Plenary Lecture

1-PL

Joy of Pharmacology; exploring and discovering body mechanisms with drugs

Shuh Narumiya[1]

[1]Dept. Drug Discovery Medicine, Kyoto Univ. Grad. Sch. Med.

Studying drug actions often takes you to new, completely unexpected, research avenues. This is joy of pharmacology. I first became aware of pharmacology as such science when I worked as a postdoctoral fellow in John Vane's laboratory at Wellcome Research Laboratories in England, where people used bioassay, blocked the actions of known substances with respective antagonists and searched for new bioactive substances. I also experienced drug development process there, because Wellcome Research Laboratories were the research institute of the Burroughs-Wellcome Company. I realized that the whole process of drug development from basic research to clinical trial is an enormously big scale of experiment. My research thereafter on the prostanoid receptors and Rho GTPases both originated from the analysis of drugs (compounds and toxin, respectively), and took me to enjoy exploration of almost all body functions and in the world of cell biology. Quite recently, I have been associated with research collaboration with Astellas Pharma and have been using drug candidates and enjoying research avenues new to me and new to all. Joy of pharmacology never ceases.

Plenary Lecture

2-PL

The endoribonuclease Regnase-1: key molecule in inflammatory and immune responses

Shizuo Akira[1]

[1]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University

Immune responses are accompanied by dynamic changes in gene expression. Many transcription factors including NF-κB and AP-1 are involved in induction of genes involved in inflammatory and immune responses. However, recent studies have revealed that control of gene expression at the mRNA level is as important as transcriptional control in the immune response. We have shown that Regnase-1 encoded by the Zc3h12a gene is an endoribonuclease involved in destabilization of a variety of mRNAs including IL-6,IL-12, and Regnase-1 itself mRNAs via the stem loop structure present in the 3'UTR of these genes

Although originally identified as LPS-inducible gene, Regnase-1 protein is present in unstimulated cells, and disappears in response to Toll-like receptor ligands via an IKK-dependent proteasome degradation pathway or in response to T cell receptor stimulation through the cleavage by Malt-1. Thus, Regnase-1 acts as a brake in unstimulated cells as well as a negative feedback regulator after cellular activation. Recently we found that IL-17 signal also inhibits the function of Regnase-1. I would like to discuss the role of Regnase-1 in the immune response.

Special Lecture

1-SL01

Unbiased whole-brain imaging to uncover molecular mechanisms and therapeutic targets for brain disorders

Hitoshi Hashimoto[1,2,3,4]

[1]Lab. Mol. Neuropharmacol., Grad. Sch. Pharmaceutic. Sci., [2]United Grad. Sch. Child Develop., [3]Instit. for Datability Sci., [4]Instit. for Open and Transdisciplinary Res. Initiatives, Osaka Univ.

Whole-brain imaging and systems analyses of entire brains at subcellular resolution and subsequent processing of the resulting images are prerequisites to investigate anatomical and functional brain networks to understand their function and dysfunction. However, it is still a challenge due to the trade-offs between imaging speed and spatial resolution irrespective of if tissue clearing methods are used. To overcome the issue, we have been attempting to increase the imaging throughput and relieve bottlenecks in the procedure, and have recently developed an automated high-speed imaging system for block-FAce Serial microscopy Tomography (termed FAST). By using this system, it became possible to perform quantitative comparisons of whole-brain structures and neuronal activities at the cellular level using the spatial coordinated alphanumeric data of brain cells and pattern recognition methods. The FAST system thus paves the way for imaging analyses of the brain and provides new opportunities for unbiased and hypothesis-free approaches that contributes to investigate molecular mechanisms and therapeutic drug targets for brain disorders.

Special Lecture

1-SL02

Structural basis of temperature sensing in vanilloid sensitive TRPV channels.

