Japan Geoscience Union Meeting 2015
(May 24th - 28th at Makuhari, Chiba, Japan)

BPT26-01 Room:104 Time:May 26 16:15-16:30

Estimation of the environmental condition at the early evolutionary periods by resurrection of ancient proteins

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It is important to clarify the environment where early life evolved to understand the origin and history of terrestrial life. It is not easy to assume the ancient environment where our extinct ancestors had lived, since geological records on the early evolution of terrestrial life are quite limited.

From the phylogenetic tree based on the 16S/18S rRNAs, Woese et al. (1990, PNAS, 87: 4576?4579) classified extant terrestrial life into three domains, Archaea, Bacteria, and Eukarya. In this tree, all extant terrestrial organisms have common ancestor (the last common universal ancestor: LUCA or Commonote). If all extant terrestrial life has the Commonote, the next question is what Commonate's nature was. The growing temperature of Commonote (or LUCA) has been interested and discussed. The LUCA (or Commonote) was poposed to be thermophilic by Pace (1991, Cell, 65: 531?533). However, there are many objections. The discussion on this issue, however, has been done mostly based on the predicted growth temperature estimated from the GC contents and amino acid frequencies of LUCA's genes and proteins inferred with molecular phylogenetic analyses. Therefore, they are not proven by the experimental data (e.g. Galtier et al. (1999, Science, 283:220?221), Boussau et al. (2008, Nature, 456:942-945), Groussin et al. (2013, Biol. Lett., 9: 21130608)). Recently, it has become to be used that experimental resurrection of ancient proteins based on the estimation of ancient amino acid sequences being possessed by ancient organisms estimated from the molecular phylogenetic analysis, as one of powerful tools to evaluate the characteristics of extinct organisms (e,g. Gaucher et al. (2003, Nature, 425: 285?288)).

There is strong correlation between the T_m of nucleoside diphosphate kinase (NDK) and optimal growth temperature of its host organism. Therefore, T_m of resurrected ancestral NDKs can be used to evaluate the growth temperature of the ancient organisms. We resurrected amino acid sequences of NDKs of the last archaeal common ancestor (LACA) and the last bacterial common ancestor (LBCA). Then, the ancestor NDKs with resurrected amino acid sequences were expressed in *Escherichia coli* cells, purified, and then temperature-dependence of their denaturation was measured. The T_m of denaturation of resurrected NDKs of LACA and LBCA were higher than 100 ℃. Therefore, both LACA and LBCA are suggested to be hyperthermophiles. We also estimated the possible Commonote NDK sequences based on the comparison of sequences of resurrected NDKs of LACA and LBCA. The Tm of the most thermally unstable Commonote's NDK we resurrected was 90 ℃ (Akanuma et al. 2013, PNAS, 110: 11067?11072). This suggests that the Commonote was thermophilic organism.

Boussau et al. (2008) suggested that the Commonote was psychrophiles and/or mesophiles. Groussin et al. (2013) suggested that the Commonote was mesophiles and/or moderate thermophiles. Their conclusions were based on the molecular phylogenetic analyses under the condition where amino acid composition is permitted to change during evolution. We resurrected amino acid sequences of NDKs of LACA, LBCA, and Commonote with same method used by Boussau et al. (2008), and then the ancestor NDKs were expressed, purified, and measured temperature-dependence of their denaturation. The T_m of these ancestor NDKs, LACA NDK, LBCA NDK, and Commonote NDK, were higher than 100 ℃, so that LACA, LBCA, and Commonote were estimated that to have been thermophiles or hyperthermphiles.

These results are experimental support on the thermophilic or hyperthermophilic nature of LACA, LBCA, and Commonote. In addition, we will discuss cellular pH of ancestral organisms based on the pH dependence of enzymatic activity of resurrected NDKs.

Keywords: Commonote, resurrection of proteins, nucleoside diphosphate kinase, thermophiles

BPT26-02 Room:104 Time:May 26 16:30-16:45

Understanding the relationship between rise of oxygen and gene expression of cyanobacterial antioxidant enzymes

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Though free oxygen (O_2) was absent from the atmosphere during the first half of Earth's 4.5-billion-year history, which is considered to have increased dramatically at the beginning and the end Proterozoic ($\sim$2.2 and $\sim$0.6 billion years ago, respectively). Recent geochemical data suggest that such transitions to an oxic atmosphere were not unidirectional, but appear to have associated with an overshoot and/or a downshoot of the O_2 levels. Such dynamic changes in the redox conditions in the atmosphere must have posed severe environmental stresses to life on Earth. However, how the changes in the O_2 levels affected the biosphere has been poorly understood. Answering this question is of fundamental importance in understanding Earth's history.

