Japan Geoscience Union Meeting 2012
(May 20-25 2012 at Makuhari, Chiba, Japan)

BPT28-01 Room:102A Time:May 24 13:45-14:00

Resurrection of ancestral genes to infer the ancient environment temperatures

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The ancient global environment is a topic that has interested many scientists. One debate regarding the ancient environment concerns the growth temperature of ancient organisms. A number of theoretical studies have argued the growth temperature of ancient organisms, but these studies remained inferential due to the lack of empirical testing. Therefore, we developed an experimental way to assess the growth temperatures of ancient organisms using an inferred amino acid sequence of a protein postulated to exist in the last universal common ancestor. Because extant genes are evolutionary descendants of ancient genes, information on ancient genes is embedded in the sequences of extant genes. Therefore, ancestral sequences of a particular protein can be inferred by comparing extant homologous protein sequences. In our experimental method, inferred ancestral residues were introduced into several extant proteins and then the thermal stabilities of the resulting mutant proteins were examined. The mutant proteins, each of which contains one or a few inferred ancestral residues, showed the trend toward enhanced thermal stability when compared to the respective wild-type protein. Because the thermal stabilities of proteins often reflect the living temperatures of host organisms, our results have supported the hyperthermophilic common ancestry hypothesis.

To further improve our knowledge of ancient living systems and of the ancient global environment where early life evolved, the ancestral sequence reconstruction method was used to predict, synthesize, and characterize the complete ancestral sequences of B subunit of DNA gyrase (GyrB) and of nucleoside diphosphate kinase (NDK). The ancestral GyrB sequence was inferred from the sequences of extant DNA gyrases and type-VI DNA topoisomerases as the member of outgroup. Genes encoding the inferred sequence and its isolated N-terminal ATPase domain were PCR constructed and expressed in *Escherichia coli*. The structural properties and thermal stability of ancestral full-length GyrB are similar to those of the extant thermophilic DNA gyrase from *Thermus thermophilus*. The thermal stability of the ancestral ATPase domain is also similar to that of the *T. thermophilus* ATPase domain. Moreover, the ancestral ATPase domain has significant catalytic activity. The fact that the thermal stabilities of the ancestral GyrB and its ATPase domain are comparable to those of the extant thermophilic proteins further supports the idea that the ancient organism lived at high temperatures.

Ancestral NDK sequences were also inferred by the phylogenetic method. For NDKs, the denaturation temperatures of the proteins are roughly correlated with the optimum growth temperatures of the host cells. The genes encoding the inferred amino acid sequences were reconstructed by a PCR-mediated gene synthesis method. The ancestral genes were expressed in *E. coli* and the resurrected proteins purified. The purified ancestral NDKs are catalytically active. Temperature-induced unfolding experiments showed that the ancestral NDKs are significantly stable even around 100^{o}C. The results are again compatible with the hyperthermophilic common ancestry. Thus, our empirical reconstruction of ancestral genes provides experimental evidences that strongly support the hypothesis that ancient organisms lived in thermophilic environments.

Keywords: phylogenetic tree, ancestral gene resurrection, last universal common ancestor, ancient environment temperature

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BPT28-02 Room:102A Time:May 24 14:00-14:15

Biological methane production and anaerobic oxidation of methane

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Approximately 140 Gt of biomass are formed globally each year from CO2 via oxygenic photosynthesis (net primary production). Of this amount, 2-3% end up in anaerobic environments such as freshwater sediments, landfills, the intestinal tract of ruminants and termites, and deeper layers of marine sediments. In these methanogenic environments, the biomass is fermented to methane, yielding approximately 1 Gt CH4 per year. In such environments, methanogenic archaea produce methane from H2 and CO2, and other substrates in an energy gaining process. The methanogenic pathways involve unique enzymes, which use novel cofactors and coenzymes to catalyze the reactions needed for methane production. Methyl-coenzyme M reductase (MCR), which contains the Ni porphinoid F430 as prosthetic group, is a key enzyme of methane formation in methanogenic archaea (1). It catalyzes the reduction of methyl-coenzyme M with coenzyme B to methane and the heterodisulfide of coenzyme M and coenzyme B. For the activation of H2, methanogens use different types of hydrogenase enzymes, namely [NiFe]-hydrogenases and [Fe]-hydrogenase, which are not phylogenetically related each other (2). The [Fe]-hydrogenase harbors a unique iron-guanylylpyridinol cofactor (FeGP cofactor). Anaerobic oxidation of methane (AOM) with sulfate is accomplished by consortia of methanotrophic archaea and sulfate reducing bacteria. Indirect evidence suggests that AOM with sulfate functions at least in part as the reverse of methanogenesis from CO2. Accordingly, the enzyme activating methane should be a methyl-coenzyme M reductase (MCR) (3). Recently, we solved the crystal structure of MCR from methanotrophic archaea in complex with coenzyme M and coenzyme B, which indicates that the same substrates for MCR from methanotrophic and methanogenic archaea are used (4). I will present our most recent results on the biochemistry of methanogenesis and anaerobic oxidation of methane.

