Mathematical analysis of dynamical robustness in biological networks
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Biological systems are highly tolerant to some types of perturbations to which the systems are usually exposed. It is thought that this robustness has been acquired through an adaptation to the environment in the evolutionary process. However, they can be extremely fragile to other types of perturbations which rarely happen. Although this 'robust but fragile' property is an essential feature of biological systems, a mathematical theory to understand this property is yet to be fully established. A potent mathematical approach to examine structural robustness of biological networks has been developed in complex network theory. It has been revealed that heterogeneously connected networks (e.g. scale-free networks) are highly robust against random removal of nodes but extremely vulnerable to targeted removal of hubs. However, dynamics is not considered in this framework. Here we focus on another framework to deal with the robustness of dynamic activity in biological networks consisting of elements having intrinsic dynamics. When some elements are inactivated, the level of dynamic activity of the whole network is lowered. There is a critical ratio of inactivated elements, at which the network dynamics vanishes. By analyzing this phase transition, we show that heterogeneously connected networks are highly fragile to targeted inactivation of low-degree elements. This is in strong contrast to the property of structural robustness. Our result implies that the interplay between dynamics and structure plays an important role in network robustness.

1SC-05 自律的酵素活発度による時間スケール調整: ホメオスタシスと記憶
Homeostasis and memory by autonomous regulation of time-scales through enzyme abundances
Kunihiko Kaneko (University of Tokyo, Center for Complex-Systems Biology)

Biological systems generally consist of a variety of time scales. These timescales also change in time depending on their internal state, according to the change in abundances of enzyme that governs the reaction speed. Here we discuss that homeostatic response and memory emerge as autonomous regulation of enzyme concentrations. First it is shown that slow relaxation process with some plateaus generally emerge in dynamics of catalytic reaction networks, where the negative correlation between the enzyme and substrate abundances is a key factor for such 'glassy' dynamics [1]. Second, we demonstrate that the enzyme-limited competition leads to a homeostasis in the system. To be specific, we study temperature compensation in period of circadian rhythm [2]: By considering a simple system consisting just of Kai proteins, the period of the rhythm is found to be kept constant against temperature change. The origin of this temperature compensation is attributed to enzyme-limited competition, where negative correlation between abundances of substrates and enzymes again plays an important role. Finally, another consequence of long-term dynamics by autonomous regulation of enzyme abundances is generation of cellular, epigenetic memory. After discussing this possibility, I will also illustrate relevance of epigenetic memory to adaptation and differentiation, if I have time.


1SD-01 Characterization of Protein-DNA complexes dynamics related to Chromatin structure regulation using Single-Molecule Techniques
Yong-Woon Han, Yoshihiro Harada (iCeMS, Kyoto University)

Eukaryotic gene expression is regulated by chromatin structures and/or DNA modification such as CpG methylation. The basic unit of eukaryotic chromatin structure is a nucleosome consisting of approximately 150 bp DNA wrapped in 1.7 superhelical turns around a histone octamer. The histone octamer consists of two copies each of H2A, H2B, H3 and H4. Post-translational histone modifications such as acetylation, methylation, phosphorylation and ubiquitination regulate chromatin structure, resulting in activation or repression of gene expression. On the other hand, CpG methylation represses gene expression and is essential for silencing of parasitic DNA, genomic imprinting and embryogenesis. During DNA replication, methylated CpGs are converted into hemi-methylated CpGs and newly replicated CpGs should be methylated in order to maintain methylation pattern. DNA methyltransferase 1 (Dnmt1) is the enzyme to methylate hemi-methylated CpG regions. Np95 is methylated CpG binding protein and interacts with Dnmt1, followed by recruitment of Dnmt1 to hemi-methylated CpG regions. SRA domain of Np95 is responsible for hemi-methylated CpG binding activity. We characterize the process of hemi-methylated CpG recognition by SRA domain using Single-Molecule technique, and in this symposium, we will show our present data.

1SD-02 The mechanism of nuclear protein searching on DNA: Coarse-Grained simulation study
Tsuoshi Terakawa (Grad. Sch. Sci., Univ. Kyoto)

Various nuclear proteins search their specific binding sites on DNA and function at proper time and location. This process is important for gene expression regulation. Previous theoretical studies have revealed that combination of one-dimensional diffusion along the DNA chain and three-dimensional diffusion in the bulk solution makes it possible for these proteins to search its cognate binding site efficiently. Although this theory is based on an assumption that these proteins can quickly diffuse on DNA, the mechanism of the quick diffusion at molecular level has been elusive. In addition, it has been controversial whether the same mechanism is valid in nucleosomal environment in which histone proteins bind to DNA and possibly hinder the one-dimensional diffusion. In order to approach such problems, we have developed coarse-grained model for protein-DNA complex where most of the parameters are derived from atomic structural information or atomic simulation results. Using this model, we conducted molecular dynamics simulations of several proteins (e.g. TF IIIA, p53, and PCNA) with DNA and got insights into the mechanism of one-dimensional diffusion on DNA. We also performed simulations of the proteins with DNA to which histone proteins bind. Then, we will discuss the validity of this mechanism in nucleosomal environment.

