PL1 Electron Tomography or the Challenge of Doing Structural Biology *in situ*

Wolfgang Baumeister
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Electron cryotomography enables the structural analysis of non-repetitive pleomorphic structures, such as organelles or even whole cells providing unprecedented insights into their supramolecular organization. In conjunction with subtomogram classification and averaging molecular structures can be studied *in situ*, i.e. in their functional cellular environments. Recent developments such as the targeted micromachining of cells embedded in amorphous ice using correlative LM-EM techniques and focused ion beam technology open up new windows of opportunity for studying cellular ultrastructure. Studies of ribosomes and proteasomes *in situ* and of neurotoxic aggregates will illustrate the potential of this new approach to structural cell biology.

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座長：久保 義弘（生理学研究所）

PL2 低温電子顕微鏡を用いた構造生理学研究

Structural physiology studied by cryo-electron microscopy

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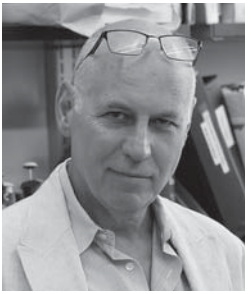
I am personally interested in molecular mechanisms, how education and experiences during human development influence the ability and personality of the adult. To challenge such a difficult question, structural and functional studies of membrane proteins are important, and thus I named this research field structural physiology. I would like to discuss mainly three topics of cell adhesive-channels. First, as an exceptional feature specific to AQP4 among 13 water channel isoforms, characteristic orthogonal arrays were observed and the array formation of AQP4 was regulated by the N-terminal palmitoylation of either Cys13 or Cys17, which was revealed by structure analysis of AQP4 2D-crystals [JMB 355, 628-39 (2006)] and subsequent freeze-fracture studies [BBA 1778, 1181-9 (2008)]. Large numbers of AQP4 molecules with cell adhesive-function are expressed in the glial lamellae of hypothalamus at which important brain functions such as thermo-, osmo- and glucose-sensory systems are thought to be carried out. For example, AQP4 might therefore be responsible for the pressure regulation in brain. The second topic is gap junction intercellular communication channels that allow a wide variety of solutes to pass through, and have critical roles in biologically important processes, such as, cardiac development, fertility, immune system and electrical signaling in the nervous system. The structures of connexin-26 were analyzed by electron crystallography [PNAS, 104 10034-9 (2007)] as well as X-ray crystallography [Nature 458, 597-602 (2009)], and we proposed plug gating model as a gating mechanism of the gap junction channel. As the third topic, we recently analyzed structure of claudin by X-ray crystallography and proposed a paracellular channel model [Science 344, 304-7 (2014)].

プレナリーレクチャー3 / Plenary Lecture 3

3月22日(日) / March 22, Sun. 10:30～11:15　　A 会場 / Room A

座長：東原 和成（東京大学）

PL3 Receptors, Neurons, and Circuits: The Biology of Mammalian Taste



Charles S. Zuker
Howard Hughes Medical Institute and Columbia University, USA

The taste system is one of our fundamental senses, responsible for detecting and responding to sweet, bitter, umami, salty, and sour stimuli. In the tongue, the five basic tastes are mediated by separate classes of taste receptor cells each finely tuned to a single taste quality. In the cortex, each taste quality is represented in its own separate cortical field, revealing the existence of a gustotopic map in the brain. We study the logic of taste coding as a platform to understand how our brain creates an internal representation of the outside world and transforms sensory signals at the periphery into percepts, actions and complex behaviors.

PL4 マウス嗅覚系における神経地図形成の基本原理
Neural Map Formation in the Mouse Olfactory System

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福井大学・医学部
Hitoshi Sakano
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In the mouse olfactory system, odorants are detected with ~1,000 different odorant receptor (OR) species expressed in the cilia of olfactory sensory neurons (OSNs). Each OSN in the olfactory epithelium (OE) expresses only one functional OR gene in a mutually exclusive and mono-allelic manner. Furthermore, OSNs expressing the same OR species converge their axons to a specific location in the olfactory bulb (OB) forming a glomerular structure. Because a given OR responds to multiple odorants and a given odorant activates multiple OR species, the odor information detected in the OE is topographically represented as the pattern of activated glomeruli in the OB[1]. A remarkable feature of axonal projection in the mouse olfactory system is that ORs play an instructive role in projecting OSN axons to the OB. For dorsal-ventral (D-V) projection, anatomical location of OSN cells within the OE regulates both OR gene choice and expression levels of axon guidance molecules, thus indirectly correlating the OR identity to the glomerular location along the D-V axis[2]. In contrast, anterior-posterior (A-P) projection is totally independent of the positional information of OSN cells, but instead dependent on the expressed OR species[3]. We have recently found that A-P targeting is regulated by the agonist-independent baseline activity of ORs using cAMP as a second messenger[4]. OR-derived cAMP signals also regulate the expression of glomerular segregation molecules for the map refinement through local sorting of OSN axons[5]. Unlike A-P projection molecules, glomerular segregation molecules are regulated by stimulus-driven neuronal activity[4].

