by our new flexible-fitting approach, in which the best-fitting atomic model for each EM density map was built by deforming the PDB structure of the 70S ribosome by using computational technique.

In this study, we analyze the 3D-EM structures of smaller molecules bound to ribosome, such as tRNA and elongation factors, which are included in the EM density maps for the 70S ribosome by taking advantage of the best-fitting atomic models that were built in the previous study. One of the difficulties in this study is to spot the small molecules in the EM density map for the large 70S ribosome. This is solved by fitting the atomic model for the 70S ribosome into the EM density map and paying attentions to the regions where atomic model is not overlapped. In this way, we are able to isolate the 3D-EM structures that may correspond to the bound molecules. Our results show that the best-fitting atomic model of the 70S ribosome built in our previous study can isolate the regions for the bound molecules more effectively than the original PDB structure. We are especially interested in the bound tRNA molecules, and analyze the structures in detail.

2P135 Analysis of translocation of tRNA molecules through ribosome using electron microscopy density map and molecular dynamics simulations

Hisashi Ishida^{1,2}, (¹Quantum Beam Science Directorate: ²Center for Computational Science and e-Systems, Japan Atomic Energy Agency)

ÅÅÅ 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, changing their positions at the A, P, and E sites. This process, called translocation of tRNAs, is catalyzed by the elongation factor G (EF-G) using energy of GTP hydrolysis.

ÅÅÅ Recent results from pre-steady-state kinetic analysis and cryo-electron microscopy (cryo-EM) suggest that there is a dynamic multistep process during translocation, where large-scale structural rearrangements of both ribosome and EF-G occur. However, the dynamic mechanism of translocation is unclear at the atomic level.

ÅÅÅ We developed an EM density-fitting refinement method using all-atom molecular dynamics simulations including water molecules while retaining the condition that the constituent biomolecules fit in the EM density map. This method was applied to construct sequential states during the translocation using the X-ray crystallography structure of 70S ribosome complexed with EF-G corresponding to the post-translocational state (PDB code: 2WRJ) and two cryo-EM density maps corresponding to the translocational states (EMID code: 1365 and 1363).

ÅÅÅ It was shown that multistep structural changes, such as ratchet-like motion between the small and large ribosomal subunits and head-rotation of the small ribosomal subunit, were observed during the translocation. The results will be discussed in detail at the conference.

2P136 タンパク質-RNA 相互作用におけるタンパク質の形状と RNA2 次構造の役割 The role of protein shape and RNA secondary structure in protein-RNA interaction

Junichi Iwakiri, Hiroki Tateishi, Naoya Kenmochi, (*Frontier Science Research Center University of Miyazaki*)

The binding of a protein to a specific RNA plays an important role in many aspects of cellular activities. Thus, understanding the mechanisms by which proteins recognize RNAs is fundamental in elucidating the diverse protein-RNA interaction network. Here we show a statistical analysis of protein-RNA interfaces using 79 protein-RNA complexes. Based on the shape (protruding/intruding) of the binding region of the protein and the secondary structure (paired/unpaired nucleotides) of the RNA, we have categorized these interfaces into six types and observed some unique features in each type of interface. If the protein interface is intruding, hydrogen bonds between the main chains of the proteins and bases of the unpaired nucleotides occurred more frequently. In contrast, when the protein interface is protruding, electrostatic interaction between arginine/lysine side chains of the proteins and phosphate groups of the nucleotides was the most frequent. Similarly for RNA, when the interaction involves in a stem-loop RNA, the unpaired nucleotides in the internal loop are often flipped out. Interestingly, the protein interface with the internal loop contained more number of aspartic acids compared to the interface with other unpaired nucleotides. These results imply the role of aspartic acids in base flipping as a unique mechanism for the specific RNA recognition. Our study highlights the role of molecular shape of proteins and secondary structure of RNA as a part of structural determinants of protein-RNA interaction.

2P137 一分子破断力測定によるリボソーム-mRNA 相互作用力の非対称性の解明

Asymmetric interaction between 30S ribosomal subunit and mRNA in translation initiation

Masatoshi Tachibana¹, Tomoaki Masuda¹, Ryo Iizuka¹, Takashi Funatsu¹, Sotaro Uemura^{1,2}, (¹Grad. Sch. of Pharm. Sci., The Univ. of Tokyo: ²Dept. of Struct. Biol., Stanford Univ. Med. Sch.)

