# The 13th Symposium of Japan Consortium for Glycobiology and Glycotechonology

## Glycoscience in Next-Generation Life Science

**October 19-20, 2015 at Winc Aichi (Nagoya)**

## Program October 19 (Monday), 2015

**Greetings from JCGG President**
**Naoyuki Taniguchi (RIKEN)**

**Opening Address of organizer**
**Koichi Kato (Okazaki Institute for Integrative Bioscience)**

### Session 1 Dynamic Ordering in Biomolecular Systems (1)

**Chair : Yoshiki Yamaguchi (RIKEN)**

**Exploration, creation, and development of dynamic ordering of biomolecular systems**
**Koichi Kato (Okazaki Institute for Integrative Bioscience)**

Living systems are characterized as dynamic processes of assembly and disassembly of various biomolecules that exhibit significant internal motions, as best exemplified by carbohydrate chains and their clusters. In order to elucidate the mechanisms underlying dynamic biomolecular organization, we are developing physicochemical measurement methods for exploring micro-macro relationships in the dynamic ordering of biomolecular systems with well-designed instability and robustness. This facilitates a better understanding of the principles governing their space-time evolution coupled with the development of their integrated functions. In this symposium, I will present a structural view of the dynamic processes of biomolecular organization, exemplified by the formation of the proteasome, a huge proteolytic machine in cells. The proteasome assembly is not a spontaneous self-organization but a chaperone-assisted ordered process. By an integrative biophysical approach including crystallography, solution scattering, and various spectroscopic methods, we characterized the chaperone-mediated arrangements of proteasome subunits and quaternary structures of proteasome assembly intermediates. Furthermore, our multidisciplinary research project, by integrating biology, chemistry, and physics, attempts to provide insights into the creation of artificial self-organizing systems that embody the design rules of the biomolecular

orchestration.

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## Structural analysis by electron microscopy from molecular to cell - Visualization of phage adsorption process to host cell -

Kazuyoshi Murata (National Institute for Physiological Science, NINS)

Cryo-electron tomography (cryoET) is a powerful tool to analyze a structure of the cell at molecular level. Here, we combine cryoET with a single particle analysis method to visualize the adsorption process of the bacteriophage to the host cell and the following conformational changes of the phage itself during this process.

P-SSP7 is a marine cyanophage that infects a photosynthetic cyanobacterium, *Prochlorococcus* MED4 living in the ocean. Though the molecular structure of P-SSP7 has been well characterized, its interaction with the host cell remains poorly defined. We use the new approach to visualize the structural snapshots during the process of phage adsorption to the host cell.

In the adsorption process, the phage tails adopt different angular inclinations to the cell surface. At the presumed moment of the DNA injection, the tail is normal to the cell surface and the majority of the tail fibers assume an extended conformation, whereas the tail fibers are in the folded conformation in the non-infecting states.

This study opens a new research field where we could analyze a sequential biological process at molecular level happening around the cell using cryo-ET and quick freezing technique in time course.

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## Dynamics of exo-type cellulases explored by single-molecule imaging analysis

**Ryota Iino (Institute for Molecular Science, NINS)**

Cel7 and Cel6 are cellulases that directly hydrolyze crystalline celluloses into water-soluble cellobioses. They have drawn attention as a tool that could be used to convert cellulosic materials into biofuels. However, detailed mechanisms of action, including elementary reaction steps such as binding, processive hydrolysis, and dissociation, have not been thoroughly explored because of the inherent challenges associated with monitoring reactions occurring at the solid/liquid interface. By applying single-molecule imaging with super-resolution fluorescence microscopy and high-speed atomic force microscopy, we have successfully measured and compared the kinetic parameters for elementary reaction steps of Cel7 and Cel6 from *Trichoderma reesei* (TrCel7A and TrCel6A) and *Cellulomonas fimi* (CfC el6B). In this talk, we will discuss origin of different susceptibilities of cellulose 10 and III, against TrCel7A, and structural basis of determinants of kinetic parameters for TrCel6A and CfCel6B by comparing their crystal structures.

