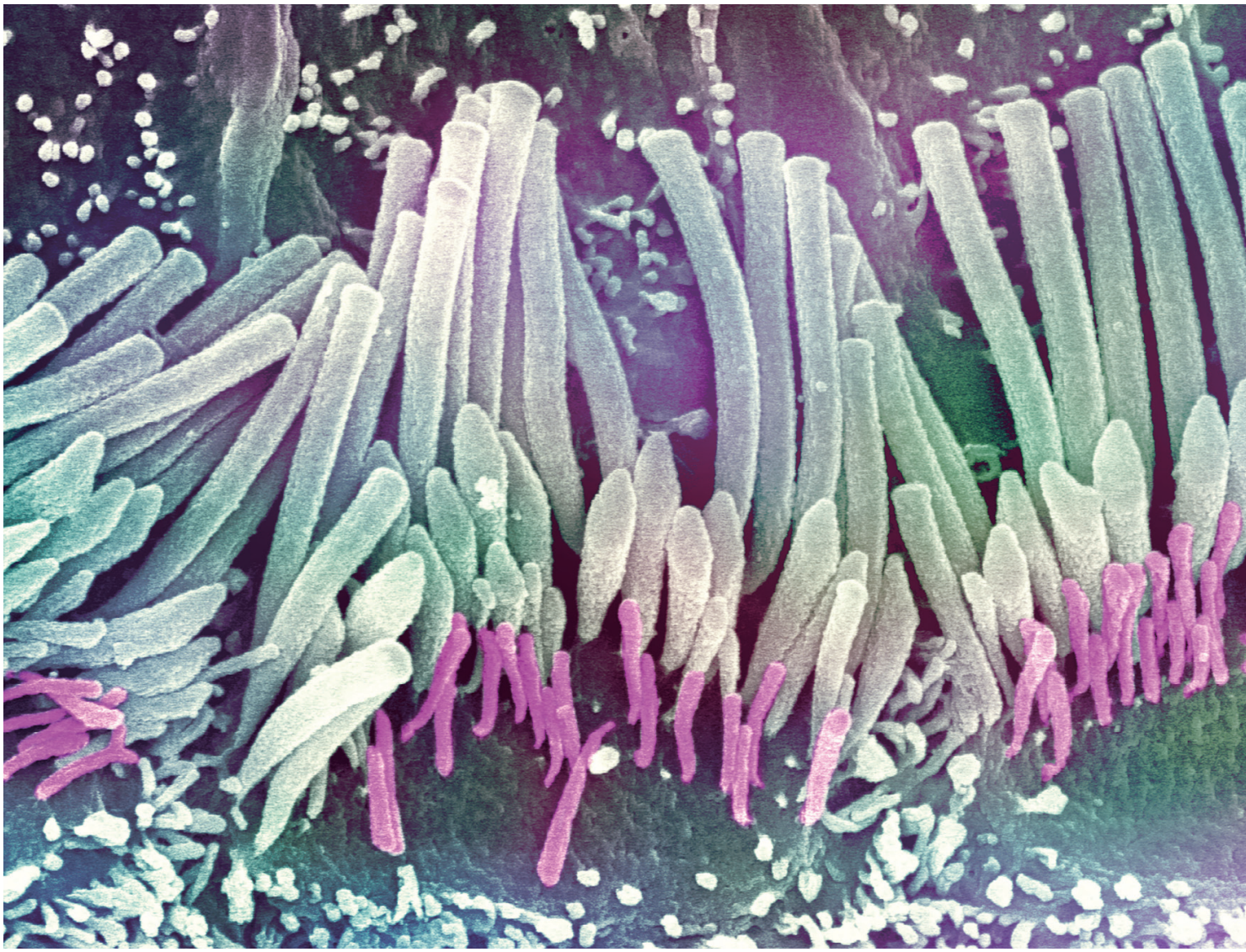
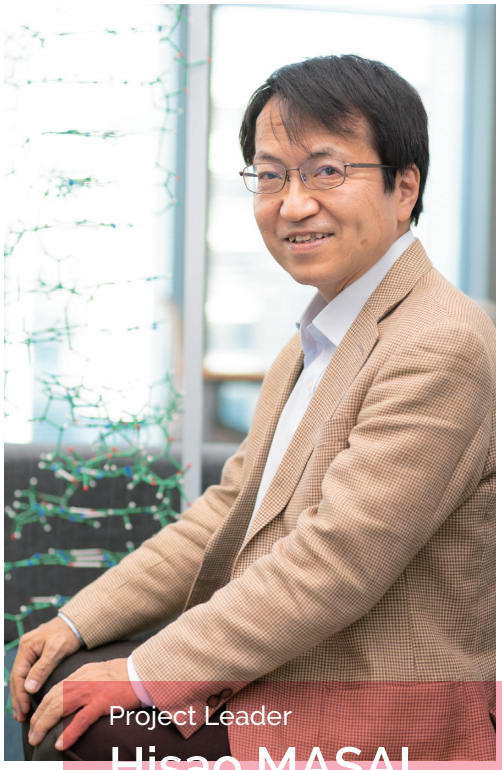


Mouse cochlear inner hair cells, conventional sensory receptors that transmit most of the acoustic information to the brain. Red cilia represent those lost during aging.

