3PT004 蛍光相互相関分光法を用いた転写因子-DNA 間相互作用の定量**Determination of dissociation constant between glucocorticoid receptor and DNA using FCCS**

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Glucocorticoid receptor (GR) is a transcriptional factor that controls broad range of physiological gene networks. By stimulation of ligand, not only GR activates gene transcription by association to the specific genomic glucocorticoid response elements (GRE) as a dimer, but also GR represses transcription by association to negative GRE as a monomer in nucleus. Hence, it is important to understand quantitatively association of GR to GRE.

For measurement of affinity between DNA and DNA binding protein, such as a transcriptional factor, the electrophoresis mobility shift assay (EMSA) is well used in vitro. However, in EMSA, their interaction is enhanced by the cage effect of gel matrix. Therefore, it is difficult to discuss about the affinity acquired from EMSA as in living cells, although EMSA is excellent as a method for detecting their interaction.

So, we established the determination of the affinity (dissociation constant, Kd) between GR and DNA contained GRE sequence using fluorescence cross correlation spectroscopy (FCCS). For FCCS measurement, the full length human GR was expressed in insect cells as EGFP fused protein (EGFP-GR) and purified by using combination of His-tag and cation exchange column chromatography. By FCCS measurement of purified EGFP-GR with Alexa647-labeled GRE, the dissociation constant of GR-GRE was estimated as 2.71 μ M. Furthermore, we will discuss the differences of affinity of GR to several different sequence of GRE.

3PT005 Quantitative study of Homo-Dimer Glucocorticoid Receptor interaction by using Fluorescence Cross Correlation Spectroscopy in living cell

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The Glucocorticoid Receptor (GR) is a member of a superfamily of ligand inducible transcription factors that control variety of physiological and metabolic processes. Upon ligand binding, GRs translocate from cytoplasm to nucleus, then associate with glucocorticoid response element (GRE) as a dimer. This dimerization involves in regulation of variety of genes; however it is not clear that GR dimerization takes place in cytoplasm or nucleus in living cell. To understand the dimerization mechanism of GR, Fluorescence Cross Correlation Spectroscopy (FCCS) was performed in living U2OS cells transiently expressed mCherry tandem dimer and EGFP fused GR wild type. The positive cross correlation was obtained after addition of Dexamethasone (Dex) which is an agonist for GR. Then, the Kd value of dimerization of GR wild type was estimated as 13.2 μ M. Moreover, we measured A458T and C421G mutants which were mutated to disable for dimerization and DNA binding, respectively, for further understanding dimerization of GR. In nucleus after addition of Dex, almost no cross correlation was observed in A458T mutant. On the other hand, positive cross correlation was obtained in C421G mutant as wild type. Then, the Kd value of dimerization of C421G was estimated as 12.3 μ M. These results suggest that DNA binding is unnecessary to dimerization for GR, and GR is dimerized before association with GRE. Furthermore, we will discuss the dimerization mechanism in cytoplasm by FCCS measurement of nuclear translocation deficiency mutant.

3PT006 Transcription factor p53 searching dynamics on nucleosomal DNA: Coarse-grained molecular dynamics simulation study

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Transcription factor p53 has to search its specific binding site on DNA to activate transcription of several proteins that are involved in tumor suppressing process. Two of four major domains of p53 [Core domain (Core) and C-terminal domain (CTD)] are known to bind to DNA non-specifically. The roles and interplay of these two DNA binding domains during the search have been controversial. Furthermore, the searching mechanism on nucleosomal DNA in which histone proteins bind to DNA has been largely unknown. In this work, we

performed coarse-grained molecular dynamics simulations of tetrameric full-length p53 on duplex DNA with and without histone proteins to elucidate such searching mechanisms.

First, we conducted coarse-grained simulations of p53 on DNA without histone proteins at various ion concentrations. The result shows that only CTDs keep bound to DNA and the Cores frequently hop on DNA during the search. This is in perfect agreement with a recent single molecule experiment. The result also shows that the sliding via the CTD does not follow the helical pitch of DNA (rotation-uncoupled diffusion mechanism) at physiological ion concentration. This finding indicates that p53 can bypass the obstacles on DNA such as histone proteins.

Second, in order to get insight into how p53 bypasses the histone proteins, we conducted coarse-grained simulations of p53 on DNA with histone proteins. The result indicates the importance of rotation-uncoupled diffusion mechanism to bypass the obstacles on DNA.

3PT007 粗視化分子シミュレーションを用いた T4 sliding clamp の DNA 上での動きの解析**Movement of T4 sliding clamp on DNA studied by coarse-grained molecular simulations**

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DNA sliding clamp proteins increase enzymatic processivity of DNA polymerases during DNA synthesis. DNA sliding clamp also play a key role in controlling several reactions, such as DNA repair, translesion DNA synthesis, DNA methylation and chromatin remodeling, through the coordination and organisation of different proteins. DNA sliding clamp forms a closed circle around double stranded DNA and is able to move along DNA in a random walk, but the molecular nature of this diffusion process along DNA is poorly understood.

Then we studied movement of the DNA T4 sliding clamp (bacteriophage) on double strand DNA at different ion strength by using a coarse-grained molecular dynamics method of CafeMol. We used Clementi's Go model for proteins, de Pablo model for DNA and included electrostatic and excluded volume interactions between DNA and protein. From a series of simulations, we measured the diffusion constant of the DNA T4 sliding clamp, its dependence on the ion strength, coupling between the rotation around DNA and the diffusion. We also analyze the tilt angle toward DNA.

3PT008 分子シミュレーションと電子顕微鏡像を用いて解析された tRNA 転位の自由エネルギー地形**Free-energy landscape of tRNA translocation through ribosome analysed using MD simulations and cryo-EM density maps**

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Ribosome is one of the supra-biomolecules used in the process of translating genetic information for the synthesis of polypeptides. In the course of its synthesis, two tRNA molecules move with mRNA through ribosome. This process, called translocation of tRNAs, is catalyzed by the elongation factor G (EF-G). Recent results from cryo-electron microscopy (cryo-EM) suggest that there are large-scale structural rearrangements of both ribosome and EF-G occur. However, the dynamic mechanism of translocation is unclear at the atomic level. We used all-atom molecular dynamics (MD) simulations including water molecules to direct 70S ribosome complexed with EF-G at the post-translocational state towards the pre-translocational states by fitting 70S ribosome into cryo-EM density maps. Additionally, the simulations were assisted by umbrella sampling simulations, in which biased potentials were imposed on the centers of masses of the protein molecules in the 70S ribosome, to relax the transitional conformations and to construct the free-energy landscape of the translocation. Multistep structural changes, such as a ratchet-like motion between the small and large ribosomal subunits were observed during the translocation. The free-energy landscape shows that there are semi-stable states between two stable states at the pre- and post-translational states. It was shown that a loop of nucleic acids from the small ribosomal subunit, which is located near the P- and E-sites, plays an important role in the translocation of P-tRNA and E-tRNA.

3PT009 粗視化シミュレーションによる単 / 多ヌクレオソーム系の構造ダイナミクス**Mono- and poly-nucleosome structural dynamics by coarse-**