currently, a cDNA library of *Nematodinium* is constructed and nematocyst-related genes can be screened out of the library. Finally, the approach of comparative study between hydra and *Nematodinium* would give insights in the evolution of cnidarian nematocyst.

(10) To search the origin of neural tube: Gene expression analysis of ciliary band in sea urchin embryo

Sonoko Kinjo, Kazuho Ikeo and Takashi Gojobori

Echinoderms and chordates are thought to be a monophyletic and derived from common ancestor. It has been thought that the neural tube of chordates has originated from the ciliary band of Echinoderm larva because the concentration of neural cells is seen in both organs. To know whether the ciliary band of echinoderm larva is homologous to the neural tube of chordates, we have been analyzing the expressed genes in the ciliary band of sea urchin embryo. For this purpose, we isolated the ciliary band tissue from 420 samples of sea urchin larvae, and we constructed cDNA library of those.

(11) Evolutionary studies of the nervous system based upon comparative genomics

Akiko Ogura Noda

To elucidate the evolutionary process of the nervous system (NS) in metazoa, the relationship between human genes specifically expressed in the NS (NS-specific genes) and the time of their evolutionary emergence were examined. Two hundred and fifty-five human NS-specific genes were obtained from the gene expression data of the Human Full-length cDNA Annotation Invitational (H-invitational) database. To determine when these genes emerged for the first time during evolution, orthologues of the 255 NS-specific genes in 13 species (excluding human) were searched for by homology searches against their complete genome sequences. we found that 14% of the NS-specific orthologous genes had already emerged before the divergence between yeast and human. This finding suggests that a common ancestor, which should have no nervous system, already possessed a portion of the genes homologous to human NS-specific genes, implying that 14% of the NS-specific genes should have changed differentially their original functions during evolution. If this is the case, then the remaining 86% of the 255 NS-specific human genes have newly emerged during evolution. In particular, we found that the largest portion (24%) of the 255 NS-specific genes had emerged after divergence of urochordata and human but before divergence of fishes and human. These results suggest that the main cause of the NS evolution was the addition of new genes which took place most actively just before or at the evolutionary emergence of vertebrates. We also found that there are regions where the NS-specific genes are concentrated (NSC regions) in human chromosomes 1, 4, 11, 14, 17, 19, 21 and X and that most of the NS-specific genes in these NSC regions emerged after divergence of urochordata and human but before divergence of fishes and human. In particular, most of functions of the gene products in the NSC region of chromosome X are related to binding to other molecules such as DNAs and proteins. It suggests that the NS-specific genes in the eight NSC regions may have contributed to the formation of network of gene interactions to other molecules. From these results, we concluded that the formation of gene network was crucial to the evolution of the vertebrate NS.

PUBLICATIONS

Papers

1. International Rice Genome Sequencing Project: Iwama, H., Gojobori, T., Itoh, T., Niimura, Y., et al. (2005) The map-based sequence of the rice genome, **Nature**, *436*, 793 - 800
2. The FANTOM Consortium and RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group): Gojobori, T., Ikeo, K., et al. (2005) The transcriptional landscape of the mammalian genome, **Science**, *309 (5740)* 1559 - 1563
3. Chiusano, M. L., Gojobori, T. and Toraldo, G. (2005) A C++ computational environment for biomolecular sequence management, **CMS**., *2 (3)* 165 - 180
4. Fujii, Y., Itoh, T., Sakate, R., Koyanagi, KO., Matsuya, A., Habara, T., Yamaguchi, K., Kaneko, Y., Gojobori, T. and Imanishi, T. (2005) A web tool for comparative genomics: G-compass, **GENE**, *364C*, 45 - 52

5. Hanada, K., Suzuki, Y., Nakane, T., Hirose, O. and Gojobori, T. (2005) The origin and evolution of porcine reproductive and respiratory syndrome viruses, **Mol. Biol. Evol.**, *22 (4)* 1024 - 1031

6. Kijimoto, T., Watanabe, M., Fujimura, K., Nakazawa, M., Murakami, Y., Kuratani, S., Kohara, Y., Gojobori, T. and Okada, N. (2005) cimp1, a novel astacin family metalloproteinase gene from East African cichlids, is differentially expressed between species during growth, **Mol. Biol. Evol.**, *22 (8)* 1649 - 1660

7. Koyanagi, KO., Hagiwara, M., Itoh, T., Gojobori, T. and Imanishi, T. (2005) Comparative genomics of bidirectional gene pairs and its implications for the evolution of a transcriptional regulation system, **GENE**, *353 (2)* 169 - 176

8. Matsumura, Y,. Shimokawa, K,. Hayashizaki, Y,. Ikeo, K,. Tateno, Y,. Kawai, J. (2005) "Development of a spot reliability evaluation score for DNA microarrays.", **GENE**, *350 (2)* 149 - 160

9. Ogasawara, M., Imanishi, T., Moriwaki, K., Gaudieri, S., Tsuda, H., Hashimoto, H., Shiroishi, T., Gojobori, T. and Koide, T. (2005) Length variation of CAG / CAA triplet repeats in 50 genes among 16 inbred mouse strains, **GENE**, *349*, 107 - 119

10. Ogura, A., Ikeo, K. and Gojobori, T. (2005) Estimation of ancestral gene set of bilaterian animals and its implication to dynamic change of gene content in bilaterian evolution, **GENE**, *345 (1)* 65 - 71

11. Osada, N., Hirata, M., Tanuma, R., Kusuda, J., Hida, M., Suzuki, Y., Sugano, S., Gojobori, T., Shen, CK., Wu, CI. and Hashimoto, K. (2005) Substitution rate and structual divergence of 5'UTR evolution: Comparative analysis between human and cynomolgus monkey cDNAs, **Mol. Biol. Evol.**, *22 (10)* 1976 - 1982

12. Shirai, T., Nakato, A., Izutani, N., Nagahisa, K., Shioya, S., Kimura, E., Kawarabayasi, Y., Yamagishi, A., Gojobori, T. and Shimizu, H. (2005) Comparative study of flux redistribution of metabolic pathway in glutamate production by two coryneform bacteria, **Metab. Eng.**, *7 (2)* 59 - 69

13. Tamiya, G., Shinya, M., Imanishi, T., Ikuta, T., Makino, S., Okamoto, K., Furugaki, K., Matsumoto, T., Mano, S., Ando, S., Nozaki, Y., Yukawa, W., Nakashige, R., Yamaguchi, D., Ishibashi, H., Yonekura, M., Nakami, Y., Takayama, S., Endo, T., Saruwatari, T., Yagura, M., Yoshikawa, Y., Fujimoto, K., Oka, A., Chiku, S., Linsen, S., Giphart, M., Kulski, J., Fukazawa, T., Hashimoto, H., Kimura, M., Hoshina, Y., Suzuki, Y., Hotta, T., Mochida, J., Minezaki, T., Komai, K., Shiozawa, S., Taniguchi, A., Yamanaka, H., Kamatani, N., Gojobori, T., Bahram, S. and Inoko, H. (2005) Whole genome association study of rheumatoid arthritis using 27, 039 microsatellites, **Hum. Mol. Genet.**, *14 (16)* 2305 - 2321

14. Tanaka, T., Tateno, Y. and Gojobori, T. (2005) Evolution of vitamin B6 (Pyridoxine) metabolism by gain and loss of genes, **Mol. Biol. Evol.**, *22 (2)* 243 - 250

15. Tanaka, Y., Hanada, K., Orito, E., Akahane, Y., Chayama, K., Yoshizawa, H., Sata, M., Ohta, N., Miyakawa, Y., Gojobori, T. and Mizokami, M. (2005) Molecular evolutionary analyses implicate injection treatment for schistosomiasis in the initial hepatitis C epidemics in Japan, **J Hepatol.**, *42 (1)* 47 - 53

16. Tanino, M., Debily, M.-A., Tamura, T., Hishiki, T., Ogasawara, O., Murakawa, K., Kawamoto, S., Itoh, K., Watanabe, S., Souza, S. J. d., Imbeaud, S., Graudens, E., Eveno, E., Hilton, P., Sudo, Y., Kelso, J., Ikeo, K., Imanishi, T., Gojobori, T., Auffray, C., Hide, W. and Okubo, K. (2005) The Human Anatomic Gene Expression Library (H-Angel), the H-Inv integrative display of human gene expression across disparate technologies and platforms, **Nucleic Acids Res.**, *33 (Database Issue)* 567 - 572

17. Tateno, Y., Saitou, N., Okubo, K., Sugawara, H. and Gojobori, T. (2005) DDBJ in collaboration with mass-sequencing teams on annotation, **Nucleic Acids Res.**, *33 (Database Issue)* 25 - 28

18. Yamasaki, C., Koyanagi, KO., Fujii, Y., Itoh, T., Barrero, R., Tamura, T., Yamaguchi-Kabata, Y., Tanino, M., Takeda, J., Fukuchi, S., Miyazaki, S., Nomura, N., Sugano, S., Imanishi, T. and Gojobori T. (2005) Investigation of protein functions through data-mining on integrated human transcriptome database, H-Invitational database (H-InvDB), **GENE**, *364C*, 99 - 107

19. Yokomine, T,. Shirohzu, H,. Purbowasito, W,. Toyoda, A,. Iwama, H,. Ikeo, K,. Hori, T,. Mizuno, S,. Tsudzuki, M,. Matsuda, Y,. Hattori, M,. Sakaki, Y,. Sasaki, H. (2005) Structural and functional analysis of a 0.5-Mb chicken region orthologous to the imprinted mammalian Ascl2 / Mash2-Igf2-H19 region., **Genome Res.**, *15 (1)* 154 - 165

20. 鈴木　善幸，池尾　一穂，五條堀　孝　(2005)ゲノムネットワーク，日本薬理学雑誌，126 (1) 55 - 59

21. 五條堀　孝(2005)特集　遺伝学会レポート　日本遺伝学会第77回大会と公開記念講座　『遺伝』をプラスイメージに，生物の科学　遺伝，*60 (1)* 17

22. 谷野　元彦，鈴木　穣，今西　規，日紫喜　光良，大久保　公策，五條堀　孝，菅野　純夫　(2005)ゲノムから人間　ヒトのトランスクリプトーム　ゲノムから生命システムへ，**蛋白質核酸酵素**，*50 (16)* 2059 - 2064

Books

23. Nishio, Y., Usuda, Y., Gojobori, T. and Ikeo, K. (2005) Comparative whole genome sequence analysis of corynebacteria, **Bioinformatics of Genome Regulation and Structure** II, 121 - 129

24. 五條堀　孝，菅原　秀明，斎藤　成也，舘野　義男，宮崎　智，深海ー小林　薫，鈴木　善幸，福地　佐斗志，阿部　貴志，金城　玲，飯塚　高康，渡邉　康司，大久保　公策(2005)配列データの登録・検索・解析，DDBJの利用法，(五條堀　孝，菅原　秀明)，共立出版，1 - 181