Here, we discuss the recent progress in the neural map and circuit formation in the mouse olfactory system.

References
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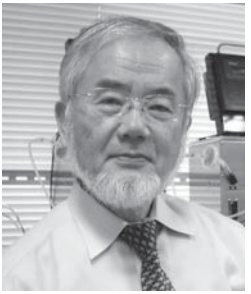
4) Nakashima, A., et al.: *Cell* **154**, 1314 (2013).
5) Serizawa, S., et al.: *Cell* **127**, 1057 (2006).

座長：内山 安男（順天堂大学）

PL5 酵母によるオートファジーの分子機構

Molecular Dissection of Autophagosome Formation in Yeast



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東京工業大学・フロンティア研究機構
Yoshinori Ohsumi
Integrated Research Institute, Tokyo Institute of Technology, Japan

Autophagy is well a conserved degradation process of cytoplasmic constituents in the lysosome/vacuole. Recently it is getting clear that autophagy plays important roles in so many physiological events and is related to diseases. More than 26 years ago we first found autophagy in yeast induced by nutrient starvations by light microscopic observation. Taking advantage of the yeast system, we started genetic approach to dissect the process, and successfully isolated many autophagy-defective mutants. Subsequent identification of ATG genes revealed unique set of genes involved in membrane dynamics during autophagy. These genes were mostly conserved in mammals and plants and most other eukaryotes. These findings triggered a vast of autophagy research in various organisms. We know now that 18 *ATG* genes are essential for starvation-induced autophagy in yeast. They consist of six functional units, namely the Atg1 protein kinase and its regulators, the PI3 kinase complex, the Atg2-Atg18 complex, the membrane protein Atg9, and two unique ubiquitin-like conjugation systems. Then we have been focusing to elucidate the structure and function of each Atg protein. Atg proteins function concertedly in membrane dynamics during the formation of autophagosome. Recent studies on the Atg proteins, especially early steps of the PAS assembly will be presented. In addition recent physiological roles of autophagy in yeast will be discussed.

3月21日(土) / March 21, Sat. 14:00～14:45　　A 会場 / Room A

座長：久場 博司（名古屋大学）

EL1 生命の要、モーター分子群、KIFs と細胞内輸送：記憶・学習及び発生の制御から疾患まで

Kinesin Superfamily Molecular Motors, KIFs: Intracellular Transport, Regulation of Higher Brain Function, and Development and Diseases

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東京大学・医学系研究科
Nobutaka Hirokawa
Graduate School of Medicine, The University of Tokyo, Japan

神経細胞を初めとする全ての細胞内で多くの蛋白質は、様々な膜小胞や蛋白複合体として直径25nm の微小管の上を、特定の方向へ異なる速度で輸送される。この輸送機構は、細胞の機能や形作りに必須であり、その主役は、微小管上を様々な荷物（cargo）を輸送するモーター分子である。

私達は、哺乳類（ヒト、及びマウス）のモーター分子群、Kinesin superfamily proteins（KIFs）の遺伝子 45 個を全て同定し、細胞内輸送機構を解明してきた。すなわち、各々の KIFs のカーゴ、輸送の方向性と速度、KIF によるカーゴの認識、目的地でのカーゴの乖離、そして異なる方向への輸送の機構を明らかにした。

さらに個体レベルでの各々の KIF の働きを解明する為、transgenic mouse, knockout mouse の作成と解析を行った。この解析を通して、多種類の KIF が、記憶・学習などの高次脳機能や、脳の回路網の形成、活動依存性の神経細胞の生と死、神経の分化増殖の制御や、腫瘍の抑制あるいは、私たちの体の左右非対称性の決定などの重要な生命現象を司っているという驚くべき事実が明らかになり、さらに、KIFs の障害により、神経変性症、癲癇、不安神経症、水頭症、女性不妊、腫瘍、糖尿病等の疾患が起こる事も明らかとなった。さらに Cryo EM, X- 線結晶解析、生物物理学を駆使して KIF の動く機構を明らかにした。