Here we focused on the changes in the expression of genes encoding antioxidant enzymes of cyanobacteria through history. Changes in environmental O_2 levels must have affected the production of reactive oxygen species, which in turn affected antioxidant gene expression. Gene expression is regulated by the nucleotide sequence in the promoter regions. Thus we hypothesized that the ancestral promoter sequences of the antioxidant enzymes may reflect the environmental O_2 levels at the time the ancestor existed. In this study, we resurrected the ancestral promoter sequences of the antioxidant enzymes, and discuss its relationship to the O_2 evolution through time. In this presentation, we will first introduce recent models of the atmospheric O_2 evolution through Earth's history, then we will report our progress in resurrecting ancestral promoter sequences of cyanobacterial antioxidant enzymes.

Keywords: rise of oxygen, antioxidant enzymes, cyanobacteria

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BPT26-03 Room:104 Time:May 26 16:45-17:00

Dynamics of Proterozoic oceanic euxinia and its impact on the biosphere

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Proterozoic is characterized by substantial changes in the redox state of atmosphere and oceans at both ends of the eon and stasis between them. Accumulating geological/geochemical records demonstrate that the first major oxygenation of the Earth's surface at 2.45-2.22 billion years ago (Ga) (known as "Great Oxidation Event") may have been followed by a subsequent drop of atmospheric oxygen level at the end of the Lomagundi-Jatuli event (LJE) (~2.08-2.05 Ga). Such waxing and waning of the oxygenation state of Earth's surface would have caused substantial changes in oceanic chemical composition and would surely have impacted the biosphere. In this context, the evidence for strongly sulfidic (euxinic) oceanic environments in the LJE aftermath in Gabon and Karelia are notable because their low molybdenum isotopic values (less than 0.95 ‰ and 0.85 ‰, respectively) imply widespread euxinia at that time. The spatiotemporal variation of euxinia should have played a crucial role in Proterozoic biological and geochemical evolution not only because of its toxicity to eukaryotes but also because of its fundamental role on bioessential trace metal availability in the ocean interior. However, the nature of oceanic biogeochemical dynamics and its impact on the biosphere in the Proterozoic remains unclear.

To investigate the dynamics of oceanic biogeochemical response during the Paleoproterozoic eon, we improved upon the CANOPS model, in which coupled C-N-O-P-S marine biogeochemical cycles and a series of redox reactions were adequately taken into account. We newly developed an open system modeling approach for marine sulfur cycling in which the oceanic sulfur balance is explicitly evaluated.

In this study we show that a plausible cause and effect chain of events that is consistent with the geological records of the LJE aftermath can be obtained when a dramatic decrease in atmospheric oxygen level (from $>$50%PAL to $<$1%PAL; PAL=present atmospheric level) is assumed: the deoxygenation of an atmosphere-ocean system stimulates the microbial sulfate reduction, resulting in a transitional (~20 Myr) expansion of euxinia until the ocean reaches a new steady state. A mass balance calculation of Mo also demonstrates that LJE aftermath is marked by an expansion of euxinia to ca. 9-40% of the whole seafloor. Under such conditions Mo levels would decrease to as low as ca. 2.0-6.5 nM where nitrogen fixation by Fe-Mo nitrogenase is very sensitive to Mo concentration. We therefore suppose that waxing and waning of the atmospheric oxygenation state in the Paleoproterozoic could have affected evolution and diversification of the aerobic bacteria and/or eukaryotes through dynamic oceanic euxinia.

Keywords: Proterozoic, oceanic redox states, ocean biogeochemical cycle model, euxinia

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BPT26-04 Room:104 Time:May 26 17:00-17:15

Nitrogen isotope chemostratigraphy from the Ediacaran to early Cambrian in South China

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The earth is only the planet where higher forms of life exist. The appearance and evolution of metazoans are the most important issue of the evolution of the earth and life, but the causes are still obscure. It is considered that increase in oxygen content of atmosphere and seawater resulted in the evolution, but the evidence for correlation between the increase in the oxygen content and biological evolution is poor. This study focuses on nitrogen that is one of the most important nutrients at present. Preservation of continuous and fossiliferous strata from the Ediacaran to the Cambrian, South China is suitable for reconstruction of secular change of compositions of seawater through the time. This study presents secular change of nitrogen cycle from the Ediacaran to the Early Cambrian including shallow marine and deep-sea environments based on chemostratigraphies of organic nitrogen isotopes of shallow marine and deep-sea environments.