1. Ermler, U., Grabarse, W., Shima, S., Goubeaud, M. & Thauer, R.K. (1997) Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. Science 278, 1457-1462.
2. Shima, S., Pilak O., Vogt, S., Schick, M., Stagni, M.S., Meyer-Klaucke, W., Warkentin, E., Thauer, R.K., & Ermler, U. (2008) The crystal structure of [Fe]-hydrogenase reveals the geometry of the active site. Science, 321, 572-575.
3. Kruger, M., Meyerdierks, A., Glockner, F.O., Amann, R., Widdel, F., Kube, M., Reinhardt, R., Kahnt, J., Bocher, R., Thauer, R.K. & Shima, S. (2003) An conspicuous nickel protein in microbial mats that oxidize methane anaerobically. Nature, 426, 878-881.
4. Shima, S., Krueger, M., Weinert, T. Demmer, U., Kahnt, J., Thauer, R.K. & Ermler, U. (2012) Structure of a methyl-coenzyme M reductase from Black Sea mats that oxidize methane anaerobically. Nature 481, 98-101.

Keywords: Methanogenesis, Anaerobic oxidation of methane, methyl-coenzyme M reductase, X-ray crystallography, Biochemistry, [Fe]-hydrogenase

Japan Geoscience Union Meeting 2012
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BPT28-03 Room:102A Time:May 24 14:15-14:30

Evolution of multicellularity in cyanobacteria: molecular genetic and genomic approaches

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Multicellularity is one of major innovation in organismic evolution, which lead to morphological and functional diversification. When and how organisms evolved multicellularity is one of key questions in an understanding of the biological history.

Cyanobacteria form a morphologically diverse group among prokaryotes. Especially in filamentous cyanobacteria, vegetative cells can mature in four developmental directions (vegetative cells, heterocysts, akinetes and hormogonia) in response to environmental growth conditions. Therefore they provide an interesting experimental system to study mechanisms and evolution of prokaryotic development. Here we performed molecular genetic study of the model filamentous cyanobacterium *Nostoc punctiforme* ATCC29133 to identify genes that control hormogonia formation. We further investigate evolution of the cell differentiation in cyanobacteria, by integrating molecular-phylogenetic and genomic analyses.

Recent advance in next-generation sequencing technology allows us to study taxonomically diverse organisms on genome basis. Multidisciplinary approaches using genomic, physiological, and ecological data together with geologic information would shed light on the early history of life on earth.

Keywords: multicellularity, evolution, cyanobacteria, genome, molecular biology, fossil

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BPT28-04 Room:102A Time:May 24 14:30-14:45

Microbial sulfur cycle in aquatic ecosystems

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Sulfur is essential for all organisms as major components of cell materials. There are also a variety of abundant inorganic sulfur compounds in the biosphere, and prokaryotic respiratory processes depending on these chemical species are major driving force of sulfur cycle in ecosystems. In the sulfur cycle, reductive processes are mainly mediated by sulfate-reducing prokaryotes (SRP). SRP are capable of dissimilatory sulfate reduction coupled with oxidation of organic matter, and this reaction is thought to contribute largely to anaerobic mineralization in aquatic ecosystems. Shen et al. (2001) demonstrated that microbial sulfate reduction had evolved by 3.47 Gyr ago, and suggested the earlier emergence of biological sulfur oxidation. Activity of SRP results in generation of sulfide, which supports growth of sulfur-oxidizing prokaryotes (SOP). Both SRP and SOP are polyphyletic, and their diversities in natural environments are drastically affected by temperature. We will discuss the relationship between microbial sulfur cycle and temperature.