Kenton J. Swartz[1]

[1]Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA

Protein toxins from venomous organisms have been valuable tools for investigating the structure and gating mechanisms of voltage-activated ion channels. Transient Receptor Potential (TRP) channels are a large family of ion channels that are activated by diverse stimuli and ligands, including second messengers, temperature, voltage and natural products such as capsaicin, menthol and wasabi. We have begun to investigate the structure and gating mechanisms of the heat-activated TRPV1 channel using the double-knot toxin (DkTx) from tarantula venom. I will present the structure of DkTx that we solved using NMR, and show how we have docked DkTx into the electron density maps from the recent single particle EM structure of the toxin bound TRPV1 channel to reveal a range of interesting features of the toxin-channel interaction. In particular, our results reveal that DkTx binds to the perimeter of the external pore of TRPV1 at the interface of the channel with the surrounding lipid membrane. I will also talk about functional experiments suggesting that DkTx and extracellular ions profoundly alter activation of TRPV1 by heat, implicating the external pore in the mechanism of temperature-sensing.

Special Lecture

1-SL03

Next-generation proteomics unveils a global landscape of cancer metabolism

Keiichi I. Nakayama[1]

[1]Dept. Mol. Cell. Biol., Med, Inst. Bioreg., Kyushu Univ.

We have developed a next-generation proteomics approach—in vitro proteome–assisted multiple reaction monitoring for protein absolute quantification (iMPAQT)—that allows genome-wide absolute quantification of the human proteome and is reliant on the production of ～18,000 recombinant proteins. We applied iMPAQT to delineate the metabolic landscape of human diploid fibroblasts. Oncogenic transformation of these cells gave rise to relatively small but global changes in metabolic pathways that account for aerobic glycolysis (Warburg effect) and increased rates of macromolecule synthesis. Modulation of metabolic enzyme expression revealed an unexpected functional interaction between glycolysis and the pentose phosphate pathway that facilitates nucleic acid synthesis. Furthermore, integration of proteomic and metabolomic data allowed construction of a mathematical model for identification of key enzymes responsible for the metabolic shift in cancer. We found that substantial remodeling in glutamine metabolism, which we call the "second" Warburg effect, is essential for malignant phenotypes of cancer cells. Our results thus provide a global view of metabolic restructuring in cancer that underlies adaptation to a rapid growth state.

Special Lecture

1-SL04

Structural basis for molecular mechanisms of novel membrane transporters and channels

Osamu Nureki[1]

[1]Dept. Biological Sciences, The Univ. Tokyo, Grad. Sch. Sci.

Plant cells have specific organelle distinct from moving animals, vacuoles, which store toxic chemicals such as ferric ions. Vacuolar iron transporter 1 (VIT1) transports cytoplasmic ferric ions into vacuoles. We solved crystal structure of VIT1, which acts as an H^+-dependent antiporter for Fe^{2+} and other transition metal ions. VIT1 adopts a novel protein fold forming a dimer of five-membrane-spanning domains, forming an ion-translocating pathway constituted by the conserved methionine and carboxylate residues at the dimer interface. The second transmembrane helix protrudes from the lipid membrane by about 40 Å and forms a three-helical-bundle triangular cytoplasmic domain, which binds to the substrate metal ions and stabilizes their soluble form, thus playing an essential role in the transport.

Recent progress in cryo-EM single particle analysis enables us to solve large membrane protein structure rapidly. I would like to present here new structures of heterodimeric amino-acid transporter and two physical stimuli-sensing channels from human to uncover their molecular mechanisms.

Special Lecture

2-SL05
Titin folding powers muscle contraction

Julio M. Fernández[1]

[1]Department of Biological Sciences Columbia University, USA

By combining springs with motors and chemical switches biological systems can achieve great scales of mechanical power amplification, for example when jumping or launching a projectile. However, the molecular mechanisms of how this is achieved remain unknown. The giant muscle protein titin, composed of hundreds of tandem Ig domains, is a complex elastic protein whose role in muscle function is still poorly understood. A crucial recent discovery that we have made is that titin domains do a surprisingly large amount of mechanical work when they fold against an opposing mechanical force. We have shown that the amount of mechanical work done by a folding titin Ig domain can be 2-3 times larger (～120 zJ) than that of the chemically powered motor myosin II; ～38 zJ. Titin molecules store mechanical energy by unfolding and extending under force. Elastic energy is stored this way by stretching caused by gravitational pulling during locomotion, inertia, chemical modifications, and ATP powered sources to name a few. Titin unfolding occurs at varying rates over a very wide range of forces above >8 pN. By contrast, most of the stored mechanical energy is delivered back only over a small range of forces where the folding probability increases from 0 to 1 (< 6 pN) and the folding protein does large amounts of mechanical work. Thus, protein folding/unfolding is likely to operate as a mechanical battery where different types of energy sources are stored, and then converted back into contractile power.

