bicelles. In summary, we found that the fibril structures and fibrillation kinetics in the presence of bicells were different significantly from those in the absence of bicells.

2P-102 超分子複合体タンパク質の微小重力条件下での結晶化

Crystallization of supramacromolecular complexes of proteins under microgravity condition

Yu Hasegawa (1), Nobutaka Numoto (1),(2), Koji Inaka (3), Taro Nakagawa (2),(4), Yoshihiro Fukumori (2) and Kunio Miki (1). (1: Dept. Chemistry, Graduate School of Science, Kyoto Univ.; 2: School of Natural System, College of Science and Engineering, Kanazawa Univ.; 3: MARUWA Foods and Biosciences, Inc.; 4: Research Center for Micro-Nano Technology, Hosei Univ.)

High-quality single-crystals are essential to obtain structures of proteins by means of X-ray crystallography. Crystallization of supramacromolecular protein complexes is more of a challenge than any other small protein. Crystallization in space is expected to have some significant advantages to obtain better crystals, because microgravity environment could reduce some unfavorable effects on crystallization caused by convection and sedimentation. We carried out crystallization of two supramacromolecular complexes as target proteins in space experiments. One is a kind of giant hemoglobin composed of 180 subunits with molecular mass of 3,600-kDa (complex I) and the other complex having ATPase activity consists of 8 subunits with 400-kDa (complex II). Crystallization in space was performed using the counter diffusion method and some crystals of both protein complexes were successfully obtained. As for the complex I, the maximum resolution of the crystals was improved and we succeeded in determining phases with the molecular replacement method. There was a remarkable change in the size of the lattice of the complex II. The unit-cell constants were approx. 2% shorter in all the axes than those of the crystals grown by the vapor diffusion method. These results indicate that crystallization under microgravity using the counter diffusion method is useful on the crystallization of supramacromolecular protein complexes. This work is performed in collaboration with the members of JAXA and Confocal Science, to whom the authors' thanks are due.

2P-103 分子シミュレーションによる分子モーター RuvB の分岐点移動メカニズム解析

Analysis of mechanism of branch migration by RuvB using molecular dynamics simulation

Hisashi Ishida, Quantum Beam Science Directorate, Japan Atomic Energy Agency

Homologous recombination occurs in all organisms, allowing for genetic exchange, the reassortment of genes along chromosomes, and the repair of broken DNA strands. A four-stranded DNA structure called a Holliday junction (HJ) is a central intermediate state in homologous recombination, and one of the crucial steps in the process of recombination is branch migration of the HJ. In *Escherichia coli*, branch migration of the HJ is promoted by proteins RuvA and RuvB. The RuvA tetramer specifically recognizes and binds to the HJ. The RuvB hexamer works as a molecular motor to drive branch migration at the expense of the energy of ATP hydrolysis. It is thought that the constituent RuvB monomers undergo conformational changes, according to their state: ATP-bound, ADP-bound, and nucleotide-free states. To understand the relationship between the conformational changes in RuvB and the translocation of the HJ DNA, molecular dynamics (MD) simulations of the RuvA/RuvB/HJ DNA complex were carried out at a constant pressure of one bar and a temperature of 350 K. Principal component analysis was performed to analyze what kinds of concerted motions in RuvB contribute to the translocation of the HJ DNA. It was found that several low frequency modes showed the motions of the RuvB hexamer were coupled with the translational motion of DNA. These motions implied that the C-terminal domain of RuvB is important for the translation motion of DNA. The results will be discussed in detail at the conference.

2P-104 網膜前駆細胞で働く bHLH 型転写因子の in vitro 解析

In vitro analyses of bHLH transcription factors expressed in retinal progenitor cells

Osamu Hisatomi (1), Koji Hasegawa (1), Daisuke Kitano (1), Tatsushi Goto (1), Fumio Tokunaga (1) and Fumio Hayashi (2). (1: Graduate School of Science, Osaka Univ.; 2: Graduate School of Science, Kobe Univ.)

