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 聚光鮮明分光法を用いた細胞内グルココルチゾ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. Besides homodimer of GR can act as repressor by association to negative GRE, GR can tether other transcriptional factors, such as NFkB, as monomer. These complicated regulation can be unraveled by determination of affinity properties of GR and/or associated molecules in living cell. Fluorescence correlation spectroscopy (FCS) provides two molecular properties such as the diffusion time and the number of molecules in defined region. Then, molecular interaction is accessible from the changing of the diffusion time. FCS measurement revealed interaction between GR and DNA in nucleus could be modified by difference of ligand.

To understand more detailed of GR dynamics in nucleus, the dimerization process of GR that is occur before binding to GRE was evaluated by using Fluorescence Cross Correlation Spectroscopy (FCCS) measurement of transiently expressed mCherry tandem dimer (mCh2) and EGFP fused GR in living cell.

In our experiment, the positive cross correlation was obtained after addition of DEX. Then, Kd (association constant) value of dimerization of GR wild type and mutant was estimated and compare in living cell.

1SE-01 蛋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, 2Korea 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 those 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 conformational 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 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 保存アミノ酸性で構成されたジンキフィンの構造と分子認識
New Functions of Zinc Fingers Revealed by Substitution of Conserved Residues
Miki Imanishi (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 function 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, CDH2-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)2PO4. 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フォールディングとコンフォメーション動力学
Folding and conformational dynamics of multi-domain proteins
Shoji Takada (Dept Biophys, Kyoto Univ.)