Basic Medical Sciences



Project Leader
Hisao MASAI

Hisao Masai is the director of TMIMS and the head of the Genome Dynamics Project. After graduating from the University of Tokyo in 1981, he worked as a graduate student under the supervision of Dr. Ken-ichi Arai at DNAX Research Institute in Palo Alto, California, USA, and received his Ph.D. in 1987 from the University of Tokyo. He has spent his career studying how genetic information is duplicated and inherited, and what happens when these processes fail. His current interests include understanding diversified modes of DNA replication, how failure to respond to replication stress leads to cancerous growth, and the roles of unusual nucleic acid structures, including G-quadruplexes and RNA-DNA hybrids, in shaping chromosomes, copying and reading genetic information, and in causing detrimental diseases.

Genome Dynamics

Laboratory HP: https://www.igakuken.or.jp/genome/

Staff

Researchers
Zhiying YOU
Kenji MORIYAMA
Taku TANAKA
Yutaka KANOH
Tomohiro IGUCHI
Yoichi TAJIMA

Postdoctoral fellows
Sayuri ITO
Chi-Chun YANG

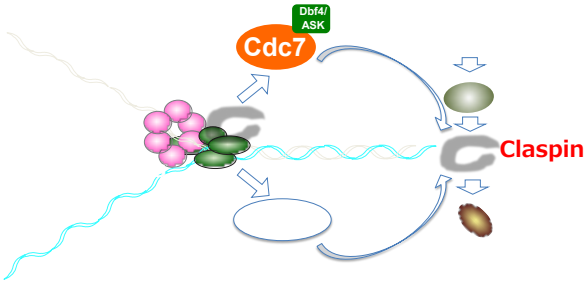
Research Assistants
Naoko KAKUSHO
Rino FUKATSU
Akiko MINAGAWA

Students
Karin HORI
Shunsuke KOBAYASHI
Tomoko SAGI
Hao-Wen HSIAO
Kaho TAKASAWA
Naoya INOUE
Hikari MIYAMOTO
Shoha KINOSHITA
Trinh Thi To NGO

Research Summary

Our goal is to understand the molecular mechanisms responsible for faithful inheritance of genetic materials and stable maintenance of the genome. To achieve this, we are studying various aspects of chromosome dynamics with particular emphasis on regulation of DNA replication during S-phase in E. coli, fission yeast, and mammalian cells. We work to elucidate how chromosomes replicate and how the inheritance of replicated chromosomes is regulated to enable stable maintenance of the genome through generations. Answers to these questions will shed light on how defects in these processes contribute to the development of diseases, including cancers, and to senescence. They will also help to identify novel target proteins for cancer therapies. We are addressing the following questions.

1) How are the timing and location of DNA replication determined, and how are these coordinated with other chromosomal processes?
2) What are the biological functions of G-quadruplex structures, particularly in regulating DNA replication?
3) How are cellular responses to replication stress regulated, and how are these responses related to other cellular stress response pathways?
4) What are the roles of replication factors in development of individual organs and tissues, and how are these replication systems diversified to regulate development of different parts of our bodies?



Different mechanisms of replication stress responses in cancerous and non-cancerous cells. In cancer cells, Cdc7 is primarily responsible for phosphorylation of Claspin, a mediator of replication checkpoint, whereas in non-cancer cells, casein kinase 1γ1 is the primary kinase. This differential mechanism can be exploited to develop a strategy for cancer cell-specific cell killing by targeting Cdc7 kinase.

Selected Publications

Masai H, et al. (2020) "Detection of cellular G-quadruplex by using a loop structure as a structural determinant." ***Biochemical and Biophysical Research Communications,*** 531, 75-83.

Yang C-C, et al. (2019) "Cdc7 activates replication checkpoint by phosphorylating the Chk1 binding domain of Claspin in human cells." ***E-life***. 8. pii: e50796

Kobayashi S, et al. (2019) "Both a unique motif at the C terminus and N-terminal HEAT repeat contribute to G4 binding and origin regulation by Rif1 protein." ***Mol Cell. Biol.*** 39(4). pii: e00364-18

You Z and Masai H (2017) "Potent DNA strand annealing activity associated with mouse Mcm2-7 heterohexamer complex." ***Nucleic Acids Res.*** 45, 6495-6506.

Yang C-C, et al. (2016) "Claspin recruits Cdc7 kinase for initiation of DNA replication in human cells." ***Nature Communications*** 7:12135

Kanoh Y, et al. (2015) "Rif1 binds to G-quadruplexes and suppresses replication over long distances." ***Nature Struct. Mol. Biol.*** 22, 889-897.

Project Leader
Yoshiaki KIKKAWA

Yoshiaki Kikkawa has been leading the Deafness Project since 2020. Dr. Kikkawa completed his Ph.D. on animal genetics and evolution in 1998 from the Tokyo University of Agriculture. He then worked in mouse genetics and genomics under the supervision of Dr. Hiromichi Yonekawa at TMIMS where he identified key genes involved in several diseases by positional cloning. In particular, he focused on using mouse models to elucidate the molecular basis for genetic deafness, and identified *Sans*, one of the few genes identified to date that are associated with human deafness. Subsequently he conducted research on protein-protein interactions associated with deafness with Prof. Steve Brown at the MRC, Harwell, UK, where he discovered protein complexes associated with stereocilia elongation in hair cells in the inner ear.