The helix-loop-helix (HLH) family of transcriptional regulatory proteins play key roles in a wide array of developmental processes. Basic HLH (bHLH) transcription factors form a homo-dimer or a hetero-dimer with other bHLH factors, and control the transcription of genes by binding to a specific DNA sequence, E-box. Inhibitor of differentiation (Id) that lacks the basic domain is also important as a negative regulator of bHLH factors. We explored bHLH factors and their negative regulators expressed in retinal progenitor cells of newts, and isolated three kinds of genes encoding, HEB (E-protein), neuroD4 (ND4 or Ath3), and ID2. We prepared recombinant ID2 protein, and bHLH domains of HEB and ND4 proteins. It was suggested from gel-shift assays that recombinant HEB protein selectively form a complex with an oligonucleotides containing an E-box sequence (E-oligo). ND4 protein binds to HEB protein and forms HEB-ND4/E-oligo complex, but ND4-ND4/E-oligo complex was not observed in our experiments. At low temperature, HEB-HEB/E-oligo complex was stable, but its dissociation was observed at high temperature. Our results suggested that dynamic behaviors of bHLH-bHLH/E-box complexes were important to elucidate the construction of neural tissues.

2P-105 電子顕微鏡法による出芽酵母 Rap1 とテロメア DNA 複合体の構造解析

Structure analysis of *S. cerevisiae* Rap1-telomeric DNA complex by Electron microscopy

Saori Maki-Yonekura (1), Tanya L. Williams (2), Elizabeth H. Blackburn (2) and Koji Yonekura (1). (1:RIKEN SPring8 Center harima Institute; 2: Dept Biochem & Biophys, UCSF)

Telomeres are specialized nucleoprotein complexes that cap the ends of eukaryotic linear chromosomes, stabilizing them and protecting their integrity. Telomeric DNA of most eukaryotes is composed of tandemly repeated sequence units whose number is tightly regulated. Telomeres in budding yeast *Saccharomyces cerevisiae* cells are maintained between 250 to 350 bp through a dynamic balance of lengthening and shortening activities. Normal telomere length regulation requires telomerase as well as a telomeric protein-DNA complex. Rap1 is the major telomere binding protein and binds duplex telomeric DNA. Rap1 also recruits the Rif1 and Rif2 proteins, which are negative regulators of telomere length. To understand telomere length regulation, we have observed complexes of Rap1 with a DNA array of 10 or 37 consensus Rap1 binding sites by electron microscopy, and have revealed unique helical structures. The length of these structures varied and we interpret this as varying Rap1 occupancy on a given DNA molecule. Hence the heterogeneity of complexes, we carried out electron tomography and three-dimensional reconstructions were calculated to better visualize these Rap1-DNA complexes. When we visualized Rap1-DNA complexes using DNA with 37 Rap1 binding sites, only partial occupancy of Rap1 binding sites was observed. In some images, we observed bent or coiled Rap1-DNA structures. Taking all of the structural data together, we propose a model of Rap1 binding to DNA as a clamp.

2P-106 計算機シミュレーションによる RNA 結合タンパク質の塩基配列特異的認識機構の解明

Simulation study on sequence recognition mechanism of RNA-binding protein

Ikuo Kurisaki (1), Astushi Mastumoto (2), Kei Yura (3), Shigenori Tanaka (4,5). (1:Kobe Univ. Graduate School of Science; 2: Center for Computational Science and Engineering, Japan Atomic Energy Agency; 3: Ochanomizu Univ. Graduate School of Humanities and Sciences 4: Kobe Univ. Graduate School of Human Development and Environment; 5: CREST)

The quality and quantity of RNAs in the cells are managed by RNA-binding proteins (RBPs). The RBPs can recognize and bind their target RNAs specifically and express the functions, such as stabilization and destabilization of RNA, translational control, splicing and so forth. Most of the RBPs specifically recognize RNA sequences and/or 3D structures to bind RNAs. The binding sequence and binding constant of each RBP have been determined by binding assays and RBP-RNA complex structures have been solved by X-ray crystallographic analysis and NMR. Some NMR studies reported that an RNA changed its dynamics when it bound to RBP. As RBP expresses its function when it binds to RNA, the relationship between the RNA dynamics and the mechanisms of specific recognition is of interest. However, only a few research results on the relationship have been reported. In this study, we employed molecular dynamics simulation to characterize the mechanism of specific recognition from the dynamical aspect. We attempted a systematic and global identification of the change of the complex stability and dynamics by mutations depending on the sites, the types and the number of mutation sites. We used Pumilio as a model RBP, which specifically recognizes and binds UGU triplet. It is expected that the crystal structure reflects the mechanism of recognition, because Pumilio utilizes mostly hydrogen bonds to recognize RNA bases. The details will be reported at the meeting.