Given that titin is now known to be the third filament of muscle, determining if protein folding can deliver work quickly enough to match the power output of the myosin motors, is a central question to be answered. The mechanical power output of protein folding is a novel concept and thus has never been studied before.

Cryptic cysteine residues are common in the elastic I band region of titin where they can oxidize to form intra-domain disulfide bonds, limiting the extensibility of an unfolding Ig domain.

Here we use magnetic tweezers force spectroscopy to study the folding dynamics of a disulfide bonded modular titin protein operating in the physiological range, with the ability to control the oxidation state of the protein in real time. We show that the midpoint folding probability of the parent Ig domain reversibly shifts up from 4.0 pN to 12.8 pN upon oxidation. In this force range, the folding contraction dominates the elastic recoil of the protein, delivering stepwise mechanical work which depends on the oxidation state in an all-or-none manner. For example, the output power of a folding contraction at 6 pN goes from 0 zW to 6,000 zW upon introduction of the disulfide bond. This large amount of power is delivered by folding at forces where single molecular motors are typically stalled. From our observations we predict that during muscle contraction, activation of myosin II motors by Ca^{++} leads to a drop in the force experienced by titin, triggering delivery of mechanical power by titin folding. Thus, it seems inevitable now that the three filaments of the muscle sarcomere act in concert to both store and deliver mechanical power, revolutionizing our understanding of the molecular mechanisms of muscle contraction.

Special Lecture

2-SL06

Dynamic regulation of signaling pathways in dopamine neurons: the intracellular actions of amphetamines

Susan G. Amara

[1]National Institute of Mental Health, National Institutes of Health, USA

Neurotransmitter transporters present at the cell surface are well-established as the primary targets for psychostimulant drugs of abuse and for drugs such as methylphenidate and amphetamines, which are used to treat attention deficit disorders. In recent studies, we have observed that once amphetamines enter neurons they can activate multiple intracellular signaling pathways. Within the cell, amphetamines activate the small GTPases, Rho and Rac1, and trigger endocytosis of the dopamine transporter (DAT) and a neuronal glutamate transporter (EAAT3) by a RhoA-dependent internalization pathway. These events depend upon the expression of an intracellular G-protein coupled trace amine receptor (TAAR1) that signals through at least two types of G-protein alpha-subunit within the cell. Using a series of subcellularly-targeted genetic fluorescence resonance energy transfer (FRET) sensors to detect RhoA or PKA activation, we have been able to characterize the subcellular membrane compartments where TAAR1 signaling events initiate. These results imply that amphetamine-like drugs not only inhibit monoamine transport and potentiate neurotransmitter action, but they also activate signaling pathways through their direct action on an intracellular GPCR target. This lecture will highlight the role of TAAR1- and other GPCR-mediated signaling events in amphetamine action and will consider how they are linked to the action of a variety of drugs that modulate monoamine signaling.

Special Lecture

2-SL07
Drama of challenge to create a new medicine by chemist

Osamu Uchikawa[1]

[1]TOWA Pharmaceutical Co., Ltd.

Focusing on melatonin secreted from the pineal body of the brain, melatonin receptor agonist Ramelteon (Product name: Rozerem) which was totally different from conventional hypnotics was discovered in Japan. Drug discovery does not come true overnight. Starting with disease and target selection, construction of screening system, drug design, optimization study, toxicity testing, clinical trials and many years of trial and error are repeated tremendously. Only one compound that has been thought out and carefully nurtured by the single-minded many researchers finally reach the goal (product). The burden of the pharmaceutical company is heavy under the limitations of low molecule drug discovery and the exhaustion of drug discovery targets, and unless new strategies to enhance the success rate are taken, the discovery of new medicine can't be hoped at all. In order to continue to maintain world-class drug discovery capabilities and to make drug discovery a reality, aggressive cooperation of industry, government and academia will create highly original results in Japan. I would like to mention how to accomplish the drug discovery through my thorns path leading to drug discovery. I really hope that one grain of drug created by many hard work researchers will present a smile to many patients.

