O₂ evolving is carried out by Mn-cluster in Photosystem(PS) II. In the enzyme cycle a Mn-cluster goes through five sequential redox states denoted S_n, where n varies from 0 to 4, upon the absorption of four successive photons. In the S₄ state two molecules of water are oxidized, the S₀-state is regenerated, and O₂ is released. By low-pH treatment, one Ca ion per PS II is selectively released concurrently with the loss of the capability of O₂ evolution which is restored by exogenously added Ca. The S₀ and S₂ states are paramagnetic with S=1/2, giving rise to a broad EPR multiline signal that can be detected at low temperature. Low-pH treatment leads to the formation of an S₂ state that is characterized by EPR multiline signal with distinctly altered multiline patterns. In order to investigate the structural modification of Ca²⁺-depleted Mn-cluster, we measured S₀-state and higher oxidized state EPR signal in Ca²⁺-depleted PS II. The S₀-enriched untreated membranes were prepared by 3 flash illumination of the S₁-enriched membranes after Y_D[•] radical was reduced chemically. The low-pH treatment has performed in S₀-enriched membranes. We detected modified S₀ multiline signal similar to that observed in untreated S₀ multiline signal. By addition of Ca²⁺ ion, normal S₀ multiline signal was appeared. Based on these observations, we will discuss the modified structure in Ca²⁺-depleted PS II.

3TP2-05 Photosystem II の蛋白質環境がどのようにしてクロロフィルペア P680 の強力な酸化力を引き出しているか

How photosynthetic reaction centers control oxidation power in chlorophyll pairs P680 in Photosystem II

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At the heart of photosynthetic reaction centers (RCs) are pairs of chlorophyll *a* (Chl*a*), P700 in photosystem I (PSI) and P680 in photosystem II (PSII) of cyanobacteria, algae, or plants, and a pair of bacteriochlorophyll *a* (BChl*a*), P870 in purple bacterial RCs (PbRCs). These pairs differ greatly in their redox potentials for one-electron oxidation, *E*_m. For P680, *E*_m is 1,100-1,200 mV, but for P700 and P870, *E*_m is only 500 mV. Calculations with the linearized Poisson-Boltzmann equation reproduce these measured *E*_m differences successfully. Analyzing the origin for these differences, we found as major factors in PSII the unique Mn₄Ca cluster (relative to PSI and PbRC), the position of P680 close to the luminal edge of transmembrane alpha-helix d (relative to PSI), local variations in the cd loop (relative to PbRC), and the intrinsically higher *E*_m of Chl*a* compared with BChl*a* (relative to PbRC).

3TP3-01 DNA—ヘリカーゼ相互作用の1分子観察

3P-253 Single—molecule observation of DNA-helicase interaction

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Escherichia coli UvrD is a superfamily 1 DNA helicase which plays a crucial role in nucleotide excision repair and methyl-directed mismatch repair. There is a general consensus that the enzyme unwinds a double-stranded DNA (dsDNA) from a 3' end single-stranded DNA (ssDNA) tail, a gap, or a nick and translocates along ssDNA in a 3'-5' direction. However, conflicting models for the detailed unwinding mechanism have been proposed.

To understand the unwinding and translocation mechanisms of the helicase in more detail using single-molecule fluorescence microscopy, we have been carried out fluorescence labeling of the helicase with a couple of fluorophores i.e. fluorescent dye, quantum dot or fluorescent bead and making improvements to our single-molecule force-fluorescence microscope in which single-molecule fluorescence imaging and single-molecule DNA manipulation by magnetic tweezers can be simultaneously performed.

We clearly visualized the binding modes of the helicase to various DNA substrates (18-nt ssDNA, 18-bp dsDNA, 18-bp dsDNA with a 20-nt 3' ssDNA tail, 4.7-kbp dsDNA and 4.7-knt ssDNA) immobilized on a PEGylated silicate coverslip, demonstrating that the helicase has low affinity with the 18-nt ssDNA and blunt dsDNA but high affinity with ss/ds DNA junction and that multiple helicases can attach to the 4.7-knt ssDNA¹. We then determined the number of the helicase interacting with DNA (18-bp dsDNA with various 3' ssDNA tail lengths (≥ 12 nt)) using a Cy5 labeled Cys-Ala mutant of the helicase, revealing that oligomeric form is the active form of the helicase and that dynamic interaction between them is necessary for efficient DNA unwinding.