Deafness

Laboratory HP: https://www.igakuken.or.jp/mammal/english/index.html

Staff

Researchers
Kunie MATSUOKA
Shumpei YASUDA
Yuta SEKI

Research Assistants
Takafumi OUCHI

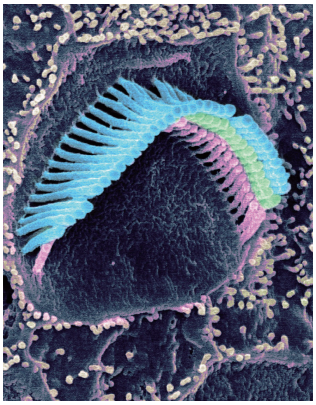
Students
Ikuo MIURA
Xuehan HOU
Yuichi KOSHIISHI

Research Summary

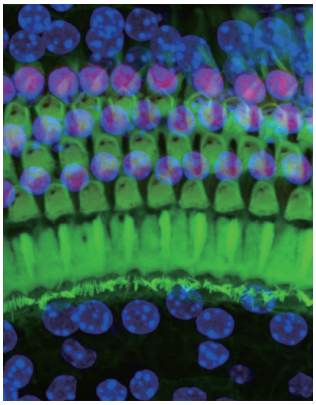
Hearing loss is a very common sensory disorder that severely affects human quality of life. In order to develop effective therapeutic strategies for deafness, it is critical to understand the mechanisms regulating its onset. Our aim is to discover novel genes associated with deafness. In particular, we are focused on identifying genes responsible for age-related hearing loss (ARHL). While genes responsible for congenital hearing loss have been identified, genes associated with ARHL, which affects a far greater number of people, have not.

Many types of hearing loss are associated with loss of outer hair cells (OHCs), which are responsible for the amplification of sound. Thus, we study the development and maintenance of OHCs. OHCs form a characteristic V-shaped stereocilia architecture. However, the genetic and molecular mechanisms involved in OHC development and death are poorly understood. To better understand OHCs and ARHL, we are:

1) Identifying genes causing and modifying ARHL in mouse models using forward genetics approaches.
2) Functionally analyzing proteins involved in the development of the OHC V-shaped stereocilia architecture.
3) Investigating the molecular mechanisms involved in OHC deaths using an OHC-specific depletion system.



The V-shaped stereocilia architecture of OHCs in 1-month-old mice. Stereocilia bundles are arranged in rows (blue, green, and magenta) of increasing height and form a staircase-shaped configuration.



OHC-specific expression of oncomodulin. Ocomodulin signals (red) were specifically labeled in the nuclei of OHCs. Green and blue signals indicate phalloidin and DAPI staining.

Selected Publications

Yasuda SP et al. (2020) "c.753A>G genome editing of a *Cdh23*^{ahl} allele delays age-related hearing loss and degeneration of cochlear hair cells in C57BL/6J mice." ***Hear. Res.*** 389: 107926.

Matsuoka K et al. (2019) "OHC-TRECK: A novel system using a mouse model for investigation of the molecular mechanisms associated with outer hair cell death in the inner ear." ***Sci. Rep.*** 9:5285.

Yasuda SP, et al. (2018) "Effects of genetic background on susceptibility and the acceleration of hearing loss in mice." ***An Excursus into Hearing Loss*** 3–23.

Seki Y, et al. (2017) "A novel splice site mutation of myosin VI in mice leads to stereociliary fusion caused by disruption of actin networks in the apical region of inner ear hair cells." ***PLoS One*** 12, e0183477.

Miyasaka Y, et al. (2016) "Heterozygous mutation of *Ush1g/Sans* in mice causes early-onset progressive hearing loss, which is recovered by reconstituting the strain-specific mutation in *Cdh23*." ***Hum. Mol. Genet.*** 25: 2045-2059.

Kikkawa Y and Miyasaka Y. (2016) "Genetic modifiers of hearing loss in mice: The case of phenotypic modification in homozygous *Cdh23*^{ahl} age-related hearing loss." ***Monogr. Hum.*** Genet. 20: 97–109.

Project Leader
Yasuko ONO

Calpain

Laboratory HP: https://www.igakuken.or.jp/calpain/indexEnglish.html

Yasuko Ono has been the leader of the Calpain Project since 2018. As a graduate student she studied the roles of calpains, a family of intracellular cysteine proteases, in muscle functions, and received her Ph.D in 1999 from the University of Tokyo, Graduate School of Science. She then studied mechanisms of sarcomere assembly as a postdoctoral fellow at the University of Arizona. Her current research includes studying the physiological impact of calpain-mediated proteolysis on cellular functions and understanding mechanisms of calpain regulation.