2P-107 Musashi-1 が有する二つの RNA 結合ドメインによる RNA 認識機構の常磁性効果 PRE、PCS、RDC を利用した解析

A simultaneous RNA-binding mode of two RNA-binding domains of Musashi-1 analyzed by paramagnetic effects, PRE, PCS, and RDC

Takashi Nagata (1), Takako Ohyama (1,2), Ayako Furukawa (1), Takashi Sugiyama (1), Tsukasa Mashima (1), Takao Imai (3), Hideyuki Okano (3), Toshio Yamazaki (2) and Masato Katahira (1,4). (1: International Graduate School of Arts and Sciences, Yokohama City Univ.; 2: Systems and Structural Biology Center, RIKEN; 3: Dept Physiology, Keio Univ. School of Medicine; 4: PRESTO, JST)

Mammalian Musashi-1 (Msi1) is an RNA-binding protein that regulates differentiation of central nervous system via translational repression of its target, m-numb RNA. Msi1 consists of two tandem RNA-binding domains (RBD1 and RBD2), both of which bind to a target sequence within m-numb RNA in a highly cooperative manner. In order to gain structural insight into how RBD1 and RBD2 simultaneously recognize the target RNA, we have performed an NMR analysis based on paramagnetic effects. Three Msi1 mutants, each of which possesses a single Cys residue, were prepared. The Cys residue of each Msi1 mutant was decorated with MTSL, which carries a stable paramagnetic nitroxide radical. The long-range distance restraints (20-40 Å) between two RBDs were derived from paramagnetic relaxation enhancement (PRE) caused by the paramagnetic center. Comparison of the PRE data obtained for Msi1 in free and complexed forms showed that the two RBDs approaches to each other upon binding with RNA. Moreover, the relative location of two RBDs in the complex was successfully determined on the basis of these long-range distance restraints. In addition, rigid lanthanide binding tag was attached to the Cys residue of some of the Msi1 mutants (in collaborating with Prof. Otting), then pseudo contact shifts (PCSs) and residual dipolar couplings (RDCs) caused by lanthanide ions were recorded.

These methods enabled us to determine the structure of Msi1 in the complex, which suggested how Msi1 recognizes its target mRNA.

2P-108 bHLH 因子の E-box 配列認識機構の解析

Analyses of recognition mechanism of E-box sequence by bHLH transcription factors

Koji HASEGAWA(1), Tatsushi GOTO(1), Mari KOTOURA(1), Daisuke KITANO(1), Fumio TOKUNAGA(1), Osamu HISATOMI(1). (1: Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan)

bHLH transcription factors containing a basic-helix-loop-helix domain are one of the representative transcription factors for eukaryotes. They form a homo-dimer or a hetero-dimer with other bHLH factors, and control the transcription of genes by binding to the specific DNA sequence, E-box. It was suggested that a glutamic acid (Eb9), the ninth residue in the basic region of bHLH factors, played a crucial role in the recognition of the E-box sequences. However, the dynamics of the formation of the bHLH-dimer/DNA complex remains to be clarified. We expressed a recombinant HEB protein, a bHLH transcription factor expressed ubiquitously in most cell types, and a HEB-GFP fusion protein. When HEB-GFP mixed with the Cy5-labeled oligonucleotides containing the E-box sequence (Cy5-Eoligo), the FRET signal was observed, suggesting that HEB-GFP bound to Cy5-Eoligo. The time-course of the FRET efficiency suggested that this reaction consisted of at least two components. Also, we replaced the Eb9 of HEB protein into glycine, glutamine and aspartic acid, and analyzed the effect of the replacements.