1. Yokota *et al. Chem. Lett.* **38** 308-309 (2009).

3TP3-02 II型 DNA トポイソメラーゼの手品のような反応を直接みる

3P-261 Direct Observation of DNA Unlinking Magic by a Type-II DNA Topoisomerase.

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Type-II DNA topoisomerases (topoII) are molecular machines that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and unlink the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell

death. TopoII unlinks DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience.

We have developed a system that enables us to observe directly this unlinking magic under an optical microscope with fluorescently labeled DNA substrates. A pair of single molecules of DNA fixed on a chamber surface is mechanically braided with dual optical tweezers. Advantages of this method against bulk assay are that we can 1) observe unlinking reaction by a single molecule of topoII, 2) control the geometry of and the tension in the DNA braid in real time, and 3) observe the reaction repeatedly for the same DNA substrates. We have observed a DNA braid a few micrometer long is unlinked in one burst, in several tenths of a second after a waiting time typically a few minutes long. Apparently a single-molecule of topoII can unlink a braid of DNA in a processive fashion. We have also examined the dependence of unlinking rate of topoII on the DNA geometry and tension. Chiral preference for left- or right-handed DNA braid is also studied.

3TP3-03 細胞内 mRNA の長時間観察を可能にする蛍光核酸プローブ

3P-262 Long-term monitoring of intracellular mRNA by hybridization-sensitive fluorescent nucleotide probe

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We recently developed a doubly thiazole orange-labeled probe to achieve high fluorescence intensity for a hybrid with the target DNA or RNA, and effective quenching for a single-strand state of the probe. Emission from the probe was controlled by interdy excitonic interaction. Here we evolved the probe for long-term live cell imaging. HPLC analysis showed that newly designed probes with a 2'-O-MeRNA structure had high resistance against exo- or endo- nucleases, which is in contrast to the low resistance of the former DNA probes. These modified probes retained the function on the on-off switching of fluorescence emission in sensitive response to RNA recognition. For live cell imaging, the probe was introduced using lipofectamine 2000 reagent, and observed for 18 hours in culturing condition. The probe fluorescence was detected mainly from nuclei, and translocation of fluorescence to the cytoplasm region was also observed. Cell life events, for example, migration and division of probe loaded cells, were also observed in a long-term monitoring and were similar to those observed for normal cells. Therefore, the probe showed no severe cytotoxicity. We concluded that it will be a practical tool for effective monitoring of the spatiotemporal characteristics of RNAs in living cells.

3TP3-04 軸対称偏光子を用いた GFP1 分子発光双極子モーメントの3次元計測

Three dimensional measurement of emission dipole orientation of single GFP molecules by the use of axisymmetric polarizers

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Fluorescent single molecule imaging is one of the potential approaches to monitor structural dynamics and the function simultaneously. We employ this approach to monitor the structural dynamics of TrkA during its activation process. TrkA is a founding member of neurotrophin receptors that possess a single transmembrane domain and tyrosine kinase domain at its cytoplasmic region. Upon binding to its ligand, nerve growth factor (NGF), receptor forms a homo-dimer and the following activation of kinase domains occurs through the auto/trans phosphorylation of kinase domains. This phosphorylation is thought to be accompanied with the dynamic structural changes as has been seen in the crystal structural analysis of phosphorylated and non-phosphorylated forms of insulin receptor's kinase domain (Hubbard, 1997). We have been trying to detect this structural change of TrkA by monitoring the orientation of GFP that is connected to the cytoplasmic domain of the receptors. Defocused single molecule orientation imaging is suitable for immobilized proteins, however, for membrane proteins, fast two-dimensional and rotational diffusions makes it difficult to capture the clear defocused single molecule images. Here we present a novel method to detect the components of emission dipole moment in 3D by using a pair of polarizers which have radial and azimuthal transmission axes. Fluorescence from single GFP molecule is decomposed into polarization component parallel to the optical axis (z axis) and that perpendicular to the optical axis (x and y axes) in a dual-view apparatus. We could acquire the 3D orientation information of GFP dipole with the sampling rate as fast as 30 ms.

3TP3-05 光学顕微鏡（対物外アポダイズド位相差）と電子顕微鏡のインタ

3P-260 ラクティブ観察による、細胞核内部を運動する構造の同定

Interactive observation of optical and electron microscopy revealed moving particles in the nucleus.

