Gunma University
Institute for Molecular and Cellular Regulation

The 4th IMCR Symposium on Endocrine and Metabolism:

At the Cutting Edge of Metabolic Regulation Research

Program & Abstracts

Date：November 8th（Thu）－9th（Fri）, 2018
Venue：Tojo Hall
Gunma University（Showa Campus）

November 8th (Thursday)

13:00	Opening Remarks Hiroshi Hiratsuka (President of Gunma University)	
Session 1		**Chair:Tsutomu Sasaki**
13:05-13:35	**How does zinc signaling control the fate determination of beige fat cells?** Ayako Fukunaka (Gunma University)	(p.1)
13:35-14:05	**Transcriptional Regulation of Brown Adipocytes and Its Implication for Humans** Hironori Waki (The University of Tokyo)	(p.3)
14:05-14:35	**Role of autophagy in pancreatic beta cells and atherosclerosis** Hirotaka Watada (Juntendo University)	(p.5)
14:35-14:50	Coffee Break	
Session 2		**Chair:Akiko Hayashi - Takagi**
14:50-15:20	**Selective autophagy of paternal mitochondria for maternal inheritance of mitochondrial DNA** Miyuki Sato (Gunma University)	(p.7)
15:20-15:50	***AUTS2* gene in the brain development and psychiatric disorders** Mikio Hoshino (National Center of Neurology and Psychiatry)	(p.9)
15:50-16:20	**Epigenetic control of mature β cell identity** Hail Kim (KAIST INSTITUTE, South Korea)	(p.11)
16:20-16:35	Coffee Break	
Session 3		**Chair:Yoshio Fujitani**
16:35-17:10	**ASK family kinases in Stress Signaling and Disease** Hidenori Ichijo (The University of Tokyo)	(p.13)
17:10-17:15	Short Break	
Special Lecture		**Chair:Takeshi Inagaki**
17:15-18:15	**The gut-liver axis in liver inflammation and hepatocarcinoma: focus on the FXR-FGF19 duo** Antonio Moschetta (University of Bari, Italy)	(p.15)
18:30-21:00	**Poster & Reception**	(p.29)

November 9th (Friday)

Session 4		**Chair:Tohru Ishitani**
9:00-9:30	**Understanding neuro-immune interactions by the gateway reflex** Daisuke Kamimura (Hokkaido University)	(p.17)
9:30-10:00	**The war between p53 corps and Akt in tumor** Yingjie Zhang (Hunan University, China)	(p.19)
10:00-10:30	**Acute HSF1 depletion induces cellular senescence through the MDM2-p53-p21 pathway in human diploid fibroblasts** Takayuki Yamashita (Gunma University)	(p.21)
10:30-10:45	Coffee Break	
Session 5		**Chair:Tadahiro Kitamura**
10:45-11:15	**Roots of risky decisions in addiction** Hiroyuki Mizoguchi (Nagoya University)	(p.23)
11:15-11:45	**Molecular crosstalk between cholesterol metabolism and immune responses** Ayaka Ito (Nagoya University)	(p.25)
11:45-12:15	**A histone demethylase JMJD1A mediates acute and chronic thermogenic responses through complementary Mechanisms** Juro Sakai (The University of Tokyo / Tohoku University)	(p.27)
12:15-12:20	**Closing Remarks** Ken Sato (Gunma University)	

Instruction to Chairs and Presenters

1. For Chairs

Arrival

Please come to the "Time keeper's desk" at the left-front of the room and let the staff know by 15 minutes before the starting time.

Process and Timing

Chairs are expected to ensure that all presentations start and finish punctually as scheduled.

Time allocation for each presentation is as follows. Staff will assist with timing by bell signal.

	Total	Presentation	Discussion	1 ring	2 rings	3 rings
Oral Presentations (Session 1,2,4,5)	30 min.	25 min.	5 min.	2 min. left to the end of presentation	End of presentation	End of discussion
Oral Presentations (Session 3)	35 min.	30 min.	5 min.	3 min. left to the end of presentation	End of presentation	End of discussion
Special Lecture	60 min.	50 min.	10 min.			

2. For Oral Presenters

Time allocation

Please see above.

Presentation Method

- Projector (connected to a laptop) will be used for presentations.
- Please bring your own computer.
- Please see the following "Technical Requirements for your laptop".

Preview

Please come to the "Preview desk" at the left-front of the room with your laptop at the following time.

Presenters in **Session 1**: Please come to the desk **by 12:45**.

Presenters in **Session 2**: Please come to the desk **on the coffee break in Session 1. (14:35-14:50)**

Presenters in **Session 3 and Special Lecture**: Please come to the desk **on the coffee break in Session 2. (16:20-16:35)**

Presenters in **Session 4**: Please come to the desk **by 8:45**.

Presenters in **Session 5**: Please come to the desk **on the coffee break in Session 5. (10:30-10:45)**

【Technical Requirements for your laptop】

① Ensure that your computer is equipped with the proper monitor connector (mini D-sub 15pin or HDMI terminal). If your computer does not have this connection, please bring an appropriate converter with you.

② Be sure to bring an AC adaptor. Please note that voltage in Japan is 100V and the frequency ranges 50-60 Hz depending on the area (50 Hz in Maebashi). The socket is type A, which has two flat plug holes. If your laptop is not convertible, transformers and/or plug adaptors are necessary.

③ **Set up your laptop not to show screensaver or be on the power saving mode.**

3. For Poster Presenters

- Please set up your poster on the panel in Tojo Hall between 2:00 p.m. and 4:00 p.m. on Wednesday, November 7th or between 9:00 a.m. and 11:00 a.m. on Thursday, November 8th. Please remove your poster by yourself as soon as the symposium ends.
- Each panel is 86 cm wide and 168 cm high.
- Please refer to the Poster Layout (on page 30) to find your panel.

Session 1
Chair: Tsutomu Sasaki (Gunma University)

How does zinc signaling control the fate determination of beige fat cells?

Ayako Fukunaka[1], Toshiyuki Fukada[2], Shingo Kajimura[3], Hirotaka Watada[4], Yoshio Fujitani[1]
([1]Lab. of Developmental Biology & Metabolism, IMCR, Gunma University, [2]Tokushima Bunri University, [3]UCSF Diabetes Center, [4]Juntendo University Graduate School of Medicine)

Obesity and its associated metabolic diseases are caused by a prolonged imbalance between energy intake and energy expenditure. Adipose tissues are the major control site of energy balance, which comprises two functionally distinct cell types; white fat cells and brown fat cells. White fat cells store excess energy, whereas brown fat cells specialize in energy expenditure. In addition, brown fat-like cells have also been found within white adipose tissue (WAT). These inducible brown fat-like cells are known as beige fat cells, which are increased by cold exposure (a process also known as adipocyte browning). Therefore, the identification of signaling pathways that regulate the acquisition of beige fat cell properties by WAT have gained attention from a therapeutic viewpoint against obesity.

We have previously reported that zinc signaling via the zinc transporter ZIP13 negatively regulates the adipocyte browning pathway (Fukunaka A, et al, *PLoS Genet*., (2017)). Here, we demonstrated that the unique amino acid region of ZIP13 facing the cytosol, named intracellular loop2 (IntL-2), which is distinct from the other ZIP family members, is involved in the inhibition of adipocyte browning. Furthermore, we identified binding molecules that associate with the IntL-2 of ZIP13. We are now clarifying the molecular mechanism as to how these IntL-2 binding proteins regulate adipocyte browning via ZIP13.

Memo

Transcriptional Regulation of Brown Adipocytes and Its Implication for Humans

Hironori Waki
(Department of Diabetes and Metabolic Diseases, Department of Molecular Science on Diabetes, Graduate School of Medicine The University of Tokyo)

The development of high-throughput sequencing technology lets us investigate the epigenome in a global and comprehensive manner, and provides previously unrecognized findings and insights. We identified nuclear factor I-A (NFIA) as a transcriptional regulator of brown fat by a genome-wide open chromatin analysis of murine brown and white fat followed by motif analysis of brown-fat-specific open chromatin regions. NFIA and the master transcriptional regulator of adipogenesis, PPARγ, co-localize at the brown-fat-specific enhancers. Moreover, the binding of NFIA precedes and facilitates the binding of PPAR, leading to increased chromatin accessibility and active transcription. Knockdown and knockout experiments as well as human studies indicate that NFIA is a physiological regulator that controls the brown-fat-specific gene program. I will also introduce a result of a clinical study in which we investigated supraclavicular brown fat density by using near-infrared time-resolved spectroscopy and discuss a potential role of brown fat for obesity in humans.

Memo

Role of autophagy in pancreatic beta cells and atherosclerosis

Hirotaka Watada
(Juntendo University Graduate School of Medicine Department of Metabolism & Endocrinology)

In the early phase of natural history of Type 2 diabetes mellitus (T2DM), insulin resistance elicited by metabolically abnormal obesity appears and pancreatic islets compensates for the insulin resistance by enhancing insulin secretary capacity and expanding islets mass. In the patients with T2DM, pancreatic islets become to be unable to compensate for insulin resistance and to show hyperglycemia. Insulin resistance and the resultant hyperglycemia synergistically accelerate atherosclerosis in the patients with T2DM and eventually elicit cardiovascular diseases. Thus, in order to develop new strategy for T2DM, it is important to clarify the mechanism underlying the islet dysfunction and the progression of atherosclerosis. One of the important research themes of our lab is to elucidate the role of "autophagy" in various cells on the pathophysiology of T2DM. Previously. we have found that autophagy failure is observed in the beta cells of T2DM and plays a causal role in the dysfunction of beta cells. We furthermore, found that autophagy is important to prevent the beta cell dysfunction caused by enhanced ER stress and accumulation of islet amyloid polypeptide. Currently, we are now trying to search for the molecular mechanism of autophagic failure in T2DM. In addition, we recently found that autophagy failure in smooth muscle cells in artery causes acceleration of atherosclerosis and morphological changes similar to human atherosclerotic lesion in mice model of atherosclerosis. Taken together, we propose that autophagic failure in pancreatic beta cells and smooth muscle cells could be possible defects in T2DM and atherosclerosis, respectively and that activation of autophagy in each cells could be a possible new strategy for the treatment of T2DM.

