

The 20th Hot Spring Harbor Symposium
and joint with the 6th Global COE International Symposium

New Horizons for Modern Science

Biology and Medicine at the Crossroads

August 19-20, 2010

The Luigans Spa & Resort (Fukuoka, Japan)

主催

九州大学・生体防御医学研究所
グローバル COE プログラム

共催

戦略的創造研究推進事業(CREST)・科学技術振興機構(JST)

文部科学大臣認定・共同利用・共同研究拠点

「多階層生体防御システム研究拠点」

Program

August 19, 2010 (Thu)

13:00~13:30 Registration

13:30~13:40 Opening Remark Yukio Fujiki (Kyushu Univ., Japan)

Session I. Protein Modification Systems* (Chair : Yasunobu Yoshikai)

- 1 13:40~14:10 Aaron Ciechanover (Technion, Israel)
Why our proteins have to die so we shall live or the ubiquitin proteolytic system - From basic mechanisms and onto disease mechanisms and drug development
- 2 14:10~14:40 Keiichi Nakayama (Kyushu Univ., Japan)
Comprehensive elucidation of enzyme-substrate relationship by proteomics: Say good-bye to western blotting
- 3 14:40~15:10 Yue Xiong (Univ. North Carolina, USA)
Acetylation regulation of metabolism
- 4 15:10~15:40 Noboru Mizushima (Tokyo Medical and Dental Univ., Japan)
Physiological roles of autophagy and its regulation mechanism

15:40~16:00 Coffee Break

Session II. Cutting-edge Technologies (Chair : Daisuke Kohda)

- 5 16:00~16:30 Tohru Natsume (AIST, Japan)
Challenge to ultra-high sensitive mass spectrometry for protein network analysis
- 6 16:30~17:00 Katsuhiko Shirahige (Univ. Tokyo, Japan)
Regulation of Cohesin dynamics by acetylation and deacetylation
- 7 17:00~17:30 Atsushi Miyawaki (RIKEN/JST, Japan)
New fluorescent probes and new perspectives in bioscience
- 8 17:30~18:00 Hiroki Ueda (RIKEN/JST, Japan)
Systems biology of mammalian circadian clocks: The role of delay in feedback repression

18:30~20:30 Dinner (Toast Takehiko Sasazuki)

20:30~22:30 Meet the Speakers (Wine & Cheese Party)

August 20, 2010 (Fri)

Session III. Cell Structure and Function* (Chair : Yoshinori Fukui)

- 9 8:30~9:00 Yukio Fujiki (Kyushu Univ., Japan)
Peroxisome: Biogenesis and homeostasis - Membrane assembly, matrix protein import, morphogenesis, and turnover
- 10 9:00~9:30 Hisao Kondo (Kyushu Univ., Japan)
p97ATPase-mediated Golgi membrane fusion
- 11 9:30~10:00 Tom Rapoport (Harvard Medical School, USA)
Mechanisms of protein transport across membranes
- 12 10:00~10:30 Tadashi Uemura (Kyoto Univ., Japan)
Linking global tissue asymmetry to cell polarity on the plane

10:30~10:50 Coffee Break

Session IV. Stem Cell and Cancer (Chair : Akira Suzuki)

- 13 10:50~11:20 Koichi Akashi (Kyushu Univ., Japan)
Targeting cancer stem cells: TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells
- 14 11:20~11:50 Peter Sicinski (Dana Farber Cancer Inst., USA)
Molecular functions of cyclins in mouse development
- 15 11:50~12:20 Yukiko Gotoh (Univ. Tokyo, Japan)
Temporal regulation of neural stem cell fate in the developing mouse neocortex

12:20~13:30 Lunch (GCOE Council)

*Sessions I and III are held as “JST collaboration session.”

Session V. Young Investigators (Chair : Takehiko Yokomizo)

- 16S 13:30~13:50 Michiko Shirane (Kyushu Univ., Japan)
Protrudin regulates Rab11-dependent synaptic function via interaction with sphingolipid
- 17S 13:50~14:10 Takeshi Imai (RIKEN/JST, Japan)
Olfactory map formation directed by the odorant receptor-derived cAMP signals
- 18S 14:10~14:30 Midori Shimada (Nagoya City Univ., Japan)
Histone binary switch mediated by Chk1-HAT and PPI-HDAC regulates DNA damage induced transcriptional repression
- 19S 14:30~14:50 Gohta Goshima (Nagoya Univ., Japan)
Mechanism of microtubule generation during cell division

14:50~15:10 Coffee Break

Session VI. Transcriptional Control and Epigenetics

(Chair : Yusaku Nakabeppu)

- 20 15:10~15:40 Yi Zhang (Univ. North Carolina, USA)
Identification and characterization of enzymes involved in DNA demethylation
- 21 15:40~16:10 Yoichi Shinkai (Kyoto Univ., Japan)
Regulation and biological function of histone lysine methylation
- 22 16:10~16:40 Hiroyuki Sasaki (Kyushu Univ., Japan)
Small RNAs, retrotransposons and genomic imprinting in the mammalian germline

16:40~16:50 Closing Remark Kenzaburo Tani (Kyushu Univ., Japan)

Session I.

(August 19, 2010, 13:40~15:40)

Protein Modification Systems

Why our proteins have to die so we shall live or the ubiquitin proteolytic system - From basic mechanisms and onto disease mechanisms and drug development

Aaron Ciechanover

Cancer and Vascular Biology Research Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Between the 50s and 80s, most studies in biomedicine focused on the central dogma - the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared - was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

**Education:**

- 1965-1972 Graduate School of Medicine, Hebrew University, Jerusalem (M.D.)
1969-1970 School of Medicine, Hebrew University, Jerusalem (M.Sc.)
1976-1981 Faculty of Medicine, Technion-Israel Institute of Technology (D.Sc.)

Research Positions:

- 1977-1979 Research Fellow, Technion-Israel Institute of Technology
1979-1981 Lecturer, Technion-Israel Institute of Technology
1984-1987 Senior Lecturer (with tenure), Technion-Israel Institute of Technology
1987-1992 Associate Professor, Technion-Israel Institute of Technology
1992-2002 Full Professor, Technion-Israel Institute of Technology
2002- Distinguished Research Professor, Technion-Israel Institute of Technology

Awards:

- 2000 The Albert Lasker Award for Basic Medical Research
2003 The Israel Prize for Biology
2004 Nobel Prize in Chemistry

Academies:

- 2004 Israeli National Academy of Sciences and Humanities
2005 Member (Foreign), American Philosophical Society
2007 Associate (Foreign), National Academy of Sciences of the USA (NAS USA)
2008 Fellow (Honorary; Foreign), American Academy of Arts and Sciences (AAAS)
2008 Associate (Foreign), Institute of Medicine of the National Academies of the USA (IOM)

Memo

Comprehensive elucidation of enzyme-substrate relationship by proteomics: Say good-bye to western blotting

Keiichi I. Nakayama^{1,3}, Kanae Yumimoto^{1,3} and Masaki Matsumoto^{2,3}

¹Department of Molecular and Cellular Biology, and ²Department of Proteomics, Medical Institute of Bioregulation, Kyushu University, Japan, ³CREST, Japan Science and Technology Agency (JST), Japan

A bottleneck of the modern biology is a lack of universal method to identify substrates from enzymes. In most instances, enzyme-substrate relations have been discovered by substrate-to-enzyme approaches, with opposite (enzyme-to-substrate) approaches having been rarely applied and mostly unsuccessful. On the other hand, analysis of the genome sequences of various organisms has led to the identification of large numbers of enzymes such as protein kinases (>500) and ubiquitin ligases (>1000) on the basis of their conserved catalytic domains. However, universal approaches to the identification of substrates for a given enzyme have not been established to date, and many enzymes remain “orphans” in this regard. It is especially difficult to identify substrates for ubiquitin ligases, given that such substrates are immediately ubiquitylated and degraded as a result of their association with the enzyme.