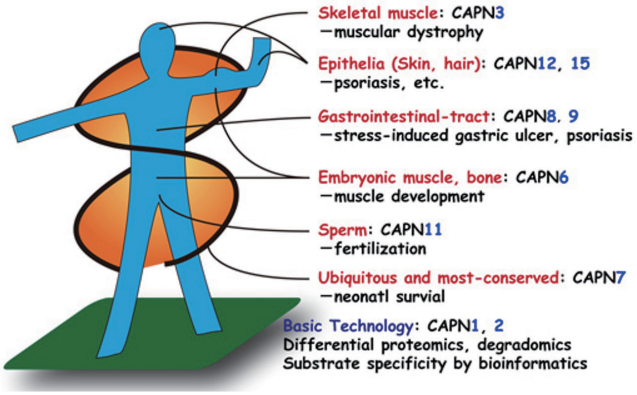
Staff

Researchers
Shoji HATA
Atsushi IRIE
Fumiko SHINKAI-OUCHI
Aya NOGUCHI

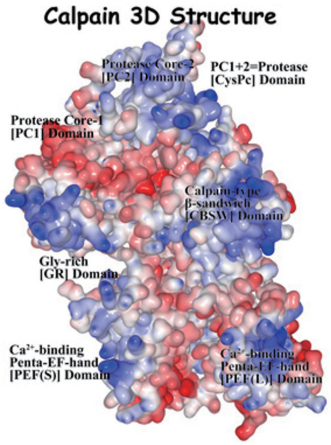
Research Assistants
Naoko DOI

Research Summary

Proteins are chains of amino acids, and their functions change when they are cut or partially cut. Calpains are proteolytic enzymes that perform such cuts or limited proteolytic processing in cooperation with calcium. Humans have 15 calpain species. Defects of these species cause various deficiencies, such as muscular dystrophy, stomach ulcers, and embryonic lethality.



In this project, we aim to understand the biology of calpains, and translate this knowledge into improvements in health.



Selected Publications

Shinkai-Ouchi F, et al. (2020) "Calpain-2 participates in the process of calpain-1 inactivation." ***Biosci. Rep.***, 40: BSR20200552.

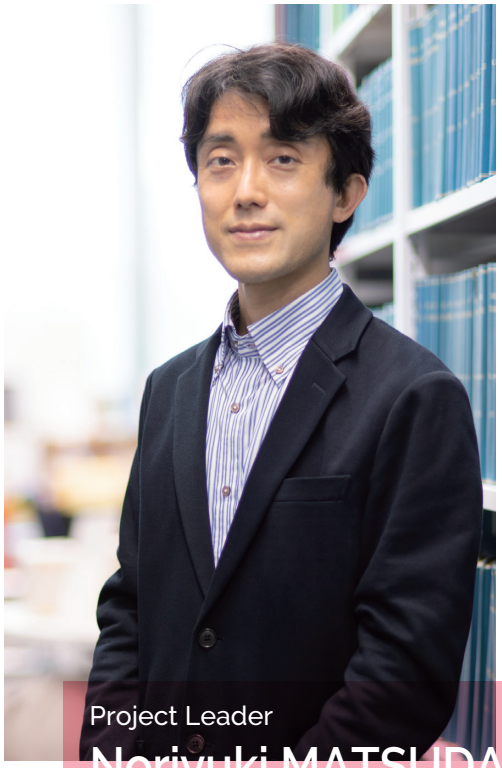
Hata S, et al. (2020) "A muscle-specific calpain, CAPN3, forms a homotrimer." ***Biochim. Biophys. Acta, Proteins Proteomics***, 7: 140411.

Hata S, et al. (2016) "A gastrointestinal calpain complex, G-calpain, is a heterodimer of Capn8 and Capn9 calpain isoforms, which play catalytic and regulatory roles, respectively." ***J. Biol. Chem.***, 291: 27313-27322.

Ono Y, et al. (2016) "Calpain research for drug discovery: challenges and potential." ***Nature Reviews: Drug Discovery***,15: 854-876.

Shinkai-Ouchi F, et al. (2016) "Predictions of cleavability of calpain proteolysis by quantitative structure-activity relationship analysis using newly determined cleavage sites and catalytic efficiencies of an oligopeptide array." ***Mol. Cell. Proteomics***, 15: 1262-1280.

Ono Y, et al. (2014) "The N- and C-terminal autolytic fragments of CAPN3/p94/calpain-3 restore proteolytic activity by intermolecular complementation." ***Proc. Natl. Acad. Sci. USA***, 111: E5527-5536.



Project Leader
Noriyuki MATSUDA

Noriyuki Matsuda has been the leader of the Ubiquitin Project since 2015. He received his Ph.D in 2001 from the University of Tokyo Graduate School of Science for identification of the membrane-bound RING finger-type ubiquitin ligase, Rma1/Rnf5, from *H. Sapiens* and *A. thaliana* (Matsuda, *J. Cell. Sci.* 2001). He then worked as a postdoctoral fellow studying mechanisms and functions of ubiquitylation under the supervision of Dr. Keiji Tanaka at the Tokyo Metropolitan Institute of Medical Science. His current interests are to understand how ubiquitin is conjugated on damaged mitochondria, how these mitochondria are degraded in a mitochondria-specific autophagic process known as mitophagy, and how mitophagy prevents detrimental diseases such as Parkinson's disease.