Memo

Session 2
Chair: Akiko Hayashi - Takagi (Gunma University)

Selective autophagy of paternal mitochondria for maternal inheritance of mitochondrial DNA

Miyuki Sato
(Lab. of Mol. Memb. Biology, IMCR, Gunma University)

Mitochondrial DNA (mtDNA) encodes genes essential for mitochondrial functions. Interestingly, mtDNA is maternally inherited in many organisms. Using *Caenorhabditis elegans* as a model system, we have shown that paternal mitochondria are selectively eliminated via autophagy in embryos and this explains why mtDNA is maternally inherited (Sato & Sato, Science 2011). In addition to paternal mitochondria, sperm-derived lysosome-related organelles called MOs are also degraded in this autophagy. Therefore we named this autophagy allophagy (allogeneic organelle autophagy). However, how paternal organelles are recognized and targeted for autophagy remained elusive. Recently, we identified a novel autophagy receptor ALLO-1 and a worm homologue of the TBK1/IKKε family kinase, IKKE-1, as regulators essential for allophagy. After fertilization, ALLO-1 and IKKE-1 localize to the paternal organelles prior to autophagosome formation, and recruitment of autophagy machineries around the paternal organelles depends on this ALLO-1 and IKKE-1 localization, suggesting that ALLO-1 and IKKE-1 control local autophagosome formation around the paternal organelles. We also showed that ALLO-1 and IKKE-1 physically interact with each other, and the IKKE-1-dependent phosphorylation of ALLO-1 is important for allophagy (Sato, *et al*., Nature Cell Biol. 2018). This ALLO-1 and IKKE-1-dependent mechanism of allophagy is reminiscent of xenophagy and mitophagy in mammalian cells, implying that these autophagic pathways may have the same origin.

Memo

AUTS2 gene in the brain development and psychiatric disorders

Mikio Hoshino
(Department of Biochemistry and Cellular Biology, National Center of Neurology and Psychiatry (NCNP))

Mutations in the *Autism susceptibility candidate 2 gene (AUTS2)* have been shown to be associated with multiple psychiatric illnesses including autism spectrum disorders (ASD), intellectual disability, schizophrenia, depression and drug addiction. However its function was largely unclear until recently.

We have recently revealed that AUTS2 protein reorganizes actin cytoskeleton via regulating Rho-family GTPases, Rac1 and Cdc42, in neural cells. Furthermore, we also clarified that AUTS2 participates in important developmental processes of the brain, such as cortical neuronal migration, neurite extension and synapse formation. AUTS2 localizes at pre- and post-synapses of excitatory synapses and restricts the number of excitatory synapses, while this protein does not affect inhibitory synapses. Loss of *AUTS2* causes excessive excitatory synapses leading to E/I (excitation/inhibition) imbalance, which may underlie pathology of various psychiatric illnesses, including epilepsy. Furthermore, *AUTS2* is also involved in development of cerebral cortex, hippocampus and cerebellum, probably related to cognitive and motor dysfunctions in patients with *AUTS2* mutations. It is also suggested that *AUTS2* is somewhat related to the evolution of the human brain.

Thus, *AUTS2* contributes to neural network formation and is critical for the acquisition of higher brain function and, therefore, this study gives insights into the pathology of human psychiatric disorders with *AUTS2* mutations.

Memo

Epigenetic control of mature β cell identity

Hail Kim
(Korea Advanced Institute of Science and Technology, KAIST)

Although the progression of type 2 diabetes initiates with the development of insulin resistance, hyperglycemia does not develop as long as pancreatic β-cells compensate for insulin resistance. Type 2 diabetes can be diagnosed when β-cells fail to come up with the increased insulin demand due to insulin resistance. Thus, β-cell failure along with insulin resistance is an essential feature of type 2 diabetes. β-Cell death has been thought as a primary cause of β-cell failure. However, given that clear deficiency in glucose stimulated insulin secretion is observed in type 2 diabetes despite the absence of marked differences in β-cell mass, β-cell seems to experience more dynamic changes before β-cell death develops. In fact, recent studies in human and mice indicate that fully differentiated β-cell retains more plasticity than we appreciated before and the loss of β-cell identity results in dedifferentiation of β-cell into alternate endocrine cell fates. Although β-cell dedifferentiation is characterized by the changes in the expression of β-cell specific transcription factors as well as the decrease in insulin production, it remains to be elucidated how β-cell dedifferentiation develops.

Here, we show a novel function of PRMT1-dependent histone H4 arginine 3 asymmetric dimethylation (H4R3me2a) in maintaining β cell identity. Our results reveal that H4R3me2a functions as an active histone code that increases chromatin accessibility at the binding sites for transcription factors including CTCF, NKX6.1, MAFA, PDX1 and NEUROD1. Deletion of *Prmt1* in mature β cells results in the immediate loss of H4R3me2a and the subsequent dedifferentiation of β cells. β cell-specific *Prmt1* knock-out mice developed diabetes phenotype and PRMT1-dependent open chromatin regions show a strong association with the risk of diabetes in humans. In conclusion, our results indicate that PRMT1 plays an essential role in maintaining β cell identity by regulating chromatin accessibility.

Memo

Session 3
Chair: Yoshio Fujitani (Gunma University)

ASK family kinases in Stress Signaling and Disease

Hidenori Ichijo
(Lab. of Cell Signaling, Grad. Sch. of Pharm. Sci., The University of Tokyo)

Stress-responsive signaling pathways mediate transduction of cellular stresses to various physiological responses such as cell proliferation, apoptosis and inflammation. Their dysfunction thus induces abnormal cellular behaviors, which may lead to tumorigenesis and tumor progression including tumor metastasis. It has been revealed that tumors are more than homogenous masses of proliferating tumor cells; instead, they consist of multiple and heterogeneous cell types including normal host cell types, interacting with one another in a heterotypic fashion. Stress-responsive signaling cascades play pivotal roles also in these heterotypic interactions. Therefore, stress-responsive signaling cascades and cancer are very closely related and could be very promising drug targets for cancer therapy.

Mitogen-activated protein kinase (MAPK) cascades are one of these pathways and are intricately involved in both tumor promotion and suppression. In these cascades, upstream MAP3K phosphorylates and activates MAP2K and MAP2K similarly activates MAPK. Activated MAPK regulates various effector molecules including transcription factors by phosphorylation and outputs physiological responses. Here, we present the diverse roles of apoptosis signal-regulating kinase (ASK) family molecules in tumor formation and progression. ASK family is a member of MAPK kinase kinases (MAP3Ks) in the JNK and p38 MAPK pathways and is composed of three family members, ASK1, ASK2 and ASK3. Accumulating evidence indicates that ASK1 controls tumorigenesis through regulation of innate immunity, apoptosis, DNA-damage response and cell cycle. ASK2 also regulates tumorigenesis via apoptosis. Furthermore, analysis of the experimental lung metastasis model in mice suggests that host ASK1 deficiency attenuates tumor lung metastasis. In this presentation, we would like to discuss the potential roles of ASK family signal pathways in tumor progression and other cellular functions.

Memo

Special Lecture
Chair: Takeshi Inagaki (Gunma University)

The gut-liver axis in liver inflammation and hepatocarcinoma: focus on the FXR-FGF19 duo

Antonio Moschetta
(Department of Interdisciplinary Medicine, University "Aldo Moro" of Bari, Italy)

While it has long been recognized that bile acids (BAs) are required to facilitate lipid digestion and cholesterol absorption in the intestine, the discovery that they serve as ligands for the nuclear Farnesoid X receptor (FXR), opened a new chapter in the characterization of BAs as signalling molecules. FXR is a transcription factor and the master regulator of BAs homeostasis. In the gut-liver axis, FXR regulates BA synthesis, influx, efflux, and detoxification. The landmark discovery of the role of a FXR target gene, the fibroblast growth factor 15/19 (murine and human, respectively – FGF15/19), in the feedback regulation of hepatic BA synthesis shed light on the physiological relevance of the crosstalk of the gut-liver axis in the context of BA homeostasis. FGF15/19 is an atypical fibroblast growth factor that functions as a hormone. Via the enterohepatic circulation it travels from the small intestine to the liver, where it acts on a cell surface receptor complex to repress BA synthesis and gluconeogenesis and activate glycogen and protein synthesis. Deregulation of BA homeostasis has been linked to cholestasis and hepatocellular carcinoma (HCC), and spontaneous hepatocarcinogenesis has been observed in FXR-null mice. We have demonstrated that intestinal selective FXR reactivation is sufficient to restore the enterohepatic BA homeostasis via fibroblast growth factor 15 (FGF15)/cholesterol-7alpha-hydroxylase (Cyp7a1) axis and eventually provide protection against progression of liver damage, even in the absence of hepatic FXR. Intestinal-selective FXR modulators could stand as potential therapeutic intervention in patients with impaired BA homeostasis-associated hepatic diseases, even in patients carrying a somatic FXR mutation.