Here we show development of new technology designated DiPIUS (differential proteomics-based identification of ubiquitylation substrates) to discover substrates for Skp1-Cul1-F-box protein (SCF) complex. We applied DiPIUS to Fbxw1 (β -TrCP1), Fbxw7, Fbxl1 (Skp2) and Fbxl5, four of the most well-characterized F-box proteins, as well as VHL, a receptor subunit of Cul-2-based SCF-like complex. We successfully identified their known substrates and many candidates for the new substrates. Some of the candidates were subjected to validation studies, and confirmed as authentic substrates for each ubiquitin ligase. These observations indicate that DiPIUS is a powerful tool for unbiased and comprehensive screening of substrates for ubiquitin ligase.

We have also developed a targeted proteomics approach based on multiple reaction monitoring (MRM) to detect and quantify proteins in total HeLa cell digests. All human recombinant proteins are now being produced to establish information-based MRM (IB-MRM) system. We illustrate the power of this technique by the consistent and fast measurement of all human proteins. We therefore demonstrate the potential of IB-MRM-based proteomics to provide assays for the measurement of any set of proteins of interest in humans at high-throughput and quantitative accuracy.

**Education:**

- 1980-1986 Faculty of Medicine, Tokyo Medical and Dental University (M.D.)
1986-1990 Graduate School of Medicine, Juntendo University (Ph.D.)

Research Positions:

- 1990-1990 Post-doctoral Fellow, RIKEN
1990-1992 Post-doctoral Fellow, Washington University Medical School
1992-1995 Research Associate, Howard Hughes Medical Institute, Washington University Medical School
1995-1996 Senior Scientist, Nippon Roche Research Center
1996- Professor, Kyushu University
2009- Distinguished Professor, Kyushu University

Awards:

- 1990 Medical Alumni Award, Tokyo Medical and Dental University
2005 JSPS (Japan Society for the Promotion of Science) Prize
2007 JCA-Mauvernay Award

Memo

Acetylation regulation of metabolism

Yue Xiong

Molecular & Cell Biology Lab, Fudan University, Shanghai, China
Department of Biophysics and Biochemistry, Lineberger Comprehensive Cancer Center,
University of North Carolina, USA

Alterations in cell growth and cell cycle regulations are commonly associated with the development of several human proliferative diseases, in particular cancer, and are believed to link with changes in cellular metabolisms. While most metabolic pathways have been well established several decades ago, how extracellular metabolic fuels signal to different metabolic pathways remain poorly understood. Recent proteomic analyses on protein acetylation identified potentially a large number of acetylated proteins in the cytoplasm, including most enzymes involved in intermediate metabolisms. Substantial amount of enzymes can be acetylated in vivo and the levels of acetylation change dynamically in response to nutrients change and during calorie restriction. Acetylation regulates metabolic enzymes by multiple mechanisms, including enzymatic inhibition, activation, and protein stability. Furthermore, acetylation of metabolic enzymes is highly conserved from prokaryotes to eukaryotes. Given the frequent occurrence of metabolism dysregulation in diabetes, obesity, and cancer, enzymes modulating acetylation may provide attractive targets for therapeutic intervention for these diseases.

**Education:**

- 1978-1982 Department of Biology, Fudan University (B.Sc.)
1982-1983 Guangzhou English Language Center, Zhongshan University/University of California at Los Angeles
1983-1984 Graduate School, Shanghai Institute of Plant Physiology Chinese Academy of Science
1984-1989 Department of Biology, The University of Rochester (Ph.D.)

Research Positions:

- 1989-1990 Postdoctoral Fellow, The University of Rochester
1990-1993 Postdoctoral Research Associate, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory
1993-1999 Assistant Professor, The University of North Carolina at Chapel Hill
1999-2003 Associate Professor, The University of North Carolina at Chapel Hill
2003- Professor, The University of North Carolina at Chapel Hill
2005- William R. Kenan Professor of Biochemistry & Biophysics
2006- Leader, Cell Biology Program, Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill

Awards:

- 1995 Jefferson-Pilot Fellowship
1995 Life & Health Insurance Medical Research Fund Award
1995 American Cancer Society Junior Faculty Research Award
1995 Pew Scholar in the Biomedical Sciences
1999 United States Department of Defense Breast Cancer Research Career Development Award
1999 AACR Gertrude B. Elion Cancer Research Award
1999 UNC Hettleman Award for Scholarly Achievement
2005 William. R. Kenan Professor, UNC-Chapel Hill
2006 American Lung Association Diane Emdin Sachs Lung Cancer Award

Memo

Physiological roles of autophagy and its regulation mechanism

Noboru Mizushima

Department of Physiology and Cell Biology, Tokyo Medical and Dental University, Japan

Autophagy is the primary pathway for the degradation of cytoplasmic constituents in the lysosome. When autophagy is induced, a portion of cytoplasm is sequestered by autophagosomes, and then delivered to lysosomes. We have analyzed physiological roles of autophagy using several mouse models. Using autophagosome-indicator mice (GFP-LC3 transgenic mice) and conventional Atg5 knockout mice, we have shown that autophagy is up-regulated during starvation and is critically important to tide over starvation such as during the early neonatal period. We also discovered that Autophagy is essential for preimplantation development of mouse embryos. Analysis of oocyte-specific Atg5 knockout mice revealed that autophagy-defective embryos failed to develop beyond the 4-8-cell stages. We suggest that degradation of maternal proteins by autophagy is critical to produce necessary amino acids during preimplantation development in mammals.

On the other hand, we found that constitutive “basal” autophagy is important for intracellular protein quality control, because multiple protein aggregates accumulate in the cytoplasm of neural cell- and liver-specific Atg5 knockout mice. Finally, to further analyze the effects of defects in autophagy for a long period in vivo, we generated mice with mosaic deletion of Atg5. In these mice, multiple tumors develop only in the liver, which are derived from Atg5KO cells. These results suggest that autophagy is important for suppression of spontaneous tumorigenesis in the liver.

We have also conducted studies on molecular mechanism of autophagy regulation and autophagosome formation. We determined hierarchical relationships among mammalian autophagy factors including several novel proteins and found that the ULK1-mAtg13-FIP200-Atg101 complex is the most upstream unit and is directly regulated by mTORC1. Upon starvation, the ULK1 complex localizes to punctate structures associated with the ER. We hypothesize that these structures represent autophagosome formation sites in mammalian cells.

**Education:**

- 1985-1991 Faculty of Medicine, Tokyo Medical and Dental University (M.D.)
1991-1996 Graduate School of Medicine, Tokyo Medical and Dental University (Ph.D.)

Research Positions:

- 1996-1998 Research Fellow (PD), The Japan Society of the Promotion of Science
1998-1999 Post-doctoral Fellow, National Institute for Basic Biology
1999-2002 PRESTO, Japan Science and Technology Corporation (JST)
2002-2004 Assistant Professor, National Institute for Basic Biology
2004-2006 Laboratory Head, The Tokyo Metropolitan Institute of Medical Science
2006- Professor, Tokyo Medical and Dental University

Awards:

- 1999 Medical Alumni Award, Tokyo Medical and Dental University
2001 Young Investigator Award, The Japanese Biochemical Society
2005 Mitsubishi Chemical Award, The Molecular Biology Society of Japan
2006 The Young Scientists' Prize, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
2007 FEBS Letters Young Scientist Award
2008 JSPS (Japan Society for the Promotion of Science) Prize
2008 Tsukahara Award, Brain Science Foundation
2009 Inoue Prize for Science, Inoue Foundation for Science

Memo

Session II.