Keywords: sulfate reduction, sulfur oxidation, microorganism, genome, aquatic environment, functional gene

Japan Geoscience Union Meeting 2012
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BPT28-05 Room:102A Time:May 24 14:45-15:00

Complex evolutionary histories of actin, tubulins and elongation factor protein in Rhizaria based on Retaria hypothesis

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Radiolaria and Foraminifera are important pelagic organisms in the field of paleontology due to their good and long fossil record of shells. Recent molecular phylogenetic analyses have shown that these two protist groups are sister to each other (known as Retaria hypothesis) and belong to one of large taxonomic assemblages of eukaryotes Rhizaria, togather with two other groups Endomyxa and Filosa.

Cytoskeletal proteins (actin, alpha-tubulin, and beta-tubulin) and translation elongation factor proteins are essential in a eukaryotic cell. Phylogenies of genes encoding actin, alpha-tubulin, beta-tubulin, and elongation factor proteins are frequently coincident with the organismal phylogeny. However, the evolutionary processes of these protein-coding genes in the rhizarian lineage remain unclear due to less availability of radiolarian gene sequences. In the present study, the genes encoding actin, alpha-tubulin, beta-tubulin, and one of elongation factor proteins were identified from diverse groups of Radiolaria, and the evolutionary histories of these genes are discussed based on the phylogenetic analyses.

Two paralogues of actin, alpha-tubulin, and beta-tubulin were identified from radiolarian species examined. The phylogenies reconstructed in this study suggest that the actin gene was duplicated in a common ancestor of Radiolaria and Foraminifera and that one of two alpha-tubulin paralogues was laterally transferred from an unknown organism to a common ancestor of Radiolaria. It is also suggested that the highly divergent one of two beta-tubulin paralogues originated from a common ancestor of Radiolaria and Foraminifera. Furthermore, the gene encoding elongation factor-like (EFL) protein, one of two paralogues of elongation factors (elongation factor-1alpha and EFL), were identified from several species of Radiolaria. Based on the EFL phylogeny the radiolarian homologues could have been vertically inherited from a common ancestor of Radiolaria, Foraminifera and an endomyxan species Gromia. In summary, our present study unveils the complex evolutionary scenarios of these essential protein-coding genes within the lineage of Rhizaria.

Keywords: Polycystina, Acantharia, actin, tubulin, elongation factor-like protein, phylogenetic analysis

Japan Geoscience Union Meeting 2012
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BPT28-06 Room:102A Time:May 24 15:00-15:15

Can molecular phylogeny of protein-coding genes provide new insights for foraminiferal morphology?

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Multi-gene phylogenetic studies have revealed one of big eukaryotic group, Rhizaria include Foraminifera, Radiolaria, Filosa, and Endomyxa. Foraminifera is known diverse group having two distinct life-styles: planktic and benthic. Recently, molecular phylogeny of foraminifera showed the polyphyletic origins of planktic foraminifera that they were diverged from bentic foraminiferal lineages at least twice (Ujiie et al., 2008). Moreover, high genetic diversity at intra-species level has been inferred based on the SSU rDNA and ITS rDNA sequences (e.g., Darling and Wade, 2008). However, these studies have not been challenged to understand the evolutionary processes according with cell-structures.

This present study shows the foraminiferal phylogenies of two protein-coding genes (actin 2 and b-tubulin 2), which are corresponded to actin granules and microtubules in cell structure. Especially foraminiferal b-tubulin 2 forms a helical filament, which is involved in rapid microtubule assembly/disassembly system resulting in the quick movement of pseudopodia (Habura et al. 2005). Both phylogenies of actin 2 and b-tubulin 2 show two robust clades according with tube- and fan-shaped pseudopodia which are observed in attaching new chamber during their growth processes. Rotaliida including planktic foraminifera have fan-shaped pseudopodia, whereas some of benthic groups have tube-shape one. This preliminary data suggests that the hylogenetic analyses of the protein-coding genes potentially implicate the mechanisms of morphological traits in shell-bearing proists. Future assessments are required increasing taxonomic sampling of benthic tube-shaped types.

(References)

Darling, K.F., Wade, C.M., 2008. The genetic diversity of planktic foraminifera and the global distribution of ribosomal RNS genotypes. Mar. Micropaleontol. 67, 216?238.

Habura, A., Wegener, L., Travis, J.L., Bowser, S., 2005. Structural and functional implications of an unusual foraminiferal b-tubulin. Mol. Biol. Evol., 22, 2000-2009.