Special Lecture

3-SL08

Physiological function of thermosensitive TRP channels and their significance as potential drug targets

Makoto Tominaga[1,2]

[1]Div. Cell Signaling, National Int. Physiol. Sci., [2]Thermal Biology Group, Explor. Res. Cent. Life and Living Systems

Animals survive by detecting and adapting to the ambient temperatures. One of the important molecules involved in the temperature detection is TRP channel which consists of 28 members in 6 subfamilies in mammals. Eleven among the 28 TRP channels are activated by temperature changes, and thus called ‘thermosensitive TRP channels' Cation influx through temperature-sensitive channels like TRP channels causes some depolarization leads to activation of voltage-gated Na^+ channels, followed by action potential generation. Because TRP channels have relatively high Ca^{2+} permeability, Ca^{2+} entering the cells activates Ca^{2+}-activated Cl^- channels, causing further depolarization. Thermosensitive TRP channels are activated in the different temperature ranges and involved in various physiological phenomena in many species. I will introduce structures and functions of thermosensitive TRP channels together with significance of the complex of TRP channels and Ca^{2+}-activated Cl^- channels, anoctamin 1. I will also discuss the TRP channels as potential drug targets. Furthermore, the evolutional aspects of thermosensitive TRP channels will be mentioned since animals are thought to have evolved by changing the expression and function of thermosensitive TRP channels through the changes in ambient temperatures.

Special Lecture

3-SL09

Calcium signaling and potassium channel diversity as a potential target of drug discovery

Yuji Imaizumi[1]

[1]Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ.

Intracellular Ca^{2+} signaling plays obligatory roles in the regulation of cellular physiological functions, and also the proliferation and/or death of cells. Hyper-activation of Ca^{2+} signaling often initiates and/or facilitates the pathological conditions in many kinds of diseases and has been studied as the potential target for new therapeutic approaches and drug discovery. The generation of Ca^{2+} signaling has two major pathways; the Ca^{2+} inflow and the Ca^{2+} release from intracellular stores, which are controlled in extensively different manners depending on cellular excitability. In excitable cells, membrane potential changes due to ion channel activities and Ca^{2+} signaling are bidirectionally regulated to maintain the homeostasis of physiological functions efficiently by Ca^{2+} microdomain formation, which has been revealed by molecular imaging. Even in non-excitable cells, the resting membrane potential substantially modulates Ca^{2+} signaling via the inflow through non-voltage-dependent Ca^{2+} channels, such as store-operated Ca^{2+} entry. The crucial significance of K^+ channel in Ca^{2+} signaling is attributable to its inverse functions in excitable and non-excitable cells. Based on the large molecular diversity, the heterologous multimer-formation and the tissue-specific expression pattern, K^+ channels particularly in non-excitable cells are now recognized as a hot target of drug discovery research and development.

Special Lecture

3-SL10
Primary cilia and other mysteries

David E. Clapham[1]

[1]Janelia Group Leader, Professor, Harvard Medical School, USA

Primary cilia are solitary, generally non-motile, hair-like protrusions that extend from the surface of cells between cell divisions. Defects in primary cilia formation and function result in many developmental diseases and some adult ciliopathies. In this lecture, I will discuss the function of primary cilia in neurons, glia, kidney, and cell lines. The focus will be on measurements of ion currents in primary cilia, their underlying physiology, and the structures of these channels.

Lecture Special Lecture

1-JAL

Combining Pharmacology and Genetics to Study and Treat Human Diseases

Lorraine J. Gudas[1]