25. 櫻井　仁美，ロベルト・バレロ，五條堀　孝(2005) miRNAの標的遺伝子の予測，RNA工学の最前線　シーエムシー出版，114 - 125

26. 鈴木　善幸(2005)相同性検索，DDBJの利用法，(五條堀　孝，菅原　秀明)，共立出版・東京，75 - 88

27. 鈴木　善幸(2005)同義置換速度・非同義置換速度の比較による自然選択の検出，DDBJの利用法，(五條堀　孝，菅原　秀明)，共立出版・東京，127 - 133

28. 花田　耕介，五條堀　孝(2005)ウイルスの多様性と系統，バイオディバーシティ・シリーズ4　菌類・細菌・ウイルスの多様性と系統，(杉山　純多)，裳華房，370 - 395

Database

29. Genome Network Platform　http://genomenetwork.nig.ac.jp/

30. BSD　http://bsd.genes.nig.ac.jp/bsd_web/

31. EMBEST　http://cibexsv.genes.nig.ac.jp/cibest/embest.html

32. CIBEX　http://cibex.nig.ac.jp/index.jsp

33. RAP DB　http://rapdb.lab.nig.ac.jp/

34. H-ANGEN　http://jbirc.jbic.or.jp/hinv/h-angel/

35. H-ZNU　http://www.h-invitational.jp/

EDUCATION

講義・セミナー

1. IKEO, K.　遺伝子発現データベース，DDBJ ing 講習会，国立遺伝学研究所生命情報研究センター

2. IKEO, K.　遺伝子発現データベース　ー遺伝発現データ活用・将来展望の観点からー，DDBJ ing講習会in東京農大，東京農業大学世田谷キャンパス

3. 鈴木　善幸　Inferring positive selection operating on viral proteins，名古屋市立大学

4. 鈴木　善幸　分子進化解析入門，第12回DDBJing講習会，国立遺伝学研究所

5. 鈴木　善幸　分子進化解析入門・実習：ClustalWから系統樹作成へ，第13回DDBJing講習会，東京農業大学

6. 鈴木　善幸　ミニシンポジウム「正の自然選択による分子進化」，第77回日本遺伝学会大会，代々木

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

Gojobori, T.　Meeting of Bioinformatics/Database group(司会)，Jeju, Korea

Gojobori, T.　JBIRC H-Invitational Disease Edition，東京都江東区

Gojobori, T.　「バイオインフォマティクス人材養成のあるべき姿とは？」(司会)，東京都江東区

Gojobori, T.　第77回日本遺伝学会大会(大会委員長)，東京都渋谷区

Gojobori, T.　「Integrative Annotation of the Genome, Transcriptome and Proteome」, Session 6, Transcriptome 2005(座長), Shanghai, China

Gojobori, T.　「Genome Sequence Annotation and Comparative Genomics」, Session II, 1st International Biocurator Meeting(座長), Asilomar, California, USA

各種受賞

五條堀　孝：全米科学振興協会フェロー(AAAS Fellow)

五條堀　孝：日本遺伝学会木原賞受賞

学会活動

五條堀　孝：日本遺伝学会第77回大会委員長

五條堀　孝：日本遺伝学会評議員

五條堀　孝：(財)遺伝学普及会常務理事

五條堀　孝：Vice-Chairman of the International Society of Molecular Evolution

五條堀　孝：Editor of GENE

五條堀　孝：Editor of FEBS Letters

五條堀　孝：Associate Editor of PLoS Genetics

五條堀　孝：Associate Editor of Molecular Biology and Evolution

五條堀　孝：Editorial board of Genome Research

五條堀　孝：Editorial board of BMC Genomics

五條堀　孝：Editorial board of Briefings in Bioinformatics

五條堀　孝：Editorial board of Gene Therapy and Molecular Biology

五條堀　孝：Editorial board of LNCS Trans actions on Computational Systems Biology

I-b. Laboratory for Gene-Product Informatics
Ken Nishikawa Group

RESEARCH ACTIVITIES

(1) Predicting absolute contact numbers of native protein structure from amino acid sequence

Akira R. Kinjo, Katsuhisa Horimoto and Ken Nishikawa

The contact number of an amino acid residue in a protein structure is defined by the number of Cb atoms around the Cb atom of the given residue, a quantity similar to, but different from, solvent accessible surface area. We present a method to predict the contact numbers of a protein from its amino acid sequence. The method is based on a simple linear regression scheme and predicts the absolute values of contact numbers. When single sequences are used for both parameter estimation and cross-validation, the present method predicts the contact numbers with a correlation coefficient of 0.555 on average. When multiple sequence alignments are used, the correlation increases to 0.627, which is a significant improvement over previous methods. In terms of discrete states prediction, the accuracies for 2-, 3-, and 10-state predictions are, respectively, 71.4%, 54.1%, and 18.9% with residue type-dependent unbiased thresholds, and 76.3%, 59.2%, and 21.8% with residue type-independent unbiased thresholds. The difference between accessible surface area and contact number from a prediction viewpoint and the application of contact number prediction to three-dimensional structure prediction are discussed (Kinjo, A.R. et al., 2005).

(2) Recoverable one-dimensional encoding of three-dimensional protein structures

Akira R. Kinjo and Ken Nishikawa

One-dimensional (1D) structures of proteins such as secondary structure and contact number provide intuitive pictures to understand how the native three-dimensional (3D) structure of a protein is encoded in the amino acid sequence. However, it is still not clear whether a given set of 1D structures contains sufficient information for recovering the underlying 3D structure. Here we show that the 3D structure of a protein can be recovered from a set of three types of 1D structures, namely, secondary structure, contact number and residue-wise contact order which is introduced here for the first time. Using simulated annealing molecular dynamics simulations, the structures satisfying the given native 1D structural restraints were sought for 16 proteins of various structural classes and of sizes ranging from 56 to 146 residues. By selecting the structures best satisfying the restraints, all the proteins showed a coordinate RMS deviation of <4 A from the native structure, and, for most of them, the deviation was even <2 A. The present result opens a new possibility to protein structure prediction and our understanding of the sequence-structure relationship (Kinjo, A.R. and Nishikawa, K., 2005a).

(3) Genome-wide survey of transcription factors in prokaryotes reveals many bacteria-specific families not found in archaea

Yoshiaki Minezaki, Keiichi Homma and Ken Nishikawa

Assignment of all transcription factors (TFs) from genome sequence data is not a straightforward task due to the wide variation in TFs among different species. A DNA binding domain (DBD) and a contiguous non-DBD with a characteristic SCOP or Pfam domain combination are observed in most members of TF families. We found that most of experimentally verified TFs in prokaryotes are detectable by a combination of SCOP or Pfam domains assigned to DBDs and non-DBDs. Based on this finding, we set up rules to detect TFs and classify them into 52 TF families. Application of the rules to 154 entirely sequenced prokaryotic genomes detected more than 18,000 TFs classified into families, which have been made publicly available from the "GTOP-TF" database. Despite the rough proportionality of the number of TFs per genome with genome size, species with reduced genomes, i.e., obligatory parasites and symbionts, have only a few if any TFs, reflecting a nearly complete loss. Also the number of TFs is significantly lower in archaea than in bacteria. In addition, all but one of the 19 TF families present in archaea are present in bacteria, whereas 33 TF families are found exclusively in bacteria. This observation indicates that a number of new TF families evolved

in bacteria, making the transcription regulatory system more divergent in bacteria than in archaea (Minezaki, Y. et al., 2005).

(4) Predicting secondary structures, contact numbers and residue-wise contact orders of native protein structure from amino acid sequence using critical random networks

Akira R. Kinjo and Ken Nishikawa

Prediction of one-dimensional protein structures such as secondary structures and contact numbers is useful for the three-dimensional structure prediction and important for the understanding of sequence-structure relationship. Here we present a new machine-learning method, critical random networks (CRNs), for predicting one-dimensional structures, and apply it, with position-specific scoring matrices, to the prediction of secondary structures (SS), contact numbers (CN), and residue-wise contact orders (RWCO). The present method achieves, on average, Q3 accuracy of 77.8% for SS, correlation coefficients of 0.726 and 0.601 for CN and RWCO, respectively. The accuracy of the SS prediction is comparable to other state-of-the-art methods, and that of the CN prediction is a significant improvement over previous methods. We give a detailed formulation of critical random networks-based prediction scheme, and examine the context-dependence of prediction accuracies. In order to study the nonlinear and multi-body effects, we compare the CRNs-based method with a purely linear method based on position-specific scoring matrices. Although not superior to the CRNs-based method, the surprisingly good accuracy achieved by the linear method highlights the difficulty in extracting structural features of higher order from amino acid sequence beyond that provided by the position-specific scoring matrices (Kinjo, A.R. and Nishikawa, K., 2005b)

(5) Intrinsically disordered loops inserted into the structural domains of human proteins

Satoshi Fukuchi, Keiichi Homma, Yoshiaki Minezaki and Ken Nishikawa

Much attention has been paid recently to proteins with partially or fully disordered structures, which are found to exist mostly in eukaryotes and are involved mainly in pivotal cellular processes such as transcriptional regulation, translation and cellular signal transduction. Long disordered sequences are sometimes inserted within the single structural domains of proteins, forming loops from the molecular surface. Such intrinsically disordered loops (IDLs) either are invisible in X-ray crystallography, or hamper protein crystallization itself due to great flexibility. Perhaps because of this, such long disordered sequences have not been characterized adequately. Here, we propose an informational method that stringently identifies IDLs in the structural domains of proteins using the amino acid sequence alone. A genome-wide survey of human proteins conducted with the method identified 50 IDL-containing proteins, several of which have experimentally determined 3D structures. Similar searches in other entirely sequenced organisms revealed that IDLs are prevalent in eukaryotes, while they are much less so in prokaryotes. As there is a statistically significant coincidence between the boundaries of IDLs and those of exons, we suggest that IDLs were produced mainly by exon addition in eukaryotes. IDLs are almost always located at the surface of proteins and are enriched with hydrophilic residues, and IDL-containing proteins tend to be intracellular. Some of the well-characterized proteins with IDLs illustrate that IDLs play pivotal roles in the switching of intracellular signaling or regulatory functions, suggesting that IDL insertion is an effective way to create functionally different domain variants (Fukuchi, S. et al., 2006).