(August 19, 2010, 16:00~18:00)

Cutting-edge Technologies

Challenge to ultra-high sensitive mass spectrometry for protein network analysis

Tohru Natsume

Biomedical Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Japan

Functional proteomics aims to discover gene functions at the protein level. Mass spectrometry (MS)-based proteomic approaches are powerful tools for identification and quantification analysis of the protein components of complexes in cells. Therefore, a variety of sample preparation techniques for the MS approach has been developed and reported. Although these affinity purification methods are indeed useful, the problem is that the purified samples for proteome-wide analysis tend to be uneven in quality because of human elements. In large-scale protein interaction analysis, even though using partially-automated system, researchers or technicians are involved in laborious repetitive work of sample preparation, in which they must handle tens of culture dishes at a time and perform sequentially cell harvest and extraction, affinity purification of protein complexes, and enzymatic protein digestion. During the preparation of a number of samples, there must be difference in conditions between the first and last treated samples. Denaturation of the component proteins of the complexes and proteolysis are progressive over time, and the denaturated proteins are thought to be the cause of non-specifically binding. Furthermore, the difference of the sample quality is also caused by the personal skills.

To solve the problem, we have developed an automated sample preparation system for mass spectrometry-based functional proteomics. Since this robotic affinity purification system enables the isolation of protein complexes at short times under equal mild conditions, we successfully identify minor proteins reproducibly with non-specific proteins minimized. Using the well-characterized Wnt signaling pathway proteins, β -catenin and Axin1, we could identify interaction partners, including novel component proteins, in mammalian cells.



Education:

- 1984 Institute of Agriculture and Forestry, University of Tsukuba (B.Sc.)
- 1986 Graduate School of Agricultural and Life Sciences, University of Tokyo (M.Sc.)
- 1993 Graduate School of Medicine, Kyoto University (Ph.D.)

Research Positions:

- 1996-2000 Research Fellow, University of Tokyo
- 2000-2001 Section Chief, Science and Technology Agency
- 2001-2006 Team Leader of Protein Network Team, National Institute of Advanced Industrial Science and Technology (AIST)
- 2006- Team Leader of Biological Systems Control Team, National Institute of Advanced Industrial Science and Technology (AIST)

Memo

Regulation of Cohesin dynamics by acetylation and deacetylation

Katsuhiko Shirahige¹, Masashige Bando¹ and Takehiko Itoh²

¹Research Center for Epigenetic Disease, The University of Tokyo, Japan, ²Tokyo Institute of Technology, Graduate School of Bioscience, Japan

Cohesin regulates sister chromatid cohesion during the mitotic cell cycle, with Nipped-B-Like (NIPBL) facilitating its loading and unloading onto chromosomes. In addition to this canonical function, cohesin also plays a critical role in regulating gene expression as a transcriptional insulator in postmitotic cells. Heterozygous mutations in NIPBL or cohesin structural components SMC1A and SMC3 result in the multisystem developmental disorder Cornelia de Lange Syndrome (CdLS). Genome-wide localization analyses (ChIP-seq) of cohesin in three mutant cell lines from severely affected CdLS probands showed that >60% of cohesin binding sites are absent. Transcriptome analyses of 16 CdLS probands identified a unique profile of dysregulated gene expression that correlated significantly with lost cohesin sites in CdLS. Upregulated genes in CdLS tend to lose cohesin sites within 5 kb from the transcription start site, suggesting that cohesin may have a more direct role than insulator in transcriptional regulation. Esco1 and Esco2 are cohesin-specific acetyltransferases that are required for establishment of sister chromatid cohesion. We found that Esco2 is required for cohesin acetylation only in S-phase, whereas Esco1 is required for cohesin acetylation in both G1 and S-phase. Heterozygous mutations in the gene *ESCO2* causes the developmental disorder, Roberts Syndrome, further suggesting that cohesin acetylation plays a crucial role in transcriptional regulation. Using RNA interference-based screening, we identified a cohesin-specific deacetylase gene (*Hdac8*). Knockdown of *Hdac8* levels or inhibition of its activity led to an increase of the amount of acetylated cohesin, the number of acetylated cohesin binding sites, and induced cohesion defects. FRAP analysis showed that *Hdac8* knockdown destabilized cohesin–chromosome interactions, suggesting that the acetylation state of cohesin is important for controlling its dynamics. The biological significance of cohesin acetylation and deacetylation will be discussed.

**Education:**

1986-1988 Faculty of Arts and Sciences, University of Tokyo (B.Sc.)
1988-1990 Graduate School of Medicine, Osaka University (M.Sc.)
1990-1993 Graduate School of Medicine, Osaka University (Ph.D.)

Research Positions:

1993-1994 Post-Doctoral Fellow, Nara Advanced Institute of Science and Technology
1994-2002 Research Associate, Nara Advanced Institute of Science and Technology
2002-2004 Senior Researcher, RIKEN Genomic Science Center
2004-2007 Assistant Professor, Tokyo Institute of Technology
2007-2010 Professor, Tokyo Institute of Technology
2010- Professor, The University of Tokyo

Awards:

2003 Mitsubishi Chemical Award, the Molecular Biology Society of Japan
2008 NAIST Award for Academic Achievements
2009 JSPS (Japan Society for the Promotion of Science) Prize
2010 Prizes for Science and Technology (Research Category), The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology

Memo

NEW FLUORESCENT PROBES AND NEW PERSPECTIVES IN BIOSCIENCE

Atsushi Miyawaki

RIKEN Brain Science Center, Japan

The behavior of biochemical molecules moving around in cells makes me think of a school of whales wandering in the ocean, captured by the Argus system on the artificial satellite. When bringing a whale back into the sea --- with a transmitter on its dorsal fin, every staff member hopes that it will return safely to a school of its species. A transmitter is now minute in size, but it was not this way before. There used to be some concern that a whale fitted with a transmitter could be given the cold shoulder and thus ostracized by other whales for “wearing something annoying.” How is whale’s wandering related to the tide or a shoal of small fish? What kind of interaction is there among different species of whales? We human beings have attempted to fully understand this fellow creature in the sea both during and since the age of whale fishing.

In a live cell imaging experiment, a fluorescent probe replaces a transmitter. We label a fluorescent probe on a specific region of a biological molecule and bring it back into a cell. We can then visualize how the biological molecule behaves in response to external stimulation. Since fluorescence is a physical phenomenon, we can extract various kinds of information by making full use of its characteristics. For example, the excited energy of a fluorescent molecule donor transfers to an acceptor relative to the distance and orientation between the two fluorophores. This phenomenon can be used to identify interaction between biological molecules or structural change in biological molecules. Besides, we can apply all other characteristics of fluorescence, such as polarization, quenching, photobleaching, photoconversion, and photochromism, in experimentation.

Cruising inside cells in a supermicro corps, gliding down in a microtubule like a roller coaster, pushing our ways through a jungle of chromatin while hoisting a flag of nuclear localization signal --- we are reminded to retain a playful and adventurous perspective at all times. What matters is mobilizing all capabilities of science and giving full play to our imagination. We believe that such serendipitous findings can arise out of such a sportive mind, a frame of mind that prevails when enjoying whale-watching.

**Education:**

- 1981-1987 School of Medicine, Keio University (M.D.)
1987-1991 Graduate School of Medicine, Osaka University (Ph.D.)

Research Positions:

- 1991-1993 Research Fellow (PD), The Japan Society of the Promotion of Science
1993-1998 Research Associate, University of Tokyo
1995-1997 HFSP Long-term Fellowship, University of California San Diego
1997-1999 Research Pharmacologist, University of California San Diego
1999- Team Leader, RIKEN Brain Science Center

Awards:

- 2004 The Yamazaki-Teiichi Prize, Foundation for Promotion of Material Science and Technology of Japan
2006 Woodward Visiting Scholar, Harvard University
2006 JSPS (Japan Society for the Promotion of Science) Prize
2007 Brain Science Foundation, Tsukahara Award
2007 Kitazato Prize, Keio University
2008 Prizes for Science and Technology (Development Category), The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology

Memo

SYSTEMS BIOLOGY OF MAMMALIAN CIRCADIAN CLOCKS: THE ROLE OF DELAY IN FEEDBACK REPRESSION

Hiroki R. Ueda

Laboratory for Systems Biology and Functional Genomics Unit, Center for Developmental Biology, RIKEN, Japan.