Memo

Session 4
Chair: Tohru Ishitani (Gunma University)

Understanding neuro-immune interactions by the gateway reflex

Daisuke Kamimura, Masaaki Murakami
(Molecular Psychoimmunology, Institute for Genetic Medicine, Graduate School of Medicine, Hokkaido University)

The blood-brain barrier (BBB) limits the exchanges of substances and the migration of immune cells to the central nervous system (CNS). Despite the BBB, immune cells can enter the CNS even at the steady state. In CNS inflammatory diseases such as multiple sclerosis (MS), the BBB is damaged and immune cell invasion occurs at a much greater degree. We found a mechanism operated by a specific local neuroimmune interaction that explains how autoreactive CD4+ T cells transverse the BBB to invade the CNS, which is now known as the gateway reflex. The gateway reflex is induced by the activation of specific neural pathways by various stimulations including gravity, electric, pain, and chronic stress etc., which enhances chemokine expressions at specific vessels and establishes specific gateways for autoreactive CD4+ T cells toward the CNS. Mechanistic analysis showed that neurotransmitters such as norepinephrine or ATP and cytokines at the specific vessels induce a simultaneous activation of NF-kB and STAT3 followed by enhanced expression of various NF-kB targets including chemokines to establish the gateway of immune cells in the BBB. While these gateways are localized at the vessels of the fifth lumbar spinal cord at the steady state due to stimulation by gravity, the location can be changed with the type of sensory stimulations. For example, pain induces an immune cell gateway at the ventral vessels of spinal cord, and electric stimulation creates gateways depending on where to stimulate. Recently, we unexpectedly found that chronic stress-mediated neural activation changes the location of immune cell gateway to from the fifth lumbar cord to the specific blood vessels of the brain, followed by a fatal dysregulation in the gastrointestine and heart. I will discuss the discovery and recent advances about the gateway reflex.

Memo

The war between p53 corps and Akt in tumor

Yingjie Zhang
(College of Biology, Hunan University)

Wild type p53 is an important pro-apoptotic factor and tumor suppressor. PUMA (p53 up-regulated modulator of apoptosis) has been identified as a critical downstream regulator of p53, whose activation is proposed as a biomarker for colon cancer therapy. Based on this, we screened series of new drugs and inhibitors to induce PUMA activation and colon cancer cell apoptosis. As a result, we found four of them are excellent, which activated PUMA in p53-dependent and -independent manner (through inhibiting Akt and activating FoxO3a), and suppressed colon cancer growth *in vitro* and *in vivo*. For the mechanism, PUMA promoted Bax translocation directly and indirectly to induce mitochondrial apoptosis. However, mutant p53 proteins promote tumor progression and metastasis through a gain-of-function activity, whose mechanism underlying remains incompletely understood. We identify that mutant p53R172H (equivalent to human p53R175H) activates the Akt-mTORC1 signaling through down-regulation of PHLPP2 mRNA. Genetic and pharmacological studies demonstrate that the activated Akt-mTORC1 signaling is required for mutant p53 to exert its gain-of-function in vivo. Importantly, our results reveal that Akt-mTORC1 signaling feedback sustains mutant p53, and accordingly targeting the Akt-mTORC1 signaling remarkably reduces mutant p53. Thus, targeting the Akt-mTORC1 signaling that mediates the gain-of-function properties of mutant p53 and controls mutant p53 protein synthesis provides an attractive therapeutic strategy for a subset of cancer patients carrying mutant p53.

Memo

Acute HSF1 depletion induces cellular senescence through the MDM2-p53-p21 pathway in human diploid fibroblasts

Takayuki Yamashita
(Lab. of Molecular genetics, IMCR, Gunma University)

In many types of cells, various stresses induce a complex state termed “cellular senescence”, characterized by a permanent cell cycle arrest and secretion of inflammatory cytokines. Importantly, cellular senescence serves as an anti-tumor barrier and contributes to the development of age-related pathologies *in vivo*. Heat shock transcription factor 1 (HSF1) plays critical roles in the regulation of proteostasis, cell metabolism and growth through controlling expression of heat shock proteins (HSPs) as well as many non-HSP proteins. Because HSF1 deficiency suppresses tumorigenesis and accelerates aging at the organismal level, it is attractive to hypothesize that these phenotypes are partly based on cellular senescence. However, little is known about the role of HSF1 for cellular senescence. Here, we report that HSF1 depletion-induced senescence (HDIS) of human diploid fibroblasts (HDFs) was independent of HSP-mediated proteostasis but dependent on activation of the p53-p21 pathway, partly because of the increased expression of dehydrogenase/reductase 2 (DHRS2), a putative MDM2 inhibitor. We observed that HDIS occurred without decreased levels of major HSPs or increased proteotoxic stress in HDFs. Moreover, pharmacological inhibition of HSP70 family proteins increased suppressed cell growth, but failed to induce senescence. Importantly, we found that activation of the p53-p21 pathway due to reduced MDM2-dependent p53 degradation was required for HDIS. Furthermore, increased DHRS2 expression contributed to p53 stabilization and HDIS. Collectively, our observations uncovered a molecular pathway in which HSF1 depletion-induced expression of DHRS2 leads to activation of the MDM2-p53-p21 pathway and HDIS. These findings provide important insights into the regulatory functions of HSF1 in tumorigenesis and aging.

Memo

Session 5

Chair: Tadahiro Kitamura (Gunma University)

Roots of risky decisions in addiction

Hiroyuki Mizoguchi

(Research Center for Next-Generation Drug Development, Research Institute of Environmental Medicine, Nagoya University)

Drug dependence is defined as a chronically relapsing disorder that is characterized by compulsive drug taking, inability to limit intake, and intense drug cravings, which may be associated with neural plasticity and remodeling of specific brain circuits caused by repeated exposure to drugs of abuse. Addicts, especially methamphetamine users, also show altered decision-making. Addicts are less able to flexibly adapt their behavior to changes in reward contingencies, have difficulties in integrating reinforcements to guide future behavior, and have a greater tendency to engage in risk-taking behaviors and to choose actions that confer short-term rewards at the cost of long-term disadvantages. These impairments seem to contribute to compulsive drug taking and relapse, and curing these impairments may lead to improve the quality of life of patients. Thus, a better understanding of the mechanisms underlying impaired decision-making should provide insights into novel and successful treatments for drug dependence.

In this symposium, we will talk about the mechanism of altered decision-making in methamphetamine-treated animal based on our results.

Memo

Molecular crosstalk between cholesterol metabolism and immune responses

Ayaka Ito
(Research Institute of Environmental Medicine, Nagoya University)

The liver X receptors α and β (LXRα and LXRβ) are members of the nuclear receptor superfamily that regulate cholesterol, fatty acid and phospholipid metabolism. LXRs are also known to modulate immune responses. One of the best-characterized roles of LXRs in immune cells is to inhibit inflammatory gene expression triggered by toll-like receptors (TLRs). We recently demonstrated that this anti-inflammatory effect is attributable at least in part to primary effects of LXRs on cellular lipid metabolism. We showed that transcriptional induction of *Abca1* by LXRs promotes cholesterol efflux and alters plasma membrane cholesterol distribution, resulting in the attenuation of MAPKs and NFκB signaling downstream of TLRs. These findings suggest that the dual role of LXRs in lipid homeostasis and immune cell function have common mechanistic underpinnings.
Our previous work revealed that inactivation of LXRα and LXRβ in mice leads to autoimmunity, however, how the regulation of cholesterol metabolism contributes to autoimmunity is unclear. We recently elucidated that cholesterol loading of CD11c+ cells triggered the development of autoimmunity, whereas preventing excess lipid accumulation by promoting cholesterol efflux was therapeutic. LXRβ-deficient mice crossed to the hyperlipidemic ApoE-deficient background or challenged with a high-cholesterol diet developed autoantibodies. Cholesterol accumulation in lymphoid organs promoted the production of the B cell growth factors Baff and April. Conversely, B cell expansion and the development of autoantibodies in ApoE/LXRβ-deficient mice were reversed by ApoA-I expression. These findings implicate cholesterol imbalance as a contributor to immune dysfunction and suggest that stimulating HDL-dependent reverse cholesterol transport could be beneficial in the setting of autoimmune disease.

Memo

A histone demethylase JMJD1A mediates acute and chronic thermogenic responses through complementary Mechanisms

Juro Sakai
(Tohoku University Faculty of Medicine, Division of Molecular Physiology
The University of Tokyo, RCAST, Division of Metabolic Medicine)

In acute cold exposure in mammals, JMJD1A, an H3K9 demethylase, is known to up-regulate thermogenic gene expression in response to β-adrenergic signaling in brown adipose tissue (BAT). Recently, we showed that JMJD1A activity depends on phosphorylation of a serine residue (S265) but not on its demethylation activity. Mammals also have another type of thermogenesis in response to long-term cold stress, which induces the browning of subcutaneous white adipose tissue (scWAT) referred to as "beige-ing" of scWAT. Previous studies have suggested that the long-term cold stress is epigenetically memorized, although the mechanism remains unclear. Here we show that S265 phosphorylation of JMJD1A is also pivotal for beige-ing. Ablation of S265-phosphorylation of JMJD1A impaired beige fat function and insulin sensitivity in mice. In the first step (signal sensing) cold stress (which generates β-adrenergic signal) causes the phosphorylation of JMJD1A at S265, which in turn, causes JMJD1A to target beige-selective genes through the formation of a transcriptional protein complex containing PGC1α, PRDM16, and DNA-bound sequence PPARγ. In the second step (epigenetic rewriting) under prolonged stress, phosphorylated JMJD1A demethylates H3K9me2 to turn on transcription in scWAT.