Ujiie, Y., Kimoto, K., Pawlowski, J., 2008. Molecular evidence for an independent origin of modern triserial planktonic foraminifera from benthic ancestors. Mar. Micropaleontol. 69, 334-340.

Keywords: Foraminifera, protein-coding gene, cell structure

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BPT28-07 Room:102A Time:May 24 15:30-15:45

Amino acid sequence specifying eukaryotic species

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Japanese only

Keywords: Eukaryote, evolution, PolA1 gene, RNA polymerase I

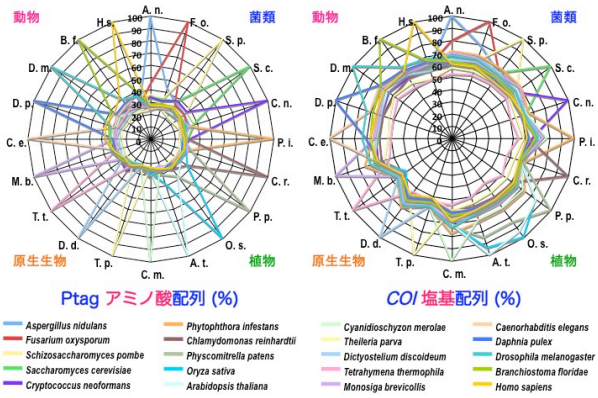

図　真核生物におけるPtagアミノ酸配列およびCOI 塩基配列の種間変異（%）

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BPT28-08 Room:102A Time:May 24 15:45-16:00

Chemotaxonomy of Cretaceous plant fossils from compositions of molecular units in resistant macromolecule

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Resistant macromolecules such as cutin and suberin polymers constituting living plants are known to be stable and much resistant to microbial degradation and diagenesis. Furthermore, the composition of molecular unit (monomer) constituting resistant macromolecules are various according to taxonomy. If variability of these compositions preserved in plant fossils that had undergone diagenetic alteration, these compositions can be useful as chemotaxonomic indicator. In the present study, we analyzed plant carbonized fossils collected from three Cretaceous coal layers to investigate variability of composition of molecular units in their resistant macromolecules, and to examine applicability of these compositions for chemotaxonomic study.

We analyzed plant fossils of angiosperms and gymnosperms collected from three locations; 1) Hirono, Fukushima Prefecture (Ashizawa Formation, Futaba Group), 2) Mukawa, Hokkaido (Hakobuchi Formation, Yezo Group), and 3) Mikasa, Hokkaido (Mikasa Formation, Yezo Group), Japan. For example, we used fruit fossils of *Hironoia fusiformis* and *Archaefagacea futabensis*, flower fossils of *Esgueiria futabensis*, leaf fossils of *Juniperus* and *Platanus*, a stem fossil of *Ephedra*, as well as some fossils of fruits, seeds and woods that were taxonomic uncertain. Powder samples of above fossils were extracted with methanol and dichloromethane, and were subsequently refluxed under high temperature to remove free compounds completely. Finally, the residues were saponified by KOH/methanol to obtained ester-bound compounds. GC-MS analysis was performed for identification and quantification of compounds.

As ester-bound molecular units in resistant macromolecule of all fossil samples, C_{10}-C_{28} *n*-alkanoic acids and C_{10}-C_{28} *n*-alkanols were mainly detected. It was found that distributions of carbon number of n-alkanoic acids were clearly different between woody and non-woody fossils in the Futaba samples. In the non-woody fossils (e. g. flowers, fruits and leaves), which were organs that have cuticles, C_{18} /C_{16} ratios of n-alkanoic acids were lower. In the Hakobuchi plant fossils, we could obtain such difference for the C_{18} /C_{16} alkanoic acid ratios between woody and non-woody fossils. On the other hands, the C_{18} /C_{16} ratios of n-alkanoic acids are higher in wood fossils. In addition, C_{14} /C_{16} ratios of n-alkanoic acids in non-woody fossils tended to be higher than those in woody fossils. These results imply that the characteristics of the compositions in the n-alkanoic acid units might be attributed to monomer compositions of cutin and suberin. From scatter diagram for relationships between C_{18} /C_{16} and C_{14} /C_{16} ratios as independent variables, a linear function which can distinguish non-woody fossils from wood fossils was obtained. From scatter diagram for the relationship between C_{20} /C_{18} and C_{20} /C_{16} ratios of n-alkanols as independent variables, wood fossils could be roughly separated from flower, fruit and leaf fossils. The higher ratios of C_{20} n-alkanol in woody fossils suggested high contribution to suberin-derived monomer in the fossils. From these results, we propose that the alkanoic acid and alkanol units from polyesters of resistant macromolecule can be powerful chemotaxonomic indicators for ancient plant fossil, although further examination is necessary.