PUBLICATIONS

Papers

1. Kinjo, A. R., Horimoto, K. and Nishikawa, K. (2005) Predicting absolute contact numbers of native protein structure from amino acid sequence., **Proteins**, *58*, 158 - 165
2. Kinjo, A. R. and Nishikawa, K. (2005) Recoverable one-dimensional encoding of protein three-dimensional structures., **Bioinformatics**, *21 (10)* 2167 - 2170
3. Kinjo, A. R. and Nishikawa, K. (2005) Predicting secondary structures, contact numbers and residue-wise contact orders of native protein structure from amino acid sequence using critical random networksc, **Bioinformatics**, *1*, 67 - 74
4. Minezaki, Y., Homma, K. and Nishikawa, K. (2005) Genome-wide survey of transcription factors in prokaryotes reveals many bacteria-specific families not

found in archaeac, **DNA Res**, *12 (5)* 269 - 280

5. Saka, K., Tadenuma, M., Nakade, S., Tanaka, N., Sugawara, H., Nishikawa, K., Ichiyoshi, N., Kitagawa, M., Mori, H., Ogasawara, N. and Nishimura, A. (2005) A complete set of Escherichia coli open reading frames in mobile plasmids facilitating genetic studiesc, **DNA Res**, *12*, 63 - 68

6. Yamasaki C, Koyanagi KO, Fujii Y, Itoh T, Barrero R, Tamura T, Yamaguchi-Kabata Y, Tanino M, Takeda J, Fukuchi S, Miyazaki S, Nomura N, Sugano S, Imanishi T, Gojobori T. (2005) Investigation of protein functions through data-mining on integrated human transcriptome database, H-Invitational database (H-InvDB)., **Gene**, *364*, 99 - 107

Books

7. 金城玲，西川建(2005)第２章５：タンパク質の構造予測　タンパク質科学ー構造・物性・機能ー　(後藤祐児，桑島邦博，谷澤克行)　化学同人　87 - 100

I-c. Laboratory for Gene Function Research Yoshio Tateno Group

The laboratory consists of five members: Dr. Yoshio Tateno (Professor), Dr. Roberto Antonio Barrero Gumiel (Assistant Professor), Dr. Tokumasa Horiike (Postdoc), Dr. Lihua Jin (Postdoc) and Ms. Naoko Murakata (Secretary). We aim at the elucidation of the origin, evolution and function of genes and proteins in view of molecular evolution, structural biology and information biology in collaboration with other research groups in the institute and out. Besides the research activities summarized below, we take part in the DDBJ activity in particular in the database construction and public relations.

RESEARCH ACTIVITY

(1) Genomic evolution of MHC class I region in primates

Fukami-Kobayashi, K., Shiina, T., Anzai, T., Sano, K., Yamazaki, M., Inoko, H. and Tateno, Y

To elucidate the origins of the MHC-B-MHC-C pair and the MHC class I chain-related molecule (MIC)A-MICB pair, we sequenced an MHC class I genomic region of humans, chimpanzees, and rhesus monkeys and analyzed the regions from an evolutionary stand-point, focusing first on LINE sequences that are paralogous within each of the first two species and orthologous between them. Because all the long interspersed nuclear element (LINE) sequences were fragmented and nonfunctional, they were suitable for conducting phylogenetic study and, in particular, for estimating evolutionary time. Our study has revealed that MHC-B and MHC-C duplicated 22.3 million years (Myr) ago, and the ape MICA and MICB duplicated 14.1 Myr ago. We then estimated the divergence time of the rhesus monkey by using other orthologous LINE sequences in the class I regions of the three primate species. The result indicates that rhesus monkeys, and possibly the Old World monkeys in general, diverged from humans 27-30 Myr ago. Interestingly, rhesus monkeys were found to have not the pair of MHC-B and MHC-C but many repeated genes similar to MHC-B. These results support our inference that MHC-B and MHC-C duplicated after the divergence between apes and Old World monkeys (Fukami-Kobayashi *et al.*, 2005).

(2) Development of a spot evaluation score for DNA microarrays.

Matsumura, Y., Shimokawa, K., Hayashizaki, Y., Ikeo, K., Tateno, Y., and Kawai, J.

We developed a reliability index named SRED (Spot Reliability Evaluation Score for DNA microarrays) that represents the probability that the calibrated gene expression level from a DNA microarray would be less than a factor of 2 different from that of quantitative real-time polymerase chain reaction assays whose dynamic quantification range is treated statistically to be similar to that of the DNA microarray. To define the SRED score, two parameters, the reproducibility of measurement value and the relative expression value were selected from nine candidate parameters. The SRED score supplies the probability that the expression level in each spot of a microarray is less than a certain-fold different compared to other expression profiling data, such as QRT-PCR. This score was applied to approximately 1,500,000 points of the expression profile in the RIKEN Expression Array Database (Matsumura et al., 2005).

(3) Large-scale analysis of human alternative protein isoforms: pattern classification and correlation with subcellular localization signals.

Nakao M, Barrero RA, Mukai Y, Motono C, Suwa M, and Nakai K.

We investigated human alternative protein isoforms of >2600 genes based on full-length cDNA clones and SwissProt. We classified the isoforms and examined their co-occurrence for each gene. Further, we investigated potential relationships between these changes and differential subcellular localization. The two most abundant patterns were the one with different C-terminal regions and the one with an internal insertion, which together account for 43% of the total. Although changes of the N-terminal region are less common than those of the C-terminal region, extension of the C-terminal region is much less common than that of the N-terminal region, probably because of the difficulty of removing stop codons in one isoform. We also found that there are some frequently used combinations of co-occurrence in alternative isoforms. We interpret this as evidence that there is some structural relationship which produces a repertoire of isoformal patterns. Finally, many terminal changes are predicted to cause differential subcellular localization, especially in targeting either peroxisomes or mitochondria. Our study sheds new light on the enrichment of the human proteome through alternative splicing and related events. Our database of alternative protein isoforms is available through the internet (Nakao *et al.*, 2005).

(4) Evolution of vitamin B6 (pyridoxine) metabolism by gain and loss of genes.

Tanaka T, Tateno Y, Gojobori T.

Vitamin B(6) (VB6) functions as a cofactor of many diverse enzymes in amino acid metabolism. Three metabolic pathways for pyridoxal 5'-phosphate (PLP; the active form of VB6) are known: the *de novo* pathway, the salvage pathway, and the fungal type pathway. Most unicellular organisms and plants biosynthesize VB6 using one or two of these three biosynthetic pathways. However, animals such as insects and mammals do not possess any of the pathways and, thus, need to intake VB6 in their diet to survive. It is conceivable that breakdowns of these pathways occurred in the evolutionary lineages of insects and mammals, and one of the major reasons for this would be the loss of pertinent genes. We studied the evolution of VB6 biosynthesis from the view of the gain and loss of 10 pertinent genes in 122 species whose genome sequences were completely determined. The results revealed that each gene in the pathways was lost more than once in the entire evolutionary lineages of the 122 species. We also found the following three points regarding the evolution of PLP biosynthesis: (1) the breakdown of the PLP biosynthetic pathways occurred independently at least three times in animal lineages, (2) the de novo pathway was formed by the generation of pdxB in gamma-proteobacteria, and (3) the order of the gene loss in VB6 metabolism was conserved among different evolutionary lineages. These results suggest that the evolution of VB6 metabolism was subject to gains and frequent losses of related genes in the 122 species examined. This dynamic nature of the evolutionary changes must have been responsible for the breakdowns of the pathways, resulting in profound differentiation of heterotrophy among the species (Tanaka *et al.*, 2005).

(5) DDBJ in collaboration with mass-sequencing teams on annotation.

Tateno Y, Saitou N, Okubo K, Sugawara H, Gojobori T.

In the past year, we at DDBJ (DNA Data Bank of Japan; http://www.ddbj.nig.ac.jp) collected and released 1,066,084 entries or 718,072,425 bases including the whole chromosome 22 of chimpanzee, the whole-genome shotgun sequences of silkworm and various others. On the other hand, we hosted workshops for human full-length cDNA annotation and participated in jamborees of mouse full-length cDNA annotation. The annotated data are made public at DDBJ. We are also in collaboration with a RIKEN team to accept and release the CAGE (Cap Analysis Gene Expression) data under a new category, MGA (Mass Sequences for Genome Annotation). The data will be useful for studying gene expression control in many aspects (Tateno *et al.*, 2005).

(6) Investigation of protein functions through data-mining on integrated human transcriptome database, H-Invitational database.

Yamasaki C, Koyanagi KO, Fujii Y, Itoh T, Barrero RA, Tamura T, Yamaguchi-Kabata Y, Tanino M, Takeda J, Fukuchi S, Miyazaki S, Nomura N, Sugano S, Imanishi T, and Gojobori T.

H-Invitational Database (H-InvDB) is a human transcriptome database, containing integrative annotation of 41,118 full-length cDNA clones originated from 21,037 loci. H-InvDB is a product of the H-Invitational project, an international collaboration to systematically and functionally validate human genes by analysis of a unique set of high quality full-length cDNA clones using automatic annotation and human curation under unified criteria. Here, 19,574 proteins encoded by these cDNAs were classified into 11,709 function-known and 7865 function-unknown hypothetical proteins by similarity with protein databases and motif prediction (InterProScan). The proportion of "hypothetical proteins" in H-InvDB was as high as 40.4%. In this study, we thus conducted data-mining in H-InvDB with the aim of assigning advanced functional annotations to those hypothetical proteins. First, by data-mining in the H-InvDB version of GTOP, we identified 337 SCOP domains within 7865 H-Inv hypothetical proteins. Second, by data-mining of predicted subcellular localization by SOSUI and TMHMM in H-InvDB, we found 1032 transmembrane proteins within H-Inv hypothetical proteins. These results clearly demonstrate that structural prediction is effective for functional annotation of proteins with unknown functions. All the data in H-InvDB are shown in two main views, the cDNA view and the Locus view, and five auxiliary databases with web-based viewers; DiseaseInfo Viewer, H-ANGEL, Clustering Viewer, G-integra and TOPO Viewer; the data also are provided as flat files and XML files. The data consists of descriptions of their gene structures, novel alternative splicing isoforms, functional RNAs, functional domains, subcellular localizations, metabolic pathways, predictions of protein 3D structure, mapping of SNPs and microsatellite repeat motifs in relation with orphan diseases, gene expression profiling, and comparisons with mouse full-length cDNAs in the context of molecular evolution. This unique integrative platform for conducting in silico data-mining represents a substantial contribution to resources required for the exploration of human biology and pathology (Yamasaki *et al.*, 2005).

(7) Novel mammalian microRNA targets identified by an extensive transcriptome-wide affinity screening

Barrero RA, Tamura T, Sakurai H, Hayakawa S, Tateno Y, Ikeo K, Imanishi T and Gojobori T.

MicroRNAs (miRNAs) act as repressors of target mRNAs via translation inhibition or messenger degradation. In mammals, miRNAs were shown to associate with haematopoietic cell differentiation1, downregulation of HOXB82 and control of insulin secretion3. Identification of reliable miRNA targets has become one of the key issues in the understanding of miRNA regulatory networks. Here we report a large scale affinity screening of miRNA targets, which utilizes structural information of accessibility to miRNA-recognition elements (MREs). Our method predicted 920 non-redundant miRNA-MRE pairs, representing 688 human and mouse genes, for 142 miRNAs. We validated ~71% of the predicted MREs subjected to reporter assays, which have at least 5.5 kcal higher affinities to bind miRNAs than to make potential internal secondary structures. Most of the identified targets (85.5%) are new for mammals, and 195 of these are involved in multiple biological processes, which support the notion that miRNAs may regulate complex gene networks.