Memo

Poster Session & Reception

1. "Polη, a Y-family translesion synthesis polymerase, promotes cellular tolerance of Myc-induced replication stress"

Takayuki Sekimoto[1], Kiminori Kurashima[1], Tsukasa Oda[1], Tsuyoshi Kawabata[2], Fumio Hanaoka[3] and Takayuki Yamashita[1]
([1]Lab. of Molecular Genetics, IMCR, Gunma University, [2]Dept of Stem Cell Biology, Nagasaki University, [3]Dept of Life Science, Gakushuin University)

2. "An exploratory research for the regulation of adipocyte differentiation by artificial editing of a histone methylation"

Tomohiro Suzuki[1], Silvia Bolzani[1], Diana Vargas Trujillo[1], SumiyoMorita[2], Takuro Horii[2], Izuho Hatada[2], Hiroshi Shibata[1], Takeshi Inagaki[1]
([1]Lab. of Epigenetics & Metabolism, IMCR, Gunma University, [2] Laboratory of Genome Science, IMCR, Gunma University)

3. "PP cells as stage-specific precursors of endocrine pancreas development"

Takahiro Fukaishi[1], Takashi Sato[1], Yuko Nakagawa[1], Ayako Fukunaka[1], Azumi Noguchi[1], Akemi Hara[2], Hirotaka Watada[2], and Yoshio Fujitani[1]
([1]Lab. of Developmental Biology & Metabolism, IMCR, Gunma University, [2]Juntendo University Graduate School of Medicine)

4. "Proliferative regulation of pancreatic polypeptide producing cells by the proglucagon gene"

Yuko Nakagawa[1], Yoshitaka Hayashi[2], Tadahiro Kitamura[3] and Yoshio Fujitani[1]
([1]Lab. of Developmental Biology and Metabolism, IMCR, Gunma University, [2]Division of Stress Adaptation and Protection, Research Institute of Environmental Medicine, Nagoya University, [3]Metabolic Signal Research Center, IMCR, Gunma University)

5. "Physiological analysis of mammalian small GTPase Rab35"

Ikuko Maejima[1], Tomoko Akuzawa[1], Rika Hirai[1], Hisae Kobayashi[1], Inoya Isobe[1], Taichi Hara[1,2] and Ken Sato[1]
([1]Lab. of Molecular Traffic, IMCR, Gunma University, [2]Lab. of Cellular Regulation, Faculty of Human Sciences, Waseda University)

6. "Role of iron in adipogenic differentiation of 3T3-L1 cells"

Hiroshi Shibata[1], Tomohiro Suzuki[1], Kyoko Tanimura[2], Diana Vargas Trujillo[1], Tasuku Hirayama[3], Hideko Nagasawa[3]
Takeshi Inagaki[1]
([1]Lab. of Epigenetics and Metabolism, IMCR, Gunma University, [2]Dept. of Diabetes, Endocrinology and Metabolism, Nippoin Medical School, [3]Lab. of Pharmaceutical & Medicinal Chemistry, Gifu Pharmaceutical University)

7. "Single cell imaging of ATP level reveals physiological importance of ATP homeostasis"

Masakatsu Takaine[1,2], Hiromi Imamura[3], Satoshi Yoshida[1,2,4]
([1]GIAR, Gunma University, [2]Lab. of Cellular Signaling, IMCR, Gunma University, [3]Graduate School of Biostudies, Kyoto University, [4]School of International Liberal Studies, Waseda University)

8. "Epigenome editing: manipulating DNA methylation of specific DNA loci using CRISPR/Cas9 and a repeating peptide array-based amplification."

Sumiyo Morita
(Biosignal Genome Resource Center, IMCR, Gunma University)

9. "Efficient generation of conditional knockout mice via sequential introduction of loxP sites"

Takuro Horii, Ryohei Kobayashi, Eriko Suetomo, Yuika Kawada, Mika Kimura, Sumiyo Morita, Izuho Hatada
(Biosignal Genome Resource Center, IMCR, Gunma University)

10. "Melanophilin mediates insulin granule fusion without stable docking to the plasma membrane through interaction with open form of syntaxin-4"

Hao Wang[1], Kouichi Mizuno[1], Noriko Takahashi[2], Eri Kobayashi[1], Haruo Kasai[3], Katsuhide Okunishi[1] and Tetsuro Izumi[1]
([1]Lab. of Molecular Endocrinology and Metabolism, IMCR, Gunma University, [2]Department of Physiology, Kitasato University School of Medicine, [3]Laboratory of Structural Physiology, Center for Disease Biology and Integrative Medicine, Faculty of Medicine, The University of Tokyo)

11. "Mutant p53 switches the fate of senescent RasV12 cells from apical elimination to tumor priming."

Yukinari Haraoka[1,2], Yuki Akieda[1], Tohru Ishitani[1,2]
([1]Lab. of Integrated Signaling System, Department of Molecular Medicine, IMCR, Gunma Univ., [2]MIB, Kyushu Univ.)

12. "Development of innovative wide-view mapping of synaptic ensemble in the psychiatric model"

Kisho Obi[1], Masayasu Sato[1], Junko Sugi[1] and Akiko Hayashi-Takagi[1]
([1]Lab of Medical Neuroscience, IMCR, Gunma University)

13. "Analysis of iron-dependent cell death ferroptosis in cancer cells"

Seiji Torii[1], Ryosuke Shintoku[1,2], Yuta Takigawa[3], Keiichi Yamada[4], Chisato Kubota[1], Yuhei Yoshimoto[2], Toshiyuki Takeuchi[1], Ichiro Koshiishi[3]
([1]Secretion Biology Lab, IMCR, [2]Department of Neurosurgery, Graduate School of Medicine, [3]Department of Laboratory Sciences, Graduate School of Health Sciences, [4]Department of Chemistry and Chemical Biology, Graduate School of Science and Technology, Gunma University)

14. "Progressive synapse deterioration in the DISC1 knockdown mice and its relationship with the pathophysiology of schizophrenia"

Fukutoshi Shirai and Akiko Hayashi-Takagi
([1]Lab. of Medical Neuroscience, IMCR, Gunma University)

15. "Tryptophan degradation by producing kynurenic acid in Saccharomyces cerevisiae"

Kazuto Ohashi
(Tenure track, Institute for Molecular and Cellular Regulation, Gunma University)

16. "Insulin-GDF3-ALK7 axis accelerates fat accumulation."

Yun Bu[1], Katsuhide Okunishi[1], Satomi Yogosawa[1], Kouichi Mizuno[1], Maria Johnson Irudayam[2], Chester W. Brown[2], Tetsuro Izumi[1]
([1]Lab. of Molecular Endocrinology & Metabolism, IMCR, Gunma University, [2]University of Tennessee Health Science Center)

17. "Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice"

Yuhkoh Satouh[1,*], Kaori Nozawa[1,2], Masahito Ikawa[1,3]
([1]Research Institute for Microbial Diseases, Osaka University, Japan, [2]Baylor College of Medicine, Houston, USA, [3]The Institute of Medical Science, The University of Tokyo, Japan.
*present address: Laboratory of Molecular Traffic, Institute for Molecular and Cellular Regulation, Gunma University, Japan)

18. "Neuronal SIRT1 regulates simple sugar selection through FGF21 and oxytocin signalling in mice"

Sho Matsui, Tsutomu Sasaki, Daisuke Kohno, Hiromi Yokota-Hashimoto, Masaki Kobayashi, Tadahiro Kitamura
(Lab. of Metabolic Signal, Metabolic Signal Research Center, IMCR, Gunma University)

19. "SGLT1 in pancreatic α cells regulates glucagon secretion in mice, possibly explaining the distinct effects of SGLT2 inhibitors on plasma glucagon levels"

Takayoshi Suga[1,2], Osamu Kikuchi[1], Masaki Kobayashi[1], Sho Matsui[1], Hiromi Yokota-Hashimoto[1], Eri Wada[1], Daisuke Kohno[1], Tsutomu Sasaki[1], Kazusane Takeuchi[3], Satoru Kakizaki[2], Masanobu Yamada[2], Tadahiro Kitamura[1]
([1]Metabolic Signal Research Center, IMCR, Gunma University, [2]Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, [3]Cosmic Corporation Co., Ltd.)

20. "How cellular senescence causes individual aging?"