2P-109 粗視化 DNA モデルに基づく真核生物の転写調節における考察

A coarse-grained wormlike chain DNA model for transcriptional control in eukaryote

Naoko Tokuda(1), Masaki Sasai(1) and George Chikenji(1). (1: Dept. of Computational Science and Technology, Grand Sch. of Engineering, Nagoya Univ.)

It is well established that gene expression in eukaryote is controlled by sequence-dependent binding of trans-acting proteins to regulatory elements like promoters or enhancers. A less well understood level of gene regulation is governed by the various conformational and physical properties of DNA or chromatin. Recently, experimental clues have been reported on the relationship between the DNA conformation and gene expression. First, it has been shown that the long-range control of gene expression can be mediated through direct physical interactions between promoters and enhancers (Tolhuis et al., Mol. Cell (2002)). Second, structural change of DNA, enclosure of an enhancer in a DNA loop, has been shown to reduce activation of gene expression (Ameres et al., EMBO J. (2005)). Physical origins of such DNA structure dependent gene regulation, however, are still elusive. To address this issue, we perform Monte Carlo simulations of linear DNA by developing a wormlike chain model of the regulation of gene expression in eukaryote. We calculated the proximity probability between an enhancer and a promoter which are separated by 2.1 kbp from each other. The proximity probability is reduced to one several when an enhancer is enclosed by the DNA loop. This decay of the probability is caused by increase in the DNA bending energy in the enhancer-enclosing loop, which might be a physical origin of the DNA structure dependent gene regulation. In this presentation, I will also discuss the system in which multiple genes are included.

2P-110 タンパク質-DNA 複合体の疎視化モデルによるシミュレーション

Coarse-grained molecular simulation of protein-DNA complex

Hiroo Kenzaki (1) and Shoji Takada (1,2). (1: Faculty of Science, Kyoto University; 2: CREST, JST)

Proteins play roles of regulation of gene expression; for example, helicases unwind the double helical DNA and histone proteins act as spools for DNA. To investigate these large-sized system, coarse-grained molecular simulation is very useful because it is very difficult to perform all-atom simulation. Recently, functional dynamics of protein has been investigated by Go-like model, and some coarse-grained DNA models that are able to treat both single- and double-stranded DNA have been suggested. Coarse-grained model containing both protein and DNA is, however, needed to be introduced. Here, we develop the simulation tool for protein-DNA complex. Go-like model is used for protein, a coarse-grained model is employed for DNA, and inter protein-DNA interactions is added.

2P-111 DNA ヘアピンの確率共鳴

Stochastic resonance in DNA hairpins

Kumiko Hayashi (1), Nuria Forns (2), Maria Manosas (2), Josep Maria Huguet (2), Felix Ritort (2). (1: Dept Physics., Waseda University; 2: Dept Physics., University of Barcelona)

We performed single molecule experiments of DNA hairpins by using optical tweezers [1]. By pulling a DNA hairpin 20 bps long with the tension about 13 pN, the DNA hairpin switches between a folded state and an unfolded state. Its thermodynamic properties and folding and unfolding kinetics have been investigated [2].

In our study, we added an oscillating force to a DNA hairpin and observed stochastic resonance. Stochastic resonance is a well known phenomena observed in nonlinear systems when a weak signal can be amplified and optimized by assistance of noise. Although stochastic resonance has been observed in a large variety of systems, including Brownian particle systems, semiconductor devices, there are few studies on stochastic resonance in

bio-molecules.

In the absence of oscillation forces, we first measured residence times of folded and unfolded states, τ_f and τ_u . Then, with oscillations, the folding and unfolding kinetics was investigated by measuring the signal to noise ratio (SNR) as a function of the oscillating frequency under several conditions. We have checked that the SNR has a peak at the resonance frequency which corresponds to $(\tau_f + \tau_u)^{-1}$ measured without oscillations.

[1] J. M. Huguet, N. Forns, S. B. Smith, C. Bustamante, F. Ritort, DNA thermodynamics inferred from DNA unzipping experiments using optical tweezers. Preprint (2008).

[2] F. Ritort, Nonequilibrium fluctuations in small systems: from physics to biology, Advances in Chemical Physics, vol. 137, 31-123 (2008). Ed. Stuart. A. Rice, Wiley publications.