During translation, ribosome moves towards the 3'-end along mRNA driven by the hydrolysis of GTP. Although structural and single-molecule studies revealed the dynamics of translating ribosome, little is known about how ribosome works as a molecular motor. To investigate the mechanism, we measured the rupture force for ribosome-mRNA complex in translation initiation using optical tweezers. 30S ribosomal subunit complex with biotinylated mRNA was immobilized on a glass surfaces via streptavidin-biotin linkage. An oligonucleotide labeled with Cy3 and digoxigenin was designated to hybridize to the extension loop on 30S subunit, which is genetically engineered. A bead coated with anti-digoxigenin antibody was conjugated to the oligonucleotide. The interaction between 30S subunit and mRNA was ruptured by moving the piezo stage at a constant velocity during the bead is trapped. The rupture force was determined to be 8.04 pN when the ribosome complexes are pulled toward the 3' end of mRNA (forward load), whereas, an external load imposed against the 3' end (backward load) increased it to be 15.8 pN. Likewise, in the presence of tRNA^{Met}, the rupture force was estimated to be 16.2 pN (forward load) and 22.5 pN (backward load). These results suggest that 30S subunit asymmetrically interacts with mRNA. The asymmetric interaction can account for the unidirectional movement of ribosome toward the 3' end of mRNA during the translation. Now, we are conducting demonstration experiments to verify the hypothesis.

2P138 リボソームの粗視化 MD による翻訳での動的振舞の研究 Dynamical Behavior of Ribosomes in Translation Process by Coarse-Grained Molecular Dynamics Simulation

Shigenori Matsumoto, Fumiko Takagi, Takashi Shimada, Nobuyasu Ito, (*Department of Applied Physics, School of engineering, The University of Tokyo*)

We propose a dynamical model of the translation elongation cycle of ribosomes. In the translation process, proteins are synthesized in ribosomes under the influence of heat fluctuations and densely-packed biological molecules. For this process, some scenarios have been proposed, however it remains theoretically unclear how correct proteins are synthesized under a given environment. In our approach, we simulate that translation process by using the coarse-grained molecular dynamics technique, which involves effects of a temperature and a density of molecules.

In our simulation, we find the maximum speed of protein synthesis because of traffic jam of ribosomes on mRNA. This feature consists with previous studies using stochastic models in which the ribosomes hops to the next codon stochastically on mRNA. Using the dynamical model, the hopping probability adopted in the stochastic modes will be estimated from the temperature and the density of amino acyl-tRNA. We also find that the elongation speed is enhanced by adding extra molecules, which may indicate the feature of the translation in a cell.

2P139 周期骨格 DNA アレイを用いた大規模二次元非周期パターンの構築

Non-periodic and large-scale two-dimensional nano-patterns constructed on periodic DNA tile arrays

Kohsuke Ara¹, Miho Tagawa², Koh-ichiroh Shohda¹, Kenzo Fujimoto³, Akira Suyama¹, (¹Department of Life Sciences and Institute of Physics, Graduate School of Arts and Sciences, The University of Tokyo: ²Brookhaven National Laboratory: ³School of Materials Science, Japan Advanced Institute of Science and Technology)

Due to a self-assembling nature, DNA is utilized to create various programmable nano-constructions called DNA nanostructures. One goal of the DNA nanostructure research is to generate scaffolds for bottom-up assembly of functional biomolecules and nano-electronic components. Toward this goal, the functional nanomaterials must be placed in a non-periodic manner. DNA origami is well known as the non-periodic DNA nanostructure. However, the size of a single DNA origami is 100 nm at the maximum, which is too small to construct non-periodic scaffolds of practical use.

Here we demonstrate a new method to construct non-periodic and large-scale two-dimensional (2D) DNA tile arrays with a potential of expanding to micrometer-scale. DNA double-crossover AB-staggered (DXAB) tiles, which can self-assemble into periodic 2D DNA arrays of micro-mere scale, are applied to this method [1]. To construct a non-periodic 2D scaffold we attached a DNA tag sequence on a DXAB tile. The tag sequence represents the tile position in a 2D array. The tiles with the tag were first self-assembled into a 1D-DXAB tile string using a messenger DNA (mDNA) strand as a template,