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Gene expression is probably integrated into architectural framework of cell nucleus. How is dynamic change in the intranuclear structures correlated with nuclear functioning? Presently, it is difficult to answer the question well, because intranuclear structure is not well observed with conventional microscopy. We, therefore, developed pupil projection apodized phase contrast (PPAPC) microscopy. PPAPC is a combination of apodized phase contrast (APC) and pupil projection phase contrast (PPPC). Using PPAPC, we observed fine structures within the interphase nucleus in living Hela cells without staining. The fine structures were moving dynamically within the nucleus. Simultaneous observation of the nucleus with PPAPC and with fluorescence showed that particles were really moving inside the nucleus.

To identify the moving particles in the nucleus, we introduced interactive observation of optical and electron microscopy. We recorded particle movements in the nucleus with PPAPC microscope with optical slice thickness of 700-800nm. During the observation, we applied fixative to the cell under the optical microscope. The cell was fixed within 100msec after the application of fixative. The identical nucleus was sliced sequentially with thickness of 70nm and observed with electron microscopy. 10-12 sequential images of electron micrographs revealed the moving particles in the living nucleus observed with PPAPC. Mechanism of the intranuclear movement will also discussed in this presentation.

3TP3-06 ストレプトアビジン二次元結晶基板の高速 AFM 観察への適用

Streptavidin 2D crystals as solid supports for the visualization of biomolecular processes by high-speed AFM

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For the direct observation of biomolecular processes by atomic force microscopy (AFM), solid supports are required to fulfill several criteria, such as surface flatness, a capability to control the orientation of the immobilized molecules and absence of nonspecific protein adsorption. Here, we characterized streptavidin two-dimensional crystals as substrate surface. In this study, three types of streptavidin crystals with C222, P2 and P1 symmetries were obtained on the supported lipid bilayers. The surface of these streptavidin crystals is flat enough to identify the individual molecules on these supports. Proteins were specifically immobilized through streptavidin-biotin linkage on the crystal surfaces, while nonspecific adsorption of non-biotinylated proteins was highly restricted as revealed by fluorescent microscopy. These properties of the streptavidin crystal surfaces ensure the visualization of specifically immobilized proteins in a multi-component sample. Importantly, the inert surfaces towards nonspecific protein adsorption possibly guarantee the immobilized proteins against disfunction. As test samples, calmodulin (CaM), GroEL-GroES and actin filaments were observed by high-speed AFM. The conformational change of CaM upon binding to calcium was detected as a change in height of this protein. Furthermore, controlled orientation of immobilized GroEL-GroES complex was achieved by conjugating biotin at a specific site of GroEL. Finally, a polymerization process of a single actin filament was traced.

3TP4-01 フォトンフォースによる水溶液中のアミノ酸の分子捕捉

Optical Trapping of Amino Acids in Aqueous Solution by Photon Force

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The optical trapping technique would be the only methodology in a non-contact-mode to capture and 3D-manipulate a molecule in solution phase. Manipulation of bioactive functional molecules has potential applications in bio/medical science. The target of molecular trapping has hitherto been limited only to a few large macromolecular systems: Natural polymers such as DNA and artificial polymers (Y. Tsuboi, M. Nishino, Y. Matsuo, K. Ijiri, N. Kitamura, *Bull. Chem. Soc. Jpn.*, **Vol. 80**, (2007), 1926 - 1931). Small molecules have never been optically trapped in solution, but instead their translational diffusion has been observed to be suppressed at a focal point due to a shallow optical potential. In the present study, we examined optical trapping of several amino acids in aqueous (D2O) solution by focusing a near-infrared CW laser beam ($\lambda=1064$ nm). Photon-force-induced molecular assembling of amino acids was revealed by means of microscopic imaging and Raman microspectroscopy. We experimentally demonstrated that photon force can trap amino acids dissolved in aqueous solutions. Amino acid molecules assemble in a few minutes at the focused area. A growth rate of aggregate increases with increases in concentration and laser power. The origin of the present optical trapping of amino acids was ascribed to the cluster trapping mechanism (Y. Tsuboi, T. Shoji, N. Kitamura, *Jpn. J. Appl. Phys.*, **Vol. 46**, (2007), L1234 - L1236), and the technique could be extended to other small molecules or small oligomers. Such molecular trapping and manipulation based on photon force has a wealth of application such as selective drug delivery to a living cell, molecular separation, targeted synthesis of bioactive molecules (protein).

