2SB-02 スイッチ機能を有した化学プローブのデザイン・合成によるマルチモードイメージング

DESIGN, SYNTHESIS AND BIOLOGICAL APPLICATION OF MOLECULAR IMAGING PROBES WITH TUNABLE CHEMICAL MATCHES

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One of the great challenges in the post-genome era is to clarify the biological significance of intracellular molecules directly in living cells. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output. Magnetic resonance imaging (MRI) is an imaging modality adequate for in vivo studies. Therefore, many scientists are interested in the development of MRI probes capable of detecting enzyme activities in vivo. Because background signal is hardly detectable, 19F-MRI probes are promising for in vivo imaging. A novel design strategy for 19F-MRI probes to detect protease activities is proposed. The design principle is based on the paramagnetic relaxation effect from Gd3+ to 19F. A peptide was synthesized, Gd-DOTA-DEVD-Tfb, attached to a Gd3+ complex at the N-terminus and a 19F-containing group at the C-terminus. The 19F-NMR transverse relaxation time (T2) of the compound was largely shortened by the paramagnetic effect of intramolecular Gd3+. The peptide was designed to have a sequence cleaved by an apoptotic protease, caspase-3. When the peptide was incubated with caspase-3, the peptide was cleaved and subsequently the Gd3+ complex and the 19F-containing group were separated from each other. T2, after cleavage, was extended to cancel the intramolecular paramagnetic interaction. T2 is a parameter that can be used to generate contrasts in MRI images. Using this probe as a positive contrast agent, the probe could detect caspase-3 activity spatially using 19F MRI.

2SB-03 細胞内送達ツールとしての導入ペプチド

Cell-penetrating peptides as a tool for intracellular delivery

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Intracellular delivery using cell-penetrating peptides is a recently developed methodology that has been employed successfully to transport various bioactive molecules into cells to modify cell functions. The efficient delivery of proteins, peptides, nucleic acids, liposomes, and so on has been accomplished using this methodology by conjugation of a peptide vector with the cargo molecules. Although the potentials of this approach for medical and pharmaceutical applications have attracted our attention, this methodology is also a promising tool for the intracellular delivery of biosensors for the analysis of cell function and its dynamics. Arginine-rich peptides, including a basic peptide segment derived from the human immunodeficiency virus type 1 (HIV-1) Tat protein and oligoarginine peptides, are categorized into one of the most frequently used peptide vectors. We have shown that counterions with high hydrophobicity such as pyrenebutyrate can greatly accelerate the internalization of the R8 peptide into various cells. Using this counterion-mediated technique, we have succeeded in direct and rapid delivery of green fluorescent protein (GFP) into cytosols. This methodology was also applied to cell NMR observation of proteins. On the other hand, we found that the R12 peptide can directly translocate through plasma membranes even in the absence of pyrenebutyrate, presumably due to its higher affinity to cell surfaces than the R8 peptide.

2SB-04 忍耐性タンパク質プロセッジングディバイスを利用した核-細胞質移動ペプチドの創製

Development of nucleocytoplasmic shuttle peptide using stimulus-responsive processing device


The methodology for temporal and spatial control of the function of peptides/proteins by a stimulus has received increased attention due to its potential in various fields such as chemical biology. Recently, we have developed the amino acid derivatives with stimulus-responsive processing ability (peptide bond cleavage) and successfully applied to control the peptide function [1]. In this system, the peptide containing the stimulus-responsive amino acid can release a functional peptide after the stimulus-induced peptide bond cleavage. In this presentation, we will discuss the applications of the stimulus responsive amino acid to chemical biology use including development of nucleocytoplasmic shuttle peptide.


2SB-05 光応答性ナノカテゴロドの設計と合成

Design and synthesis of caged nucleotides

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Caged compounds of oligonucleotides would serve as new functional molecules that can be activated by applying external triggers, thereby enabling manipulation of gene expression, transcription activation, and translation inhibition with high spatio-temporal resolution. We designed new precursors of caged nucleotides having an affinity tag for purification, targeting and molecular recognition. To demonstrate utility of the compound, two new nucleotide caging agents, Bio-Bhc-diazio and PNA-Bhc-diazio were synthesized. Bio-Bhc-diazio has biotin as an affinity tag and Bhc-diazio group as a caging agent for nucleotides, such as cyclic nucleotides, DNAs and RNAs. We tested the compound in reactions with dsDNAs and found that the dsDNAs were covalently modified by the Bio-Bhc group and the caged DNAs were separated from the unmodified starting materials using streptavidin magnetic beads. The expression levels of luciferase from the purified caged linear dsDNAs (linear RL) were monitored in cultured mammalian cells and found to be nearly one fifth of those from crude mixtures of non-purified caged linear RLs. The purified caged linear RLs were reactivated after exposure to ultraviolet light. PNA-Bhc-diazio has a peptide nucleic acid (PNA) tag complementary to a target gene for sequence-selective caging. To test the compound, DNAs having PNA binding sequences were reacted with the PNA-Bhc-diazio. Both ssDNAs and dsDNAs were covalently modified with the PNA-Bhc-diazio in a sequence selective manner.