Reference

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## Industry-Academia Joint Seminar (sponsored by Promega Corporation)

## Bioluminescent glycosyltransferase assays: New nucleotide detection tools for glycobiology research

**Hicham Zegzouti (Promega Co.)**

Glycosyltransferases (GTs) play a pivotal role in many biological processes, including cell-cell interactions, cell signaling and bacterial cell wall biosynthesis. Many GTs attracted the interest of scientists in diverse therapeutic areas, including diabetes, inflammation, infectious diseases and lysosomal storage diseases. Because of the importance of this class of enzymes, there is a need for biochemical assays to monitor their activity, their mode of regulation, and to search for selective and potent inhibitors. Activity based assays have been utilized successfully in the discovery of small-molecule inhibitors for many classes of enzymes like kinases or other drug targets. However, monitoring glycosylation reactions was hampered by the lack of robust non-radiometric assays. Traditional assays for GT activity are not easily configured for rapid GT activity detection nor for high throughput screening

because they rely on detection of radiolabeled substrate which requires product isolation, the use of non-homogenous antibody based assays or mass spectrometry. In a typical Glycosyltransferase reaction, after sugar transfer from the donor nucleotide sugar substrate, the nucleotide moiety is released as the reaction product. Therefore, an assay that detects the nucleotide molecule could be generically used to assess all Glycosyltransferases activity in vitro. We developed three homogenous bioluminescent assays for measuring Glycosyltransferase activities based on UDP, GDP, CMP and UMP detection. Each of these assays is performed in one-step detection that relies on converting simultaneously any of these nucleotide products to ATP, then to light in a robust luciferase reaction. The light output is proportional to the nucleotide concentration produced ranging from low nM to 2SμM. These assays are highly sensitive, robust, and resistant to chemical interference; three features that are highly desirable and essential for monitoring the activity of the majority of GT classes and screening for their modulators. Because of its versatility, this nucleotide assay platform can be used with GTs that are tagged, native, pure or affinity bound to beads. Examples of various applications of these nucleotide detection assays (UDP-Glo, GDP- Glo and CMP/UMP-Glo) will be presented, including studies on specificity of transfer of different sugars to different acceptors by diverse GTs. We will show their utility in screening for specific GT inhibitors and the study of their mode of action. The development of the UDP, GDP, CMP and UMP detection assays will enable the investigation of a large number of GTs and PGTs and may have significant impact on diverse areas of Glycobiology research.

### A novel assay system for glycosyltransferase activity and its application for high-throughput analysis

**Yasuhiko Kizuka (RIKEN)**

Reference
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## JCGG Luncheon Seminar (sponsored by Nihon Waters Corporation)

### Multi-dimensional structural analysis of antibody drug by mass spectrometry

**Susumu Uchiyama (Osaka University)**

Therapeutic proteins such as antibodies are susceptible to different levels of structural changes. Whereas, mass spectrometry is a powerful and effective method for the proper and precise monitoring of structural alternations of antibody drugs. We found cysteine residue located at the hinge region of IgG1 is racemized during storage at neutral pH condition. We also found histidine residue located at the CH2

region of IgG1 is oxidized under a light stressed condition. Because these changes in the primary structure have possibility to result in the cause of adverse effect, our findings alert researchers to properly monitor changes of the primary structures including above modifications. Structure analysis by mass spectrometry at a higher level can be achieved by hydrogen/deuterium exchange mass spectrometry (HDX-MS). I'll introduce examples of HDX-MS where higher-order structure change during storage was properly analyzed using Waters HDX system. Finally, as a future prospect, it will be important to conduct researches to quantitatively reveal the relation among structure, interaction, biological activities and adverse effect.