3TP4-02 一周一細胞の上皮性チューブの形成

Formation of an epithelial tube of single cell-size circumference

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Tracheal branches of *Drosophila* are simple tubes consisting of an epithelial monolayer wrapped around a lumen. A thin tracheal tube is a tube of single cell-size circumference. This type of tube develops by transformation of a tube of multiple cell-size circumference, in which all adherence junctions (AJs) are intercellular, into a thinner tube of single cell-size circumference including auto-cellular AJs. The transformation consists of cell rearrangement and cell intercalation.

...The cell intercalation can be described by a cell model of vertex dynamics, which we have been constructing previously.^{2,3} Using the cell model, we computer-simulated the tube transformation into the tube with auto-cellular AJs. The transformation proceeded under the perturbation of polarized extension of cell bodies.⁴ In the present paper, we report a successful result in simulation under repeated contraction of AJs. The result of simulation is compared with an actual observation of tracheal morphogenesis.

1. Ribeiro et al., *Curr. Biol* 14: 2197-2207, 2004.
2. Nagai & Honda, *Philosophical Magazine B* 81: 699-719, 2001.
3. Honda, Nagai & Tanemura, *Devel. Dyn.* 237: 1826-1836, 2008.
4. Honda & Nagai, *41st Ann. Meeting JSDB*, 1P029, 2008.

3TP4-03 枯葉に擬態した蛾の翅模様に見られる揺らぎ構造とモジュールデザイン

Variational and Modular Design of Moth Wing Pattern Mimicking a 'Dead Leaf'

Takao Suzuki (1), **Shigeru Kuratani** (1) ((1) RIKEN CDB Lab. for Evol. Morph.)

How an enormous organismal diversity and complexity could have evolved is a fundamental question of design principles of living organisms. In general, natural selection shapes the variational/covariational organization of morphological parts. However, it has never been referred to the variational aspects of the pattern arrangements. Here, we report the morphological design of moth wing pattern mimicking a dead leaf.

As a model organism, we picked up a noctuid moth, *Oraesia excavata*, whose forewings have all the appearances of a detailed resemblance to a leafy pattern with one main and left-/right-sided leaf veins. By employing morphometrics, we found that the leafy pattern established a precise and sophisticated organization: a lower variation against perturbation and the modular architecture concordant with sub-functional tasks. To satisfy ecological requirements, these characteristics are likely to improve a camouflage strategy for an effective resemblance in a wild life. In addition, we found that when the leafy pattern evolved, inter-dependency of pattern bands were restructured through coupling and uncoupling between them. The complex and intriguing pattern was generated by an extremely flexible developmental system that allows independent changes of these pattern bands free from mutual constraints. To gain the further insight into the variational organization, we developed an analytical method for distinguishing between developmental noise and genetical variation. We would discuss ideas on how to proceed from here.

3TP4-04 ニワトリ砂胃の平滑筋は cSWiP-1 によって間充細胞から分化する

Differentiation of smooth muscle in chicken gizzard is induced by cSWiP-1 protein

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During development of the chicken gizzard, a thick layer of mesenchymal cells is first constructed, and then, they differentiate into smooth muscle cells or connective tissues. We (Ko et al.1996) succeeded to differentiate smooth muscle cells from cultured mesenchymal cells. The differentiation of smooth muscle cells in chicken gizzard occurred first near the outer surface of gizzard, and the differentiated area spreads to the inside of the gizzard. Therefore, it was assumed that the differentiation of most of the smooth muscle cells in the gizzard is induced by the differentiated smooth muscles. In fact, the extract of differentiated gizzard induced the differentiation of smooth muscle cells from undifferentiated cells in culture (Kofuji et al.2002). The cDNA cloning of active factor for the differentiation of smooth muscle cell was performed using library of embryonic chicken gizzard, and we isolated a clone containing a part of cSWiP-1 gene as a candidate. It has been reported that cSWiP-1 is SOCS box-containing WD-protein associated with Shh signaling. This protein is synthesized in embryonic chicken gizzard or limb bud, but the physiological function is this protein is little known. We obtained full size cSWiP-1 gene by PCR method and obtained cSWiP-1 protein. This protein induced the differentiation of smooth muscle cells in culture. Therefore cSWiP-1 protein or related protein may induced the differentiation of smooth muscle in chicken gizzard.