Shohei Ogamino, Masayuki Oginuma, Kota Abe, Chihiro Mogi, Tohru Ishitani
(Lab of Integrated Signaling Systems, Department of Molecular Medicine Institute for Molecular & Cellular Regulation, Gunma University)

Poster Layout

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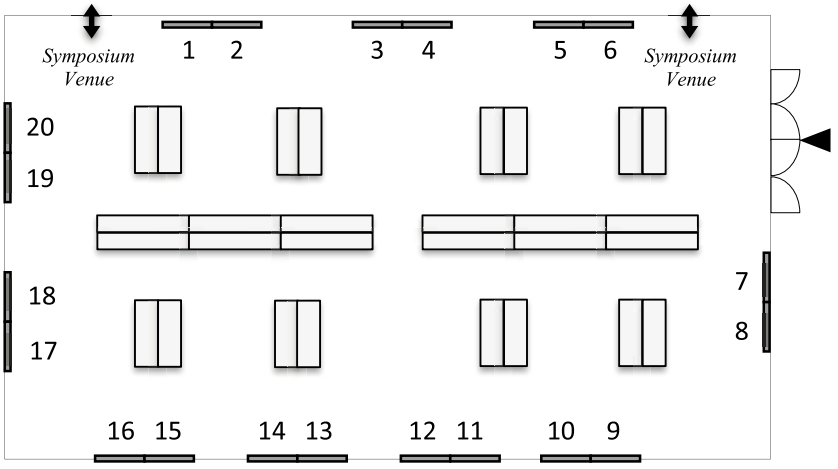

Poster Session

No. 1

"Polη, a Y-family translesion synthesis polymerase, promotes cellular tolerance of Myc-induced replication stress"

Takayuki Sekimoto[1], Kiminori Kurashima[1], Tsukasa Oda[1], Tsuyoshi Kawabata[2], Fumio Hanaoka[3] and Takayuki Yamashita[1]

([1]Lab. of Molecular Genetics, IMCR, Gunma University, [2]Dept of Stem Cell Biology, Nagasaki University, [3]Dept of Life Science, Gakushuin University)

DNA replication is hampered by various factors, such as template damages. The resultant slowing or stalling of the replication fork is referred to as "replication stress" (RS), leading to generation of double-strand breaks (DSBs) and genomic instability. Normal cells have evolved multiple pathways to overcome RS. Some of these pathways are also useful for neoplastic cells to survive oncogene-induced RS, thus providing potential targets for cancer therapy. Translesion synthesis (TLS) plays an essential role in cellular tolerance to various types of RS and bypasses replication barriers by employing specialized low-fidelity polymerases. However, limited information is available about the role of TLS polymerases in oncogene-induced RS. Here, we report that Polη, a Y-family TLS polymerase, prevents Myc-induced RS. Polη was recruited to Myc-induced RS sites, and Polη depletion enhanced the Myc-induced slowing and stalling of replication forks and the subsequent generation of DSBs. Overexpression of a catalytically-dead Polη also promoted Myc-induced DSB formation. In the absence of Polη, Myc-induced DSB formation depended on MUS81-EME2 (the S-phase specific endonuclease complex), and concomitant depletion of MUS81-EME2 and Polη enhanced RS and cell death in a synergistic manner. Collectively, these results indicate that Polη facilitates fork progression during Myc-induced RS, thereby helping cells tolerate the resultant deleterious effects. Additionally, the present study highlights the possibility of synthetic sick or lethal interaction between Polη and MUS81-EME2 in cells experiencing Myc-induced RS.

No. 2

"An exploratory research for the regulation of adipocyte differentiation by artificial editing of a histone methylation"

Tomohiro Suzuki[1], Silvia Bolzani[1], Diana Vargas Trujillo[1], SumiyoMorita[2], Takuro Horii[2], Izuho Hatada[2], Hiroshi Shibata[1], Takeshi Inagaki[1]

([1]Lab. of Epigenetics & Metabolism, IMCR, Gunma University, [2] Laboratory of Genome Science, IMCR, Gunma University)

Histone methylation is considered to regulate gene expression in a long-lasting manner and work as a memory of environmental stimuli, which could be a pathogenesis of lifestyle-related diseases such as obesity, diabetes, and hypertension. Although it is expected that the artificial modification of histone methylation would be a therapeutic target for lifestyle-related diseases, the technical evidence for the approach is not well established. Thus, we are challenging to manipulate a histone modification. Employing the CRIPSR-dCas system-based SunTag method, we try to regulate adipocyte deafferentation by recruiting a histone methyl-modifying enzyme to the genomic regions of *Pparg* and *Cebpa*, which are the master regulatory factors of adipocyte differentiation. In this poster session, we will present our system, recent results, and the technical difficulties of artificial regulation of adipocyte differentiation by mediating histone modification.

No. 3

"PP cells as stage-specific precursors of endocrine pancreas development"

Takahiro Fukaishi[1], Takashi Sato[1], Yuko Nakagawa[1], Ayako Fukunaka[1], Azumi Noguchi[1], Akemi Hara[2], Hirotaka Watada[2], and Yoshio Fujitani[1]
([1]Lab. of Developmental Biology & Metabolism, IMCR, Gunma University, [2]Juntendo University Graduate School of Medicine)

Pancreatic polypeptide (PP) is a 36-amino acid peptide encoded by the *Ppy* gene, and is produced by PP cells, which are a small population of cells located in the periphery of the islets of Langerhans. PP cells have been proposed to be the precursor of endocrine cells by lineage tracing analysis, but experimental limitations in the accuracy and specificity of the PP-antibody and transgenic mice used has made it difficult to assess the precise role for PP cells. Recently, we established a PP-specific monoclonal antibody and knock-in Cre driver mice within the *Ppy* locus, which have enabled us to accurately investigate the role of PP cells. Here, we demonstrate that *Ppy* lineage cells account for a significant proportion of all endocrine cell types in adult islets, and contribute to endocrine development, particularly in the embryonic stages. Surprisingly, lineage-tracing analysis showed that almost all insulin-, glucagon-, and PP-producing cells and about half of all somatostatin-producing cells at E15.5 are derived from *Ppy*-expressing precursors. On the other hand, at later time points in adult islets, non-*Ppy*-expressing endocrine precursors dominantly contribute to the development of α, β and δ cells. These results suggest that there are two distinct waves of endocrine development; the first involving *Ppy*-expressing precursors at embryonic stages, and the next when these endocrine cells are progressively replaced by non-*Ppy*-lineage cells. Our data demonstrate an unprecedented role of PP cells in endocrine development, which may provide valuable insight into new therapies for diabetes.

No. 4

"Proliferative regulation of pancreatic polypeptide producing cells by the proglucagon gene"

Yuko Nakagawa[1], Yoshitaka Hayashi[2], Tadahiro Kitamura[3], and Yoshio Fujitani[1]
([1]Lab. of Developmental Biology and Metabolism, IMCR, Gunma University, [2]Division of Stress Adaptation and Protection, Research Institute of Environmental Medicine, Nagoya University, [3]Metabolic Signal Research Center, IMCR, Gunma University)

Endocrine cells are organized in islets of Langerhans comprising mainly four cell types, namely, α, β, δ, and pancreatic polypeptide (PP) cells, which produce the hormones glucagon, insulin, somatostatin, and PP, respectively, and contribute to blood glucose regulation. An imbalance of the production of these hormones may cause abnormal glucose metabolism and the development of diabetes. Thus, the amount of each cell type and the maintenance of their cell fates is thought to be strictly controlled, but the mechanisms involved remain unclear.
PP cells constitute only a few percent of islet endocrine cells, and their function has been largely unknown. Recently, we found that PP cells are the precursors of β cells during pancreas development, and we hypothesized that they may play an important role in maintaining homeostasis of pancreatic islet function. We found that the *proglucagon* gene negatively regulates the proliferation of PP cells, and further plays an important role in maintaining the fate of the four islet endocrine cell types. We are currently analyzing *proglucagon* gene-deficient mice, to clarify the regulatory mechanism of PP cell proliferation under conditions of disrupted glucagon signaling.

No. 5

"Physiological analysis of mammalian small GTPase Rab35"

Ikuko Maejima[1], Tomoko Akuzawa[1], Rika Hirai[1], Hisae Kobayashi[1], Inoya Isobe[1], Taichi Hara[1,2] and Ken Sato[1]
([1]Lab. of Molecular Traffic, IMCR, Gunma University, [2]Lab. of Cellular Regulation, Faculty of Human Sciences, Waseda University)

Rab35 is a member of small GTPase Rab protein family and plays an essential role in the recycling of protein cargos to the plasma membrane. Rab35 has been shown to regulate many cellular functions, including cytokinesis, cell migration, phagocytosis, immune synapse formation, exosome release, neurite outgrowth and neurotransmission release. However, its physiological functions in mammalian remain unclear. To reveal this, we generated Rab35 knockout mice. Rab35 knockout mice were embryonic lethal. We also generated brain-specific Rab35 knockout mice to analyze the roles of Rab35 in brain development and function.

No. 6

"Role of iron in adipogenic differentiation of 3T3-L1 cells"

Hiroshi Shibata[1], Tomohiro Suzuki[1], Kyoko Tanimura[2], Diana Vargas Trujillo[1], Tasuku Hirayama[3], Hideko Nagasawa[3], Takeshi Inagaki[1]

([1]Lab. of Epigenetics and Metabolism, IMCR, Gunma University, [2]Dept. of Diabetes, Endocrinology and Metabolism, Nippoin Medical School, [3]Lab. of Pharmaceutical & Medicinal Chemistry, Gifu Pharmaceutical University)

Adipogenesis is regulated by specific epigenetic factors including histone methylases/demethylases. Among them, jmjC domain-containing histone demethylases are Fe(II)/α-ketoglutarate-dependent dioxygenases, which requires Fe(II) ion as a co-factor. Here we investigated the role of iron in adipogenic differentiation of 3T3-L1 cells. Chelation of extracellular iron with deferoxamine (DFO) dose-dependently inhibited triglyceride accumulation at Day 8 of differentiation. To inhibit differentiation, DFO was necessary only during the first 48 hours after the induction of differentiation. DFO-induced inhibition was associated with reduced expression of PPARγ and C/EBPα at Day 2. In addition, DFO also inhibited the reduction of H3K27me3 staining in the nuclei at Day 2. Consistent with these findings, triglyceride accumulation at Day 8 was inhibited when cells were cultured in the iron-free defined medium for the first 48 hours after induction, which was rescued by the addition of iron-saturated transferrin. These results suggest that iron plays a critical role in the early period of adipogenic differentiation of 3T3-L1-cells.