(8) Development of a method for constructing a phylogenetic tree by using many different orthologs

Tokumasa Horiike, Daisuke Miyata, Kazuo Hamada, Satoshi Saruhashi, Takao Shinozawa, Yoshio Tateno

To compare the topologies between a pair of phylogenetic trees, we developed a method, which can accommodate the difference in the number of OTUs between the pair in question. Applying this method to a number of orthologous gene sets, we constructed the corresponding number of phylogenetic trees and pair-wisely compared the constructed trees. This method further enables to cluster a group of the constructed trees among which no inconsistency of tree topology exists irrespective of the number of OTUs. Since the one with the largest trees among the clusters

is considered to show the closest phylogenetic relationships of the species involved to the true ones, we also developed a statistical method to single it out. In reality, however, we have faced the difficulty in refining and redefining orthologous genes in the extant orthologous databases not mention to selecting those from genome sequence data in general. We will thus find a way of selecting orthlogous genes and compiling reliable orthologous gene sets.

(9) The analysis of the evolutionary relationship between duplication and alternative splicing

Jin LH., Ikeo K., Suzuki Y. and Gojobori T.

Living things increase their functional diversification by variable evolutionary mechanisms. Among these mechanisms, gene duplication and alternative splicing (AS) are known as the two of major evolutionary ways that can bring the functional diversification by increasing gene variations. Our research interest is to clarify the evolutionary relationship between these two different phenomena by utilizing all available data resources. The results of this study showed that gene duplication and age of gene both give positive influence on the occurrence of AS, in both human and mouse genomes. Furthermore, when combine the effect of gene duplication and age of gene by using a new parameter ADE (Accumulated Duplication Effect), the AS isoform number tends to increase in early stage of ADE, remain stable in the middle stage, and decrease in the later stage. Since large ADE generally resulted from either large size or old age of a gene family, therefore, we can conclude that these two factors cause increase of AS isoform numbers only to a certain level.

(10) Evolutionary study of small-RNA-mediated gene silencing pathways by investigating the evolution of Rnase III enzymes

Jin LH., Ikeo K., Suzuki Y. and Gojobori T.

The small-RNA-mediated gene silencing pathways are evolutionarily conserved processes. It highlights a fundamental role of short RNAs in eukatyotic gene regulation and antiviral defense. Recently, 3 distinct small-RNA-mediated silencing pathways are observed, such as the destruction of mRNA via siRNA, inhibition of mRNA translation *via* miRNA, and epigenetic gene silencing *via* siRNA. It was also found that in these pathways, the members of ribonclease III family play important roles in diverse RNA maturation and decay. Here we investigated the evolution of Rnase III nucleases, Dicer as representative, to further figure out the evolutionary relationship of 3 gene silencing pathways. With the advantage of using genomic sequences as the subject in homologue search, in un-annotated genomic regions, we were able to detect possible candidates for several functional domains and genes of Rnase III nuclease members. Moreover, we found that 150 prokaryotes including eubacteria and archbacteria lack completely the PAZ domain of Dicer. These results show the taxonomic-dependent evolution of the small-RNA-mediated gene silencing pathways.

(11) A Novel Arabidopsis Gene Causes Bax-like Lethality in *Saccharomyces cerevisiae*

Kawai-Yamada M., Saito Y,, Jin LH., Ogawa T., Kim KM., Jin LH., Tone Y., Hirata A., Umeda M., and Uchimiya H.

Overexpression of the mammalian proapoptotic protein Bax induces cell death in plant and yeast cells. The Bax inihibitor-1 *(BI-1)* gene rescues yeast and plant from *Bax*-mediated lethality. Using the *Arabidopsis BI-1 (AtBI-1)* gene controlled by the *GAL1* promoter as a cell death suppressor in yeast, *Cdf1* (cell growth defect factor-1) was isolated from *Arabidopsis* cDNA library. Overexpression of *Cdf1* caused cell death in yeast, whereas such an effect was suppressed by co-expression of *AtBI-1*. The *Cdf1* protein fused with a green fluorescent protein was localized in the mitochondria and resulted in the loss of mitochondrial membrane potential in yeast. The *Bax*-resistant mutant BRM1 demonstrated tolerance against *Cdf1*-mediated lethality, whereas the Δ *atp4* strain was sensitive to Cdf1. Our results suggest that *Cdf1* and *Bax* cause mitochondria-mediated yeast lethality through partially overlapped pathways.

(12) Collaboration with Prof. Tadao Saito of Tohoku University and his laboratory on the function and evolution of glucosidase and galactosidase genes in *Lactobcillus.*

Saito, T, Yamazaki Y and Tateno Y

(13) Collaboration with Prof. Shintou Eguchi of the Institute of Mathematical Statistics and his laboratory on the statistical analyses of SNP and gene expression data.

Eguchi S, Ikeo K and Tateno Y

(14) Collaboration with Dr. Kaoru Fukami-Kobayashi of RIKEN on evolutionary analysis of MHC genes in primates.

Fukami-Kobayashi K and Tateno Y

(15) Collaboration with Prof. Naoko Takezaki of Kagawa Medical College on the evolution of genes expressed in brains.

Takezaki N and Tateno Y

(16) Collaboration with AHG-Evolutional Curation System teams on annotation of expressed human genes.

Ikeo K., Suzuki Y., Ohta S., Jin LH., Yamazaki C., Suzuki M., Kanno M., Saichi N., Sanbonmatsu R., Koyanagi K., Sakate R., Itoh T., and Imanishi T.

PUBLICATIONS

Papers

1. Fukami-Kobayashi, K., Shiina, T., Anzai, T., Sano, K., Yamazaki, M., Inoko, H. and Tateno, Y. (2005) Genomic evolution of MHC class I region in primates., **Proc Natl Acad Sci USA**, *102*, 9230 - 9234
2. Matsumura, Y., Shimokawa, K., Hayashizaki, Y., Ikeo, K., Tateno, Y., and Kawai, J. (2005) Development of a spot evaluation score for DNA microarrays, **Gene**, *350*, 149 - 160
3. Nakao M, Barrero RA, Mukai Y, Motono C, Suwa M, Nakai K. (2005) Large-scale analysis of human alternative protein isoforms: pattern classification and correlation with subcellular localization signals., **Nucleic Acids Res**, *33*, 2355 - 2363
4. Tanaka, T., Tateno, Y. and Gojobori, T. (2005) Evolution of vitamin B6 (pyridoxine) metabolism by gain and loss of genes, **Mol Biol Evol**, *22*, 243 - 250
5. Tateno, Y., Saitou, N., Okubo, K., Sugawara, H. and Gojobori, T. (2005) DDBJ in collaboration with mass-sequencing teams on annotation, **Nucleic Acids Res**, *33*, D25 - D28
6. Yamasaki C, Koyanagi KO, Fujii Y, Itoh T, Barrero RA, Tamura T, Yamaguchi-Kabata Y, Tanino M, Takeda J, Fukuchi S, Miyazaki S, Nomura N, Sugano S, Imanishi T, Gojobori T (2005) Investigation of protein functions through data-mining on integrated human transcriptome database, H-Invitational Database (H-InvDB)., **Gene**, *364*, 99 - 107
7. ロベルト・バレロ, 櫻井仁美(2005)「miRNAのターゲット同定の現状」, **バイオテクノロジージャーナル**, *5 (4)* 403 - 405

Books

8. 櫻井仁美, ロベルト・バレロ, 五條堀孝(2005)「miRNAと標的遺伝子の予測」『RNA工学の最前線』 シーエムシー出版 113 - 125
9. 舘野義男(2005)「バイオインフォマティクス」,「プロテオーム」 細胞生物学事典 (石川統, 黒岩常祥, 永田和宏) 朝倉書店 338 - 376
10. 舘野義男(2005)「国際塩基配列データファイルの構造」 DDBJの利用法 (五條堀孝・菅原秀明) 共立出版 21 - 31
11. 舘野義男(2005)国際DNAデータベース 予防医学事典 (松島綱治, 酒井敏行, 石川昌, 稲寺秀朗) 朝倉書店 201 - 202

Database

12. DDBJデータベース http://www.ddbj.nig.ac.jp/Welcome-e.html
13. CIBEXデータベース http://cibex.nig.ac.jp/index.jsp

EDUCATION

講義・セミナー

1. 舘野義男 講義「分子系統学講義」, 実習「分子系統樹の作成」 独立行政法人科学技術振興機構 サイエンスプラザ
2. Barrero RA. Introduction to the DDBJ database and use of it for bioinformatic research Yang-Ming University (Taipei, Taiwan)
3. Barrero RA. Introduction to the DDBJ database and use of it for bioinformatic research National Health Research Institute (Hsin Chu, Taiwan)
4. Y. Tateno The fundamental of population genetics and molecular evolution National Yang-Ming University, Taipei, Taiwan
5. Y. Tateno The fundamental of population genetics and molecular evolution National Health Research Institute, Hsin-chu, Taiwan
6. 舘野義男 「バイオインフォマティクス講座ゲノムネッ

トワーク遺伝子の発現・制御，ゲノムネットワークデータベース」 沼津地域産業振興協議会主催「バイオインフォマティクス入門講座」 ぬまづ産業振興プラザ，静岡県沼津市

7. 舘野義男 「バイオインフォマティックスの現状」 青山学院大学 理工学部 化学・生命科学科

8. 舘野義男 「系統樹作成基礎(Ⅰ)」集団遺伝学と分子進化学の基礎 東京国際フォーラム会議場，東京都千代田区

9. 舘野義男 「遺伝子発現とデータベース」 東海大学開発工学部 生物工学科

10. 舘野義男 「ゲノムからみた霊長類の進化」 東京農業大学応用生物科学部

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

Center for Information Biology and DNA Data Bank of Japan, NIG, National Genome Information Center, KRIBB, Korea The 4th Korea-Japan-China Bioinformatics Training Course Deajon, Korea
日本遺伝学会 第77回日本遺伝学会大会 東京

特許

特願2005-330761，「コンピュータによる径路関係探索法」，舘野義男，堀池徳祐，宮田大輔，猿橋智

学会活動

舘野義男 Dr. Y. Tateno served the Genetics Society of Japan as Representative member of the Union of Academic Societies for Natural History
舘野義男 Dr. Y. Tateno served the Microarray Gene Expression Data Society as an Advisory Board Member
舘野義男 Dr. Y. Tateno served the Society of Evolutionary Studies as a Member of the Committee of Genetics under the Science Council of Japan
舘野義男 Dr. Y. Tateno served the Genetics Society of Japan as a Member of Editorial Board

I-d. Laboratory for Research and Development of Biological Databases
Hideaki Sugawara Group

RESEARCH ACTIVITIES

(1) Information systems for molecular biology and its related disciplines

1) From Web services to a Bioportal

Yasumasa Shigemoto[†], Haruka Sakai, Takshi Abe, Satoru Miyazaki[††], Hideaki Sugawara.
[†]Hitachi soft, [††]Tokyo Univ. of Sci.