2P-112 BrG 置換されたヒトテロメア DNA の単一分子 FRET 計測に基づく G-quadruplex 立体構造の考察

Examination of G-quadruplex Structures of Human Telomere DNA Based on the Single Molecule FRET Measurements with BrG-substitutions

Kenji Okamoto(1), Yuta Sannohe(1), Tomoko Mashimo(1), Hiroshi Sugiyama(1) and Masahide Terazima(1). (1: Department of Chemistry, Graduate School of Science, Kyoto University)

The human telomere, which consists of tandem repeats of a short G-rich sequence GGGTTA, is thought to play important roles in biological processes, including cell aging, death and proliferation of cancer. Telomere is known to form the three-dimensional G-quadruplex structure. Recently, two stable structures, so-called parallel-antiparallel hybrid structures, were specified in physiological K^+ solution. Both models consist of three parallel and an antiparallel G-tracts connected with three TTA-linkers, and glycosidic conformations of guanines were also determined uniquely. However, it is still unknown why only those structures can be stable among numerous possible structures. The structural dynamics is also unknown, although the G-quadruplex is thought to be in equilibrium among different structures in solution.

We investigated the G-quadruplex structures by the single molecule fluorescence resonance energy transfer (FRET) measurements. The G-quadruplex structure was modified by the bromoguanine (BrG)-substitutions at specific positions. The FRET distributions were analyzed by a new scheme, which was based on cumulative distribution function instead of histogram and enabled the curve-fitting independent of bin size. Five components were resolved and the dependence of those populations on the BrG-substitutions were examined.

We propose the triple-strand-core model to explain experimental results. The model predicts the equilibrium between the hybrid and the antiparallel structures.

2P-113 一本鎖切断を含むクラスター損傷の分子動力学シミュレーション

Molecular dynamics simulation of clustered DNA damage site including single strand break

Mariko Higuchi and Miroslav Pinak, (Research Group for Radiation Effect Analysis, Japan Atomic Energy Agency)

Ionizing radiation leads to clustered DNA damage sites, which are defined as two or more single lesions induced within 10-20 base pairs (bp). These sites are repaired with less efficiency than single lesions. So far the detail mechanism of repair of multiple lesions is not known. A lesion which locates within a few base pairs opposite to a 8-oxo-7,8-dihydroguanine (8oxo-G) tends to inhibit excision of the 8oxo-G by repair enzyme MutM. The strength of inhibition effect is difference according to a kind of lesion. The inhibition effect of β - δ single strand break (β - δ SSB) is stronger than that of reduced abasic (rAP) site. And, the inhibition effect of β SSB is about the same strength as that of rAP site.

In this study, we present the results of molecular dynamics (MD) simulation, by comparing 1) β - δ SSB, 2) β SSB and 3) the AP site in cluster damaged DNA which is containing 8-oxoG. According to observed results, tilt and roll are similar among all three lesions. While twist of β SSB and AP site is as same as that of native structure, twist of β - δ SSB differs from that of native structure and fluctuates largely. It is because either hydrogen or covalent bonds link main chain in β SSB and AP site region, while there is no link observed in β - δ SSB region.

2P-114 分子動力学シミュレーションによる DNA の柔らかさの塩基配列依存性と水和パターンの関係解析

Sequence-dependent DNA deformability and hydration studied by molecular dynamics simulations

Yoshiteru Yonetani(1), Satoshi Fujii(2), Akinori Sarai(2), Hidetoshi Kono(1) and Nobuhiro Go(1). (1: Computational Biology Group, Neutron Biology Research Center, Japan Atomic Energy Agency; 2: Department of Bioscience and Bioinformatics, Kyushu Institute of Technology)

Sequence dependence of DNA conformational deformability is one of important factors in the sequence-specific recognition by regulatory DNA-binding proteins. Several causes for this sequence effect can be considered. Intrinsic mechanical properties of DNA will make a dominant contribution, but the other effect of hydration cannot be neglected because it is inevitable for realizing DNA helix structures. A well known feature of DNA hydration is the formation of *water spine*, which is composed of highly ordered water molecules in the minor groove of specific DNA sequences. Emergence of such a hydration pattern depends on