2SC-01 カタクシレーゼ抑制剤全体の1次構造レベルメイニングと3Dコンピュータ・モデリングによる形態解析

Imaging of normal tailbud-stage Ciona intestinalis embryo at single cell level and Analysis of anatomy by constructing 3D Virtual Embryo

Kohji Hotta, Mitsuru Nakamura, Jun Terai, Reiko Okuhoro, Kotaro Oka (Dept. of Sci. & Tech., Keio Univ.)

Tailbud feature binds various chordates into one common group because all chordates have tails at the larval stage. In this study, to understand a comprehensive anatomy of tailbud embryo at single-cell-level, we reconstructed three-dimensional computer model from confocal image stacks of Ciona tailbud embryo (stage 22, 10.9 hpf at 18°C). As a result, we characterized unique cell arrangement in each tissue and revealed that the tailbud embryo consists of 1583 cells. Among them, the epidermis, nervous system, mesenchyme, muscle, notochord, and endoderm consist of 840, 227, 219, 36, 40, and 203 cells, respectively. Moreover, four pairs of undeveloped cells were found at trunk lateral side of tailbud embryo. Our comprehensive manually-annotated model of tailbud embryo will become a standard of cellular-level anatomy as well as geometry when considering molecular mechanism behind such unique tissue morphology.

2SC-02 細胞分裂と形態形成の協調：神経管形成過程における伸縮化したG2期の役割

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Coordination of mitosis and morphogenesis: Role of a prolonged G2 phase during chordate neural tube closure

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Development of multi-cellular organisms relies on execution of cell division and cell movement in proper space and time. It is thought that these two cellular behaviors are incompatible because each of them requires different cytoskeletal arrangement. Therefore embryonic cells must regulate their timing for coordinated embryogenesis. Using a fluorescent ubiquitination-based cell-cycle indicator (Fucci), we investigated spatio-temporal cell cycle progression patterns during neural tube closure in the embryos of an ascidian, Ciona intestinalis. Epidermal cells of Ciona divide 11 times as the embryos progress from 1-cell to the tadpole larval stage. We detected a long G2 phase between the 10th and 11th cell divisions, during which fusion of the left and right epidermal layers occurred to complete neural tube closure. During the G2 phase, epidermal cells performed the characteristic changes of cell shape that is dependent on F-actin regulation. cdc25 is probably a key regulator of the cell-cycle progression of epidermal cells. Artificially shortening the G2 phase by overexpressing cdc25 caused precocious cell division before or during neural tube closure, thereby disrupting the characteristic changes of cell shape. Delaying the precocious cell division by prolonging the S phase with a DNA replication inhibitor aphidicolin ameliorated the effects of cdc25. These results suggest that the long interphase during the 11th epidermal cell cycle is required for epidermal cells to change their shapes to accomplish neural tube closure.

2SC-03 光変換型発光タンパク質 Kaece を用いたホヤの変態過程における中枢神経系の通路

Tracing of the central nervous system of ascidian larva during metamorphosis with photovariable fluorescent protein, Kaece

Takeo Horie, Yasunori Sasaki (Shimoda Marine Research Center, University of Tsukuba)

Ascidians are primitive chordates, and their tadpole like larvae share basic body plan with vertebrate. Ascidian tadpole larvae change its shape to be sessile adult through metamorphosis. During metamorphosis, the larval central nervous system (CNS) is subjected to extensive rearrangement to construct the adult CNS. In this study, we examined how the larval CNS contributes to construct the adult CNS, by using a photovariable fluorescent protein, Kaece. The fluorescence of Kaece is green when it is translated, and the fluorescence is converted into red after irradiation with UV light. This fluorescence change is irreversible, and thus it enables us to trace the cell fates of labeled cells. We established various transgenic lines which expressing Kaece in the larval nervous system and traced larval neural cells during metamorphosis by utilizing Kaece’s photoconversion system. We show first that most of the ascidian larval CNS are maintained during metamorphosis and recruited to form the adult CNS. Second, most of the larval neurons disappear and only a subset of cholinergic motor neurons and glutamatergic neurons are retained. Third, epidermal cells of the larval CNS contribute to construct the adult CNS and some differentiate into neurons in the adult CNS. An unexpected role of epidermal cells highlighted by the present study is that they serve as neural stem-like cells to reconstruct the adult nervous network during chordate metamorphosis.