References
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## Session 2　Dynamic Ordering in Biomolecular Systems (2)

**Chair : Koichi Kato (Okazaki Institute for Integrative Bioscience)**

### Clustering and functions of cell surface receptor proteins

**Yasushi Sako (RIKEN)**

Receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) are the two major super-families of cell surface receptors and important targets of current medical drugs. We are analyzing behaviors of EGF receptor (EGFR), one of the RTKs, and metabotropic glutamate receptor (mGluR), one of the GPCRs, using single-molecule imaging in living cells. EGFR and mGluR tagged with fluorescent proteins can be visualized as a single-molecule on the surface of living cells using fluorescence microscopy to detect their movements, oligomerizations, and interactions with the ligands and cytoplasmic proteins. EGFR is known to dimerize after association with EGF for its activation. In our observation, EGFR formed various sizes of oligomers before EGF association. These oligomerizations were highly dynamic. EGF induced oligomerization and immobilization of EGFR molecules. Oligomers of EGFR were the major sits of signal transduction to a cytoplasmic protein, Grb2. mGluR, which forms constitutive dimers, also formed higher-order

oligomers up to octamer. An agonist for mGluR induced an increase of slow-mobile receptors in higher oligomers that led to endocytosis from the coated pits. Thus, importance of higher-order oligomerization has been revealed for the function of both receptor species.

## Spatiotemporal control of transport vesicle formation from the endoplasmic reticulum

Ken Sato (The University of Tokyo)

The secretory and endocytic pathways in eukaryotic cells act as major routes for protein and lipid transport out of and into the cell. Transport from one organelle of these pathways to another is mediated by vesicular carriers, which are generate by coat protein complexes regulated by the small GTPases. The endoplasmic reticulum (ER) is the starting point for the secretory pathway, where newly synthesized proteins are concentrated at specialized ER exit sites. In these distinct zones of the ER, the coat protein complex II (COPII} and the small GTPase Sar1 generate COPII vesicles through a sequence of events under the control of multiple regulatory mechanisms. The essential and evolutionally conserved component Sec16 has been described as the organizer of ER exit sites. Although COPII vesicle formation and the organization of ER exit sites are clearly related, the molecular basis for a COPII organizing activity of Sec16 has remained a long-standing question. Our recent results provide the evidence that the Sec16 serves to fine-tune Sar1 activity during COPII coat assembly and ER exit sites organization. From these and other findings, I will discuss how the COPII machinery along with Sec16 is involved in ER exit sites formation.

## Development and application of siRNA modified with acyclic artificial nucleic acid

Yukiko Kamiya (Nagoya University)

RNA interference (RNAi) is an endogenous gene silencing system that has been utilized to inhibit expression of specific genes through the introduction of short double-stranded RNAs, called small interfering RNAs (siRNAs) in cells. siRNA is expected to be a clinical agents due to its remarkable gene-silencing ability. However, unmodified siRNAs have serious problems including low resistance to nuclease degradation, off-target effect and difficulty of delivery to target cells for practical application. Chemical modification is one of the solutions to improve their performance. Our group has been developed artificial nucleic acid, serinol nucleic acid (SNA), in which ribose backbone is substituted by acyclic backbone. We also developed a method for modification of oligonucleotide by functional molecules through D-threoninol scaffold (TN). In this presentation, we will introduce the

modified siRNAs for improving the performance and for investigation of their intracellular trafficking.

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## Dynamic ordering and biologically-relevant functions of well-defined sugar clusters

Sota Sato (Tohoku University)

Sugar clusters on cellular membranes play essential roles in biological systems, where very weak recognition ability of each monomeric sugar unit is dramatically enhanced by the accumulation to show cooperative effect: typical examples are a series of gangliosides, sialylated glycosphingolipids as acceptors for bacterial toxins and viruses. Ganglioside GM1 clusters are especially important because they recognize amyloidogenic proteins and trigger the formation of amyloid fibrils, which are the cause of Alzheimer's disease. In the present work, we synthesized a cluster of the GM1 pentasaccharide through the chemical functionalization of a self-assembled metal-organic spherical complex with the biological systems, where very weak recognition ability of composition of 36 building units, i.e., 12 metal ions and 24 bidentate ligands. A discrete, well-defined sphere bearing just 24 GM1 penta-saccharides was synthesized in an efficient synthetic route, to show the recognition abilities toward amyloid $\beta$ and $\alpha$-synuclein. We designed the cluster structure to lack hydrophobic lipid moieties, which otherwise would stably trap the intermediary species of the target proteins, and the artificial cluster enabled NMR spectroscopic observation of the early encounter stage of the protein-sugar cluster interactions, which has not been found in previous works.