Ubiquitin

Laboratory HP: https://www.igakuken.or.jp/english/project/detail/ubiquitin.html

Staff

Researchers
Yukiko YOSHIDA
Koji YAMANO
Fumika KOYANO

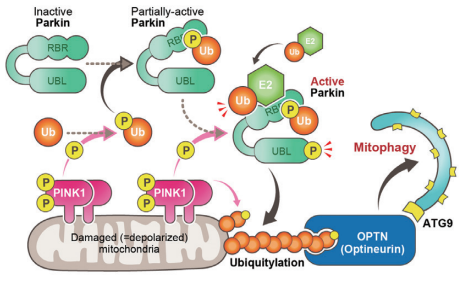
Postdoctoral fellows
Waka KOJIMA

Students
Anni HUO
Chisato UDAGAWA
Rhota HAYASHIDA

Research Summary

Parkinson's disease (PD) is a common movement disorder characterized by loss of dopaminergic neurons. The majority of PD cases are sporadic, however, the discovery of genes linked to hereditary forms has provided important insights into molecular mechanisms associated with PD pathology. For example, functional analysis of recessive familial PD-related genes has identified a link between PD and mitochondrial quality control. However, the molecular mechanisms underlying this relationship have been obscure.

We focused on two genes associated with hereditary recessive PD, PINK1 and PARKIN. PINK1 encodes a Ser/Thr kinase and PARKIN encodes a RING-IBR protein. We found that when the mitochondrial membrane potential decreases, a sign of mitochondrial damage, PINK1 phosphorylates ubiquitin at Ser65. Phosphorylated ubiquitin activates the ubiquitin ligase (E3) function of Parkin (Koyano *Nature* 2014). Moreover, ubiquitin chains phosphorylated by PINK1 function as Parkin receptors and recruit Parkin to damaged mitochondria (Okatsu *J.Cell.Biol.* 2015). Consequently, the trio of PINK1, Parkin, and phospho-ubiquitin induced rapid ubiquitination of mitochondrial outer membrane proteins. Since a bewildering array of substrates are ubiquitinated by Parkin during this process, Parkin substrate specificity remained poorly understood. We found, using artificial mitochondria-targeted proteins, that substrate specificity of Parkin is not determined by specific amino acid sequences but instead by mitochondrial localization (Koyano *J.Biol.Chem.* 2019). Ubiquitin is well-known for directing proteins for degradation. However, increasing evidence indicates that ubiquitination is also involved in quality control of larger structures including organelles, by tagging and directing damaged organelles for autophagic degradation. We found that ubiquitin chains on depolarized mitochondria are recognized by OPTN, an adaptor protein that recruits ATG9, a downstream autophagic protein, to damaged mitochondria (Yamano *J.Cell.Biol.* 2020). Impairment of this process prevents mitochondrial degradation and induces a predisposition to familial PD. Our work identifies a mechanism for PD pathology.



Schematic model for how PINK1, Parkin, and ubiquitin cooperate in the degradation of damaged mitochondria.

Selected Publications

Yamano K, et al.(2020) "Critical role of mitochondrial ubiquitination and the OPTN-ATG9A axis in mitophagy." ***J. Cell Biology*** 219: e201912144.

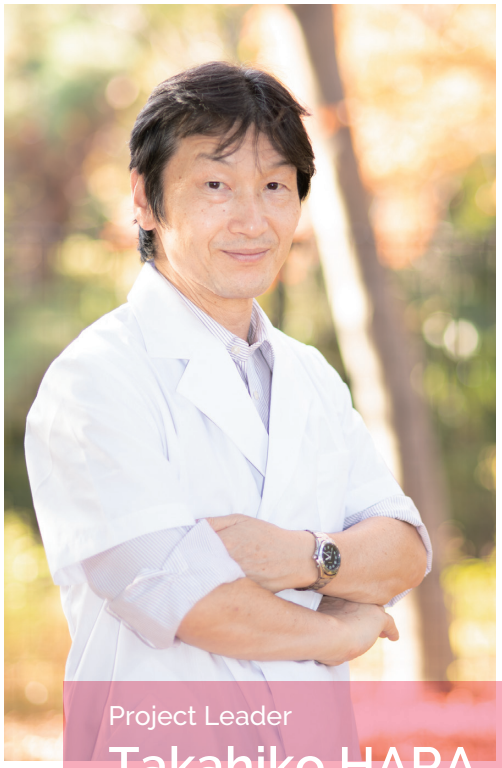
Koyano F, et al.(2019) "Parkin recruitment to impaired mitochondria for nonselective ubiquitylation is facilitated by MITOL." ***J Biol Chem*** 294: 10300-10314.

Koyano F, et al.(2019) "Parkin-mediated ubiquitylation redistributes MITOL/March5 from mitochondria to peroxisomes." ***EMBO Rep***. 20: e47728.

Yamano K, et al.(2018) "Endosomal Rab cycles regulate Parkin-mediated mitophagy." ***eLife*** 7: e31326

Okatsu K, et al.(2015) "Phosphorylated ubiquitin chain is the genuine Parkin receptor." ***J. Cell Biology*** 209: 111-128

Koyano F, et al.(2014) "Ubiquitin is phosphorylated by PINK1 to activate Parkin." ***Nature*** 510: 162-166.



