measurements demonstrated that the BL-induced change of the hydrodynamic radius (R_H) of LOV domain was slight. However, the R_H of FL and ZL in the light-adapted states were about 5% larger than those in the dark-adapted states. The R_H values were reverted to the dark-adapted level when illuminated FL and ZL were kept in the dark. Our results suggested that FL and ZL underwent a reversible conformational change upon illumination. Furthermore, the non-reducing SDS-PAGE assay indicated that ZL existed as dimers through intermolecular disulfide bonds in bZIP domains. The conformational change and dimer formation may play a crucial role for AUREO1 function.

1. Takahashi, F., et al. *Proc. Natl. Acad. Sci. USA* 104, 19625-19630 (2007).

2PT201 インシリコハイブリダイゼーション：新しい原核生物種の分類手法 *In silico* hybridization: A new tool for the classification of prokaryotic cells

Kunio Ihara¹, Tomomi Kitajima-Ihara¹, Masahiro Kamekura², Akinobu Echigo³ (¹Nagoya Univ., ²Halophiles Lab., ³Toyo Univ.)

What does species mean in prokaryotic cell? We, now, know even a single mutation might cause a drastic phenotypic or biochemical changes. Therefore, it is very difficult to delineate the species by comparing two prokaryotic cells only in phenotypic or biochemical point of view. At present, further including of DNA-DNA hybridization (DDH) studies should be essential for establish the species concept. However, DDH studies are laborious and their reproducibility among different laboratory would be marginal. Recently, it comes to easy to make complete genome sequencing by using next generation sequencer. We have tried to make a quick and simple method for classification of prokaryotes using SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing system. For the first trial, we have investigated the genus *Haloarcula*, because of our interesting of rhodopsin patchy distribution. We have sequenced 7 *Haloarcula* strains including the type strain *Haloarcula marismortui*. Several millions of 50bp short reads produced by the SOLiD sequencer were *de novo* assembled using the software velvet to produce thousands of contigs. Total short reads from one strain were mapped against assembled contigs. Mapping process is to search for the closely related region from the total genome, what we call, *in silico* hybridization (ISH). The mapping values obtained in ISH were compared with the experimental DDH values and their correlation constant was 0.97. We will present in detail in the meeting.

2PT202 Homologous proteins with different fold: how were they raised ? Shintaro Minami¹, Kengo Sawada¹, George Chikenji² (¹Grad. Sch. Eng., Nagoya Univ., ²Dept. Eng., Nagoya Univ.)

Currently some examples of protein pairs with evidence for evolutionary relationships that fold into topologically different but share the same secondary structure packing arrangement have been known. One of the most interesting examples of such protein pairs is that of KH domains of hnRNP K and ribosomal protein S3 [1]. They show statistically significant sequence similarity (38% identity) implying homology. However, their structures are quite different in topology while converging to the same architecture.

Such examples suggest that proteins have changed their folds by segment shuffling or rearrangement events while conserving the same core packing arrangements. Further investigation of such protein pairs would give us a hint as to how proteins can change their fold in the course of evolution.

In this work, we performed comprehensive database search for all possible pairs which was classified into same superfamily in SCOP to encompass such interesting pairs of homologous protein. After the comprehensive search, we found a number of homologous protein pairs which show non-topological structural similarity. These pairs were investigated at a level of structure, amino acids, and DNA, and we found some clues as to how these proteins can arise in the course of evolution.

[1]N. V. Grishin, J. Str. Biol. (2001) 134:167-85

2PT203 全生物共通祖先以前のタンパク質のアミノ酸組成に実験的に迫る Experimental approach to the amino acid usage of pre-last universal ancestor

Masami Shimada, Satoshi Akanuma, Kozue Shinozaki, Yoshiki Nakajima, Akihiko Yamagishi (Dept. of Mol. Biol., Tokyo Univ. of Pharm. Life Sci.)