The logic of complex and dynamic biological networks such as circadian clocks is difficult to elucidate without (1) comprehensive identification of network structure¹⁻³, (2) prediction and validation based on quantitative measurement and perturbation of network behavior^{4,6}, and (3) design and implementation of artificial networks of identified structure and observed dynamics⁵.

In the previous studies, we tried to reveal the complex structure of transcriptional circuits underlying mammalian circadian clock and found that it is composed of 20 transcription factors, and three type of DNA elements; "morning" element (E-box), "day-time" element (D-box) and "night-time" element (RevErbA/ROR binding element, RRE)^{1,2}. To derive and prove transcriptional logic of mammalian circadian clocks, we then developed *in vitro* mammalian cell culture system, where we can design and implement artificial transcriptional circuits composed of synthetic transcriptional regulators and promoters to physically simulate natural circadian transcriptional circuits. Using that system, we found transcriptional logic to execute "day-time" and "night-time" transcriptional program. However, "morning" transcriptional program still remains to be solved⁵.

In this symposium, we will introduce the current progress on the understanding of "morning" transcriptional program and discuss the role of transcriptional delay in feedback repression.

Reference

1. Ueda, H.R. et al, *Nature* 418, 534-539 (2002).
2. Ueda, H.R. et al, *Nat. Genet.* 37, 187-192 (2005).
3. Sato T K, et al, *Nat. Genet.* 38:312-9 (2006).
4. Ukai H, et al, *Nat. Cell Biol.* 9:1327-34 (2007).
5. Ukai-Tadenuma M, et al, *Nat. Cell Biol.* 10, 1154-63 (2008).
6. Isojima Y. et al, *PNAS* 106, 15744-49 (2009).

**Education:**

- 1994-2000 Faculty of Medicine, University of Tokyo (M.D.)
2000-2004 Graduate School of Medicine, University of Tokyo (Ph.D.)

Research Positions:

- 1997-1998 Student Trainee, University of Tokyo
1998-1999 Research Assistant, Sony Computer Science Laboratories
1999-2000 Research Assistant, ERATO Kitano Symbiotic Project
1999-2000 Technical Staff, Yamanouchi Pharmaceutical Co., Ltd.
2000-2001 Researcher, Yamanouchi Pharmaceutical Co., Ltd.
2001-2002 Postdoctoral Fellow, Yamanouchi Pharmaceutical Co., Ltd.
2002-2004 Group Leader, NEDO project, Yamanouchi Pharmaceutical Co., Ltd.
2003- Laboratory Head, RIKEN Center for Developmental Biology
2004- Subunit Leader, RIKEN Center for Developmental Biology

Awards:

- 2001 Atamasukkiri Award, Japanese Drosophila Research Conference/Moriwaki-Daigoro
2002 SRBR Joint Meeting Travel Award, Sleep Research Society
2004 Japan Innovator Award, Nikkei Business Publications Inc.
2005 Gold Medal, Tokyo Techno-Forum 21
2006 The Young Scientists' Prize, The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
2006 Tomorrow's PI Award, Genome Technology
2007 Young Investigator Promotion Awards, Japanese Society for Chronobiology
2009 IBM Science Award

Memo

Session III.

(August 20, 2010, 8:30~10:30)

Cell Structure and Function

Peroxisome: Biogenesis and Homeostasis - Membrane Assembly, Matrix Protein Import, Morphogenesis, and Turnover

Yukio Fujiki

Department of Biology, Faculty of Sciences, Kyushu University Graduate School, Japan, CREST, Japan Science and Technology Agency (JST), Japan

Peroxisome is a ubiquitous organelle that functions in numerous essential metabolic pathways including β -oxidation of very long chain fatty acids and biosynthesis of ether-glycerophospholipids, plasmalogens. Human fatal peroxisome biogenesis disorders such as Zellweger syndrome are autosomal recessive, comprising 13 genotypes. All of 13 pathogenic genes are recently delineated. Functional roles of the peroxins may be addressed in four distinct aspects.

1) Pex3p, Pex16p, and Pex19p are peroxisome membrane assembly factors. Pex19p forms a complex with peroxisomal membrane proteins (PMPs) including C-tail anchored proteins in the cytosol and transports it to Pex3p, the membrane receptor (class I pathway). Pex19p also forms a complex with newly synthesized Pex3p and translocates it to Pex16p on the peroxisomal membranes (class II pathway).

2) Peroxisome targeting signal 1 (PTS1) receptors, Pex5pS and Pex5pL, function in matrix protein import in mammals. Pex5pL also translocates the PTS2 receptor Pex7p-PTS2 complexes. Pex5p-cargo complexes initially dock with Pex14p of the import machinery. Pex5p and Pex7p are imported in an ATP-independent manner whereas the import of PTS1 proteins is ATP-dependent. Pex5p requires the AAA ATPase Pex1p-Pex6p complexes on the membrane peroxin Pex26p, a novel protein p40, and ATP-driven energy at the export-step in its peroxisome-cytoplasmic shuttling.

3) Fis1 plays a pivotal role in peroxisome division and maintenance of peroxisome morphology in a concerted manner with Pex11p β and DLP1. Our most recent study suggests that peroxisome morphogenesis is also regulated by polyunsaturated fatty acids such as DHA.

4) Our recent findings show that in mammalian cells peroxisomal proteins are degraded by two degradation systems involving autophagy and proteasomes depending on various cell-culture conditions, where Pex14p serves as a prerequisite factor for the degradation of peroxisomal proteins via interaction with LC3, a mammalian Atg8.

I will discuss several issues in these regards from our recent findings.



Education:

- 1967-1971 Faculty of Agriculture, Kyushu University (B.Sc.)
- 1971-1973 Graduate School of Agriculture, Kyushu University (M.Sc.)
- 1973-1976 Graduate School of Agriculture, Kyushu University (Ph.D.)

Research Positions:

- 1976-1979 Post-doctoral Fellow, Cornell University Medical College
- 1979-1980 Research Associate, The Rockefeller University
- 1980-1985 Assistant Professor, The Rockefeller University
- 1985-1988 Division Head, Meiji Institute of Health Science
- 1988-1994 Head and Chief Scientist, Meiji Institute of Health Science
- 1994-1999 Professor, Kyushu University
- 1999-2009 Professor, Kyushu University Graduate School
- 2009- Distinguished Professor, Kyushu University

Memo

p97ATPase –mediated Golgi membrane fusion

Kaori Tamura¹, Go Totsukawa¹, Kumi Matuura-Tokita¹, Hiroyuki Toh²
and **Hisao Kondo**¹

¹ Division of Molecular Cell Biology, Faculty of Medical Sciences, Kyushu University, Japan

² Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Japan

p97ATPase functions in Golgi and ER biogenesis and has been shown to use two distinct cofactors, p47 and p37, for its membrane fusion function. p47 is specialized for the reassembly of organelles at the end of mitosis, and p37 is required for organelles maintenance during interphase as well as for their reassembly during mitosis. p97 also requires a deubiquitinating enzyme, VCIP135, for its fusion function.

We have identified a novel essential factor, p87, in p97-mediated Golgi membrane fusion. p87 localizes to the Golgi and is required for p97/p47-mediated Golgi membrane fusion. The p97/p47 pathway in Golgi membrane fusion requires the deubiquitinating activity of VCIP135. p87 binds to VCIP135 and activates its deubiquitinating function.



Education:

1982-1988 Faculty of Medicine, Kyoto University (M.D.)