Project Leader
Takahiko HARA

Takahiko Hara, the department chief of the Institute since April of 2018, has been the leader of the Stem Cell Project since 2005. After receiving Ph.D from the Graduate School of Science, Univ. of Tokyo in 1990, he conducted researches at DNAX Research Institute in Palo Alto, California, USA, as a postdoctoral fellow under the supervision of Dr. Atsushi Miyajima. He molecularly cloned a cDNA encoding mouse IL-3 receptor alpha subunit. Next, he developed *ex vivo* culture system of hematopoietic stem cells (HSCs) in the aorta-gonad-mesonephros region of mouse embryo. Since then, molecular mechanism of HSC development has been his major research interest. In the mean while, he started to investigated regulators of spermatogonial stem cells and muscle regeneration factors. Subsequently, he focused on a RNA helicase DDX1 and a CXC-type chemokine CXCL14, as they are involved in tumorigenesis and anti-tumor immunity, respectively.

Stem Cell

Laboratory HP: https://www.igakuken.or.jp/english/project/detail/stem-cell.html

Staff

Researchers
Kenji KITAJIMA
Kosuke TANEGASHIMA
Teruhiko SUZUKI
Masatoshi MURAOKA

Research Assistants
Tsuruyo TANIGUCHI

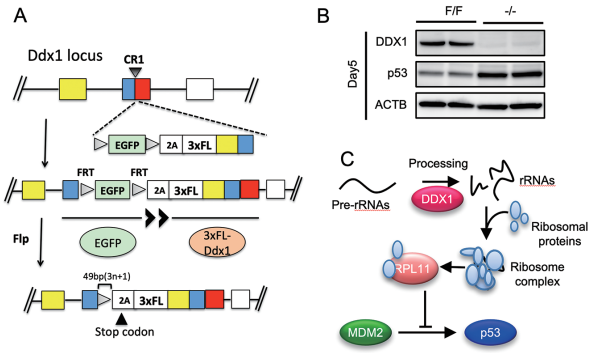
Students
Miho NAKAGAWA
Chihiro NAKATA
Miyu TANIKAWA
Kaho ISHIGE
Mako HAMASAKI
Satoko TAKAGI
Shota HOYANO
Shoma YAMAGUCHI
Risa SAITO

Research Summary

Dr. Yamanaka's inducible pluripotent stem cell (iPSC) technology has opened a new avenue to overcome incurable diseases by cell transplantation. In 2011, we discovered that overexpression of Lhx2 (transcription factor) in hemogenic mesodermal cells resulted in ex *vivo* expansion of transplantable HSCs from mouse embryonic stem cells (ESCs) and iPSCs. Since then, we have been making efforts for applying this method to human iPSCs. We believe that comparison of the *in vitro* differentiation capacity of hematopoietic cells between mouse and human iPSCs will uncover novel and fundamental aspects of human HSC development.

We discovered that CXCL14 is one of the causative factors for obesity-associated diabetes. In contrast, CXCL14 is known to possess tumor-suppressive activity against lung and oral carcinomas. In 2017, we found that CXCL14 carries CpG DNA into dendritic cells. This causes activation of the TLR9 signaling pathway, which is effective in immune-suppression of cancers. We are vigorously investigating physiological roles of CXCL14 and its action mechanisms. CXCL14 is a promising tool for developing novel anti-cancer and anti-diabetes drugs.

The presence of cancer stem cells has been proposed in various types of human cancer. Presumably, both tissue and cancer stem cells commonly express critical transcriptional regulators and signal transducers. We have identified DDX1 (RNA helicase) and PTPN23 (tyrosine phosphatase) as essential molecules for the onset of testicular tumors. In 2020, we discovered that DDX1 is essential for ribosome RNA metabolism in ESCs and cancer cells. In the absence of DDX1, these cells stop proliferation and undergo apoptosis by p53 activation (Figure).



Conditional knockout system of ES cells uncovered a novel role of DDX1 in ribosome RNA processing which is linked to p53-mediated cell growth control.

Selected Publications

Suzuki T, et al. (2020) "A novel all-in-one conditional knockout system uncovered an essential role of DDX1 in ribosomal RNA processing." ***Nucl. Acid Res.,*** (in press.)

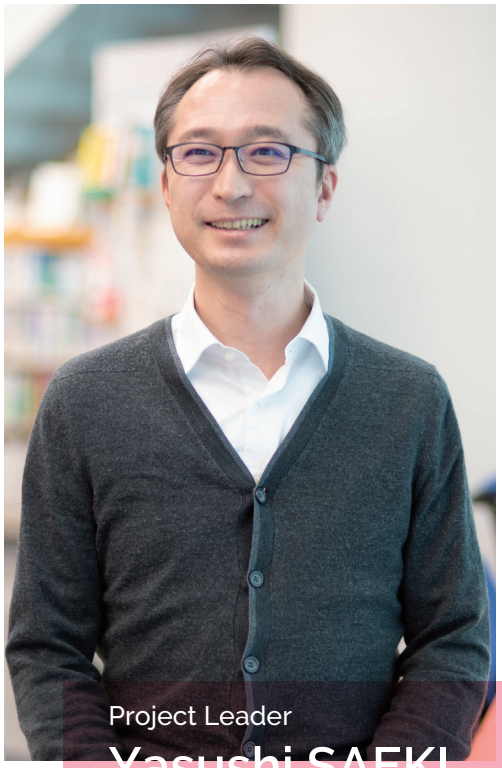
Sato K, et al. (2020) "Nitric oxide and a conditioned medium affect the hematopoietic development in a microfluidic mouse embryonic stem cell/OP9 co-cultivation system." ***Micromachines,*** 11: 305.