No. 7

"Single cell imaging of ATP level reveals physiological importance of ATP homeostasis"

Masakatsu Takaine[1,2], Hiromi Imamura[3], Satoshi Yoshida[1,2,4]

([1]GIAR, Gunma University, [2]Lab. of Cellular Signaling, IMCR, Gunma University, [3]Graduate School of Biostudies, Kyoto University, [4]School of International Liberal Studies, Waseda University)

Adenosine triphosphate (ATP) is essential for many enzymatic processes, hence, cellular concentration of ATP should be tightly controlled. However little is understood about the physiological consequence of ATP homeostasis failure (ATP crisis). Using an ATP biosensor QUEEN, we found that cellular concentration of ATP is stably and homogenously maintained at a constant level in yeast cells regardless of the growth conditions. We identified several ATP homeostasis mutants that exhibit large heterogeneity in the ATP level within a clonal population. Live cell imaging revealed that the heterogeneity is due to cell autonomous fluctuation which often leads to transient depletion of ATP. The transient depletion of ATP can induce abnormal protein aggregation and the ATP homeostasis mutants accumulated aggregated proteins and exhibited hypersensitivity to proteotoxic stresses.
Our study reveals the physiological requirement of ATP homeostasis and provides a functional link between ATP homeostasis and proteostasis, suggesting a possible involvement of ATP crisis in deteriorating protein aggregation.

No. 8

"Epigenome editing: manipulating DNA methylation of specific DNA loci using CRISPR/Cas9 and a repeating peptide array-based amplification."

Sumiyo Morita

(Biosignal Genome Resource Center, IMCR, Gunma University)

DNA methylation, one of the most studied epigenetic modifications, regulates many biological processes. Dysregulation of DNA methylation is implicated in the etiology of several diseases, such as cancer and imprinting diseases. Accordingly, technologies designed to manipulate DNA methylation at specific loci are very important and many epigenome editing technologies have been developed, based on Zinc finger proteins, TALEs, and CRISPR-dCas9 targeting. We developed a method to induce and assess DNA demethylation on a target gene. It is based on a modification of the dCas9-SunTag system for efficient, targeted demethylation at specific DNA loci. The original SunTag system consists of ten copies of the GCN4 peptide separated by 5-amino-acid linkers. To achieve efficient recruitment of an anti-GCN4 scFv fused to the ten-eleven (TET) 1 hydroxylase, an enzyme that demethylates DNA, we changed the linker length to 22 amino acids. We show that the modified system can induce DNA demethylation efficiently in cell culture (embryonic stem cells, cancer cell lines, primary neural precursor cells) and *in vivo* in mouse fetuses, and that its application in vivo could lead to its future use in the clinic.

No. 9

"Efficient generation of conditional knockout mice via sequential introduction of loxP sites"

Takuro Horii, Ryohei Kobayashi, Eriko Suetomo, Yuika Kawada, Mika Kimura, Sumiyo Morita, Izuho Hatada
(Biosignal Genome Resource Center, IMCR, Gunma University)

There is a large demand for conditional knockout mice to analyze gene functions. The most commonly-used system for conditional knockout is Cre/loxP, which uses a site-specific Cre recombinase and its target sequence loxP with unique 34-bp sequences. In this system, a region of interest flanked by two loxP sites (flox) is deleted by Cre-mediated recombination, leading to gene knockout only in a Cre-expressing cell. CRISPR/Cas9 in combination with two sets of guide RNAs and a single-stranded oligonucleotide including a loxP sequence enables simultaneous insertion of two loxP sequences into fertilized embryos. However, this method induces double-strand breaks at two sites on the same chromosome, which causes an undesirable chromosomal deletion and reduces the flox rate. To solve this problem, we investigated a method that sequentially introduces each loxP sequence at the 1-cell and 2-cell embryonic stages, respectively. The sequential method was applied to both microinjection and electroporation systems. Sequential electroporation improved the flox efficiency compared with ordinary simultaneous microinjection, leading to a high yield of offspring with floxed alleles. Finally, we directly produced Cre/loxP mice containing both the Cre transgene and floxed allele via sequential electroporation using Cre zygotes, which accelerated the generation of conditional knockout mice compared with the ordinary method.

Reference

Horii T, *et al*. Efficient generation of conditional knockout mice via sequential introduction of lox sites. *Sci Rep*. 7:7891 (2017).

No. 10

"Melanophilin mediates insulin granule fusion without stable docking to the plasma membrane through interaction with open form of syntaxin-4"

Hao Wang[1], Kouichi Mizuno[1], Noriko Takahashi[2], Eri Kobayashi[1], Haruo Kasai[3], Katsuhide Okunishi[1] and Tetsuro Izumi[1]
([1]Lab. of Molecular Endocrinology and Metabolism, IMCR, Gunma University, [2]Department of Physiology, Kitasato University School of Medicine, [3]Laboratory of Structural Physiology, Center for Disease Biology and Integrative Medicine, Faculty of Medicine, The University of Tokyo)

Melanophilin/Exophilin3/Slac2-a is a Rab27a effector and makes the link between Rab27a and myosin-Va and allows the interaction of melanosomes with the actin network. Although the absence or dysfunction of melanophilin leads to abnormal pigmentation reflecting the dysfunction of lysosome-related organelles, phenotypes resulting from the defective exocytosis of endocrine secretory granules have not been reported. Here we show that melanophilin is expressed in pancreatic beta cells and promotes their exocytosis. Loss of melanophilin in melanophilin mutant (leaden) mice induces glucose intolerance and impaired glucose-stimulated insulin secretion. TIRF microscopy analysis shows that melanophilin specifically affects fusion events without stable docking to the plasma membrane. We newly find that melanophilin interacts with t-SNARE protein syntaxin-4 on the plasma membrane. Furthermore, in contrast to the wild type melanophilin, its mutant disrupting interaction with syntaxin-4 fails to rescue the decreased "undocked" granule exocytosis in leaden β cells. Because melanophilin binds with the fusion-competent, open-form of syntaxin-4, it seems that melanophilin mediates exocytosis of "undocked" granules recruited from the cell interior after the stimulation through its interaction with the open form of syntaxin-4.

No. 11

"Mutant p53 switches the fate of senescent RasV12 cells from apical elimination to tumor priming."

Yukinari Haraoka[1,2], Yuki Akieda[1], Tohru Ishitani[1,2]
([1]Lab. of Integrated Signaling System, Department of Molecular Medicine, IMCR, Gunma Univ., [2]MIB, Kyushu Univ.)

Tumor is generally believed to arise from a single oncogenic cell, which has the abnormal activities of oncogenes (e.g. Ras and Erbb2) or tumor-suppressor genes (e.g. p53 and APC). However, at the initial step of tumorigenesis, it is unclear how single oncogenic cells grow up to tumors in our bodies. In addition, although accumulation of multiple mutations in oncogenes and tumor-suppressor genes is thought to drive tumor formation, the detailed mechanisms of how accumulation of these mutations promotes the initial tumorigenesis are still poorly understood. Using zebrafish larval skin as a model of human epithelial tissue, we analyzed how multiple mutations affect the behaviors of a single oncogenic cell at the initial tumorigenic process. Here, we show that oncogenic Ras (RasV12) and p53 mutations cooperate to initiate tumorigenesis in zebrafish skin, while RasV12 mutation is insufficient to prime tumorigenesis. A single RasV12 cell became senescent and expressed matrix metalloproteinase 9 (MMP9), which would reduce the junctional integrity between RasV12 cell and their neighboring normal cells. Consequently, RasV12 cell left the larval skin. On the other hand, oncogenic cell with RasV12/p53-double mutation activated SASP (senescence-associated secretory phenotype)-like inflammation in neighboring tissue by inducing expressions of inflammatory cytokines (IL-1β and IL-11) and cyclooxygenase 2 (Cox2) and then formed heterogeneous tumor-like cell mass including surrounding normal cells. This RasV12/p53 cell-mediated tumor-like cell mass formation was blocked by IL-1β knockdown or Cox inhibitor treatment. Thus, we revealed new mechanisms by which p53 mutation switches senescent RasV12 cells to tumorigenic state.