Research Positions:

1988-1995 Assistant Professor, Kyoto University (Ph.D.)

1995-1999 Post-doctoral Fellow, Imperial Cancer Research Fund, UK

1999-2004 Principal Investigator, University of Cambridge

2004-2006 Principal Investigator, Mitsubishi Kagaku Institute of Life Sciences

2006- Professor, Kyushu University

Awards:

1992 Baelz Award

Memo

Mechanisms of protein transport across membranes

Tom A. Rapoport

Howard Hughes Medical Institute, Department of Cell Biology, Harvard Medical School,
Boston, USA

Many proteins in bacteria are transported during their biosynthesis across or are integrated into the plasma membrane, a process that is similar to protein translocation across the endoplasmic reticulum (ER) membrane in eukaryotes. Transport occurs through a protein-conducting channel that is formed from a conserved heterotrimeric membrane protein complex (SecY or Sec61 complex). The channel associates with different partners in different translocation pathways. In bacteria, the SecY channel can associate with the translating ribosome (co-translational translocation) or with the cytoplasmic ATPase SecA (post-translational translocation). Crystal structures of the SecY complex alone or in association with SecA provide important insight into the mechanism of translocation. Many of the predictions made on the basis of the X-ray structures have now been confirmed by biochemical experiments. Taken together, these results show how the channel is opened, how it prevents the passage of small molecules, how trans-membrane segments of membrane proteins exit laterally into lipid, and how SecA pushes polypeptide chains through the channel.

**Education:**

- 1969-1972 Institute of Biochemistry, Humboldt-University (Ph.D.)
1972-1977 Institute of Biochemistry, Humboldt-University (Habilitation)

Research Positions:

- 1972-1985 Research Associate, Central Institute for Molecular Biology, Academy of Science
1985-1990 Professor, Central Institute for Molecular Biology, Academy of Science
1991-1994 Professor, Max-Delbrück-Center
1991-1995 Professor, Harvard Medical School
1997- Professor, Howard Hughes Medical Institute, Harvard Medical School

Awards:

- 1973 Johannes-Müller-prize of the Society for Experimental Medicine
1980 Rudolf-Virchow-prize
2004 Otto-Warburg-Medaille of the GBM in the field of Biochemistry
2007 Max Delbrück Medal
2007 Sir Hans Krebs Medal
2007 AAAS Fellow
2009 A. Clifford Barger Excellence in Mentoring Award
2009- Distinguished Research Chair Professor in the Center for Biotechnology and the Center for Medical Excellence at National Taiwan University
2010 Anatrache Membrane Protein Award
2010 Van Deenen Medal
2010 Keith R. Porter Lecturer at 50th Annual Meeting of the ASCB

Memo

Linking global tissue asymmetry to cell polarity on the plane

Tadashi Uemura^{1,2}, Toshiyuki Harumoto^{1,2}, Yuko Shimada¹, Hiroki R. Ueda³, and Tetsuya J. Kobayashi^{4,5}

¹Graduate School of Biostudies, Kyoto University, ²CREST, Japan Science and Technology Agency (JST), ³Center for Developmental Biology, RIKEN, ⁴Institute of Industrial Science, the University of Tokyo, ⁵PRESTO, JST

Cells sense global axes of the tissue to which they belong and manifest polarity for specialized functions. One such example is planar cell polarity (PCP), which is seen in many animals and tissues such as some epithelia that develop unidirectionally beating cilia. To date, underlying mechanisms of PCP have been best studied in the *Drosophila* wing, where epidermal cells somehow sense the cue along the proximal-distal (P-D) axis, localize an assembly of actin filaments at the distal cell vertex, and produce single wing hairs. The pertinent molecular players have been classified into at least the 2 following categories: The first group includes atypical cadherins Dachshous and Fat that are thought to contribute to the tissue patterning information across the axis. Second, members of the “core group,” including Frizzled and the seven-pass transmembrane cadherin Flamingo, assemble into asymmetric complexes that straddle the proximodistal junctions between adjacent cells; and they specify the intracellular location of the wing hair formation.

Unsolved questions include how the above 2 categories of regulators are functionally related to each other and why Frizzled is relocalized at distal cell borders in the first place. We previously proposed that cellular mechanisms underlying this relocalization include polarized transport of Frizzled-containing vesicles along P-D-oriented non-centrosomal microtubules (MTs). We have been analyzing dynamics of the MTs and movements of the vesicles to elucidate 2 critical questions: First, how do the MTs become aligned along the P-D axis? Second, why are the Frizzled vesicles transported distally? Our quantitative *in vivo* imaging has shown that Dachshous and Fat control alignment and asymmetry of the MT growth, and it has also revealed statistical properties of the vesicle movements, which will give us insight into the asymmetric relocalization of the core group.

**Education:**

1978-1982 Faculty of Science, Kyoto University (B.Sc.)
1982-1984 Graduate School of Science, Kyoto University (M.Sc.)
1984-1987 Graduate School of Science, Kyoto University (Ph.D.)

Research Positions:

1987-1989 Postdoctoral Fellow, University California, San Francisco
1989-1999 Assistant Professor, Kyoto University
1999-1999 Associate Professor, Kyoto University
1999- Professor, Kyoto University

Awards:

2006 JSPS (Japan Society for the Promotion of Science) Prize
2009 Inoue Prize for Science

Memo

Session IV.

(August 20, 2010, 10:50~12:20)

Stem Cell and Cancer

Targeting cancer stem cells: TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells

Koichi Akashi, Yoshikane Kikushige and Toshihiro Miyamoto

Department of Medicine and Biosystemic Sciences, Kyushu University Graduate School of Medicine, Japan

Malignant hematologic disorders originate from a small abnormal cell population with deregulated self-renewal capacity, impaired differentiation activity and/or reinforced cell survival. This “cancer stem cell” hypothesis is well supported in the field of hematologic disorders. We have succeeded in reconstituting some of human hematologic neoplasms by utilizing the NOD/SCID/IL2 γ ^{null} xenograft model. Acute myeloid leukemia (AML) originates from self-renewing leukemic stem cells (LSCs), an ultimate therapeutic target for permanent cure of patients. Based on differential transcriptome analysis of prospectively-purified LSCs from AML patient samples and normal hematopoietic stem cells (HSCs), we identified T-cell immunoglobulin mucin-3 (TIM-3) as a surface molecule expressed specifically on AML LSCs. In human hematopoiesis, TIM-3 is mainly expressed in mature monocytes and their progenitors, but not in normal HSCs. In AML, TIM-3 is expressed in the LSC population in all human AML types except for acute promyelocytic leukemia. We established an anti-human TIM-3 mouse IgG2a antibody with an antibody-dependent cellular cytotoxicity, and administered it to NOD/SCID/IL2 γ ^{null} immune-deficient mice transplanted with human AML cells. This treatment markedly reduced leukemic repopulation, but did not affect reconstitution of normal HSCs. In some mice transplanted with AML bone marrow, only normal hematopoiesis was reconstituted after anti-TIM-3 antibody treatment, suggesting that the antibody selectively killed LSCs, sparing residual normal HSCs. Thus, targeting the surface molecule to kill AML LSCs is effective to selectively block leukemic repopulation in vivo, and TIM-3 might be one of the ideal targets based on this therapeutic strategy.



Education:

1979-1985 Faculty of Medicine, Kyushu University (M.D.)

Research Positions:

1985-1987 Intern, Resident, Kyushu University

1987-1991 Research Fellow, Kyushu University (Ph.D.)