The publicly available bioinformatics resources, comprising databases and analytical tools, have expanded in recent years. While the information environment for life sciences has gradually become more abounding, it is still difficult to combine multiple, heterogeneous bioinformatics resources for a specific research purpose. To set up and run an integrated system, it is often necessary to write and update custom programs. In addition, different research groups continually write programs that have overlapping functions. We need an information environment that is conducive to efficient and appropriate bioinformatics resource utilization for a wide range of users. Therefore, the Center for Information Biology and DNA Data Bank of Japan, in alliance with the National Institute of Informatics (NII) and the Mitsubisi Research Institute, Inc. (MRI) have started a three years long project since 2003, "Research and Development of the New Generation of Bio-portal", to enhance the information environment for the relevant user communities. In this project, the Laboratory for Research and Development of Biological Databases is responsible for the development of biological Web services. The project site is open at http://www.bioportal.jp/ in 2004. From there, a Web page for links to sites complete genome sequence and annotation are also prepared and accessible, in addition to the biological Web services. The former is named "Genome Menu".

2) Expansion of Genome Information Broker (GIB)

Masaki Hirahata, Naoto Tanaka, Takashi Abe, Satoru Miyazaki[†], Hideaki Sugawa.
[†]Tokyo Univ. of Sci.

GIB was originally created for the retrieval and analysis of E. coli genomic information in a set. We implemented microbial genome data into GIB whenever genome sequencing was completed and the data is made open to the public. At the GIB Web page (http://gib.genes.nig.ac.jp/), key word search, homology search, links to DBGET, KEGG and GTOP and

visualization of the data are available for more than 300 strains as of December 2005. We have utilized XML, CORBA and a distributed database in order to cope with the explosion of microbial genome information.

3) Development of Genome Information Broker for Viruses genome (GIB-V)

Masaki Hirahata, Takashi Abe, Naoto Tanaka, Hideaki Sugawa.

The importance of analyses of the viral genomes is increasing. Comparative genomic analysis is being advocated as the new analysis to discover knowledge discovery.

We developed a database of viral genomes that we call, the Genome Information Broker for Viral genomes (GIB-V). GIB-V contains data for 1487 viral and viroid genomes and can indicate, compare, and analyze the data contained therein. For the development of the database, we applied the architecture of the Genome Information Broker for Microbial genomes (GIB) that have powerful comparative genomics function. GIB-V is possible to execute the analysis for the selected viral genomes. We equipped a function to visualize the result of arraignment to compare the genome structure of each viral genomes. GIB-V is an effective and powerful tool for extracting a viral genome sequences. GIB-V is freely available at http://gib-v.genes.nig.ac.jp.

4) Development of Genome Information Broker for Insertion sequence (GIB-IS)

Takehide Kosuge, Takashi Abe, Yoshio Tateno, Hideaki Sugawara

The prediction of insertion sequence (IS) is one of the important processes for bacterial genomes annotation, and has great influence on the judgment of the gene prediction. Although huge number of IS data exist in the international nucleotide database (DDBJ/EMBL/GenBank), it is difficult to obtain accurate annotations and sequences of IS, because some the data have erroneous annotations. Additionally, there has been no public service where users can retrieve whole set of IS data. To solve the problems of current situation, it is important to provide users with exact IS data. We developed a IS database (named as GIB-IS) which includes whole bacterial IS data, such as, IS name, synonym, family name, and sequences. GIB-IS is freely available at http://bioportal.ddbj.nig.ac.jp/is/index.html.

(2) Information systems on microbes and biodiversity

1) WFCC-MIRCEN World Data Centre for Microorganisms (WDCM)

Yasumasa Shigemoto[†], Junko Nagaya, Hideaki Sugawara.
[†]Fujitsu.

WFCC and MIRCEN stand for World Federation for Culture Collections and Microbial Resource Centers network respectively. The laboratory is the host of WDCM and maintains the World Directory of microbial resource centers. The on-line World Directory contains the detailed information of 469 centers in 65 countries and also the list of their holdings. Any culture collection is able to register, update and delete the information at http://www.wdcm.org/. WDCM could promote the update of the data by culture collections funded by the American Society for Microbiology and UNESCO.

2) Development of an e-Workbench for Biological Classification and Identification (InforBIO)

Naoto Tanaka, Kouji Koorikawa[†], Takashi Abe, Satoru Miyazaki[††], Hideaki Sugawara.
[†]Hitachi soft, [††]Tokyo Univ. of Sci.

We continued the development of an e-Workbench named InforBIO by use of JAVA, XML and a relational database management system in the public domain. We have distributed InforBIO to several laboratories that study microbes and improved the utility and robustness of InforBIO based on the feedback (http://lilium.genes.nig.ac.jp/index_e.html).

3) An information system for pathogenic microorganisms

We participated in a national project for the resource center of pathogenic microorganisms. Our role is to develop an information system for pathogenic fungi and actinomycetes, and also a portal site for pathogenic microorganisms in general (http://www.wdcm.org/byogen/).

(*) The information system on pathogenic microorganisms has been supported by Special Coordination Funds for Promoting Science and Technology.

4) Global Biodiversity Information Facility (GBIF)

Hideaki Sugawara, Motomi Itoh† and Keiichi Matsuura††
†Univ. of Tokyo, ††National Science Museum,Tokyo

GBIF is an international project to establish a mechanism to aggregate data on diverse domains including abiotic ones. The GBIF data system is composed of national nodes and we now host a Japan GBIF node. This laboratory has implemented a wrapping system based on one of GBIF standards, namely, DarwinCore and DiGIR protocol and some biodiversity data in Japan are now accessible in the GBIF portal site.

(3) Applications of IT to the International Nucleotide Sequence Database

1) Development of Open Annotation System

Satoru Miyazaki†, Takashi Abe, Hideaki Sugawara.
†Tokyo Univ. of Sci.

A number of the complete genome sequences have been submitted to INSD since 1995. The annotation information, however, is not consistent among genome sequencing teams. In addition, researchers outside of the team might have more information and knowledge on some genes and biological molecules. Therefore, it is quite important to develop the system which allows any expert to evaluate the annotation given by the team to attach more valuable information. As a new feature of INSD, we develop so-called "Open Annotation System (OASYS)" as an annotation editor in the distributed environment on the Internet.
(*) OASYS project has been supported by BIRD of Japan Science and Technology Corporation (JST)

2) Development of an e-Workbench for comparative genome analysis (G-InforBIO)

Naoto Tanaka, Kouji Koorikawa†, Takashi Abe, Satoru Miyazaki††, Hideaki Sugawara.
†Hitachi soft, ††Tokyo Univ. of Sci.

We have developed the G-InforBIO system for seamless integration of browse and analysis of genomic data published in the International Nucleotide Sequence Database (INSD: DDBJ/EMBL/GenBank) and also local data in research laboratories. Major functions of the system are database management, data retrieval, sequence data analysis and the visualization for genomics. The Suite is written in Java so that it is executable in Macintosh OSX, Windows XP, Linux and Unix machines, and will be downloadable from http://wdcm.org/inforbio/.

The database accommodates multiple flat files (FFs) of entries registered in the INSD and also tab-delimited files of local data. The SOAP interface on the G-InforBIO system is used to download FFs of microbial genome data published from DDBJ. Genome information on the imported database can be readily retrievable by keyword searches. A subset of the database can be also retrieved for the further analysis and graphical representation. The G-InforBIO system includes sequence analysis programs for cluster analysis (Blastclust, ClustalW and SOM) and homology analysis (MegaBlast, Blast, Blat and Sim4). The analysis can be applied to the retrieved subset and data imported from outside. The system provides a broad-ranging utilization of genome information.

3) Exhaustive evaluation of the annotation data obtained by simultaneous annotations of whole bacterial genomes.

Takehide Kosuge, Toshihisa Okido, Yasumasa Shigemoto†, Masaki Hirahata, Naoto Tanaka, Takashi Abe, Satoru Miyazaki††, Hideaki Sugawara.
†Fujitsu, ††Tokyo Univ. of Sci.

The number of complete genomes of microorganism is increasing year by year. Currently, complete genome sequence of ~300 strains are available from INSD (International Nucleotide Sequence Database, http://www.insdc.org/) and they are summarized in our web-site, GIB (http://gib.genes.nig.ac.jp/). Users expect that they can obtain exact and up-to-date annotations from the genomes. In fact, users must take care for using the gene annotations of the publicized data because the qualities of the annotations are not uniform, e.g. difference of blast and motif database version, prediction programs, and parameters for using prediction program. To solve

the problems of current situation, it is important to supply ORF data predicted by the same protocol and databases as a third party annotations.

We have already developed an annotation protocols (named as GTPS) which aimed at bacterial genomes. GTPS protocol can classify the all predicted ORFs into several grades based on a degree of reliability. The GTPS protocol was applied to all chromosomes and plasmids of 123 microbial strains at the same time. The 123 indicates the number of strains whose complete genome sequence was publicized as of July, 2003. Our protocol could found miss-annotations which were not annotated in publicized data because some of the ORFs are registered in the current Swiss-Prot or RefSeq databases. The simultaneous annotations were updated in 2004, the number of analyzed strains increased to 182 strains. The update data will be available from our web site (URL).

(4) Genomics

1) A large-scale Self-Organizing Map (SOM) unveils sequence characteristics of a wide range of eukaryote genomes

Takashi Abe, Toshimichi Ikemura†, Hideaki Sugawara.
†SOKEN-DAI

Novel tools are needed for comprehensive comparisons of interspecies characteristics of massive amounts of genomic sequences currently available. An unsupervised neural network algorithm, Kohonen's Self-Organizing Map (SOM), is an effective tool for clustering and visualizing high-dimensional complex data on a single map. We modified the conventional SOM, on the basis of batch-learning SOM, for genome informatics making the learning process and resulting map independent of the order of data input. We generated the SOMs for tetranucleotide frequencies in 10- and 100-kb sequence fragments from 38 eukaryotes for which almost complete genome sequences are available. SOM recognized species-specific characteristics (key combinations of oligonucleotide frequencies) in the genomic sequences, permitting species-specific classification of the sequences without any information regarding the species. We also generated the SOM for tetranucleotide frequencies in 1-kb sequence fragments from the human genome and found sequences for four functional categories (5' and 3' UTRs, CDSs and introns) were classified primarily according to the categories. Because the classification power is very high, SOM is an efficient and powerful tool for extracting a wide range of genome information.

2) Phylogenetics Analyses of Environmental Samples on the Basis of Self-Organizing Map (SOM)

Takashi Abe, Toshimichi Ikemura† Hideaki Sugawara.
†SOKEN-DAI.