No. 12

"Development of innovative wide-view mapping of synaptic ensemble in the psychiatric model"

Kisho Obi[1], Masayasu Sato[1], Junko Sugi[1] and Akiko Hayashi-Takagi[1]
([1]Lab of Medical Neuroscience, IMCR, Gunma University)

The dysregulation of dendritic spines, protrusions of neural connectivity, is thought to be involved in the pathophysiology of a variety of psychiatric disorders, but the links between spines and psychiatric disorders have been largely correlational because of lacks of a technique for manipulating individual spine. To overcome this problem, we developed a novel synaptic optoprobe, AS-PaRac1 (Activated Synapse targeting PhotoActivatable Rac1), which is unique not only because it can specifically label the recently potentiated spine, but can also selectively induce shrinkage in just those spines containing AS-PaRac1. This indicates AS-PaRac1 specifically visualizes the synaptic plasticity, which can be erased by blue light.
Here, we aim to visualize the potentiated neuronal circuits by simultaneous three colour imaging of an activity-dependent expression of the presynaptic marker Vamp2-mTurquoise2, the postsynaptic neuron markers tdTomato, together with AS-mClover as a potentiated synaptic marker.To identify the ideal probe with a proper temporal regulation, the selection of promoter and protein degradation sequences were extensively compared in the mice. We transduced various kinds of constructs into layer 2/3 pyramidal neurons in the V1 cortex. Two days after dark rearing, 2 hours light exposure was performed. Time-course of an activity-dependent tdTomato and mTq2 expression and subsequent transportation into ipsilateral V1 cortex were compared and we finished the selection of ideal probes. With the development and application of these tools, studies on synaptic ensemble will pioneer new discoveries, eventually leading to a comprehensive understanding of how the brain works.

No. 13

"Analysis of iron-dependent cell death ferroptosis in cancer cells"

Seiji Torii[1], Ryosuke Shintoku[1,2], Yuta Takigawa[3], Keiichi Yamada[4], Chisato Kubota[1], Yuhei Yoshimoto[2], Toshiyuki Takeuchi[1], Ichiro Koshiishi[3]

([1]Secretion Biology Lab, IMCR, [2]Department of Neurosurgery, Graduate School of Medicine, [3]Department of Laboratory Sciences, Graduate School of Health Sciences, [4]Department of Chemistry and Chemical Biology, Graduate School of Science and Technology, Gunma University)

The specific iron-dependent cell death is induced by a number of small compounds referred to as ferroptosis-inducers (FINs). During ferroptosis, inactivation of the glutathione peroxidase 4 (GPX4) causes accumulation of lipid reactive oxygen species that leads to cell death. A recent report suggested that a therapy-resistant state of cancer cells is characterized by vulnerability to ferroptosis induced by GPX4 inhibition. In this study, we examined the expression and dynamics of several lipoxygenases (LOXs) in oncogenic RAS-expressing cancer cell lines, and assessed the contribution of their lipid peroxidation activity to ferroptosis. Both specific inhibitors and siRNA-mediated knockdown of *ALOX15* significantly decreased FIN-induced cell death. Immunofluorescence analyses revealed that the ALOX15 protein constitutively localizes to the cell membrane during the course of ferroptosis. Importantly, treatments of cells with ALOX15-activating compounds accelerated cell death at low doses of FINs, indicating that the combined use of these drugs could be effective in cancer therapy. Our data suggest that increased production of lipid hydroperoxides in cellular membranes by LOXs can promote susceptibility to ferroptosis in cancer cells.

No. 14

"Progressive synapse deterioration in the DISC1 knockdown mice and its relationship with the pathophysiology of schizophrenia"

Fukutoshi Shirai and Akiko Hayashi-Takagi

([1]Lab. of Medical Neuroscience, IMCR, Gunma University)

Schizophrenia (SZ) is a devastating mental disorder, and genetic risk factors presumably induce the disruption of neuronal circuits. Nonetheless, the detailed cellular mechanism of how the genetic factors affect the neural circuits remain unknown. In the postmortem studies of SZ, the decrease in the dendritic spine, the majority of excitatory synapse site, have been reproducibly reported in the pyramidal neuron of prefrontal cortex (PFC), implicated in the cognitive impairment, the major and untreatable symptom of SZ. Consistently, in our previous study, the knockdown of DISC1, one of a strong candidate gene for SZ, exhibited the decrease in the spine density. In addition, the prominent phenotype that we noticed was the existence of the extremely huge spines in the pyramidal neuron of DISC1 KD mice. One theory of pyramidal neuron function posits that the neuron responds best to coincident activation of multiple synaptic inputs. In other words, the pyramidal neuron functions as a co-incident detector that integrates simultaneous inputs from hundreds synapse. However, recent studies proposed that the synaptic inputs from several big spines are enough to trigger action potential (AP), the theory of which would markedly changes the view of neuronal circuits. As such, the high frequency of huge spine in the DISC1 KD mice would also have a new interpretation of neural circuit malfunction of SZ. To address this new possibility, we introduced inducible DISC1 knockdown together with GCaMP6f, an ultrasensitive Ca2+ indicator, in the pyramidal neuron of PFC. By coordination with electrophysiology, correlation between the input from the huge spine and the generation of AP would be compared before and after the ablation of the huge spine. These strategies would enable us to elucidate the progressive synapse alteration and its relationship with the deregulated generation of AP, which would be a possible cause of the malfunction of neuronal circuits of SZ.

No. 15

"Tryptophan degradation by producing kynurenic acid in Saccharomyces cerevisiae"

Kazuto Ohashi

(Tenure track, Institute for Molecular and Cellular Regulation, Gunma University)

Kynurenic acid (KA) is a tryptophan (Trp) metabolite that is synthesized in a branch of kynurenine (KYN) pathway. KYN aminotransferase (KAT) catalyzes deamination of KYN, yielding KA. KA interacts with several receptors (NMDA receptor, GPR35, AHR nuclear receptor, etc) and sulfotransferases. Although KA is found in unicellular organisms, such as bacteria and yeasts, the cellular benefits of synthesizing KA are still unclear. In this study, we constructed a KAT-null yeast mutant defective in KA synthesis to clarify the cellular function of KA. Amino acid sequence analysis and LC/MS quantification of KA revealed that Aro8 and Aro9 are the major KATs. KA was significantly decreased in the *aro8Δ aro9Δ* double mutant. We found that *aro8Δ aro9Δ* cells did not exhibited no apparent defect in cell growth. We investigated whether *aro8Δ aro9Δ* cells were sensitive to several stresses, including 3 mM H_2O_2, 1 M NaCl, heat shock at 55 °C for 50 min, and cold stress at 15 °C, but these conditions caused minor growth defects in *aro8Δ aro9Δ* cells. Next, we focused on the role of KAT in the Trp degradation. We investigated whether excess Trp causes a problem in the growth of *aro8Δ aro9Δ* cells. As expected, *aro8Δ aro9Δ* cells showed significant growth defects with 10 mM Trp compared to wild type cells. However, the growth deficiency of *aro8Δ aro9Δ* cells by 10 mM Trp was not rescued by adding 10 mM KA. These data suggested that Aro8 and Aro9 activities, but not KA, are responsible for the tolerance to 10 mM Trp. In conclusion, we propose that KAT activity is required for detoxification of Trp by converting it to the less toxic KA.

No. 16

"Insulin-GDF3-ALK7 axis accelerates fat accumulation."

Yun Bu[1], Katsuhide Okunishi[1], Satomi Yogosawa[1], Kouichi Mizuno[1], Maria Johnson Irudayam[2], Chester W. Brown[2], Tetsuro Izumi[1]

([1]Lab. of Molecular Endocrinology & Metabolism, IMCR, Gunma University, [2]University of Tennessee Health Science Center)

We previously clarified that activin receptor-like kinase 7 (ALK7), one of the type I TGF-β receptors, is mainly expressed in mature adipocytes in white adipose tissues, and that ALK7 signaling increases fat mass via suppression of lipolysis. Although growth/differentiation factor 3 (GDF3) has been suggested to function as a ligand of ALK7 under excess nutrient and obese conditions, how is GDF3 produced and regulated still remains unclear. We find that GDF3 is mainly expressed in $CD11c^+$ adipose tissue macrophages (ATMs) in stroma-vascular fraction (SVF), and that depletion of ATMs by clodronate liposome enhances lipolysis and reduces fat accumulation in ALK7-intact obese mice, but not in their ALK7-deficient counterparts. Furthermore, a physiologically low level of insulin converts $CD11c^-$ ATMs into GDF3-producing $CD11c^+$ ATMs ex vivo and directs ALK7-dependent fat accumulation in vivo. Depletion of ATMs or transplantation of GDF3-deficient bone marrow counteracts the in vivo effects of insulin on both lipolysis and fat accumulation in ALK7-intact obese mice. These results reveal a novel mechanism by which insulin regulates both fat metabolism and mass via GDF3-ALK7 axis between ATMs and adipocytes.

No. 17

"Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice"

Yuhkoh Satouh[1,*], Kaori Nozawa[1,2], Masahito Ikawa[1,3]
([1]Research Institute for Microbial Diseases, Osaka University, Japan, [2]Baylor College of Medicine, Houston, USA, [3]The Institute of Medical Science, The University of Tokyo, Japan.
***present address: Laboratory of Molecular Traffic, Institute for Molecular and Cellular Regulation, Gunma University, Japan)**

In mammal, entry of sperm into oocytes triggers a series of changes of intracellular Ca^{2+} concentration, called Ca^{2+} oscillations, that initiate resumption of the meiotic cell cycle. In this study, we identified phospholipase C zeta 1 (PLCz1) in sperm head as the sperm-borne oocyte activation factor (SOAF). We generated Plcz1 knockout (KO) mice by CRISPR/Cas9 system, and found that the KO spermatozoa completely failed to induce Ca^{2+} changes in intracytoplasmic sperm injection (ICSI), but induced atypical patterns of Ca^{2+} changes in normal fertilizations. Furthermore, most of the oocytes fertilized by KO spermatozoa stop their development at the 1 cell stage because of oocyte activation failure or polyspermy. As a result of examination of two major systems for block of polyspermy, zona pellucida block to polyspermy (ZPBP) and plasma membrane block to polyspermy (PMBP), both of them were shown to delay in the oocytes fertilized by KO spermatozoa. We then discovered that polyspermy hardly occurs in astacin-like metalloendopeptidase KO female that lack ZPBP. Thus, we conclude that PMBP plays more critical role than ZPBP in vivo, and that the delay of PMBP is a cause of polyspermy by *Plcz1* KO. Finally, we obtained healthy pups from male mice carrying human infertile *PLCZ1* mutation by single sperm ICSI supplemented with *Plcz1* mRNA injection. These results suggest that PLCz1 is the long-sought SOAF that can ensure oocyte activation for monospermic fertilization, and further, that mammalian spermatozoa have (an) oocyte activation mechanism(s) independent from PLCz1.