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## Session 3　Biochemical Aspects of Field-Formation and Functions

Chair : Koichi Furukawa (Chubu University)

## Roles of membrane microdomain-localized glycans in fertilization

## Ken Kitajima (Nagoya University)

Lipid rafts or membrane microdomains on the cell surface are recognized as hotspots of cellular interactions and signal transductions. One of unique features of rafts is an enrichment of particular glycan chains attached on glycoproteins and glycolipids. Few studies, however, have focused on those raft-localized glycan chains. In 1999, we first reported biochemical characterization of sperm rafts using sea urchin, and have demonstrated the importance of the raft-localized glycans during fertilization. One of the raft-localized proteins is flagellasialin, which is a GPI-anchored protein that contains unique $\alpha$2,9-linked polysialic acid chains (80-90% by weight) attached on a short polypeptide. In 2006, we showed that flagellasialin was related with intracellular $Ca^{2+}$ regulation and sperm motility. Although $Ca^{2+}$ regulation is an important physiological event in sperm, an involvement of such a heavily glycosylated protein has never been reported. Therefore, we asked if this type of glycoprotein ubiquitously occurred in other animals. We have so far found a highly glycosylated protein on lipid rafts of pig and chicken sperm, suggesting that a raft-localized, glycan-enriched GPI-anchored protein involved in intracellular $Ca^{2+}$ regulation is commonly present in various animal sperm.

## Spatial distribution and function of Wnt proteins regulated by specifically modified heparan sulfate proteoglycans

## Shinji Takada (Okazaki Institute for Integrative Bioscience)

Secreted signal proteins, like Wnt, BMP, and FGF, are generally considered to distribute within a tissue by forming a concentration gradient, but it still remains unclear whether these proteins also do so around a single cell. Since plasma membrane of a sing cell is rather heterogeneous in terms of lipid composition and protein localization, it seems probable that the distribution of signal proteins is not uniform around a cell. To directly observe the distribution of the signal protein in tissues and embryos, we generated antibodies specifically available for detection of endogenous Wnt proteins in paraffin sections and whole mount embryos. We found that Wnt8 is actually distributed in a gradient fashion within Xenopus embryo, but also locally accumulated by forming puncta around a cell. Of note, heparan sulfate proteoglycans (HSPG) are also localized in a punctate manner, especially N-sulfated form of HSPG is highly co-localized with Wnt8. Gain- and loss-of-function of enzyme, which is responsible for this N-sulfation, N-deacetylase/N-sulfotransferase 1, indicated that N-sulfation of HSPG is necessary and sufficient for local accumulation of N-sulfated HSPG and Wnt8 around a cell. I will also discuss the biological significance of this HSPG-mediated localization of Wnt proteins.

## Calcium dynamics in organogenesis

Naoto Ueno (National Institute for Basic Biology)

During development of central nervous system (CNS) which eventually forms brain and spinal cord, progenitor cells undergo a drastic cell shape change, called apical constriction, which triggers the neuroepithelial sheet (neural plate) to form tubular structure. Yet how the apical constriction is controlled in the neural plate and contributes to the tissue morphogenesis are not fully understood. In this study, we show that intracellular calcium ion ($Ca^{2+}$) is required for the neural tube formation, and its concentration fluctuates throughout the Xenopus neural plate at the single-cell level. The $Ca^{2+}$ fluctuation preceded a remodeling of F-actin and the apical constriction at the cellular level, and repeated accelerations of the closing movement of the neural plate, suggesting that the $Ca^{2+}$ fluctuation at the cellular level dynamically regulates the apical constriction for the efficient tube formation. In silico analysis predicts that the $Ca^{2+}$ fluctuation accelerates apical constriction and decreases tissue size independently of its frequency. However, although the dense rather than sparse fluctuation induces rapid tissue deformation, its overall effect throughout the entire course of the simulation is not effective when the total number of the pulses is constant.