Metagenomic approach, which is the genome analysis on a mixture of uncultured microorganisms, has been recently developed to search for novel and industrially useful genes and to study microbial diversity in a wide variety of environments. We previously modified the conventional SOM for genome informatics to make the learning process and resulting map independent of the order of data input[5),6)]. In the present study, we developed the SOM as a novel bioinformatics strategy to capture and visualize microbial diversity and relative abundance of microorganisms within an environmental sample. First we constructed SOMs of tri- and tetranucleotide frequencies in 1- and 5-kb sequence fragments from prokaryotic genomes for which complete sequence is available. The sequences could be classified primarily according to species and to 11 major phylogenetic groups without information regarding the species. For example, 88% of 5-kb sequences were classified into the correct phylogenetic group. Importantly, the classification could be done without orthologous sequence sets, and. therefore, SOM was especially useful to analyze novel sequences from poorly characterized species for industrial applications and scientific studies. With the SOM method, all non-rRNA sequences in the Database that were from unidentified or uncultured bacteria and longer than 1 kb were classified into major phylogenetic groups. The present method can also be developed as a tool for surveys of pathogenic microorganisms in environmental and clinical samples that can not be cultured easily and in sterilized samples.

PUBLICATIONS

Papers

1. Abe, T., Sugawara, H., Kanaya, S., Kinouchi, M., Matsuura, Y., Tokutaka, H., and Ikemura, T. (2005) A large-scale Self-Organizing Map (SOM) constructed with the Earth Simulator unveils sequence characteristics of a wide range of eukaryotic genomes, **Proceedings of Workshop (2005) on Self-Organizing Maps**, 187 - 194

2. Abe, T., Ikemura, T., Kanaya, S., Kinouchi, M., and Sugawara, H. (2005)A novel bioinformatics strategy for phylogenetic study of genomic sequence fragments: self-organizing map (SOM) of oligonucleotide frequencies, **Proceedings of Workshop (2005) on Self-Organizing Maps**, 669 - 676

3. Abe, T., Sugawara, H., Kinouchi, M., Kanaya, S., and Ikemura, T. (2005) Novel Phylogenetic Studies of Genomic Sequence Fragments Derived from Uncultured Microbe Mixtures in Environmental and Clinical Samples, **DNA Research**, 12, 281 - 290

4. Hayashi, H., Abe, T., Sakamoto, M., Ohara, H., Ikemura, T., Sakka, K., and Benno, Y. (2005) Direct cloning of genes encoding novel xylanases from human gut, **Canadian Journal of Microbiology**, *51*, 251 - 259

5. Saka, K.., Tadenuma, M., Nakade, S., Tanaka, N., Sugawara, H., Nishikawa, K., Ichiyoshyi, N., Kitagawa, Mori, H., Ogasawara, N. and Nishimura, A. (2005) A Complete Set of Escherichia coli Open Reading Frames ion Mobile Plasmids Facilitating Genetic Studies, **DNA Research**, *12*, 63 - 68

6. Sugawara, H., Miyazaki, S., Abe, T., and Shigemoto, Y. (2005) Biological Data Anelysis using DDBJ Web services, **Proceedings of BIOINFO 2005**, 379 - 382

7. Uchiyama, T., Abe, T., Ikemura, T., Watanabe, K. (2005) Substrate-induced gene-expression screening of environmental metagenome libraries for isolation of catabolic genes, **Nature Biotechnology**, *1*, 88 - 93

8. 阿部貴志，重元康昌，宮崎智，菅原秀明(2005) Webサービスによるバイオ情報資源の統合化の可能性，**第1回バイオ情報学研究会**，21 - 28

Books

9. 阿部貴志，金谷重彦，木ノ内誠，池村淑道(2005) コドン使用頻度と生産量との関連　生物工学ハンドブック　131 - 132

10. 五條堀孝，菅原秀明(2005) DDBJの利用法　DDBJの利用法　共立出版

11. 菅原秀明，深海・小林　薫，宮崎　智，丸山　穣(訳) (2005) インターネット生物学　インターネット生物学学会出版センター

Database

12. Gene Trek in Procaryote Space (GTPS)　http://gtps.ddbj.nig.ac.jp/

13. Genome Information Broker for Virus (GIB-V) http://gib-v.genes.nig.ac.jp/

14. GIB-IS　http://bioportal.ddbj.nig.ac.jp/is/index.html

15. Genome Information Broker for Environmental sequence (GIB-ENV)　http://bioportal.ddbj.nig.ac.jp/wgs/index.html

16. DDBJ Open Annotation System (OASYS)　http://althea.ddbj.nig.ac.jp/index.jsp

17. GBIF　http://bioportal.ddbj.nig.ac.jp/gbif_tx/,http://gbif.ddbj.nig.ac.jp/gbif_search/index.html

EDUCATION

1. 菅原秀明　生命科学特論　青山大学

2. 菅原秀明　Robust & advanced technologies for the information sharing & diffusion Useful tools for the data analysis from phenome to genome　Workshop on Bioresource Information Management (BIM)　バンコク

3. 阿部貴志　バイオデータベース検索の基礎1　バイオインフォマティクス講座　沼津産業振興プラザ

SOCIAL CONTRIBUTIONS AND OTHERS

会議等主催

菅原秀明　国立遺伝学研究所共同研究会　微生物情報研究会　三島

菅原秀明　国立遺伝学研究所共同研究会　生物情報資源の相互運用性　三島

その他

菅原秀明　財団法人バイオインダストリー協会　評議員

菅原秀明　(財)遺伝学普及会　評議員

I-e. Laboratory for Gene-Expression Analysis Kosaku Okubo Group

The laboratory for Gene-Expression Analysis consists of the following members:

Kousaku Okubo (Professor), Osamu Ogasawara

(Posdoc), Makiko Otsuji (Graduate student), Isao Kubota (Graduate student), Miya Shiojima (technical assistant),Takayasu Iizuka (systems engineer), Koji Watanabe (systems engineer), Hiroaki Imamura (systems engineer), Sumiyo Sugimoto (Secretary).

(1) BodyMap-Xs: Anatomical breakdown of 17 million animal ESTs for cross-species comparison of gene expression

Ogasawara O, Watanabe K, Imamura H, Iizuka T, Okubo K

BodyMap-Xs (http://bodymap.jp) is a database for cross-species gene expression comparison (Ogasawara, O., et.al., 2006) ; it was created by the anatomical breakdown of 17 million animal EST records in DDBJ by using a sorting program tailored for this purpose. In BodyMap-Xs, users are allowed to compare the expression patterns of orthologous and paralogous genes in a coherent manner; this will provide valuable insights for the evolutionary study of gene expression and identification of a responsive motif for a particular expression pattern. In addition, starting from a concise overview of the taxonomical and anatomical breakdown of all animal ESTs, users can navigate to obtain gene expression ranking of a particular tissue in a particular animal; this method may lead to the understanding of the similarities and differences between the homologous tissues across animal species. BodyMap-Xs will be automatically updated in synchronization with the major update in DDBJ, which occurs periodically.

(2) Identification of differentially expressed genes in psoriasis using expression profiling approaches.

To identify differentially expressed genes which play causal roles in pathogenesis and maintenance for psoriasis, we used BodyMapping and introduced amplified fragment length polymorphism approaches (Itho, K., et.al., 2005). From the BodyMap database, we selected 2007 genes which specifically expressed in epithelial tissues. Among 2007 genes, we surveyed genes which differentially expressed in involved or uninvolved psoriatic lesional skin samples compared with atopic dermatitis, mycosis fungoides, and normal skin samples. Using the 2007 gene set, we examined gene expression levels in five serial lesions from distal uninvolved psoriatic skin to involved psoriatic plaque. We identified seven genes such as alpha-1-microglobulin/bikunin precursor, calnexin, claudin 1, leucine zipper down-regulated in cancer 1, tyrosinase-related protein 1, Yes-associated protein 1, and unc-13-like protein (Coleonyx elegans) which show high-expression levels only in uninvolved psoriatic lesions. These seven genes, which were reported to be related to apoptosis or antiproliferation, might have causal roles in pathophysiology in psoriasis.

(3) Comparison of Gene Expression Patterns between human and mice brain.

Otsuji M, Okubo K

To elucidate gene expression patterns between human and mice brain, we construct 3'-directed cDNA libraries from several portions from human and mice brain. From now on, we measure gene expression levels using iAFLP method.

(4) A Theoretical Model for the Accumulation of Gene Expression Divergence

Ogasawara O, Okubo K

We have constructed an explicit theoretical model that describes the evolutionary changes caused by the accumulation of gene expression divergence; this model was constructed based on the assertion that a phenomenon concerning the distribution of transcript levels, called Zipf's law of transcriptome, would originate from the accumulation of gene expression divergence. Surprisingly, our model predicted that the transcript level variation within and between species would not be correlated; instead, the scatter plot of such a transcript level variation would exhibit a striking L-shaped distribution that is very similar to the observed distribution. It should be pointed out that this distribution had previously been considered as evidence "against" the effect of the accumulation of gene expression divergence. These results indicate that the process of the accumulation of gene expression divergence has a considerable effect on gene expression evolution.

PUBLICATIONS

Papers

1. Itoh K, Kawasaki S, Kawamoto S, Seishima M, Chiba H, Michibata H, Wakimoto K, Imai Y, Minesaki Y, Otsuji M, Okubo K. (2005) I dentification of differentially expressed genes in psoriasis using expression profiling approaches., **Exp Dermatol**., *14 (9)* 667 - 674

2. Kaimori JY, Takenaka M, Okubo K. (2005) Quantification of gene expression in mouse and human renal proximal tubules., **Methods Mol Biol**., *293*, 209 - 219

3. Tanino, M., Debily, MA., Tamura, T., Hishiki, T., Ogasawara, O., Murakawa, K., Kawamoto, S., Itoh, K., Watanabe, S., de Souza, SJ., Imbeaud, S., Graudens, E., Eveno, E., Hilton, P., Sudo, Y., Kelso, J., Ikeo, K., Imanishi, T., Gojobori, T., Auffray, C., Hide, W., and Okubo, K. (2005) The Human Anatomic Gene Expression Library (H-ANGEL), the H-Inv integrative display of human gene expression across disparate technologies and platforms., Nucleic **Acids Res**., *33*, 567 - 572

4. Tateno, Y., Saitou, N., Okubo, K., Sugawara, H., and Gojobori, T. (2005) DDBJ in collaboration with mass-sequencing teams on annotation., **Nucleic Acids Res**., *33*, 25 - 28

5. 大久保公策，日紫喜光良(2005)「ポストゲノム時代に高まるバイオ自然言語処理への期待：バイオ自然言語処理最新事情　5．ゲノムデータの機械解釈」，情報処理，*46 (2480)* 137 - 142

Books

6. 大久保公策(2005)「文献知識の計算機による利用がなぜ必要か」「蛋白質核酸酵素」編集部　vol. 50 №16　共立出版株式会社，2250 - 2254

EDUCATION

大久保公策　「言語から読み解くゲノムと生命システム．救世主は生まれるか？」　東京大学大学院21世紀COE特別講義　東京大学大学院　柏キャンパス

J. RADIOISOTOPE CENTER

RESEARCH ACTIVITIES

(1) Environmental genetics: Stationary phase-induced genome rearrangements

Yasuyuki Ogata

In nature bacterial cells are usually in stationary stages, in which the cells alter their genetic inheritance. We found that illegitimate recombination is induced when growth phase is changed from log to stationary phase; illegitimate recombination occurred more frequently in early-stationary phase than in mid-log phase during formation of λ*bio*-transducing phage. When we did a quantitative analysis of deletion formation using a miniF-based plasmid, we found that the stationary phase-induced illegitimate recombination can be also detected in this assay system. In addition, we indicated that the frequency of deletion of the *tag alkA1* double mutant was higher than that of its isogenic wild-type strain in early-stationary phase, thereby implying that the alkylated lesion may be responsible for the stationary phase-induced illegitimate recombination. Since illegitimate recombination results in genome rearrangements including deletion, duplication, insertion, or translocation, it seems likely that stationary phase may induce genome rearrangements. Thus, when bacterial cells confront unfavorable conditions for their growth, the cells seem to increase diverse mutations as if they struggle for adaptation to the environment. If the situation takes a favorable turn, a suitable mutant clone is likely to grow preferentially, which may lead to evolution.