We obtained the nitrogen isotope ratios of organic nitrogen in black shales and carbonate rocks of drill core samples from the Shuijingtuo and Shipai Formations. The nitrogen isotope ratios gradually increase from -1 to +3 permil in Shuijingtuo Formation whereas they are fluctuated between +2 and +4 permil in Shipai Formation. In addition, the variation of the nitrogen isotope ratios is not related with difference of lithology: carbonate rocks and black shale, respectively. In addition, no correlations between the nitrogen isotope ratios and C/N ratios or total N contents are found. The results indicate that the variation in the nitrogen isotope values is not artificial due to lithological change and secondary alteration but it was caused by environmental change in the Early Cambrian. The nitrogen isotope ratios gradually increase in the black shale of the upper Shuijingtuo Formation, suggesting decrease of the nitrate content of the seawater. In other words, it shows that the nitrate-rich environment was terminated and both nitrate and phosphate started to be limited since the beginning of the Botomian, namely mid-Cambrian Series2 possibly due to increasing primary productivity. Namely, modern-style marine nutrient cycle was established in the early Cambrian. The higher primary productivity led to increase of the oxygen content of the atmosphere and ocean, promoting the Cambrian explosion.

Carbon isotope chemostratigraphy is often used for comparison among sections because of lack of key fossils in the Ediacaran. However, it is well known that carbon isotope ratios of organic carbon were decoupled with those of carbonate carbon in the Ediacaran. In addition, the inorganic carbon isotope chemostratigraphies are highly distinct between shallow marine and deep-sea environments in the Ediacaran. They make it impossible to compare shallow water environments with and deep-sea environments based on the carbon isotope chemostratigraphies. In order to establish a new tool for the comparison, we analyzed nitrogen and carbon isotope ratios of organic matter of Yuanling section, deposited in a deep-sea environment, in South China from Ediacaran to early Cambrian. The results show that the pattern of nitrogen isotope chemostratigraphy in Yuanling section is similar to that in the Three Gorges area, platform sediments, from the Ediacaran to the Early Cambrian, indicating the nitrogen isotope is useful to compare between the shallow marine and deep-sea sediments. The nitrogen isotope ratios of the deep-sea sediments in the Ediacaran are lower than those of the shallow marine sediments. The difference may be because pelagic environment was more enriched in nitrate than platform environments in photic zone.

Keywords: Ediacaran, Cambrian explosion, Nitrogen isotopes, Nutrients, Biological evolution, History of the earth

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BPT26-05 Room:104 Time:May 26 17:15-17:30

Coupling of Imaging Mass Spectrometry and High Resolution U-Th-Pb Geochronology on Carbonates for the Earth Genomics

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Time-resolved elemental and isotopic data can provide key information about the time changes in the surface geochemical conditions of the Earth, and therefore, critical restriction for the origin and the evolutional sequence of the life on the Earth could be evaluated. To obtain reliable and exclusive information from the samples, tremendous efforts have been made to improve both the analysis spatial resolution and the precision in the resulting ages. Especially for the young samples, correction of isotope disequilibrium is still key issue to obtain reliable age data. Recently, we have developed new correction method for the initial disequilibrium, and therefore, application range for the U-Th-Pb geochronology could be remarkably extended to the young samples (e.g., <0.1 Ma). The U-Th-Pb age determinations have been widely applied for the U-Th-bearing mineral such as zircon, monazite or apatite, which would have high closure temperatures for the U-Th-Pb decay series isotopes. Despite the obvious success in obtaining reliable age data for these U-Th bearing minerals, age determination for other minerals of different closure temperature is highly desired. Many geologists are increasingly interested in processes in rocks that operate under the lower temperatures. To achieve this, we have focused on the U-Th-Pb age determinations for carbonates. The in-situ U-Th-Pb age determinations for carbonates have been retarded mainly due to very low U-contents in the carbonates (e.g., <1 ug/g). Moreover, system closure could be easily lost through the geological time. To measure precise Pb/U and Pb/Th isotope ratio from the carbonates, coupling of laser sampling and the high sensitivity ICPMS system was employed. Hence, all the ^{202}Hg, ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb and ^{238}U signals were simultaneously measured using six high-sensitivity ion multipliers. This multiple collector (MC) system provides much higher duty cycle over the traditional isotope ratio measurements. Sensitive in-situ U-Th-Pb isotope ratio measurements can be made directly from the solid minerals using the present analytical technique. Despite this, there still remains a problem of both the contamination of non-radiogenic Pb and the secondary loss of the radiogenic-Pb, which were the major source of the resulting age values. To overcome this, prior to the age determination analysis, elemental mapping or distribution feature of all U, Th and Pb isotopes were measured to evaluate the magnitude of the secondary loss or contamination of U, Th and Pb isotopes.