References
1) Suzuki M et al.: Dev Growth Differ 54, 266-76, 2012
2) Morita H et al.: Development 139, 1417-26, 2012
3) Suzuki M et al.: Development 137, 2329-39, 2010

## Alteration in lipid composition of synaptic plasma membranes responsible for region-specific amyloid deposition in human brain

Katsuhiko Yanagisawa (National Center for Geriatrics and Gerontology)

Deposition of amyloid β-protein (AB) (amyloid fibrils) is a crucial event of Alzheimer's disease (AD). Amyloid deposition emerges in distinct brain regions; however, the mechanism underlying the region specificity remains unknown. We previously performed analyses of human brains with early pathological changes of AD and discovered unique Aβ species characterized by its binding to GM1 ganglioside. Based on characteristics of GM1-ganglioside-bound Aβ (GAβ), we hypothesized that GAβ acts as a seed for Aβ assembly in brain. To elucidate an issue of region specificity of amyloid deposition, we studied two regions of human cerebral cortex; precuneus and calcarine cortex, one of the most vulnerable and one of the most resistant regions to amyloid deposition, respectively. In this study, we show that lipids extracted from synaptic plasma membranes isolated from the amyloid-bearing precuneus, but neither the amyloid-free precuneus nor the calcarine cortex, markedly accelerate the Aβ assembly *in vitro*. Furthermore, through

liquid chromatography-mass spectrometry of the lipids, we identified an increase in the ratio of the level of GD1b-ganglioside containing C20:0 fatty acid to that containing Cl8:0 as a cause of the accelerated Aβ assembly in the precuneus. This study suggests that the local glycolipid environment plays a critical role in region-specific amyloid deposition.

References
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## Special Lecture 1

### Chair : Koichi Kato (Okazaki Institute for Integrative Bioscience)

### Innate lymphoid cells and inflammation

Shigeo Koyasu (RIKEN)

The type 2 immune response, characterized by the production of IL-4, IL-5 and IL-13, is a critical immune response against helminths invading cutaneous or mucosal sites. In addition, type 2 immune responses are involved in the patho-physiology of various allergic diseases including asthma. We have identified a previously unidentified lymphocyte population producing large amounts of type 2 cytokines, which we named Natural helper (NH) cells and are now a member of group 2 innate lymphoid cells (ILC2s). We identified ILC2s in lymphoid clusters in adipose tissues, which we termed fat-associated lymphoid cluster (FALC). Stimulation by IL-33 or helminth infection activates ILC2s to produce large amounts of IL-5 and IL-13, which induce eosinophilia and goblet cell hyperplasia, both of which play an important role in anti-helminth immunity and pathophysiology of allergic diseases. We have recently found that ILC2s are involved in the steroid resistance of lung inflammation through Stats activation by TSLP, IL-7 or IL-2. We also found that IFNγ and IL-27 suppress tissue-resident ILC2s that expand *in situ* without migration during helminth infection and fungi infection in a STAT1-dependent manner. Our results suggest that IFNγ-and IL-27-mediated suppression of tissue-resident ILC2s is a negative feedback mechanism for ILC2 functions *in vivo*.

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## Program October 20 (Tuesday), 2015)

### Session 4 Synaptic Plasticity and Neuron Network Tuned Up by Glycans
**Chair : Shogo Oka (Kyoto University)**

### Prenatal stress causes loss of GABAergic interneurons and perineuronal nets in the cerebral cortex of postnatal mice
**Atsuo Fukuda (Hamamatsu University School of Medicine)**