1SD-03 Differences in dissociation free-energy profiles between cognate and non-cognate protein-DNA complexes
Yoshit实eru Yonemata, Hidetoshi Kono (Japan Atomic Energy Agency)

DNA-binding proteins recognize cognate and non-cognate DNA sequences with two different binding modes, in which proteins loosely bind to non-cognate DNA sequences, but to cognate sequences they tightly bind. Experimental structures of such complexes provides us an atomic view of the binding modes, however, it is not so simple to dissect which is cognate or non-cognate complexes by seeing the structures. In this study, free-energy profiles for dissociation of a cognate and a non-cognate Lac repressor-DNA complexes were calculated to obtain energetic views by performing atomic-level molecular dynamics simulations. We implemented an algorithm of adaptive biasing force (ABF) [E. Darve, A. Pohorille, J. Chem. Phys. 128, 144120 (2008)] into the AMBER software to calculate free energy changes in dissociation along a dissociation path. The results showed that the free energy profiles were clearly distinct between the cognate and non-cognate complexes. We found that this difference can be interpreted in terms of changes in the protein-DNA contacts and the number of interfacial hydration water. The calculated dissociation process here agrees with that suggested from an H/D exchange experiment. In the talk, we will discuss how gene regulatory proteins find their target sites on the DNA and what are determinants for distinguishing cognate and non-cognate targets.

1SD-04 細胞周期における染色体ダイナミックスの定量化解析
Quantitative analyses of chromosome dynamics in C. elegans early embryos
Takeshi Sugawara, Ritsuko Arai, Akatsuki Kimura (National Institute of Genetics)

During development and differentiation, it has been suggested that chromatin remodeling occurs and contributes to cell fate determination. However, there are few studies for chromatin dynamics during the course of development. We visualized and tracked chromatin loci in C. elegans early embryos. Based on the experimental data, we statistically analyzed dynamics of the chromatin locus, and then revealed quantitative features of chromatin dynamics for several developmental stages. We found several quantitative features that depend on
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developmental stages and the size of the nuclei at the stages. In order to characterize chromatin dynamics further, we are focusing on the anomaly of chromatin diffusion. The anomalous diffusion should be caused by uncharacterized intra-nuclear structures that disrupt free diffusion of the chromatin locus. Therefore, we may be able to extract biological information about intra-nuclear structures.

1SD-05
萤光相関分光法を用いた細胞内グロコルチコイド受容体の動態解析
Inside view of molecular dynamics of Glucocorticoid Receptor by using Fluorescence Cross Correlation Spectroscopy in living cell
Masataka Kinjo (Faculty of Advanced Life Science, Hokkaido University)

The glucocorticoid receptor (GR) is a ligand-inducible transcription factor belonging to the nuclear receptor superfamily. Upon ligand binding, GR translocates from cytoplasm to the nucleus, then associate as a dimer form with glucocorticoid response element (GRE) in a promoter region, or as a monomeric complex that operate with other transcription factors to induce transcription.

1SE-01
塩白質構造の整合性原理とその拡張
Consistency principle of protein conformation and its extension
Masaki Sasai1,2,3 (Nagoya University, 1Okazaki Institute for Integrative Bioscience, 3Korea Institute for Advanced Study)

Proteins are evolutionarily designed polymers which have the ability to fold into unique conformations (either as single molecules or as parts of molecular complexes). The design principle of proteins to facilitate such folding was summarized as “consistency principle” by N. Go in 1983. The validity of this principle was intensively examined in 1990s and 2000s, from the view point of the closely related “minimal frustration principle”, and the power of these principles in analyses of folding pathways, structure prediction, and protein design has been proven. In this talk, we revisit the consistency principle and discuss its extension to analyze protein functioning. First, the consistency principle is represented in 2 or 3 dimensional space of order parameters of conformation change. In such multi-dimensional expression, competition among multiple folding pathways and coexistence of multiple intermediates can be analyzed, and the further application of the principle is discussed. Proteins have evolved, however, not to fold but to function, so that the functional necessities may lead to inconsistent interactions in proteins. We discuss the localized inconsistent (frustrated) regions in proteins, which are important in allosteric transformations and catalytic reactions. We show the chameleon model, which was introduced by T.P. Terada and colleagues, is the efficient tool to analyze the roles of inconsistency in proteins.

