3P115 High stability of two-dimensional crystal of reconstituted bacteriorhodopsin in partially fluorinated phosphatidylcholine

Masaru Yoshino¹, Hiroshi Takahashi¹, Kohei Morita¹, Toshiyuki Takagi², Hideki Amii¹, Toshiyuki Kanamori², Masashi Sonoyama¹ (¹*Fac. Sci. Tech., Gunma Univ.*, ²*R.C. Stem Cell Eng., AIST*)

We have reported successful reconstitution of bacteriorhodopsin (bR) into novel partially fluorinated phosphatidylcholine (diF4H10-PC) vesicles, in that the reconstituted bR has native-like higher order structure and photocycle. The present study on structural stability of the reconstituted bR demonstrated that 2D crystals as well as trimeric structure of bR molecules are maintained and no light-induced denaturation is observed up to ~40 °C, which is much higher than the gel-to-liquid crystalline phase transition temperature (5 °C) of pure diF4H10-PC bilayer. The high stability of the reconstituted bR in diF4H10-PC is in stark contrast with bR in DMPC showing phase transition-induced disassembly of bR molecules and remarkable denaturation by visible light.

3P116 Reverse gyrase likely biases thermal DNA strand passage toward overwinding

Taisaku Ogawa¹, Katsunori Yogo², Shou Furuike³, Kazuo Sutoh¹, Akihiko Kikuchi⁴, Kazuhiko Kinoshita¹ (¹*Dept. Phys., Waseda Univ.*, ²*Grad. Sch. Med. Sci., Kitazato Univ.*, ³*Dept. Phys., Osaka Med. Coll.*, ⁴*Grad. Sch. Med., Nagoya Univ.*)

Reverse gyrase is a type I topoisomerase that can introduce positive supercoils into DNA only at >70°C. Here we have imaged the overwinding reaction at 71°C under a microscope and found that a single reverse gyrase molecule processively winds DNA for >100 turns. The rate is nearly two orders of magnitude higher than previous biochemical bulk results. The enzyme ceases to work when the torsional stress in DNA rises to mere ~4 pN·nm, as expected if its role is to keep DNA slightly overwound for protection against thermal melting. The activity is also highly sensitive to tension in DNA. All our results are consistent with the mechanism where strand passage relying on thermal motions, as in topoisomerase IA, is actively, but loosely, biased toward overwinding.

3P117 Single-molecule study of how RecA displaces SSB from single-stranded DNA

Hung-Yi Wu, Hung-Wen Li (*Dept. of Chem., Natl. Taiwan Univ.*)

RecA competes with SSB (single-stranded DNA binding proteins) for single-stranded DNA (ssDNA) substrates to form RecA nucleoprotein filaments in *E. coli*, essential for DNA homologous recombinational repair. In this study, we monitored individual RecA filament formation by tethered particle motion (TPM) technique in real-time. Binding of RecA on the SSB wrapped-ssDNA extends the DNA substrate, visible by the increase in bead Brownian motion in TPM imaging. By varying the ssDNA gap sizes, we prepared the SSB-ssDNA complex with different ssDNA lengths. Interestingly, we found a single amino acid mutation of RecA (RecA E38K) accelerates the SSB removal rate compared to wild-type RecA. Our data suggests the presence of direct RecA-SSB interaction on ssDNA.

3P118 クロマトソームの粗視化シミュレーション: H1 結伴に伴うヌクレオソーム構造のコンパクト化のダイナミクス

Coarse-grained simulation of chromatosome: H1-mediated dynamic compaction of nucleosome structure

Nobu C. Shirai, Shoji Takada (*Grad. Sch. Sci., Kyoto Univ.*)

The linker histone H1 has two disordered regions separated by a globular domain, and it stabilizes compact chromatin structure by binding to nucleosome and forming chromatosome.

Formation and dissociation of chromatosome dramatically change the accessibility of regulatory proteins, and thus the binding process of H1 is important to understand the indirect regulation of transcription and replication.

In this study, we performed molecular dynamics simulations using a coarse-grained model of chromatosome to analyze dynamic compaction processes.

As a result of the simulations, we observed that the positively charged C-terminal disordered domain of H1 induces compact structures of multi-nucleosome by placing itself in the middle of the negatively charged linker DNAs.

3P119 Characterization of the hemi-methylated CpG methylation process using fluorescent labeled SRA

Yubing Cui^{1,2}, Yong-Woon Han², Mariko Ariyoshi³, Kyohei Arita⁴, Isao Suetake⁵, Shoji Tajima⁵, Yoshie Harada^{1,2} (¹*Grad. Sch. Biostudies, Univ. Kyoto*, ²*iCeMS Inst., Univ. Kyoto*, ³*Grad. Sch. Tech., Univ. Kyoto*, ⁴*Grad. Sch. Medical Life Sci., Univ. Yokohama City*, ⁵*Protein Inst., Univ. Osaka*)

DNA methylation plays a central role in the epigenetic regulation of genes expression and cell differentiation. Dnmt1 (DNA methyltransferase) is the enzyme responsible for the maintenance of cytosine methylation at CpG dinucleotide in mammalian genome. It is suggested that the SRA domain of Uhrf1 recognizes the hemi-methylated CpG sites, and Dnmt1 interacts with SRA to facilitate accession of the catalytic center to hemi-methylated DNA, resulting in the maintenance of DNA methylation pattern. In this study, we prepared fluorescent labeled SRAs and characterized SRA-DNA complex formation kinetics with biochemical analysis.

3P120 蛍光標識ヌクレオソームを用いたクロマチン再構成複合体の機能解析

Characterization of ATP-dependent chromatin remodeling complexes using fluorescently labeled nucleosome

Yong-Woon Han¹, Yasuo Tsunaka^{1,2}, Hiroaki Yokota³, Kazuhiro Yamada⁴, Mai Ohnishi^{1,5}, Sayaka Yamazaki^{1,5}, Isao Suetake⁶, Shoji Tajima⁶, Hisashi Tadakuma⁷, Yoshie Harada⁷ (¹*iCeMS, Kyoto University*, ²*PREST*, ³*Bio Photonics, Grad. Sch. for the Creation of New Photonics Ind.*, ⁴*Max-Planck-Inst. for Med. Res.*, ⁵*Faculty of Human Life and Sci., Doshisha Woman's College of Liberal Arts*, ⁶*Inst. for Protein Res., Osaka Univ.*, ⁷*Grad. Sch. of Frontier Sci., Univ. of Tokyo*)

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. Posttranslational histone modifications such as acetylation, methylation, phosphorylation and ubiquitylation regulate chromatin structure, resulting in activation or repression of gene expression. ATP-dependent chromatin remodeling complexes also regulate gene expression by changing chromatin structure. In this study, we construct fluorescently labeled mono-nucleosomes and characterize ATP-dependent chromatin remodeling complexes, LSH and CHD.