Exposure to prenatal stress and mutations in GAD67 gene are risk factors for psychiatric disorders, while decrement of PY-positive GABAergic interneurons in the mPFC is often observed[1)]. However, the relationship between these factors remains unclear. So we examined GAD67-GFP knock-in mice underwent prenatal stress and monitored PV neurons to address an interaction between GAD67 disruption and stress. Neurogenesis of GABA neurons in the MGE was decreased and the density of PV-positive, but not PV-negative, GABAergic neurons was significantly decreased in the mPFC of postnatal heterozygotes. By contrast, these findings were not observed in wild type offspring, suggesting the double hits by these factors disturb the

neurogenesis of those destined to be PV-positive GABAergic interneurons[2]. In PV neurons, PNN is well developed and plays pivotal roles. Then, we evaluated PNN by using WFA staining and anti-aggrecan antibody. In prenatally stressed heterozygotes, both PV(+)WFA(+)/aggrecan(+) and PV(+)WFA(-)/aggrecan(-) cells were significantly decreased, while both WFA/aggrecan intensities and PV(-)WFA(+)/aggrecan(+) cell populations were unchanged. Then to further elucidate the rationale for compromised GABAergic neurogenesis, we performed microarray analysis for DNA methylation. Among multiple genes, Fktn responsible for Fukuyama type congenital muscular dystrophy was hypermethylated. Deletion of Fktn decreases the laminin and the perlecan bindings to and glycosylation of α-dystroglycan, causing neurogenesis and migration disorders[3]. Thus modification of Fktn expression might underlie the phenotypes demonstrated above.

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## Proteoglycans modulate functions of axon guidance protein, Draxin

Yohei Shinmyo (The University of Tokyo)

The mammalian neocortex is a complex six-layered structure that contains distinct populations of projection neurons in different layers. We previously showed that a chemorepulsive protein, draxin, is required for the midline crossing of corpus callosum axons in the neocortex. Here, we report that draxin has a critical role for the projections of thalamocortical axons. The thalamocortical tract carries sensory information to the neocortex. It has long been recognized that neocortical pioneer axons from subplate neurons are essential for the guidance of thalamocortical projections. However, the molecular mechanisms underlying interactions between neocortical pioneer axons and thalamocortical axons remain unclear. Thalamocortical axons in *draxin-/-* mice normally extend into the internal capsule, but then majority of them cannot enter to the neocortex. draxin is strongly expressed in early-born neocortical neurons and weakly expressed in the ventral telencephalon and thalamus. The thalamocortical phenotype in *draxin-/-* mice is rescued by the transgenic expression of draxin in the neocortex, suggesting that draxin expression

in neocortical neurons is critical for thalamocortical projections. In addition, we found the possibility that proteoglycans modulate draxin activity for thalamic axons. Thus, we propose that draxin from the neocortical neurons modulated by proteoglycans may be essential for proper guidance of thalamocortical axons.

References
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## Involvement of various types of glycosylation in neuronal migration during the development of cerebral cortex

Takeshi Kawauchi (Keio University)

Multi-step modes of neuronal migration play crucial roles in brain development and function. We have previously reported that Cdk5-p27(kipl)-Actin and JNK-MAP1B-Microtubule pathways are required for the morphological changes of migrating neurons in the developing cerebral cortex. We also found that Rab5- and Rab11-dependent endocytic recycling regulates the locomotion mode of neuronal migration that covers most part of neuronal migration path. However, how extracellular stimuli regulate neuronal migration via these cellular events is unclear. Here we show that the endocytosis from the GM1-rich membrane domain is required for the morphogenesis of multipolar neurons that occurs before the locomotion mode. In addition, the GM1-rich domain-mediated endocytosis is regulated by Rab21, a Rab5 subfamily member, rather than Rab5, suggesting that Rab5 and Rab21 differentially control neuronal migration. In addition to the involvement of the endocytosis from a specific glycolipid-rich membrane domain, our data suggest that a heparin-sulfate proteoglycan and specific N-glycosylation are involved in the early and final phases of neuronal migration, respectively. Taken together, these data suggest that various types of glycosylation have important roles in the multi-step modes of neuronal migration during the development of cerebral cortex.