With the LA-ICPMS technique, elemental imaging analysis can be made from fairly large-sized samples (e.g., >20 mm x 20 mm), and thus, the comprehensive information concerning the secondary distribution of the trace elements can be evaluated from whole sample bodies. Combination of elemental imaging and the U-Th-Pb age determination, together with the correction technique for the initial disequilibrium, can provide precise and reliable age data from the carbonatets. The details of the analytical procedure and the correction technique for the initial disequilibrium will be discussed in this presentation.

Keywords: U-Th-Pb dating, Multiple collector-ICPMS, Laser ablation, Elemental Imaging, Earth Genomics

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BPT26-06 Room:104 Time:May 26 17:30-17:45

Genetic variation of gene expression pattern among echinoderm populations.

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We compared the gene expression patterns among local populations of Peronella japonica, a species of Japanese sand dollar. We detected siginificant variation for several genes among populations. I discuss the impact of these findings on the evolutionary history of metazoans.

Keywords: Echinoderm, sea urchin, population, gene expression

BPT26-P01 Room:Convention Hall Time:May 26 18:15-19:30

Chemotaxonomy of plants by resistant macromolecular analysis in charred mesofossils from the Cretaceous Futaba Group

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Resistant macromolecules composing living plant tissues tend to be preserved through degradation and diagenesis, hence constitute major parts of fossil plants or sedimentary plant-derived organic matter. And their monomer compositions vary widely among different plant taxa, organs and growth stages. Thus, analysis of such macromolecule may serve as new technique for paleobotanical evaluation distinctive from classical paleobotnical studies depends on morphological preservation of fossils. However, there have been few studies of the macromolecules, especially in ancient geological samples such as the Paleozoic and Mesozoic. In the present study, we analyzed plant fossils from the Cretaceous strata in Japan to examine chemotaxonomic characteristics of fossil macromolecules.

Charred mesofossils of angiosperms and gymnosperms were separated from carbonaceous sand stone of the Cretaceous Ashizawa Formation, Futaba Group. These mesofossils include fruit fossils of *Hironoia fusiformis* and *Archaefagacea futabensis*, a flower fossil of *Esgueiria futabensis*, leaf fossil of *Juniperus*, a stem fossil of *Epfedra* and some fossils of fruits, seeds and woods. Powdered fossil samples were extracted with methanol and dichloromethane, and were subsequently refluxed under 110 ℃ to remove free compounds completely. The residues were hydrolyzed by KOH / methanol under 110 ℃. These released compounds were analyzed by GC-MS. Additionally, multivariable analysis were calculated using SPSS software. We used hierarchical clustering to group fossils with similar lipid distributions among species or organs.

n-alkanes, branched isoprenoids, sterans, hopanes, and aromatic hydrocarbons were mainly present in solvent extract fraction. Aromatic hydrocarbons contained various higher plant derived diterpenoid and triterpenoid derivatives. These compounds are commonly considered as chemotaxonomic markers of gymnosperms and angiosperms respectively. Unexpectedly, triterpenoid derivatives were detected from gymnosperm fossils abundantly, indicating that free lipids may have moved in the coal bed, thus these lipids are not suitable for chemotaxonomic use in this study. On the other hand, as main hydrolyzed products (ester-bound molecular units) from all fossils, C6-C28 *n*-alkanoic acids and C8-C28 *n*-alkanols were detected. Multivariable analysis were calculated in lipid distribution for these released alkyl lipids from each fossils. Cluster analysis revealed a recognizable pattern in released alkyl lipid distribution. All five fossils of woody tissue were present in a cluster that excluded non-woody tissues. Additionally, exclusive of Juniperus fossil, the lipid signatures were similar among angiosperms or gymnosperms. From these results, we propose that it is likely to be realized that paleolipidomics-like detailed chemotaxonomy of fossil plants by making a comprehensive evaluation for various lipid components involve bond alkyl lipids.