Nakajima M, et al. (2019) "In vitro differentiation of mouse T cell-derived hybrid cells obtained through cell fusion with embryonic stem cells." ***Biochem. Biophys. Res. Commun.*** 513: 701-707.

Kitajima K, et al. (2018) "Domain-specific biological functions of the transcription factor Gata2 on hematopoietic differentiation of mouse embryonic stem cells." ***Genes Cells*** 23: 753-766.

Tanegashima K, et al. (2017) "CXCL14 acts as a specific carrier of CpG DNA into dendritic cells and activates Toll-like receptor 9-mediated adaptive immunity." ***EBioMed.*** 24: 247-256.

Tanegashima K, et al. (2017) "Epigenetic regulation of the glucose transporter gene Slc2a1 by *β*-hydroxybutyrate underlies preferential glucose supply to the brain of fasted mice." ***Genes Cells*** 22: 71-83.



Project Leader
Yasushi SAEKI

Yasushi Saeki has been the leader of the Protein Metabolism Project since 2019. He received his Ph.D. in 2003 from the Graduate School of Pharmaceutical Sciences, Hokkaido University. After working as a JSPS research fellow at the Univ. of Tokyo, he joined the laboratory of Dr. Keiji Tanaka in 2007. He has been studying the ubiquitin-proteasome system and has identified the last proteasome subunit, multiple proteasome-specific chaperones, and key regulators for proteasomal degradation. He has also developed methods for analyzing proteasome activity and ubiquitin chain topology. Since 2018, he has also led the Grantin-Aid Scientific Research on Innovative Area 'New frontier for ubiquitin biology driven by chemo-technologies' and works to promote collaborative research on ubiquitin in Japan.

Protein Metabolism

Laboratory HP: https://www.igakuken.or.jp/pro-meta/eng/

Staff

Researchers
Akinori ENDO
Hikaru TSUCHIYA
Takuya TOMITA
Emeritus Researcher
Hiromichi YONEKAWA
Visiting Scientists
Fumiaki OHTAKE
Sayaka YASUDA

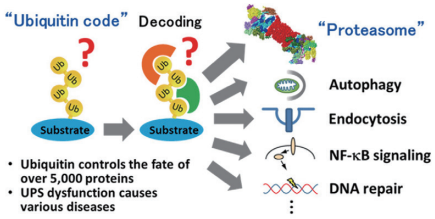
Supervisor
Keiji TANAKA
Research Assistants
Naoko ARAI
Sayaka ONO
Yasuko KAWASE
Harumi SETO
Kyoko UEDA

Students
Shota KOTANI
Miho SAKUMA
Kenta KISAI

Research Summary

The ubiquitin-proteasome system (UPS) is a crucial protein degradation system that affects almost all cellular functions in eukaryotic cells. Since protein homeostasis is essential to human health, malfunctions of the UPS cause various diseases including cancers, inflammation, and neurodegeneration. Thus, UPS regulators are attracting attention as drug discovery targets. However, there is still much unknown about the UPS. Our goal is to elucidate the fundamental mechanisms of ubiquitin signaling and proteasomal degradation and to integrate this information into pathophysiology to develop therapeutic strategies for UPS-related diseases. To this end, we are currently focusing on the following research projects.

1) Deciphering the ubiquitin code: The structural diversity of ubiquitin chains with distinct topologies, called the 'ubiquitin code,' regulates the diverse functions of ubiquitin. We have shown that the branching and length of ubiquitin chains provide additional specificity to this code (Mol Cell 2016, Nat Commun 2018). To further investigate the ubiquitin code, we are developing methods to analyze the high-order structure of ubiquitin chains using advanced mass spectrometry.
2) Decoding mechanisms for proteasomal degradation: We have identified the p97-UFD1-NPL4 complex and RAD23 family as ubiquitin decoders that direct substrates to the proteasome (Mol Cell 2017, Nat Commun 2019). Currently we are investigating the substrate selectivity of these ubiquitin decoders using advanced proteomics and by developing chemical tools to manipulate proteasomal degradation.
3) Biological significance of proteasome phase separation: Recently, we found the ubiquitin-dependent liquid-liquid phase separation (LLPS) of the proteasome under hyperosmotic stress (Nature). This compartmentalization appears to be advantageous for the rapid removal of stress-damaged proteins, and we are further investigating proteasome phase separation under various stress conditions.
4) Generation of proteasome mutant mice: Recently, gene mutations in the proteasome have been identified in patients with autism and immune disorders. To understand the pathophysiological mechanism of "proteasomopathy", we generated proteasome mutant mice and are analyzing their phenotypes.



Selected Publications

Yasuda S, Tsuchiya H, Kaiho Ai, et al. (2020) "Stress- and ubiquitylation-dependent phase separation of the proteasome." ***Nature*** 578, 296-300.

Sato Y, Tsuchiya H, et al. (2019) "Structural insights into ubiquitin recognition and Ufd1 interaction of Npl4." ***Nat. Commun.*** 10, 5708.