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## Physiological function of chondroitin sulfate proteoglycan of receptor of receptor-type tyrosine phosphatase zeta (PTPRZ) as a potential drug target

Akihiro Fujikawa (National Institute for Basic Biology)

Protein tyrosine phosphatase receptor type Z (PTPRZ) is the sole member, that is known to be expressed as chondroitin sulfate proteoglycan, in the receptor-type protein tyrosine phosphatase superfamily. Very recently, we found that after severe demyelination and axonal damage were induced in the corpus callosum by cuprizone feeding, remyelination in the lesioned area was significantly accelerated in *Ptprz*-deficient mice as compared with wild-type mice. After demyelination, the expression of pleiotrophin (PTN), an inhibitory ligand for PTPRZ, was induced in both mouse brains, particularly in the neurons involved, suggesting its role in promoting remyelination through inactivation of PTPRZ. In support of this view, oligodendrocyte differentiation was augmented in oligodendrocyte-precursor cells (OPCs) from wild-type mice in response to PTN in a primary culture. In contrast, corresponding cells from *Ptprz*-deficient mice showed higher oligodendrocyte differentiation without PTN, and the differentiation was not enhanced by PTN. Thus, PTPRZ inactivation in OPCs by PTN, which is secreted from demyelinated axons, may be the mechanism responsible for oligodendrocyte differentiation during reparative demyelination in the CNS. Our findings provide a new concept that selective inhibition of PTPRZ may represent a promising approach for multiple sclerosis (MS) treatment, a progressive neurological disorder associated with myelin destruction and neurodegeneration.

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## JCGG Luncheon Seminar (sponsored by Sysmex Corporation)

### Glycobiology of silkworm (Bmbyx mori) toward glyco-engineering

Kazuhito Fujiyama (Osaka University)

Silkworm (*Bombyx mori*) has been developed for recombinant protein production. Glycan part of the protein plays a critical role for the expression of biological activity. However, protein glycosylation depends on machineries of the host cells. The glycosylation potential in silkworm was examined. The glycan profiles of glycoproteins were determined using larvae and pupa, and some organs in larvae stage. Genes of glycosylation enzymes were also shown over the silkworm genome database. Based on this platform information, glycoengineering was applied to silkworms. This challenge developed reduction of enzyme activity by gene knock-down and creation of sialylated glycans in silkworms. These development of glycoengineering would bring small silkworms to be the promising production system for pharmaceutical protein production. Silk-industry is Japanese-traditional, but Silkbiofactory can be "renaissance".

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2) Nomura T et al.: J Biosci Bioeng 119, 131, 2015
3) Kajiura H et al.: Glycobiology in press

## Special Lecture 2

### Chair : Kenji Kadomatsu (Nagoya University)

### Cellular signaling underlying higher grain functions including emotion and memory

Kozo Kaibuchi (Nagoya University)

Dopamine (DA)-type 1 receptor (D1R) signaling in the striatum regulates neuronal excitability and reward-related behaviors presumably through PKA. However, whether and how DlR and PKA regulate neuronal excitability and behavior remains largely unknown. Here, we developed a kinase-oriented phospho-proteomic analysis method to identify known and novel PKA substrates downstream of D1R and obtained more than one hundred candidate substrates including Rap1 GEF (Rasgrp2). We found that PKA phosphorylation of Rasgrp2 activated its guanine nucleotide exchange activity on Rap 1. Cocaine exposure activated Rap1 in nucleus accumbens in mice. The expression of constitutively active Rap1 in accumbal D1R-expressing medium spiny neurons (D1R-MSNs) enhanced the firing rates and the

behavioral responses to cocaine exposure. We here discuss a novel DA-PKA-Rap1 intracellular signaling mechanism in D1R-MSNs which increases neuronal excitability to enhance reward-related behaviors.

Reference
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## Session 5　Glial Cell Communication and Glycobiology

**Chairperson : Kazuhiro Ikenaka (National Institute for Physiological Sciences)**

### A role of brain specific branched O-mannose on glia assembly

**Shinobu Kitazume (RIKEN)**

In this symposium, I would like to show how we were involved in brain biology and proceeded to study the role of branched O-mannosyl glycan using brain specific glycosyltransferase GnT-IX deficient mice.