1SE-02
Dynamic mechanism for the transcription when responses to activators

The transcription apparatus (TA) is a complex molecular machine, and involves complicated interactions between the enhancer and various proteins, and those between the related proteins themselves. The TA detects the time-varying concentrations of transcriptional activators and initiates mRNA transcripts at appropriate rates. We propose a dynamic model to describe how the TA can dynamically orchestrate reliable transcriptional response based on the general configurations of TA. We argue that there exists a relatively stable clamp-like space between the Mediator complex and the enhancer, and activators cycle rapidly in and out this space. The entry of activators into this space results in conformational transition in the Mediator complex, which gives rise to a facilitated circumstance where Pol II's can initiate-re-initiate transcription rapidly. As a result, the activators’ concentration can be encoded by a temporal occupancy rate which modulates the transcription. Furthermore, we build a stochastic model which reproduces and reconciles several experimental observations. These indicate that regulated transcription likely shares the same dynamic principles. [1] Y. L. Wang, F. Liu, and Wei Wang, “Dynamic mechanism for the transcription apparatus orchestrating reliable responses to activators”, Scientific reports, 2: 422, DOI: 10.1038/srep00422

1SE-03
保存アミノ酸位に着目したジンクフィンガーの構造と分子認識
New Functions of Zinc Fingers Revealed by Substitution of Conserved Residues
Miki Imashii (JCR, Kyoto Univ.)

The C2H2-type zinc finger motif is among the most major DNA binding motifs in eukaryotes. Each finger folds into a globular structure by coordination of a zinc ion with the conserved two cysteine (C) and two histidine (H) residues in a tetrahedral fashion. This fold is stabilized by hydrophobic interactions formed by the conserved hydrophobic amino acid residues. Usually, multiple zinc finger motifs are connected by conserved linkers. Though the Zn(II) ligands, the hydrophobic amino acid residues, and the linker are highly conserved among the C2H2-type zinc fingers, their contribution to structure and functions is not clearly understood. Here, the highly conserved amino acid residues were mutated and the function was investigated. Mutation of the conserved hydrophobic amino acids clarified the relationship between formation of a hydrophobic core and DNA binding function. On the other hand, ligand substituted zinc fingers, in which cysteine residue(s) is substituted to histidine residue(s), showed hydrolytic activity. In addition, the Zn(II)-dependent DNA binding domain, C2H2-zinc finger, has been successfully created by reducing the Zn(II) binding affinity via ligand substitution of the second cysteine to aspartic acid. The linker alteration changed the DNA binding selectivity to discontinuous target sequences. The new functions of zinc fingers revealed by substitution of highly conserved residues will be discussed.

1SE-04
ドメインスワッピングによるシトクロムcの多量化
Oligomerization of cytochrome c by domain swapping

Cytochrome c (cyt c) is a stable protein which functions in a monomeric state as an electron donor for cytochrome c oxidase. It is also released to the cytosol at the early stage of apoptosis. For nearly half a century, it has been known that cyt c forms polymers, but the polymerization mechanism remains unknown. We found that cyt c forms polymers by successive domain swapping, where the C-terminal helix is displaced from its original position in the monomer and Met-heme coordination is perturbed significantly [1]. In the crystal structures of dimeric and trimeric cyt c, the C-terminal helices are replaced by the corresponding domain of other cyt c molecules and Met80 is dissociated from the heme. The solution structures of dimeric, trimeric, and tetrameric cyt c were linear based on small-angle X-ray scattering measurements, where the trimeric linear structure shifted toward the cyclic structure by addition of PEG and (NH4)2HPO4. The absorption and CD spectra of high order oligomers (~40mer) were similar to those of dimeric and trimeric cyt c but different from those of monomeric cyt c. For dimeric, trimeric, and tetrameric cyt c, the ΔH of the oligomer dissociation to monomers was estimated to be about ~20 kcal/mol per protomer unit, where Met-heme coordination appears to contribute largely to ΔH. The present results suggest that cyt c polymerization occurs by successive domain swapping, which may be a common mechanism of protein polymerization.


1SE-05
マルチドメインタンパク質のホモフォールディングと機能イメージング動力学
Folding and conformational dynamics of multi-domain proteins
Shoji Takada (Dept Biophys, Kyoto Univ.)