[1]Dept. of Pharmacol., Weill Cornell Med. College of Cornell Univ., USA

Members of the nuclear receptor family, the retinoic acid receptors (RARs) alpha, beta, and gamma, regulate cell differentiation and epigenetic states of stem and differentiated cells. We showed that these RARs also play key roles in cancer prevention and in the inhibition of diabetes and hepatic steatosis, but the complexity of signaling by multiple receptors and the regulated production of active ligands make it challenging to establish the molecular mechanisms for these effects. To develop new pharmacological treatments, we generated unique murine models of early stage *clear cell renal cell carcinoma (ccRCC)* and *head and neck cancer (HNSCC).* These models reflect molecular and histological changes that occur in human carcinogenesis, providing us with powerful systems in which to test new drugs and increase our understanding of the early stages of carcinogenesis. In our *HNSCC model,* we are analyzing retinoic acid receptor (RAR) gamma as a tumor suppressor, and we've shown that a RAR gamma selective agonist inhibits the development of oral squamous cell carcinoma. In our ccRCC model we've found novel therapeutic targets. Using both dietary (i.e., high fat diet) and genetic (i.e., db/db mice) models of diabetes and/or hepatic steatosis, we showed that a RAR beta selective retinoid can reduce the pathological features of diabetes and hepatic steatosis. Furthermore, we reported that this RAR beta selective retinoid is useful in treatment of diabetic nephropathy and is cardioprotective. Thus, combining pharmacological studies with representative disease models provides important insights into many common human diseases (metabolic syndrome, cancer, kidney disease, and heart disease), and can lead to new therapeutics to improve human health.

2-EAL

Immunopharmacology from Japan - based on intravital imaging analyses on cellular dynamics in living systems.

Masaru Ishii[1]

[1]Dept. Immunol. Cell Biol., Osaka Univ. Grad. Sch. Med.

It is my greatest honor to receive the 12th Setsuro Ebashi Award of the Japanese Pharmacological Society. By establishing an original intravital multiphoton microscopy, I have been studying on ‘real' cellular dynamics with immune and inflammatory systems in intact tissues and organs. Especially I have first succeeded in visualizing in vivo behaviors of different cell types in bones, such as osteoclasts, which led to significant conceptual advances on dynamic bone/immune systems. This research activity with dynamic imaging technology is not only contributing to development of basic biological science in general, but also serving as a novel way for analyzing in vivo pharmacological actions of different emerging drugs. In this lecture I will outline my research so far as well as present my current trial for launching ‘immunopharmacology in Japan to the world', based on our original research trend.

3-YAL-1
Systemic regulation of the central nervous system regeneration

Muramatsu Rieko[1]

[1]Dept. Mol. Pharmacol., Natl. Inst. Neurosci., Natl. Center Neurol. Psychiat.

Injury and inflammation causes severe neurological dysfunction that can be partially reversed by spontaneous regeneration of neuronal network in the central nervous system (CNS). Although CNS environment contains the molecules that inhibit axon regeneration, recent researches pointed out that CNS neuronal network can regenerate spontaneously in the animals of some disease models. Because the CNS environment is separated by the peripheral milieu in the presence of the blood-brain barrier, CNS regeneration is thought to be controlled by the CNS microenvironment. However, we found that factors derived from peripheral tissue leak into the CNS after injury and promote remyelination in a murine model of toxin-induced demyelination. We identified that the remyelination is stimulated by the molecules which are expressed by the peripheral tissues, such as pancreas, adipose disuse, skeletal muscle, and heart. Especially, pancreas-derived FGF21 derived proliferation of oligodendrocyte precursor cells (OPCs) through interactions with beta-klotho, an essential coreceptor of FGF21, and promoted remyelination. In this presentation, I would like to show our recent reports that indicate a potentially important role for the peripheral milieu in promoting CNS regeneration.

3-YAL-2

Axonal transport through signaling endosome and its roles in neurodegenerative disorders

Naoya Yamashita[1]

[1]Dept. Pharmacol., Juntendo Univ., Sch. Med.

Long-distance axonal transport of ligand-receptor complexes from the site of endocytosis in the axon to somatodendrite acts as a signaling platform in neurons. However, the endosome-based transportations called "signaling endosomes" and its physiological significance remain to be elucidated. I discovered that retrograde axonal transport of semaphorin3A and its receptors by signaling endosomes regulates dendritic development, specifically enhances AMPA receptor GluA2 localization in dendrites. The retrograde signaling endosomes also induce anterograde delivery of nascent receptors. The axon-derived neurotrophin signaling endosomes are exocytosed to soma surface membrane where they promote anterograde transport of resident naïve receptors. As a positive feedback mechanism, this enhances the neuronal sensitivity to ligand in neurotrophin signaling. Taken together, these data suggest the antero- and retrograde communication via signaling endosomes plays critical roles in neuronal development. Since many neurodegenerative disorders are featured with impaired axonal transport, I start a project that investigates the role of signaling endosomes during the onset of these neurodegenerative disorders.