Keywords: Cretaceous plant fossil, resistant macromolecule, chemotaxonomy, suberin, cutin, fossil polyester

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BPT28-09 Room:102A Time:May 24 16:00-16:15

Allopatric speciation due to 1.55 Ma isolation of the islands of Ryukyu, Japan, based on geologic and GenBank data

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The Ryukyu island arc was originally continental margin arc, but separated from the Chinese continent by the rifting of the Okinawa trough, which began at 1.55 Ma and continued to the present. Furthermore, the Ryukyu arc was simultaneously divided into the northern Amami-Okinawa and southern Yaeyama islands by the Kerama rift valley, and consequently formed two isolated islands units. The Kuroshio warm current began to inflow from the Yonaguni strait, and outflow through the Tsushima and Tokara straits also at 1.55 Ma, and effectively acted as barrier with Taiwan, China, and Japan. Through this geologic process that we newly found, vicariant speciation to generate the Ryukyu endemic animal species is expected. We tried to justify this hypothesis by drawing lineaged phylogenetic trees of these endemic species or subspecies using GenBank data, before by ourselves DNA analyses for the many other resting Ryukyu species. We can put precise branching age in these phylogenetic trees, and show simultaneous speciation at 1.55 Ma for Amai-Okinawa and Yaeyama units, respectively. The Taiwan and Tsushima straits, barriers between Taiwan-China and Japan-Korea, was insufficient during later glacial periods, and species are intermingled. Some sea embayment barrier is expected between northern and southern China. We additionally estimate the precise DNA substitution rate and justify the molecular clock.

Keywords: Ryukyu islands, 1.55 Ma synchronous isolation, lineaged phylogenetic tree, endemic species, vicariant speciation, precisely estimated molecular evolution rate

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BPT28-10 Room:102A Time:May 24 16:15-16:30

Evolution of bivalve bodyplan

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By evolving bilaterally separate shell plates, bivalves have acquired a unique body plan in which their soft tissues are completely protected by hard shell plates. Here we asked how the unique shell morphology of bivalves was brought about by modification of their development. First, we confirmed the old descriptions on the cellular origin of shell field precursors claiming that bilaterally cleaved shell field precursor cells develop into bilaterally separated shell fields. Thus, modification of the early spiral cleavage pattern is tightly linked with the evolution of bilaterally separate shell plates. Furthermore, we found that the specific inhibition of dpp during bivalve development results in impaired development of the ligament that separates the shell plates. We conclude that the unique shell plate morphology of bivalves is a result of two distinct modifications during early embryogenesis, namely, modification of the early spiral cleavage pattern and neofunctionalisation of dpp for ligament development.

Keywords: bivalve, development, evolution

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BPT28-11 Room:102A Time:May 24 16:30-16:45

An attempt to create the animal genomic data as a Linked Data

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The pace of DNA decoding is growing faster and faster in the recent years according to improvement of the DNA sequencers. The animal genome which has relatively large size of DNA are not exceptional in this aspect. Prediction of genes, prediction of proteins and assignment of gene expression data are required tasks for more analysis of genome sequences after the genome decoding. But under the present circumstances, the pace of such predictions and annotations are not catching up to the pace of the data creation. Especially in the recent animal genome projects, the quality of gene predictions and annotations show the deteriorative because only small number of researchers can be involved.

The accuracy of genemodels depends on the accuracy of the previous gene models, because each genemodel is created by the comparative analysis with the known predicted genemodel. So in sometimes, the low quality genemodel create the next low quality genemodels, as it is called ”Junk makes Junks”. Such low quality genemodels could prompt a deadly mistakes for the comparative genome analyses although the comparative extant animal genomes are required to know the evolution of animals.

For getting away from such issues, we are now re-curating the genemodels for the variety of published animal genomes from the aspect of evolutionary biology. Additionally, we start an attempt to create the Linked data from the animal genomic data for making them convenient to the variety of comparative genomics.

Keywords: animals, genome, annotation, ontology, linked data, semantic web