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

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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 RuvB の分岐点移動メカニズム解析

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

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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

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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

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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 date 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

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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

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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.