2SC-04 器官形成を支える細胞機能の動的解析

Imaging analysis of cellular dynamics for organogenesis

Erina Kuranaga (Lab. Histicogenetic Dynamics, RIKEN CDB)

Programmed cell death or apoptosis is a process by which cells are selected for death at set times in development and is used in an adult organism to maintain homeostasis by eliminating cells that have developed abnormalities. Apoptosis plays an important role in maintaining the cellular society not only by eliminating unnecded cells at given sites and stages, but also by other functions such as regulating the proliferation and migration of neighboring cells. Our aim is to elucidate the physiological roles of apoptosis and the basic mechanisms for regulating organogenesis using molecular, genetic and bioimaging approaches. We have chosen Drosophila as our model, taking advantage of its utility in developmental studies and the wealth of genetic data available. To elucidate the role of cell death in developmental processes, we will analyze the phenotypes of mutants for apoptotic signals in which the exterior male genitalia develops abnormally. In normal Drosophila development, the terminalia rotates 360 degree as it forms, but this rotation stops prematurely when apoptotic signals were inhibited. Imaging analysis reveals that in normal flies, the speed of this rotation is variable, with distinct initiation, acceleration, deceleration, and termination stages. Inhibiting apoptotic signals result in a loss of the acceleration phase, and a failure in proper terminalia development. Through imaging analysis of the process of genitalia rotation, we hope to gain better insight into how cellular network systems function in organogenesis.

2SC-05 極めて簡素な微生物における神経網の発生・機能のイメージング

Imaging of structure, development and function of nervous system in a simple vertebrate

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Imaging of a whole vertebrate embryo at single cell resolution is still challenging. Here we discuss our efforts to visualize morphogenesis, axonal navigation, neural circuit formation and its function. For morphogenesis, Kaede/KikGR, green-to-red photo-convertible, or Dronpa, a photo-reactivatable fluorescent proteins could be effectively used. We also have developed a method to induce fluorescent proteins locally by applying focused infra-red laser pulses in the brain of transgenic animal carrying a gene regulated by HSP-promoter. This method revealed axonal navigation from area of interest in the brain. For monitoring physiological activities of muscles and neurons, we performed Ca^2+ imaging of the whole embryo at 1 fps with GCAMP3, a GFP-based Ca^2+ indicator. It was found that the spinal cord cells fired in phase with the muscles on the same side during alternating tail flaps. The method also revealed local slow Ca^2+ waves sporadically appearing during the early brain morphogenesis. Analyzing 3D structure of the embryo or brain without deformation along z-axis is difficult even by two-photon scanning microscopy. X-ray micro-tomography could be more suitable to obtain this information, but in general, soft tissues are not easily imaged even after metal staining. Here we report that, by using contrast-enhanced X-ray micro-tomography in the Springle13, arrangement of cells in the whole embryo or brain even without staining could be visualized at near-single cell resolution.

2SD-01 タンパク質機能の分子構造生物学的解析：チトクロムC defenses素による事例研究

Introduction: cytochrome c oxidase, a case study towards quantum structural biological understanding of protein functions

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Cytochrome c oxidase reduces molecular oxygen to water coupled with proton pumping. Resonance Raman analyses showed that the O2-bound form appears as the initial intermediate during O2 reduction and that the second intermediate is isoelectric form bound to Fe4S3+. These results indicate that O2 is reduced to water in one and three electron reduction steps without releasing any reactive oxygen species. X-ray structural analysis using respiratory inhibitors for probing the function of the O2 reduction site provided the possible structural bases for the O2 reduction process as follows: C60H in a trigonal plane coordination is located significantly apart from Fe3O4O and that O2 at Fe4S3+ gives O2−Fe4S3+ to induce three possible electron transfer pathways. X-ray structure of bovine enzyme showed that a pathway comprised of a hydrogen-bond network and a water channel in tandem pumps protons. The hydrogen-bond network is hydrogen-bonded to the formyl group of heme a, suggesting that the net positive charge of heme a created upon oxidation is delocalized to the formyl group to drive the proton-active transport through the hydrogen bond network. The delocalization of the net positive charge to the formyl group has been proven by resonance Raman analyses. These findings indicate that both X-ray structural and vibrational spectroscopic analyses are indispensable for understanding the reaction mechanism as the behavior of the valence electrons of the functional sites.

2SD-02 中程度の分解能でどのようなアミノ酸側鎖のイオン化状態を決定するか

How to determine ionization states of amino acid residues by X-ray diffraction method at medium resolution

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