1993-1999 Postdoctoral Fellow, Stanford University School of Medicine

2000-2004 Assistant Professor, Dana-Farber Cancer Institute and Harvard Medical School

2005-2009 Associate Professor, Dana-Farber Cancer Institute and Harvard Medical School

2008- Professor and Chair, Kyushu University

Awards:

1990 Young Investigator Award, Japanese Society of Hematology

1998 International Grant of the Jose Carreras International Leukemia Foundation

2000 Cheryl Whitlock/Pathology Memorial Prize

2001 Claudia Adams Barr Investigator Award in Cancer Research

2002 Damon-Runyon Cancer Research Scholarship Award

2004 First JSPS (Japanese Society for Promotion of Science) Prize

Memo

Molecular functions of cyclins in mouse development

Peter Sicinski¹, Frederic Bienvenu¹, Siwanon Jirawatnotai¹, Joshua E. Elias², Clifford A. Meyer³

¹Department of Cancer Biology, and ³Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, and ²Department of Cell Biology and ¹Pathology, Harvard Medical School, Boston, USA

Cyclins and their associated cyclin-dependent kinases (CDKs) represent essential components of the core cell cycle machinery. Cyclin-CDK complexes phosphorylate cellular proteins, thereby driving cell cycle progression. Consistent with their growth-promoting roles, abnormally high levels of cyclins are seen in many human cancers. For example, cyclin D1 is overexpressed in many human tumor types, including the majority of breast cancers. The full repertoire of cyclin D1 functions in normal development and in oncogenesis is currently unclear. To address this issue, we developed FLAG- and HA-tagged cyclin D1 knock-in mouse strains. We reasoned that these tagged knock-in mice would allow us to use sequential immunoaffinity purifications with anti-FLAG and -HA antibodies, followed by repeated rounds of extensive, high-throughput mass spectrometry, to determine the full repertoire of cyclin D1-interacting proteins in different mouse organs under normal conditions, or during tumorigenesis.

We utilized these mice to search for cyclin D1-binding proteins in different mouse organs and in mammary carcinomas. In addition to cell cycle partners, we observed several proteins involved in transcription. Genome-wide location (ChIP-chip) analyses revealed that during mouse development cyclin D1 occupies promoters of abundantly expressed genes. In particular, we found that in developing mouse retinas – an organ that critically requires cyclin D1 function – cyclin D1 binds the upstream regulatory region of the Notch1 gene where it serves to recruit CBP histone acetyltransferase. Genetic ablation of cyclin D1 resulted in decreased CBP recruitment, decreased histone acetylation of the Notch1 promoter region, and led to decreased levels of the Notch transcript and protein in cyclin D1-null retinas. Transduction of an activated allele of Notch1 into cyclin D1^{-/-} retinas increased proliferation of retinal progenitor cells, indicating that upregulating Notch1 signaling alleviates the phenotype of cyclin D1-deficiency. These studies reveal that in addition to its well-established cell cycle roles, cyclin D1 plays an *in vivo* transcriptional function in mouse development. Our approach, which we term “genetic-proteomic” can be used to study the *in vivo* function of essentially any protein.

**Education:**

1979-1986 Faculty of Medicine, Warsaw Medical School (M.D.)

Research Positions:

1986-1990 Researcher and Academic Lecturer, Warsaw Medical School (Ph.D.)

1990-1997 Postdoctoral Fellow, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology

1997-2003 Assistant Professor, Harvard Medical School and Dana-Farber Cancer Institute

2003-2007 Associate Professor, Harvard Medical School and Dana-Farber Cancer Institute

2007- Professor, Harvard Medical School and Dana-Farber Cancer Institute

Awards:

1986-1988 Wellcome Trust Fellowship

1988 International Federation of Societies for Histochemistry & Cytochemistry (IFSHC) Award

1988 European Molecular Biology Organization (EMBO) Fellowship

1989 Federation of European Biochemical Societies (FEBS) Fellowship

1990-1992 Fogarty International Fellowship

1992-1993 Leukemia Research Foundation Fellowship

1993-1996 Special Fellow, Leukemia Society of America

1998 Barr New Investigator Award

2002 AACR-Gertrude B. Elion Cancer Research Award

2002 Abbott Bioresearch Center Award

2005 Scholar of the Leukemia & Lymphoma Society

2005 Foreign Member, Polish Academy of Sciences

Memo

Temporal regulation of neural stem cell fate in the developing mouse neocortex

Yusuke Hirabayashi, Shohei Furutachi, Daichi Kawaguchi and **Yukiko Gotoh**

Institute of Molecular and Cellular Biosciences, University of Tokyo

One of the fundamental questions in developmental biology is how multipotent progenitors/tissue stem cells produce various cell types in an organized manner for correct tissue formation. Neural stem cells (NSCs) attract much attention since these cells give rise to neuronal and glial cell types in a defined temporal order with striking precision. In this study, we have shown that polycomb group (PcG) complex-mediated epigenetic mechanism plays a pivotal role in driving switches of NSC fate during the neocortical development. We would like to propose that PcG proteins serve as a developmental timer in embryonic NSCs. I will also discuss the regulation of adult NSC fate.

**Education:**

- 1983-1987 Faculty of Science, University of Tokyo (B.Sc.)
1987-1989 Faculty of Science, University of Tokyo (M.Sc.)
1989-1992 Faculty of Science, University of Tokyo (Ph.D.)

Research Positions:

- 1992-1993 Post-doctoral Fellow, University of Tokyo
1993-1998 Research Associate/ Assistant Professor, Kyoto University
1996-1997 Visiting Scientist, Fred Hutchinson Cancer Research Center
1997-1999 Visiting Scientist, Children's Hospital/Harvard Medical School
1998-2005 Associate Professor, University of Tokyo
2005- Professor, University of Tokyo

Awards:

- 2003 Incitement Award of Mitsubishi Chemical Corp., the Molecular Biology Society of Japan
2004 Incitement Award, the Japanese Cancer Association
2009 JSPS (Japan Society for the Promotion of Science) Prize
2009 Japan Academy Medal
2009 Tsukahara Prize

Memo

Session V.

(August 20, 2010, 13:30~14:50)

Young Investigators

Protrudin regulates Rab11-dependent synaptic function via interaction with sphingolipid

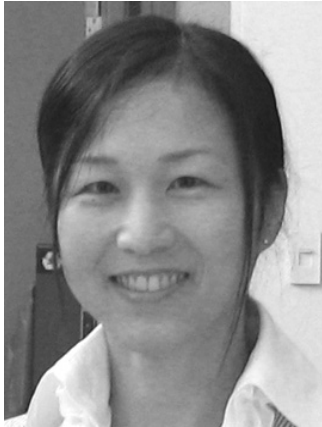
Michiko Shirane^{1,2}, and Keiichi I. Nakayama^{1,2}

¹Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Japan, ²CREST, Japan Science and Technology Agency (JST), Japan

Protrudin is a membrane protein that binds to a small GTPase Rab11 and regulates neuronal vesicular trafficking [Shirane & Nakayama, *Science* 314: 818 (2006)]. Protrudin contains two characteristic structures, a Rab11-binding domain and a FYVE domain. A typical FYVE domain is known to bind to phosphatidylinositol 3-phosphate [PI(3)P] and promote the fusion of the early endosomes, whereas the FYVE domain of protrudin was not associated with PI(3)P but specifically bound to a sphingolipid.

The protrudin-Rab11 complex promotes the anterograde vesicular trafficking to the growth cone, and this system is essential for neurite extension. A mutation in *ZFYVE27* gene that encodes human protrudin was found in the patients of autosomal dominant hereditary spastic paraplegia (AD-HSP), suggesting the role of protrudin in maintaining neuronal integrity. We generated mice deficient in protrudin, and have found that the mutant mice exhibited paraplegia as seen in human AD-HSP patients and in mice lacking the enzyme to synthesize the protrudin-binding sphingolipid.