Tsuchiya H, et al. (2018) "Ub-ProT reveals global length and composition of protein ubiquitylation in cells." ***Nat. Commun.*** 9, 524.

Ohtake F, et al. (2018) "K63 ubiquitylation triggers proteasomal degradation by seeding branched chains." ***Proc. Natl. Acad. Sci. USA.*** 115, E1401-E1408.

Tsuchiya H, et al. (2017) "In vivo ubiquitin linkage-type analysis reveals that the Cdc48-Rad23/Dsk2 axis contributes to K48-linked chain specificity of the proteasome." ***Mol. Cell*** 66, 485-502.

Ohtake F, et al. (2016) "The K48-K63 branched ubiquitin chain regulates NF-κB signaling." ***Mol. Cell*** 64, 251-266.

Laboratory Head
Kohji KASAHARA

Biomembrane

Laboratory HP: https://www.igakuken.or.jp/biomembrane/english.html

Kohji Kasahara has been the head of the Laboratory of Biomembranes at TMIMS since 2020. He obtained a BSc in Chemistry from the Tokyo Institute of Technology in 1986, a MSc in 1988, and a PhD from the University of Tokyo in 1992. After graduating, he worked at TMiMS as a research scientist from 1992 to 2003, as an independent scientist from 2003 to 2005, as a project subleader from 2005 to 2010, and as a team leader from 2010 to 2020. He also worked at PRESTO, Japan Science and Technology Agency from 2001 to 2005.

Staff

Researchers
Ikuo KAWASHIMA
Kiyoshi OGURA
Tetsuya HIRABAYASHI
Keisuke KOMATSUYA
Norihito KIKUCHI

Students
Mai KAWAGUCHI
Jun KANBE

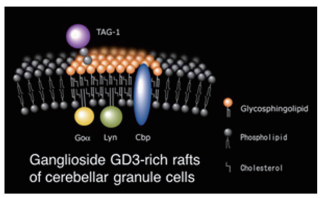
Research Summary

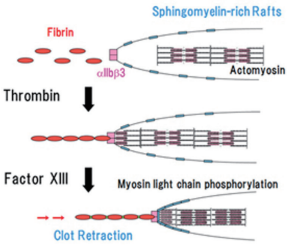
We are studying the function of lipid rafts. Lipid rafts are dynamic assemblies of glycosphingolipids, sphingomyelin, cholesterol, and proteins that can be stabilized in microdomains on cell surfaces. They are involved in the regulation of a number of cellular processes including axonal guidance, cellular migration, and blood clot formation and retraction.

In order to understand how lipid rafts receive external signals and transduce them to internal changes, we have been identifying protein interactions of glycosphingolipids in cerebellar granule cells from the nervous system, and in platelet cells from the blood.

In cerebellar granule cells we found that anti-ganglioside GD3 antibodies co-precipitate the GPI-anchored neural cell adhesion molecule TAG-1, the src-family kinase Lyn, its substrate Cbp, and the trimeric G protein Goα. TAG-1 is important for axonal guidance, and cellular migration. However, GPI anchors have no direct contact with the cytoplasm so it was unclear how TAG-1 activation causes internal cellular changes required for axonal guidance or migration. We demonstrated that TAG-1 transduces signals through interactions with Lyn/Cbp proteins found in ganglioside GD3-rich rafts of cerebellar granule cells. We further found that the chemokine SDF-1α triggers the chemoattraction of cerebellar granule cells during cerebellar development. SDF-1α stimulates GTPγS binding to Goα, and causes Goα translocation to lipid rafts, leading to growth cone collapse of cerebellar granule cells.

In blood platelets, sphingomyelin-rich lipid rafts are important for both blood clot formation and retraction through interaction with fibrin. We have identified a factor XIII-dependent fibrin-integrin αIIbβ3-myosin axis in sphingomyelin-rich membrane rafts that is important in clot retraction.





Selected Publications

Komatsuya K et al.(2020) "Function of Platelet Glycosphingolipid Microdomains/Lipid Rafts." ***Int. J. Mol. Sci.*** 21(15) :5539.

Kasahara K, et al. (2013) "Clot retraction is mediated by factor XIII-dependent fibrin-αIIbβ3-myosin axis in platelet sphingomyelin-rich membrane rafts." ***Blood*** 122, 3340-3348.

Kasahara K, et al. (2010) "Impaired clot retraction in factor XIII A subunit-deficient mice." ***Blood*** 115, 1277-1279.

Yuyama K, et al. (2007) "Translocation of activated heterotrimeric G protein Gαo to ganglioside-enriched detergent-resistant membrane rafts in developing cerebellum." ***J.Biol.Chem.*** 282, 26392-26400.

Kasahara K, et al. (2000) "Involvement of gangliosides in GPI-anchored neuronal cell adhesion molecule TAG-1 signaling in lipid rafts." ***J.Biol.Chem.*** 275, 34701-34709.

Kasahara K, et al. (1997) "Association of src family tyrosine kinase Lyn with ganglioside GD3 in rat brain. Possible regulation of Lyn by glycosphingolipid in caveolae-like domains." ***J.Biol.Chem.*** 272, 29947-29953.