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### Sulfated N-glycans regulate myelination in the peripheral nervous system

**Takeshi Yoshimura (National Institute for Physiological Sciences)**

Myelin is a multilamellar, tightly compacted membrane that surrounds axons in the peripheral nervous system (PNS) and central nervous system (CNS). Because glycoproteins are prominent components of plasma membranes, a growing number of glycoproteins have been identified and characterized in myelin. However, roles of carbohydrate chains on glycoproteins in myelin remain unknown. Here, we report that sulfated N-glycans are involved in PNS myelination. PNS myelin contained highly abundant anionic N-glycans, especially sulfated N-glycans, on glycoproteins in pigs and mice, as compared with CNS myelin. Major sulfated N-glycans in porcine and mouse PNS myelin were identified, and revealed that the sulfation at the 6-O-GlcNAc position on glycoproteins was highly conserved in PNS myelin between these species. P0 protein, the most abundant glycoprotein involved in PNS myelin compaction, had 6-0-sulfated N-glycans abundantly. Only the expression of N-acetylglucosamine 6-0-sulfotransferase-l (GlcNAc6ST-l) was detected in mouse PNS and its deficiency led to the lack of 6-0-sulfated N-glycans in PNS myelin. Further, GlcNAc6ST-1

deficiency in mice caused hypomyelination and axon degeneration in the PNS. Thus, GlcNAc6ST-1 regulates PNS myelination through the 6-O-sulfation of N-glycans on glycoproteins.

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3) Torii T, Yoshimura T, Narumi M, Hitoshi S, Takaki Y, Tsuji S, Ikenaka K; Determination of major sialylated N-glycans and identification of branched sialylated N-glycans that dynamically change their content during development in the mouse cerebral cortex. Glycoconj J 31(9), 671-83, 2014

## Regulation of synaptic functions; involvement of diffusible and adherent factors

Shuichi Koizumi (Yamanashi University)

Glial cells release so-called gliotransmitter, by which they dynamically regulate neuronal excitability. Among gliotransmitters, ATP has a central role. Here, we show an importance of glia-to-neuron communication mediated by ATP/P2 receptors, showing two examples. Firstly we show astrocyte-mediated "ischemic tolerance". Preconditioning (PC) is an attractive strategy for protecting neurons by inducing ischemic tolerance in the brain. We found that induction of ischemic tolerance was totally dependent on astrocytes. With regard to mechanisms of astrocyte-mediated ischemic tolerance, activation of P2X7 receptor was essential. After PC, P2X7 receptor was upregulated in astrocytes, and then stimulated transcription of hypoxia inducible factor la (HIF1$\alpha$), a master molecule of oxygen homeostasis. The P2X7 receptor-HIF1$\alpha$ axis in astrocytes was required for the induction of ischemic tolerance. Second, we show that glial phagocytosis mediated by P2 receptors. Both microglia and astrocytes become phagocytic after PC, and then control remodeling of neuronal circuits. We also discuss the importance of this event in regulation of brain functions.

References.
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2) Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, et al.: Nature 446, 1091-5, 2007

## Microglial regulation of neural circuits activity

Hiroaki Wake (National Institute for Physiological Sciences)

The traditional role of microglia has been in brain infection and disease, phagocytosing debris and secreting factors to modify disease progression. Recent evidence extends their role to healthy brain homeostasis, including regulation of cell death, synapse elimination, neurogenesis and neuronal surveillance. These actions contribute to the maturation and plasticity of neural circuits that ultimately shape behavior. The functional loss of microglia was indicated to result in autism spectrum disease and obsessive compulsive disorder. In this symposium, we will discuss about microglial contributions to the synaptic plasticity and maintenance of neural circuits with a focus on interactions with synapses. Our result using *in vivo* two-photon microscope in awake animals, showed that microglia effect synapse activity by their direct connection and thus regulate the learning paradigm.

References

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## Closing Remarks