No. 18

"Neuronal SIRT1 regulates simple sugar selection through FGF21 and oxytocin signalling in mice"

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Both intake amount and selection of diet influence body weight. We previously showed that in the hypothalamus controls body weight and food intake. We next assessed the role of SIRT1 in diet selection. We analyzed diet selection behavior in neuron-specific SIRT1 overexpression (NS-OE) and knockout (NS-KO) mice. The preference for high-sucrose diet was decreased in NS-OE and increased in NS-KO mice, while food intake was not affected in the absence of food choice. Hypothalamic expression analyses in these mice revealed that SIRT1 increased *oxytocin* (Oxt) expression. Oxt receptor antagonist rescued the decreased sucrose preference in NS-OE mice. Therefore, we generated Oxt neuron-specific SIRT1 overexpression (OS-OE) and knockout (OS-KO) mice and measured their sucrose selection behavior. Sucrose preference was decreased in OS-OE and increased in OS-KO mice, and Oxt receptor antagonist rescued the decreased sucrose preference in OS-OE mice. Therefore, SIRT1 in Oxt neurons is sufficient for regulating sucrose selection behavior. Next, we manipulated SIRT1 expression levels in hypothalamic cell lines, and found that SIRT1 up-regulates *Oxt* expression through -2.4~-2.1kb region of the *Oxt* promoter. We also found that FGF21, which is secreted from the liver upon simple sugar intake and suppresses simple sugar intake by acting on the hypothalamus, also promotes *Oxt* gene expression. We found that β-klotho, a co-receptor for FGF21, is expressed in hypothalamic Oxt neurons in mice, and SIRT1 up-regulated *β-klotho* gene expression in hypothalamic cells. Taken together, these data suggest that SIRT1 suppresses simple sugar selection by potentiating the negative feedback by FGF21 via Oxt.

No. 19

"SGLT1 in pancreatic α cells regulates glucagon secretion in mice, possibly explaining the distinct effects of SGLT2 inhibitors on plasma glucagon levels"

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It is controversial whether sodium glucose cotransporter (SGLT) 2 inhibitors increase plasma glucagon levels *in vivo*. Here, we showed that dapagliflozin, but not canagliflozin, increased plasma glucagon levels in mice fed a high-fat, high-sucrose diet (HFHSD) and diabetic *db*/*db* mice. A glucose clamp study revealed that the plasma glucagon increase associated with dapagliflozin could be explained as a response to acute declines in blood glucose. SGLT1 and glucose transporter 1 (GLUT1), but not SGLT2, were expressed in αTC1 cells and mouse islets. We showed that canagliflozin suppressed glucagon secretion by inhibiting SGLT1 in α cells; consequently, plasma glucagon did not increase with canagliflozin, even though blood glucose declined. We showed that the SGLT1 effect on glucagon secretion depended on glucose transport, but not glucose metabolism. Islets from HFHSD and *db*/*db* mice displayed higher SGLT1 expression and lower GLUT1 expression than the islets from control mice. These expression levels were associated with glucagon secretion levels. Furthermore, SGLT1 inhibitor and siRNA against SGLT1 suppressed glucagon secretion in isolated islets. These data suggested that a novel mechanism regulated glucagon secretion, through SGLT1, in α cells. This finding explained the distinct effects of dapagliflozin and canagliflozin on plasma glucagon levels in mice.

No. 20

"How cellular senescence causes individual aging?"

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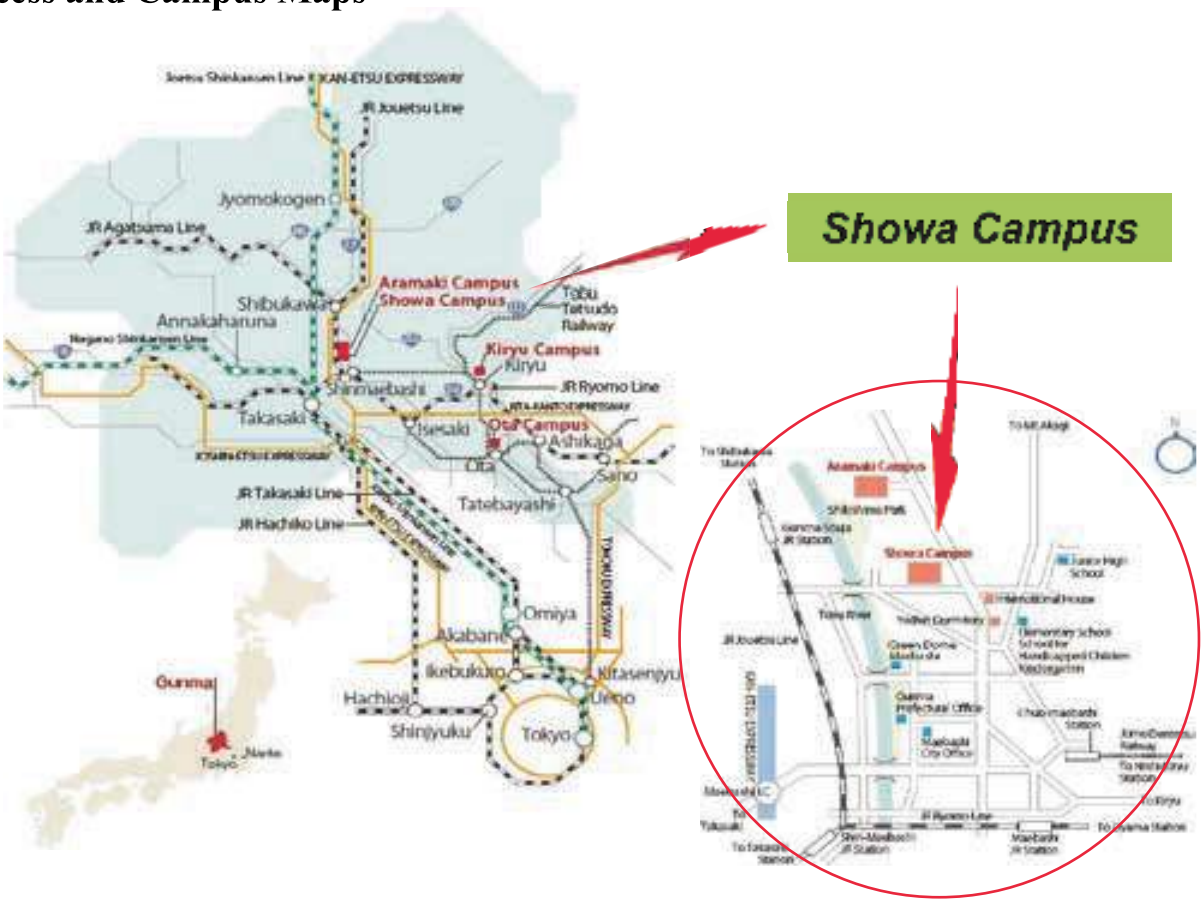
Many aging studies focus on molecular mechanisms of cell level senescence because it is assumed that cellular senescence plays a central role in systemic aging. However, it is still unclear how cellular senescence causes tissue level aging and finally systemic aging because aging study using mammal takes long time. Therefore, to clarify this question, we just started using African turquoise killifish *(Nothobranchius furzeri)* as a new model of aging research. The African turquoise killifish has an extremely short lifespan of minimal 3 months and shows the aging phenotype often seen in other organisms. And the fish model is suitable for real-time imaging of cellular event. Therefore, we take advantage of these features and are trying to create senescent cell reporter fish. We already revealed that expression level of *p15* and *p21* that are known as cellular aging marker are increased during aging prosses. Thus, we are making CRISPR/Cas9-mediated knock-in fish to visualize the expressions of *p15* and *p21*. By using these fish, the localization of aged cells in the living fish can be visualized and how cellular senescence promotes systemic aging can be observed. Furthermore, we also focus on Wnt signaling because Wnt signaling is known to contribute to individual aging. Therefore, we try to make clear the relationship between cellular senescence and individual aging by using Wnt reporter fish. In this meeting, we would like to discuss aging research that exploits the characteristics of African turquoise killifish and my progress about that.

Access and Campus Maps



By JR	Take the Joetsu Sinkansen Line train to Takasaki. From there about 30 min by taxi. Alternatively, change at Takasaki to the Ryomo Line and go to Maebashi Station. From Maebashi station about 4 km in the north direction. About 15 min by bus or 10 min by taxi. Or take the Joetsu Line train at Takasaki station to Shin-Maebashi station. From there north 5 km about 10 min by taxi.
By car	Take the Kan-Etsu Tollway to Maebashi Interchange. From there about 15 min on the ordinary road.

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