Keywords: chemotaxonomy, alkyl lipid, plant fossil, Cretaceous, resistant macromolecule, multivariable analysis

BPT26-P02 Room:Convention Hall Time:May 26 18:15-19:30

Foraminiferal psuedopodial observation during chamber formation

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Foraminifera, marine unicellular organism, have been considered as one of the major carbonate producer in ocean. Their calcareous tests are broadly utilized as paleo-environmental indicators in various studies of earth science because their tests have been archived as numerous fossil in sediment for long time and various environmental information are brought by population, morphology and geochemical fingerprints. The knowledge about the cytological process on carbonate precipitation has been described for couples of decade using by OM, SEM and TEM. Foraminiferal management of shell formation from ambient seawater are of great interest. Our study shows the potential to understanding the function of psuedopodial network for biomineralization by optical microscope.

Keywords: Foraminifera, Calcification

BPT26-P03 Room:Convention Hall Time:May 26 18:15-19:30

Comparative anatomy of molluscs

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The phylum Mollusca is characterized by a diversity of body plan. Animals of extant molluscs are categorized into seven major types, namely (1) shell-less vermiform aplacoporans, (2) polyplacophorans with eight shell plates and repetition of internal organs, (3) monoplacophorans with a single shell and internal iteration, (4) bivalves with shells divided into right and left, (5) gastropods diagnosed by the operculum and ontogenetic torsion, (6) cephalopods with the arms/tentacles modified from the foot, (7) antero-posteriorly elongated tusk-like scaphopods. In addition, novel forms have been found in fossils, and they are regarded as ancestral molluscs connecting intermediate missing links or allegedly assigned to molluscs. One of keys to understanding of diversification of molluscan body structure is comparison of organogenesis in ontogeny. This viewpoint further needs investigation of development-controlling genes and leads into comparative genomic research.

Keywords: comparative anatomy, Mollusca

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Molecular basis of shell formation and shell evolution in gastropods

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The Molluscs constitutes one of the most diverse animal phyla, and they have evolved calcified exoskeletons called “shell” ever since the Cambrian. However, the molecular basis of molluscan shell development remains unclear. Thus we sought to understand the role of the homeotic gene *engrailed* in early shell development by focusing on retinoic acid signal pathway. We examined the expression patterns of RA metabolizing enzyme *cyp26* in the limpet *Nipponacmaea fuscoviridis* and found that *cyp26* is expressed around the edge of the shell field. As a result of gain or loss functional analysis of RA, shell deformation was observed in both gain and loss of RA analyses, and engrailed is down regulated. These results suggested that the common ancestor of Mollusca likely used RA signaling system to produce the novel phenotypic trait that is to be called “shell” by recruiting the homeotic gene.

Keywords: Shell evolution, RA pathway, Mollusca

BPT26-P05 Room:Convention Hall Time:May 26 18:15-19:30

A possible coordinate system in the 3D coiling of molluscan shells

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A vast variety of forms have evolved in the molluscan shells since the Cambrian, all basing on the single and simple rules of growth, or the logarithmic spiral. Yet the biological realities underlying this mathematical regularity remained elusive except that the signal transduction protein Dpp has been demonstrated to be involved at least in the two-dimensional coiling of the shells. Here we show that another signal transduction protein is involved in the shell coiling, based on the results obtained from chemical treatments of the embryos of the pond snail Lymnaea stagnalis. We argue that those two ’morphogens’ may form a coordinate system, which grows like a moving frame of the theoretical ’growing tube’, enabling the mantle epithelial cells to form secretory three-dimensionally coiled structures.

BPT26-P06 Room:Convention Hall Time:May 26 18:15-19:30

Genetic mechanisms of shell growth and shell coiling

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Molluscan shells have been a focus of study in the global environmental changes and morphological evolution, because they have a rich and continuous fossil record throughout the Phanerozoic. The knowledge of development and growth of the shells is important to reveal their morphological evolution, which may well be related to global environmental changes. Although theoretical aspects of the shell growth have been explored extensively, the biological reality of shell growth, such as the molecules and genes related to the theoretical parameters, remained largely unexplored. However, a clue has been found in recent years, that is, Dpp. It is the transcription product of *dpp*, which is the homologous gene of *bmp 2/4* in vertebrates. It has been shown that *dpp* is an important factor for shell formation and shell coiling. In this study, we focused on another signaling protein important in development. To investigate the function of this protein to *Lymnaea stagnalis*, we used an inhibitor and an activator. We found that these signals are heavily associated with the development and growth of embryos. In particular, the phenotypes of the shell form observed when the embryos were activated at the veliger stage demonstrated that the activity of these signals likely control the speed of the shell growth and the extent of shell coiling.