(2) Archaeological genetics: Isolation of ancient bacteria from Antarctic iceberg

Yasuyuki Ogata

In Antarctica ice sheet that snow accumulates for about a million years and is transformed into ice exists. We aim to isolate ancient bacteria from a piece of iceberg and surmise the evolution of bacterial genome. We built a clean booth in our laboratory and set up a clean bench inside of it for the purpose of avoiding contamination and the iceberg piece was melted, filtrated, and particles were collected. By microscopic surveillance we obtained bacteria-like particles and DAPI staining revealed that these particles contained nuclei. Moreover, we amplified genome DNA from these particles *in vitro* and sequenced them. Self-organizing map (SOM) analysis which was developed as a novel bioinformatics strategy for phylogenetic classification of sequence fragments obtained from pooled genome samples showed that these sequence fragments originated from Firmicutes, Euryarchaeota, or Cyanobacteria.

(3) Field genetics: Obtaining of Antarctic gene resources without contamination

Yasuyuki Ogata

In Antarctica it is extremely cold and ultraviolet rays are much irradiated. I am trying to isolate the bacteria from Antarctica and obtain the genes responsible for survival under these severe environments. Novel defense systems against DNA damage or repair mechanism are expected. For this purpose, I had made an Antarctic exploring by accompanying the 47th Japanese Antarctic Research Expedition (JARE47) since November 28, 2005 to March 28, 2006 and collected deep-sea water, soil, or snow and ice without contamination; I built a clean booth on the snowfield at S17 and dug the ice core with a sterilized ice auger and I also set up a clean bench on the ground at Rundvågskollane and Ongul Islands and scooped up the soil with a sterilized spoon.

K. EXPERIMENTAL FARM

RESEARCH ACTIVITIES

Experimental Farm is responsible for preparation and cultivation of all kinds of rice strains necessary for the studies of genetic resources and of functional genomics. The facilities for plant growth in the Experimental Farm are also available to the collaborators outside the NIG. All works in the Experimental Farm have been carried out as collaborative works with the Plant Genetics Lab. One specific activity related to the wild rice collection is introduced below. For other activities, see the reports of Plant Genetics laboratory.

(1) Examination of genetic characters for accessions of wild rice core collection

Ken-Ichi Nonomura, Yukie Sano, Toshie Miyabayashi, Mitsugu Eiguchi, and Nori Kurata

For making rice genetic stocks good resources, we developed core collection of wild rice. Out of 2,000 accessions composed of twenty-one wild rice species in nine genomes maintained in the NIG, several accessions from each species were selected to prepare core collection. About 300 selected lines had already sub-grouped to Rank1, Rank2 and Rank3 categories, and a part of them were characterized for their phenotypes to record them in the Oryzabase, a rice comprehensive database.

Comparative genomics among nine rice genomes will reveal important aspects on evolution and genetic diversity in rice. For finding out genome specific genes or far-related genes different from AA, comparison of BB (W1514) and CC (W0002) genome species with the cultivated AA (Nipponbare) species have been carried out by cDNA clone sequencing. Some results of comparative analysis are presented in the report of Plant Genet. Lab.

In addition, to confirm genome sizes of each genome, species and accessions and to examine the suspicious accessions in the core collection, we have measured DNA content by flow-cytometry analysis as well as chromosome numbers by microscope observation. DNA content and chromosome number of each species and accession were well characterized as to reveal that a few accessions have been categorized to wrong species.

PUBLICATIONS

Papers

1. Ito, Y., Takaya, K. and Kurata, N. (2005) Expression of SERK family receptor-like protein kinase genes in rice, **Biochim. Biophys. Acta**, *1730*, 253 - 258

2. Itoh, J. I., Nonomura, K. I., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H. and Nagato, Y. (2005) Rice plant development: from zygote to spikelet, **Plant Cell Physiol.**, *46*, 23 - 47

3. Ito, Y. and Kurata, N.(2005) Identification of enhancer trap lines with tissue-specific GUS activities in rice, **Rice Genet. Newslet.**, *22*, 32 - 33

4. Kurata, N., Miyoshi, K., Nonomura, K. I., Yamazaki, Y. and Ito, Y. (2005) Rice mutants and genes related to organ development, morphogenesis and physiological traits, **Plant Cell Physiol.**, *46*, 48 - 62

5. Moriguchi, K., Suzuki, T., Ito, Y., Yamazaki, Y., Niwa, Y. and Kurata, N. (2005) Functional isolation of novel nuclear proteins showing a variety of subnuclear localizations, **Plant Cell**, *17*, 389 - 403

6. Xu, J-H., Kurata, N., Akimoto, M., Ohtsubo, H. and Ohtsubo, E. (2005) Identification and characterization of Australian wild rice strains of *Oryza meridionalis* and *Oryza rufipogon* by SINE insertion polymorphism, **Genes Genet. Syst.**, *80*, 129 - 134

7. Suzuki, T., Eiguchi, M., Satoh, H., Kumamaru, T. and Kurata, N. (2005) A modified TILLING system for rice mutant screening, **Rice Genet. Newslet.**, *22*, 89 - 90

EDUCATION

1. 野々村賢一　イネの減数分裂における染色体認識と動原体　東京大学大学院理学系研究科

2. 野々村賢一　減数分裂遺伝子の解析とイネ属の種分化　名古屋大学大学院

3. 倉田のり　ゲノム生物学の基礎と意味－植物ゲノム研究　京都大学大学院・農学研究科

SOCIAL CONTRIBUTIONS

1. 倉田のり：日本学術会議育種学連携委員
2. 倉田のり：日本育種学会幹事
3. 倉田のり：日本育種学会学会賞選考委員
4. 倉田のり：生物遺伝資源イネ小委員会委員長
5. 倉田のり：Rice Genetics Newsletter Editor
6. 倉田のり：NSF project advisory committee member

L. INTELLECTUAL PROPERTY UNIT

Munehiro Tomikawa

RESEARCH ACTIVITIES

This unit makes various efforts to return the outcome of research generated by NIG to the industrial world and the public while respecting the scientists' priorities in terms of scientific ideas, partnership and publications.

To be more precise, what we have done is to establish scientific results as intellectual property, explain the property rights to the industrial world intelligibly, seek collaboration with commercial industries for actualizing those results and establish partnerships. This shows the importance of carrying out both public relations and the acquisition of intellectual property.

This unit was set up in March 2003. Since April 2003, a secretary, Ms. Nozomi Yokoyama has devoted all her energy to this work from starting up ,and had assisted by creating the MTA(Material Transfer Agreement) form ,and getting it off the ground. She has also been supporting various works such as planning and promotion of publicity activities and procedures of application for intellectual property rights. The results of our work in 2005 are shown as follows.

(1) Acquirement and utilization of Intellectual Property Rights

We have continued to promote a sense of entitlement in the scientists belonging to NIG and Division of Life Science in the Graduate University for Advanced Studies (Sokendai) by giving them speeches on why entitlement is necessary to the outcome of basic research. Also, in visiting laboratories, monitoring the scientific progress and thinking of each scientist, we have explained how intellectual property rights can be gained from their results. We have also established an efficient patenting procedure. We have prepared an invention exposition format to minimize the scientists' burden in informing our unit of their scientific results. Our unit helps the scientists avoid the troublesome task of acquiring of patent rights to the practical applications of their inventions.

In terms of patent applications, we have tried to promote acquirement of the patent rights so the NIG scientist does not need to bear the costs necessary for the preparation of patent documents. At this time, 9 domestic patents have been newly applied for and 1 patents are under preparation, of which 12 as a whole will be applied for as NIG patents (including 3 joint applications and three international patents) in the Research Organization of Information and Systems (ROIS). One patent has already come into marketing as of November 2003. We got the royalty with a small amount from the second year of the business since April 2005.

Secondly, we have been involved in making guidelines for the Material Transfer Agreement which stipulates ownership, ethical codes and compensation for biological resources based on research results. At present, three kinds of agreements, depending on the positioning of biological resources as intellectual property, have been created and are in use. Each has a simple version corresponding to specific materials in the research field such as fly, yeast, (Form 1)and an intermediate version without provision for sharing of industrial property rights (Form 2) and a complete version with provision for sharing of industrial property rights (Form 3).They have been authorized and, a total of 370 agreements (approximately 80% of them are international) have been signed by the end of year.

In addition, we have participated in the Shizuoka-TLO-Yaramaika (STLO) campaign, which facilitates putting patents owned by the NIG into practical use.

(2) Acceleration of public relations activities

Ideally, this kind of work should be developed over a broad spectrum, however, we have focused our

attention on the following main points. We have given priority to our response to visitors, promotion at academic conferences, introduction of research results to the public, and the search for potential collaboration.

Firstly, we had 12 groups of visitors (total 229 persons) in 2005: prefectural university students, legal apprentices, high school students, elderly community college students and others. An overview of this institute, genetic science and current laboratory experiments was given to the visitors.

Secondly, promotion at academic conferences has been carried out in order to obtain more excellent students and scientists. We introduced the current research results obtained from NIG scientists at the Japanese Cell Biology Meeting (in Sendai), the Japanese Society for Developmental Biology meeting (in Oomiya), and the Japanese Molecular-Biology meeting (in Hakata).

Thirdly, we presented eight specifically excellent research results in the Hot News column on the website as an introduction of research results and academic awards for life science to the public. We have also had eight research results inserted in main-stream newspapers through cooperation with the media. Furthermore, we introduced two scientists to companies which were interested in their work, and fortunately, this effort led to further business-academia collaboration.

(3) Collaboration Research with academia, industry and government, and Social Action Work

I have been conducting research of Fuji Pharma Valley Cancer Diagnostics Study Group consisting of academia, industry and government. The aim of this project is to increase Research and Development potential in the Mt. Fuji area and its cities of Numazu, Mishima, Fujinomia, Fuji and Nagaizumi,, and to study together with companies operating in the field of medicine as well as the pharmaceutical industry on the development of cancer diagnostics through genomics and proteomics approaches. Fortunately, we could apply two patent regarding cancer diagnostics based on regulation of chromatin DNA this December.

