1SA-01 細胞内反応ネットワークと「少数性」問題—理論と計算によるアプローチ

Cracking Reaction Networks Involving "Minorities" in the Cell: Theoretical and Computational Approaches
YukiTogashi (Grad. Sch. Sys. Informat., Kobe Univ.)

The activities of biological systems are maintained primarily by chemical reactions, and often modeled as reaction-diffusion systems represented by partial differential equations for the "concentrations" of chemicals. However, this classical scheme requires some preconditions. First, each reaction event is instantaneously completed and not affected by previous reactions. Secondly, the molecules diffuse freely and their excluded volume can be neglected. These conditions are however not always satisfied; many of enzymes are molecular machines whose reaction cycles and motions are coupled, and cells are highly crowded with macromolecules. Moreover, recent experiments have shown that there are a number of "minority" chemicals, existing only one or a few molecules per cell. For such rare species, the notion of continuous "concentrations" is no longer valid, and the classical scheme depending on it breaks down. Using simple autocatalytic models, we previously showed that the molecular discreteness in such rare chemicals, either discreteness in numbers (i.e. integers) or spatial discreteness (i.e. finite space between molecules), may affect the reaction-diffusion behavior. Toward a theoretical framework to predict phenomena in the complex molecular networks in the cell, we have expanded the models for a number of chemical species. In some cases, the behavior strongly depends on the system size, which suggests importance of being moderately small. Relevance to cell behavior and possible experimental designs will be also discussed.

1SA-02 1分子デジタル ELISA
Single-Molecule Digital ELISA
Hiroyuki Noji (Applied Chem. U-Tokyo)

I will introduce the single-molecule digital counting assay based on the array system of a million of water-in-oil droplets that we have recently developed (Lab on a chip 2010). Especially, I will focus the digital ELISA assay as one of application methods of the digital counting. The detection principle of the single-molecule digital counting is very simple; by encapsulating a single molecule of enzyme or enzyme-conjugated antibody in a micron-sized reactor, the catalytic product molecules are highly accumulated in the micron space. Individual chambers resultanty give detectable signal, fluorescence from reaction product molecules in general. By counting the number of chambers with evidently high fluorescent signal under an optical microscope, the number of target molecule is determined. As a model reaction, we conducted digital ELISA of the marker molecule of prostate tumor (PSA). The detection limit was revealed to be 2 amol is that million-times lower than that of conventional ELISA assay of PSA, demonstrating the extremely high sensitivity of digital counting assay. In the last part of the talk, I will also discuss about the prospect of the digital counting assay for single cells assay in addition to the integration to CMOS imaging sensor for palm-top digital counting devise.

1SA-03 伝達子にコードされた発光製プローブ
Genetically encoded luminescent probes
Takeharu Nagai1,2 (ISIR, Osaka Univ., PRESTO, JST)

Optogenetic tools including ChR2, LOV domain, and CALI (chromophore-assisted light inactivation) allows us to operate biomolecule function upon light irradiation, by which we are now able to investigate protein of interest in terms of functional difference according to the existing area inside cells and expression stage with real time resolution. To understand what’s going on after the light-switching of optogenetic tools, many researcher may want to visualize some other molecular dynamics and function by fluorescence imaging. However, excitation light for the imaging shall mis-activate or mis-inactivate the optogenetic tools so that we cannot apply the fluorescence imaging in combination with the optogenetic technology. To overcome this problem, we have been developing genetically-encoded luminescent probes which are based on hybrid between a mutated chemiluminescent protein and a fluorescent protein variant. In the symposium, I will introduce our recent achievement such as development of color variant of bright luminescent protein, and the use of them in conjunction with the optogenetic technologies for understanding operation principles of bio-nanosystems.

1SA-04 協調的逆転反応に焦点を当てた光動力性モーターのシナジーとその実現

Coordination reversal of flagellar motors on a single Escherichia coli cell
Akihiro Ishijima (Tohoku University)

An E. coli cell transduces extracellular stimuli sensed by chemoreceptors to the intracellular signal molecule, which regulates the switching of the rotational direction of the flagellar motors in both direction changes. The switching is highly coordinated with a sub-second delay between motors in correlation with the distance of each motor from the chemoreceptor patch localized at a cell pole. This result suggested that a transient increase and decrease in the concentration of CheY-P caused by a spontaneous burst of its production by the chemoreceptor patch. The switching delay of the both switching was individually investigated. In wild-type cell, the switching delay clearly correlated with each motor's distance from the receptor patch in both switching. However, in a mutant cell lacking CheAs, which is required for the polar localization of CheZ, in a CW-to-CCW switching, obvious switching delay was not observed. This result suggested that the polar localization of CheZ is critical for the directed propagation of the decrease of CheY-P concentration. Next, we used caged serine and developed a photoreleasing system of serine from the caged compounds. The response time was shortened by photorelease of serine. The measured response time appeared to increase with increment of distance between receptor patch and flagellar motor. This result suggested that the directed intracellular signal was propagated in cytoplasm from receptor patch to each flagellar motor with sub-seconds time scale.

1SA-05 ヒトゲノム DNA の収納とそのダイナミクス

Human genome organization and dynamics
KazuhirO Maeshima (Structural Biology Center, National Institute of Genetics)

Human genome DNA of around 2 m in length is organized into a cell nucleus having a volume of only 1 picoliter. Each genome has only two sets of genes. In order to activate a certain gene, a protein factor, which is normally considered to be in a small number, has to search the whole genome and target the specific gene. In this process, genome organization must play an important role. In fact, how the human genome DNA is organized into a nucleus? The DNA is wrapped around histones, forming a nucleosome structure. The nucleosome had been assumed to be folded into a 30-nm chromatin fiber and other helical folding structures. However, our recent cryo-microscopy (cryo-EM) and synchrotron X-ray scattering analyses have shown almost no visible 30-nm chromatin fibers or other regular structures in interphase nuclei and mitotic chromosomes. This suggests that chromosomes consist of irregularly folded nucleosomes fibers. Thus, nucleosome fibers may be continuously moving and rearranging at the local level. Recently we observed these local nucleosome dynamics, which could be crucial for scanning genome information.

1SA-06 映乳頭胞細胞周期のシステム生物学-合成生物学

Systems and Synthetic Biology of Biological Timings
Hiroki Ueda1,2 (RIKEN, CDB, RIKEN, QBIC)

The logic of biological networks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics. Mammalian circadian clock system is such a complex and dynamic system consisting of complicatedly integrated regulatory loops and displaying the various dynamic behaviors including endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes (temperature and light cycle), and iii) temperature compensation over the wide range of temperature. In this symposium, I will take a mammalian circadian clock as an example, and introduce the systems- and synthetic-biological approaches for understanding of biological timings.

References