大会企画学術教育講演2 / Academic Education Lecture 2

3月21日(土) / March 21, Sat. 14:45～15:30　　A 会場 / Room A

座長：野田 泰子（自治医科大学）

EL2　1分子から階層を超えて生体システムにつなぐ統合的研究

Integrative research on bio-system bridging from single molecules to organ

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大阪大学・生命機能研究科
Toshio Yanagida
Graduate School of Frontier Biosciences, Osaka University, Japan

現在生命科学は飛躍的に進歩している。特に、蛋白質や遺伝子など生体分子の計測解析技術が大きく発展し、膨大なデータが得られている。問題は、これら分子レベルのデータを如何に統合してシステム、すなわち個体や臓器の生理機能につなぐかである。しかし、生体システムはあまりにも複雑でいろいろな要素が絡み合っているので、極めて難問である。最近、理研の10ペタ Flops（1秒間に10の15乗回の演算）スパコン“京”が使えるようになり、少し可能性がでてきた。我々は、1分子計測で得られたミオシン分子モータの実験データを基に、私がリーダーをしている計算機の生物応用プロジェクトに参加している久田グループが開発したUT-Heartシミュレータでスパコン“京”の中に筋肉と心臓を再現し、ミオシン分子の性質やその変異が心臓の拍動にどのような影響をあたえるかを調べている。講演では、収縮タンパク分子の変異と心臓拡張症などの病気との関係についても説明する。これとは対照的に、トップダウン的アプローチで脳機能に迫る研究についても述べる。

座長：藤田 一郎（大阪大学）

NL1 大脳 MT/MST 野による眼球運動の制御

The role of cortical areas MT/MST in short-latency ocular tracking

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京都大学・医学研究科
Kenji Kawano
Graduate School of Medicine, Kyoto University, Japan

Whenever we move around in the environment, the observer's movements activate the vestibular organs and are then compensated by the vestibulo-ocular reflexes (VORs). However, the VORs are not always perfect and the visual acuity is severely impaired if the images of interest on the retina move excessively. Recent studies revealed three distinct visual tracking eye movements with ultra-short latencies (~60 ms in monkeys), which are thought to help reduce the residual visual disturbances. One of these eye movements is 'ocular following', which deals with the visual stabilization problems confronting the observer who looks off to one side. Two other eye movements, 'disparity vergence' and 'radial-flow vergence', deal with the binocular fusion problems of the observer who looks in the direction of heading.

To understand the neural mediation of these tracking eye movements, we focused on the role of the middle temporal (MT) and medial superior temporal (MST) areas within the superior temporal sulcus (STS) of the monkey's cortex, since these areas are known to contain many neurons that respond vigorously to visual motion with directional selectivity and others that are sensitive to binocular disparity or to the patterns of optic flow experienced by the moving observer. We recorded single unit activities and made focal chemical lesions in these areas in monkeys. The results were consistent with the hypothesis that the MT/MST areas are primary sites for initiating all three visual tracking eye movements at ultra-short latencies.

3月23日(月) / March 23, Mon. 14:15～15:00　　A 会場 / Room A

座長：松田 博子（関西医科大学）

NL2 横紋筋における細胞内 Ca^{2+}；測定と生理学的意義

Intracellular Ca^{2+} in striated muscle: measurement and physiological significance

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東京慈恵会医科大学
Satoshi Kurihara
The Jikei University School of Medicine, Japan

Intracellular Ca ion (Ca^{2+}) plays a pivotal role in muscle contraction. In the present Tawara Memorial Lecture, I will present the intracellular Ca^{2+} concentration change measured with the Ca^{2+} sensitive photoprotein aequorin in mammalian cardiac muscles, and will discuss the molecular mechanism of the length-dependent change of tension in cardiac muscle (the Frank-Starling law of the heart). If the papillary muscle of the rat or ferret was stretched from a shorter length to the length to produce maximal tension (Lmax), tension was increased without a change in the peak Ca^{2+} signal (Ca^{2+} transient, CaT). However, the relaxation time was prolonged and the decay time of CaT was shortened. If muscle length was quickly shortened from Lmax to a shorter length during a twitch contraction, tension was promptly decreased and then re-developed. In response to quick release, the CaT showed a transient increase (hump) in the falling phase. The magnitude of the hump was correlated with the magnitude of tension reduction rather than with muscle length. If the preparation was treated with 2,3-butanedione monoxime, tension disappeared, but the CaT was not greatly affected. In the 2,3-butanedione monoxime-treated preparation, quick release did not induce a hump in the CaT. Thus, the change in muscle length affects the Ca^{2+} affinity of the Ca^{2+}-binding protein troponin through cross-bridge attachment and detachment. The measurement of the intracellular Ca^{2+} concentration is essential for understanding the molecular mechanism of cardiac muscle contraction.