Recently, Rab11 was also shown to promote the trafficking of AMPA receptor in the dendritic spines of post synapses, which is directly linked to higher brain functions such as learning and memory. We thus examined whether protrudin is required for the Rab11-dependent trafficking of AMPA receptor in the spine. Mice deficient in either protrudin or its ligand sphingolipid exhibited the defects in AMPA receptor transport at the dendritic spine. Furthermore, these mutant mice showed impairment in spine maturation and synaptic formation. Our results indicate that protrudin functions to tether Rab11-positive recycling endosomes to the plasma membrane via the interaction with the sphingolipid, resulting in promotion of membrane fusion. Protrudin thus facilitates the AMPA receptor trafficking in the dendritic spine at the post synapse, suggesting that protrudin possibly plays a critical role in cognitive functions of the brain.



Education:

1986-1990 Faculty of science, Osaka University (B.Sc.)
1999 Doctor of Pharmacy, Tokyo University

Research Positions:

1990-1997 Researcher, Nippon Roche Research Center (Ph.D.)
1997-2000 Post-doctoral Fellow, Kyushu University
2000-2003 Research Fellow (PD), Japan Society for the Promotion of Science
2003-2006 Research Fellow, Precursory Research for Embryonic Science and Technology
2004-2006 Assistant Professor, Kyushu University
2006- Associate Professor, Kyushu University

Awards:

2010 JSPS (Japan Society for the Promotion of Science) Prize

Memo

Olfactory map formation directed by the odorant receptor-derived cAMP signals

Takeshi Imai^{1,2,3}, and Hitoshi Sakano³

¹Laboratory for Sensory Circuit Formation, Center for Developmental Biology, RIKEN.

²Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo. ³PRESTO, Japan Science and Technology Agency (JST), Japan

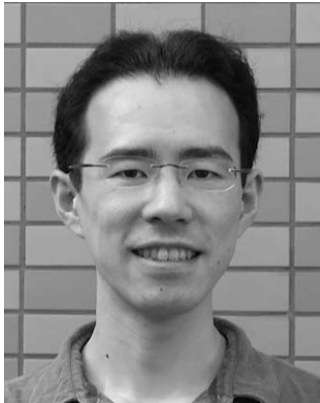
In the mouse olfactory system, odorant receptor (OR)-derived cAMP signals mediate not only the odor recognition, but also the axonal projection of olfactory sensory neurons. ORs control anterior-posterior (A-P) coarse targeting of axons through the transcriptional regulation of axon guidance molecules, Neuropilin-1 and Semaphorin 3A (Imai et al., 2006). Neuropilin-1 and Semaphorin 3A control pre-target axon sorting and thereby establishes the olfactory map topography (Imai et al., 2009). Refinement of the map is also OR-dependent, but is controlled by the extrinsic odorants. In contrast, it has been known that Golf, an essential component for odor detection, is dispensable for the coarse mapping. The nature of cAMP signals for the A-P coarse mapping has remained elusive.

Here we found that OR-instructed axonal projection is regulated by a distinct G-protein subunit, Gs. Unlike Golf, Gs is predominant in immature olfactory sensory neurons. In the Gs knockout mice, Neuropilin-1 expression was dramatically reduced, resulting in the distortion of the olfactory map along the A-P axis.

Curiously, expression level of Neuropilin-1 was not affected by naris occlusion, indicating that Gs-mediated cAMP signals are independent of extrinsic odorants. In an HEK293-derived cell line, each OR showed a unique and variable level of basal activity without odorants. Our results raise an intriguing possibility that the ligand-independent activities of ORs may determine the A-P targeting specificities of axons.

Reference

1. Imai et al., *Cold Spring Harb Perspect Biol.* (2010). doi: 10.1101/cshperspect.a001776
2. Imai et al., *Science* (2009) 325:585-590.
3. Imai et al., *Science* (2006) 314:657-661.



Education:

1997-2001 Faculty of Science, University of Tokyo (B.Sc.)
2001-2003 Graduate School of Science, University of Tokyo (M.Sc.)
2003-2006 Graduate School of Science, University of Tokyo. (Ph.D.)

Research Positions:

2006-2007 Post-doctoral Fellow, CREST program, Japan Science and
Technology Agency (JST)
2007-2008 Post-doctoral Fellow, University of Tokyo
2009-2009 Research Assistant Professor, University of Tokyo
2009- Investigator, PRESTO program, JST.
2010- Team Leader, RIKEN Center for Developmental Biology

Awards:

2007 Research Promotion Award, University of Tokyo
2007 GE & Science Prize for Young Life Scientists

Memo

Histone binary switch mediated by Chk1-HAT and PP1-HDAC regulates DNA damage induced transcriptional repression

Midori Shimada, Mayumi Haruta, Hiroyuki Niida and Makoto Nakanishi

Department of Cell Biology and Biochemistry, Graduate School of Medical Sciences, Nagoya City University, Japan

Eukaryotic cells are equipped with coordinated systems to contend with DNA damage, such as those which are used in cell cycle arrest, DNA repair and apoptosis, to maintain genomic integrity. These systems are regulated at least in part by transcriptional activation or repression. Although processes to activate transcription of specific genes have been characterized in the context of sequence-specific DNA binding factors, mechanisms of transcriptional repression have been largely unexplored. Recently, we found DNA damage-induced repression of cell cycle regulatory genes was correlated with reduction of histone H3-T11 phosphorylation (H3-pT11), which was mediated at least in part by Chk1 dissociation from chromatin. However, molecular mechanism(s) how this dephosphorylation triggers transcriptional repression remains elusive. Here, we identify an ‘acetyl/phospho’ cassette at H3-K9/T11 on E2F promoters, which is switched by replacement of Chk1-GCN5 complexes with protein phosphatase 1 γ (PP1 γ)-HDACs complexes scaffolded on Rb-family pocket proteins. PP1 γ activity toward T11 dephosphorylation is regulated by Cdk1-dependent phosphorylation of PP1 γ at T311 and interaction with NIPPI1. Under unperturbed condition, Chk1/GCN5 complex existed on E2F1 promoters. After DNA damage, PP1 γ /HDACs complex on pRb was recruited to E2F1, or E2F1 was replaced by E2F4 bound to PP1 γ /HDACs on p107 or p130, both of which ultimately lead to deacetylation/dephosphorylation at H3-K9/T11. Our results thus suggest that T11 phosphorylation may function as a phospho-acceptor targeted by ATR-Chk1 axis and accelerate K9 acetylation, which is reversed by the activation of PP1 γ and recruitment of HDACs bound to Rb-related proteins by Chk1-dependent inhibition of Cdks after DNA damage.

**Education:**

1994-1998 Faculty of Science, Osaka City University (B.Sc.)
1998-2000 Graduate School of Science, Osaka City University (M.Sc.)
2000-2003 Graduate School of Science, Osaka University (Ph.D.)

Research Positions:

2002-2003 Visiting Student, Sussex University
2003-2008 JSPS research fellow (PD), Post-doctoral Research Fellow,
Nagoya City University
2008-2009 Assistant professor, Nagoya City University
2009- Lecturer, Nagoya City University

Awards:

2003 Research Institute for Microbial Diseases Research Award,
Osaka University
2008 Award of Best Poster Prize, Gordon Research Conference

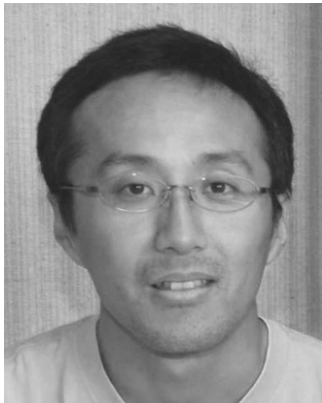
Memo

Mechanism of microtubule generation during cell division

Gohta Goshima, and Ryota Uehara

Division of Biological Science, Graduate School of Science, Nagoya University, Japan

The spindle, a bipolar structure composed of microtubules and associated proteins, ensures accurate chromosome segregation and cytokinesis. The centrosome is the main site of microtubule nucleation in the animal mitotic spindle. However, functional spindles can be formed without centrosomes in various animal cell types, suggesting that microtubules are also generated independent of centrosomes. Through a genome-wide RNAi screen and extensive follow-up experiments, we recently identified “augmin”, an 8-subunit protein complex that functions to increase spindle microtubule number within the spindle independent of centrosomes. Reduced spindle microtubule generation after augmin knockdown, particularly in the absence of functional centrosomes, has dramatic consequences on mitotic spindle formation and function, leading to reduced kinetochore fibre formation, chromosome misalignment, and spindle bipolarity defects. Furthermore, we provide evidence that augmin also plays a critical role in *de novo* central spindle microtubule generation during anaphase, which is required for completion of cytokinesis in human cells. A new model on spindle microtubule generation will be discussed.