Little is known about the nature of ancient proteins hosted by primitive organisms that lived before the last universal common ancestor emerged. However, it has been argued that primordial protein synthesis was much simpler

and involved less than 20 amino acids. Given simpler protein synthesis system, the primitive proteins, which might have comprised a reduced set of amino acids, must have had a sufficiently adequate structure for functional interactions and catalysis. To address this issue experimentally, we used "protein simplification engineering (1)" to examine whether a protein composed of less than 20 types of amino acids can retain its stable structure and biological function. Using a thermally stable, ancestral-designed nucleoside diphosphate kinase (Arc1) as the starting molecule, we constructed two reduced amino acid set variants, Arc1-s1 and Arc1-s2, in which non-conserved M, Q, K, Y and N and all of the five amino acid letters were replaced by other amino acids, respectively. Because cysteine is absent from Arc1, Arc1-s2 consists of only 14 amino acid letters. Arc1-s1 and Arc1-s2 retain thermal stability similar to that of Arc1. Arc1-s1 shows catalytic efficiency similar to that of Arc1; whereas, no detectable level of catalytic activity was observed for Arc1-s2. Therefore, the fourteen amino acid types are sufficient to encode a thermally stable protein but more amino acid types may be required for its function.

(1) Akanuma et al., PNAS 99, 13549 (2002)

2PT204 cDNA display 法による 4 種類のアミノ酸からなる機能ペプチドの創出

Generation of Functional Peptides Consisting of 4 Types of Amino Acids by cDNA Display Method

Shigefumi Kumachi¹, Miho Suzuki¹, Koichi Nishigaki¹, Yuzuru Husimi², Naoto Nemoto¹ (¹Grad. Sch. Sci. & Eng., Saitama Univ., ²Innovation Research Organization, Saitama Univ.)

In general, proteins are composed of the 20 natural amino acids. However, how many type of amino acids required for the creation of a functional protein remains unresolved. Thus, we tried to design an experiment to examine the function of primitive proteins composed of a limited set of amino acids by in vitro selection approach. Interestingly, it has been postulated by two different approaches that the most primitive protein could consist of 4 types of amino acids (i.e., glycine, alanine, aspartic acid and valine): 1) Eigen *et al.* (RNA world hypothesis); and 2) Ikehara *et al.* (protein sequences analysis). So what function would a primitive protein have in an early translation system? The first primitive protein would most likely be associated with the enhancement of Ribozyme. Furthermore, in the early stages of protein synthesis, the genotype-phenotype linking strategy, which is essential for molecular evolution, could become a virus-type, because this strategy is the most efficient for protein evolution of a single function. If this strategy is adopted, the interaction between RNA and protein is indispensable. Thus, we investigated the possibility of whether peptides consisting of these 4 amino acids could interact with RNA by cDNA display technology. Then, some peptides were selected to interact with tRNA with low affinity. This shows that a primitive protein consist of 4 types of amino acids could evolve by the virus-type strategy in the early stage of emerging translation system.

2PT205 Directed evolution of a self-encoding system using giant liposome

Takeshi Sunami^{1,2}, Norikazu Ichihashi^{1,2}, Takehiro Nishikawa¹, Yasuaki Kazuta¹, Tomoaki Matsuura^{1,3}, Hiroaki Suzuki^{1,2}, Tetsuya Yomo^{1,2,4} (¹ERATO, JST, ²Grad. Sch. Info., Univ. Osaka, ³Grad. Sch. Eng., Univ. Osaka, ⁴Grad. Sch. Biosci., Univ. Osaka)

Evolvability is a common property of living organisms, and gene replication system is essential to allow the living system to evolve. We have constructed a simplified system, in which the genetic information is replicated by self-encoded replicase in liposomes. For the self-encoding system to evolve, the genes must be compartmentalized and only a small number of them should be present in each compartment for genotype-phenotype linkage. The translated replicase needs to amplify only the gene encoding itself. Here we demonstrate the directed evolution of the self-encoding system by screening replicase mutants. Cell-sized giant liposomes were used as a micro reactor, and fluorescence-activated cell sorter (FACS) was used to analyze and sort the reacted liposomes. The antisense of the beta-galactosidase gene was inserted into the template DNA as a reporter gene. Beta-galactosidase was transcribed from the minus strand RNA replicated from plus strand RNA, and the green fluorescence from the hydrolyzed products by beta-galactosidase was evaluated as self-replication activity. Fluorescent liposomes with higher active replicase gene were sorted by FACS. The DNA templates for next selection round were amplified from the selected plus strand RNA in a test tube. After 69 rounds of selection, the self-replication activity of the selected mutants was evaluated in liposomes, and the mutants were higher active than wild-type replicase. The result indicates that the simplified gene replication system has evolvability as well as extant cells.