Recently, the participation of public facilities, such as universities and NIG, is increasing in the Government-Industry-University-Research Cooperation Conference. In June, We attended the Fourth Government-Industry-University Cooperation Conference as a panelist on behalf of NIG, which was held at Kyoto. In July, We got the matching fund with Shizuko-Sangyou Co., Ltd. from NEDO for research-development of the thinner Multi-well Plate which facilitated to transport and to reserve the biological resources such as bacteria, plasmids and DNA and succeeded in preparing one-well plate and multi-well plate with 16, 96 and 384 wells. It was quite meaningful to be able to develop a relationship with industrial people who was interested in our patent and products. In this March, we got approval of the establishment of venture business referred as BioROIS from ROIS.

In conclusion, acquiring intellectual property and engaging in returning excellent technology from scientific results to the industrial world through public relations will be important factors in gaining wide public recognition of NIG. These efforts are expected to facilitate the financial management of NIG, after turning into independent administrative entities.

M. TECHNICAL SECTION

RESEARCH ACTIVITIES

The Technical section supports the research activity of NIG in many fields. For example, we support the Radioisotope center and Experimental farm, and the Genetic strain research center where genetic and transgenic strains of mice, rice, flies, and fishes are produced, maintained, and distributed to other institutions. Members of the staff additionally support many types of experiments in various laboratories, such as developmental, cell, and molecular genetics. We continually update our technical services through attending the training and annual meetings for the technological staffs.

PUBLICATIONS

Papers

1. Ohya, Y., Sese, J., Yukawa, M., Sano, F., Nakatani, Y., Saito, T. L., Saka, A., Fukuda, T., Ishihara, S., Oka, S., Suzuki, G., Watanabe, M., Hirata, A., Ohtani, M., Sawai, H., Fraysse, N., Latge, J. P., Francois, J. M., Aebi, M., Tanaka, S., Muramatsu, S., Araki, H., Sonoike, K., Nogami, S. and Morishita, S. (2005) High-dimensional and large-scale phenotyping of yeast mutants., **Proc. Natl. Acad. Sci. USA**., *102 (52)* 19015 - 19020

2. Saka, K., Tadenuma, M., Nakade, S., Tanaka, N., Sugawara, H., Nishikawa, K., Ichiyoshi, N., Kitagawa, M., Mori, H., Ogasawara, N., Nishimura, A. (2005) A Complete Set of *Escherichia coli* Open Reading Frames in Mobile Plasmids Facilitating Genetic Studies, **DNA RESEARCH**, 63 - 68

BIOLOGICAL SYMPOSIUM 2005

Jan.12 DNA メチル化を中心としたゲノムワイドエピジェネティックス（Kunio Shiota）
Feb.16 代謝ストレス応答における膜局在に依存した転写後制御機構（Toshihumi Inada）
Mar.2 Rett 症候群の発症機構：MeCP2 によるクロマチンループを介した遺伝子発現制御機構の解明（Shinichi Horike）
Mar.7 メタゲノムライブラリーを利用した未知遺伝子スクリーニング法の開発（Taku Uchiyama）
Mar.14 GENETICS OF HUMAN CANCER SUSCEPTIBILITY（Kari Hemminki）
Mar.22 NHR-25 / Ftz-f1 / SF-1: Role during epidermal differentiation in C. elegans（Masako Asahina）
Mar.22 The steroid deficiency gene ecdysoneless（Marek Jindra）
Mar.23 細胞の生死と神経系前駆細胞の運命を制御するシグナル伝達（Yukiko Goto）
Mar.24 Age and sex effects on human mutation rates: an old problem with new complexities(James F. Crow)
Mar.25 肝臓発生を制御する分子機構のニワトリ胚を用いた解明（Yuji Yokouchi）
Apr.7 膜電位の役割を捉えなおす～細胞膜の電気的信号を細胞内情報に変換する膜タンパク～(Yasushi Okamura)
Apr.19 ゼブラフィッシュを用いた初期発生を制御する因子の機能解析：造血発生と体節形成の分子メカニズム（Atsuo Kawahara）
Apr.22 Control of Polycomb Group gene expression by Imprinting and by feed-back regulations.（Frederic Berger）
Apr.27 脊椎動物の初期体軸・神経形成の分子機構（Masahiko Hibi）
May.24 発生における自律的位置情報形成のメカニズム（Shigeru Kondo）
May.26 花粉管ガイダンスにおける細胞間コミュニケーション（Tetsuya Higashiyama）
Jun.8 Defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem / progenitor cells（Kazuhiro Aiba）
Jun.20 Molecular Dissection of the Metazoan Kinetochore（Iain M. Cheeseman）
Jun.23 How hypoxia-induced suspended animation led us to a nitric oxide mediated pathway of innate immune induction（Pat O'Farrell）
Jun.28 Cyclophilin D に依存したミトコンドリア膜透過性遷移の細胞死における役割（Takashi Nakagawa）
Jul.15 ショウジョウバエのニューロネットワーク機構解析（Akira Chiba）
Jul.20 マウス精子の形態と運動性を制御する RNA 結合タンパク質（Yasuyuki Kurihara）
Jul.25 線虫 C.elegans のシナプス形成におけるユビキチンリガーゼ RPM-1 による DLK-1-p38 MAP キナーゼ経路の制御機構（Katsunori Nakata）
Aug.16 Ran / RCC1 システムの有する多角的な生物機能（Hitoshi Nishijima）
Aug.24 カテコールアミン伝達と行動制御のメカニズム（Kazuto Kobayashi）
Sep.8 腫瘍細胞の極性喪失と増殖・浸潤とをリンクする分子メカニズム（Tatsushi Igaki）
Sep.27 ヒト DNA 組換え酵素 Dmc1 が行なう DNA 組換え反応の分子メカニズム（Takashi Kinebuchi）
Sep.30 Germ cell development in Drosophila（Ruth Lehmann）
Oct.13 ショウジョウバエにおける mRNA-like non-coding RNA の機能（Yuji Kageyama）
Oct.20 Study of Molecular Evolution: an Informal and Personal Account.（Masatoshi Nei）
Oct.24 Innate and Adaptive Immunity in the fruit fly（David Schneider）
Oct.25 Adhesion remodelling underlying tissue morphogenesis（Thomsds Lecuit）
Nov.2 Mechanism of DNA Methylation in Development（Guoliang Xu）
Nov.7 Detection and processing of color information in Drosophila（Claude Desplan）
Nov.11 Regulation of pre-RC assembly in yeast and human cells（John F.X. Diffley）
Nov.16 Asymmetric division and self-renewal of Drosophila neural stem cells（Chris Q. Doe）
Nov.21 Genomic Imprinting -Causes and Consequences（Anne C. Ferguson-Smith）
Nov.24 損傷バイパス型 DNA polymerase κ のヌクレオチド除去修復機構における新しい機能（Tomoo Ogi）
Nov.24 複製チェックポイント機構は安定な DNA 複製にいかにして貢献しているか（Kanji Furuya）
Nov.25 DNA damage checkpoint signaling in budding yeast（Katsunori Sugimoto）
Nov.28 Cell fate specification and cell shape control in the Drosophila retina（Takashi Hayashi）

Nov.30　骨の化学分析からみた先史時代人の生活（Minoru Yoneda）

Nov.30　化石から探るジャワ原人の起源と進化（Yosuke Kaifu）

Dec.1　Arabidopsis における trans-acting siRNA の生成経路（Manabu Yoshikawa）

Dec.12　The Sleeping Beauty Transposon System: A Tool for Discovering Genes Involved in Cancer and Development（David A. Largaespada）

Dec.13　Enhancer detection in zebrafish: Search for developmentally important genes and regulatory sequences（Thomas S. Becker）

Dec.14　Roles of transcription-coupled histone variant deposition in maintaining chromatin structure and epigenetic states（Kami Ahmad）

Dec.21　培養細胞株を用いた効率的な哺乳類概日時計システムのリアルタイム解析（Kazuhiro Yagita）

FOREIGN VISITORS IN 2005

Mar.2 Shinichi Horike	The Hospital for Sick Children Toronto, Canada
Mar.14 Kari Hemminki	Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, Department of Biosciences at Novum, Karolinska Institute, 141 57 Huddinge, Sweden,
Mar.22 Masako Asahina	Institute of Parasitology, Academy of Sciences of the Czech Republic
Mar.22 Marek Jindra	Institute of Entomology, Academy of Sciences of the Czech Republic, and Department of Molecular Biology, South Bohemica University, Czech Republic
Mar.24 James F. Crow	Department of Genetics University of Wisconsin-Madison
Apr.22 Frederic Berger	Temasek Lifescience Laboratory, The National University of Singapore
Jun.8 Aiba Kazuhiro	Laboratory of Genetics, National Institute on Aging, National Institutes of Health
Jun.20 Iain M. Cheeseman	Ludwig Institute for Cancer Research
Jun.23 Pat O'Farrell	Department of Biochemistry and Biophysics, UCSF
Jul.15 Akira Chiba	Department of Cell and Structural Biology, and Neuroscience University of Illinois at Urbana-Champaign
Jul.25 Katsunori Nakata	Department of MCD Biology, University of California, Santa Cruz
Sep.8 Tatsushi Igaki	Dept. Genetics, Yale Univ. Sch. Med.
Oct.20 Masatoshi Nei	Institute of Molecular Evolutionary Genetics Pennsylvania State University
Oct.24 David Schneider	Stanford University, Dept. Microbiology and Immunology
Oct.25 Thomas Lecuit	IBDM / LGPD
Nov.2 Guoliang Xu	Institute of Biochemistry and Cell Biology Shanghai Institutes for Biological Sciences Chinese Academy of Sciences
Nov.7 Claude Desplan	NYU, Dept. Biology
Nov.11 John F.X. Diffley	Cancer Research UK London Research Institute, Clare Hall Laboratories
Nov.16 Chris Q. Doe	University of Oregon, Institutes of Neuroscience and Molecular Biology
Nov.21 Anne C. Ferguson-Smith	Department of Anatomy University of Cambridge, U.K.
Nov.24 Ogi Tomoo	Genome Damage and Stability Centre, University of Sussex
Nov.24 Kanji Furuya	Genome Damage and Stability Centre, University of Sussex
Nov.25 Katsunori Sugimoto	Department of Cell Biology and Molecular Medicine University of Medicine and Dentistry of New Jersey, USA
Nov.28 Takashi Hayashi	Department of Biochemistry, Molecular Biology and Cell Biology Northwestern University
Dec.12 David A. Largaespada	Department of Genetics, Cell Biology and Development University of Minnesota Cancer Center
Dec.13 Thomas S. Becker	Sars Centre for Marine Molecular Biology Bergen, Norway
Dec.14 Kami Ahmad	Department of Biological Chemistry and Molecular Pharmacology Harvard Medical School

Annual Report of the National Institute of Genetics. No.56

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Edited by Ryu Ueda
Published by National Institute of Genetics