Education:

1993-1997 Faculty of Science, Kyoto University (B.Sc.)
1997-1999 Graduate School of Science, Kyoto University (M.Sc.)
1999-2002 Graduate School of Science, Kyoto University (Ph.D.)

Research Positions:

2002-2002 Postdoctoral Scholar, Kyoto University
2002-2007 Postdoctoral Scholar, University of California, San Francisco
2007-2010 Designated Associate Professor, Nagoya University
2010- Professor, Nagoya University

Awards:

2008 Career Development Award, Human Frontier Science Program (HFSP)
2010 Science Research Award, Inoue Foundation

Memo

Session VI.

(August 20, 2010, 15:10~16:40)

Transcriptional Control and Epigenetics

Identification and characterization of enzymes involved in DNA demethylation

Yi Zhang

Howard Hughes Medical Institute, and Department of Biochemistry and Biophysics,
University of North Carolina, USA

Epigenetic modifications play important roles in diverse biological processes that range from regulation of gene expression, embryonic development, stem cell reprogramming, and human diseases such as cancers. One of the epigenetic modifications is DNA methylation. Although enzymes responsible for DNA methylation have been well characterized, enzymes that responsible for active DNA demethylation in mammalian cells have remained a controversial topic. Using two different approaches, we have identified and characterized two different classes of enzymes that have the potential to catalyze DNA demethylation. In my talk, I will discuss our recent progress in characterizing these two families of enzymes.

**Education:**

- 1980-1984 College of Biological Sciences, China Agricultural University (B.Sc.)
1984-1987 College of Biological Sciences, China Agricultural University (M.Sc.)
1989-1995 The Institute of Molecular Biophysics, Florida State University (Ph.D.)

Research Positions:

- 1995-1999 Post-doctoral Fellowship, Howard Hughes Medical Institute and UMDNJ-Robert Wood Johnson Medical School
1999-2004 Assistant Professor, University of North Carolina at Chapel Hill
2004-2005 Associate Professor, University of North Carolina at Chapel Hill
2005- Professor, University of North Carolina at Chapel Hill
2005- Investigator, Howard Hughes Medical Institute
2009- Kenan Distinguished Professor, University of North Carolina at Chapel Hill

Awards:

- 2000 V. Scholar Award, V Foundation for Cancer Research
2001 Kimmel Scholar Award, Sidney Kimmel Foundation for Cancer Research
2003 Gertrude B. Elion Cancer Research Award, American Association for Cancer Research
2004 Ruth and Phillip Hettleman Prize for Artistic and Scholarly Achievement, University of North Carolina
2005 Investigator, Howard Hughes Medical Institute
2008 Top 10 authors of high-impact papers by ScienceWatch
2008 The Battle Distinguished Cancer Research Award, University of North Carolina

Memo

Regulation and biological function of histone lysine methylation

Yoichi Shinkai

Laboratory of Mouse Model, Experimental Research Center for Infectious Diseases, Institute for Virus Research, Kyoto University, Japan

Histone posttranslational modifications play crucial roles in epigenetic regulation of chromatin structure and function. Epigenetics-oriented approach is highly expected for elucidation and control of human diseases. To understand biological roles of histone lysine methylation, we have studied regulation and function of one of histone lysine methyl-marks, methylation of histone H3 lysine 9 (H3K9) as a model system. Our studies and others revealed that proper control of H3K9 methylation is crucial for diverse biological processes including embryogenesis, germ cell development, cellular metabolism, neural plasticity and behavior and immunological responses. To validate H3K9 methyl-modifying enzymes as novel drug targets, *in vivo* genetic validation approach is an important next step. Furthermore, elucidation of targeting mechanism(s) of different H3K9 methyl-modifying enzymes is crucial since target (not substrate) specificities of them are not determined intrinsically in general. In my talk, G9a/GLP and ESET (which are both H3K9 methyltransferase) as model enzymes, I'd like to describe how their target specificities are controlled.

**Education:**

- 1980-1984 Faculty of Science, Yamagata University (B.Sc.)
1984-1986 Graduate School of Medicine, Tsukuba University (M.Sc.)
1986-1990 Graduate School of Medicine, Juntendo University (Ph.D.)

Research Positions:

- 1990-1991 Research Associate, Howard Hughes Medical Institute;
Fellow, Columbia University
1991-1995 Research Associate, Howard Hughes Medical Institute,
Harvard Medical School
1995-1998 Senior Scientist, Nippon Roche Research Center
1998-2003 Associate Professor, Kyoto University
2003- Professor, Kyoto University
2005-2007 Director of Research Center for Infectious Diseases, Kyoto
University
2010- Vice Director of Institute for Virus Research, Kyoto
University

Awards:

- 1993-1995 Arthritis Investigator Award
1993-1995 Leukemia Special Fellow

Memo

Small RNAs, retrotransposons and genomic imprinting in the mammalian germline

Hiroyuki Sasaki^{1,2}, and Toshiaki Watanabe^{2,3}

¹Division of Epigenomics, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Japan, ²Division of Human Genetics, Department of Integrated Genetics, National Institute of Genetics, Research Organization of Information and Systems, Japan, ³Present address: Yale Stem Cell Center, Yale University School of Medicine, USA

During mammalian male germ cell development, retrotransposons and paternally methylated imprinted genes are *de novo* DNA methylated. This occurs in the prospermatogonium stage and is important for the maintenance of the integrity of the sperm genome and for the parent-specific monoallelic expression of the imprinted genes in the embryo. The *de novo* DNA methyltransferases responsible for this process have been identified, but the mechanisms underlying the sequence-specific methylation is not fully understood. In fission yeast and plants, small interfering RNAs are used as a guide to recruit the modification enzymes to specific target regions. We are therefore interested in looking at the role of small RNAs in regulation of *de novo* DNA methylation in mammalian germ cells. We found that the differentially methylated region (DMR) of the imprinted mouse *Rasgrfl* locus loses methylation in spermatogonia deficient for components of the Piwi-interacting RNA (piRNA) pathway. A retrotransposon sequence in a novel noncoding RNA spanning the DMR was targeted by piRNAs generated from a different locus. Furthermore, a direct repeat known to be required for methylation of the DMR served as the promoter for this noncoding RNA. We therefore propose a model in which piRNAs and its target RNA play a critical role in *de novo* DNA methylation and imprinting of *Rasgrfl*.

**Education:**

- 1976-1982 Faculty of Medicine, Kyushu University (M.D.)
- 1982-1983 Medical Resident, Faculty of Medicine, Kyushu University
- 1983-1987 Graduate School of Medicine, Kyushu University (Ph.D.)

Research Positions:

- 1987-1990 Assistant Professor, Kyushu University
- 1990-1993 Post-doctoral Fellow, AFRC Institute of Animal Physiology and Genetics Research & Wellcome/CRC Institute
- 1993-1998 Associate Professor, Kyushu University
- 1998-2010 Professor, National Institute of Genetics
- 2010- Professor, Kyushu University
- 2010- Distinguished Professor, Kyushu University

Awards:

- 1997 The JSHG (Japan Society of Human Genetics) Encouragement Award
- 2009 The JSHG (Japan Society of Human Genetics) Award

Memo
