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

Keywords: Commonote, resurrection of proteins, nucleoside diphosphate kinase, thermophiles
Understanding the relationship between rise of oxygen and gene expression of cyanobacterial antioxidant enzymes

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Though free oxygen (O\textsubscript{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 (~2.2 and ~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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} evolution through time. In this presentation, we will first introduce recent models of the atmospheric O\textsubscript{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
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
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 isotope ratios 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
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
We compared the gene expression patterns among local populations of Peronella japonica, a species of Japanese sand dollar. We detected significant 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
白亜系双葉層群の炭化小型植物化石の抵抗性高分子分析：結合態アルキル脂質組成による植物化石の化学分類

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

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陸上高等植物の生体組織を構成する抵抗性高分子は微生物分解や続続作用に対して抵抗性があり、植物化石や陸上植物由来の堆積有機物の主要な成分を占めていると考えられている。また、これらの構成モノマーは植物の分類群や生育環境、器官、続続作用などによって特徴的に変化することが知られている。したがって、これらの高分子の分析を行うことで古植物学的な研究のための新たな手法の確立が期待される。しかしながら、特に中生代や古生代のような古い地質時代の堆積物においては、これらの高分子を研究した例は限られている。本研究では、化石高分子の化学分類的な特徴を評価するため、白亜紀の炭質物質層から産出した植物化石の分析を行った。

試料は白亜紀ホニア属の双葉層環形が指向から産出した炭化した小型化石を用いた。分析した小型化石はHironoia fusiformisやArchaefagacea futabensisを含む双葉植物の花および果実の小型化石、裸子植物の種子・シート・材の小型化石など16試料を用い分析を行った。粉末化した試料を有機溶媒で抽出後、残渣を高圧 KRI/メタノールを用いアルカリ加水分解を行った。分解抽出された成分を GC/MS で分析・定量した。また、SPSS を用いた多変量解析を行った。植物の部位や種類において似たような脂質組成をもつ化石をグループ化するため階層クラスター分析を行った。

遊離態の抽出成分としてn-アルカン、ステレン、ホホバンおよび芳香族炭化水素が主に検出された。芳香族炭化水素はジェネルペノイドおよびトリテリペノイドの種々の誘導体が含まれ、一般的にこれらの結合種植物および被子植物の化学分類マーカーとして知られている。しかしながら、裸子植物化石の抽出成分から多量のトリテリペノイドが検出された。これは遊離態の脂質が堆積物中に移動していることを示唆していると考えられ、これらの成分が化石植物の化学分類には不向きであることがわかった。一方、加水分解性成分からはC6-C28 の飽和脂肪酸およびC8-C28のn-アルカンノールが主として検出された。それぞれの抽出試料においてこれらのアルカリ加水分解性アルキル脂質の炭素数分布を多変量解析によって詳細に分析した。階層クラスター分析によって、化石種によるアルカリ加水分解性脂質の炭素数分布の違いがみられることが明らかとなった。すべての木質組織の化石は非木質組織の化石を含まない一つのクラスターに分類された。さらに、Juniperusを除けば、被子植物化石と裸子植物化石もそれぞれ別のクラスに分けられ、分類群によって脂質構成に明確な差があることが示された。これらの結果から、エステル結合群のアルキル脂質を含む多様な脂質を網羅的に解析し、総合的に評価することで化石植物の paleolipidomics ともいえる詳細な化学分類の情報が得られることの可能性があることを指摘する。

キーワード: 化学分類, アルキル脂質, 植物化石, 白亜紀, 抵抗性高分子, 多変量解析

Keywords: chemotaxonomy, alkyi lipid, plant fossil, Cretaceous, resistant macromolecule, multivariable analysis
Foraminiferal pseudopodial 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 pseudopodial network for biomineralization by optical microscope.

Keywords: Foraminifera, Calcification
軟体動物の比較解剖学
Comparative anatomy of molluscs

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軟体動物はボディプランの多様性によって特徴づけられる。現在生き残っている軟体動物は、(1) 殻の無い虫状の無板類、(2) 8 枚の殻を持ち軟体部に繰り返し構造を示す多板殻、(3) 殻は一枚であるが軟体部には繰り返し構造を持ち単板殻、(4) 殻が左右 2 枚に分かれた二枚貝殻、(5) 盤と体のねじれによって定義される腹足類、(6) 足が腕や触手に変化した頭足類、(7) 前後に牙のように長くなった掘足類、の 7 つに類型化される体制を示す。化石では現生には見られない構造を示す種が発見されており、それらは進化の途中段階を表していると見なされるか、軟体動物であるか疑問なものも含む。これらの体の構造の成り立ちを理解する上で鍵となるのは器官形成の過程の比較である。さらに、発生段階を制御する遺伝子の研究が必要であり、本セッションの中心課題である地球ゲノム研究へつながっていく。

キーワード: 比較解剖, 軟体動物
Keywords: comparative anatomy, Mollusca
巻貝における貝殻獲得の遺伝的基盤
Molecular basis of shell formation and shell evolution in gastropods

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Keywords: Shell evolution, RA pathway, Mollusca
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.
Lymnaea stagnalisの貝殻形成におけるWntの役割
Genetic mechanisms of shell growth and shell coiling

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軟体動物の貝殻は炭酸カルシウムで形成されているため化石として残りやすく、地球環境の変遷のプロセスや生物進化の直接的証拠として研究が続けられている。貝殻形態の進化を明らかにする上では、貝殻の発生や成長に関する知見は欠かすことができない。巻貝類の貝殻成長については、理論モデルについては様々な研究が進められてきたが、実際に貝殻成長に関わる遺伝子などの生物学的実態については長年解明されておらず、近年になってようやく研究が進められてきた。その中の重要な研究として、脊椎動物のbmp2/4の相同遺伝子であるdecapentaolegic (dpp)の転写産物であるDpp分子について研究がある。軟体動物では、トコホアーパクに形成される貝殻で形成が開始される。Dppは貝殻で形成する遺伝子の1つであり、カサガイでは左右対称に、モノアラガイでは左右非対称に形成発生することが知られており (Iijima et al., 2008)、初期発生期と後期成長期でのdppの発現は前後から前に必要に (Shimizu et al., 2011)、Dppの濃度勾配も貝殻の螺旋成長について関与している (Shimizu et al., 2013)。dppが貝殻形成について重要な因子の1つであることが知られている。本研究では、Dppと並んで重要なシグナル伝達因子であるWntファミリーに注目した。脊椎動物において、背腹軸形成に関与するBmp2/4とは、直交する前後軸に沿ってWntの濃度勾配が見られ、Wntシグナルが形態形成に重要であることがすでに知られている (Niehrs, 2010)。このWntも貝殻形成の制御に何らかの働きをしているのではないかと推測し、検証することを研究目的とした。研究材料として、軟体動物腹足綱有肺類基眼目のLymnaea stagnalis (ヨーロッパモノアラガイ)を用いた。この卵を5つの発生段階 (2細胞期・胞胚期・原胚胚期・トコホアーパク・ベリジャー期) 毎に、Wntの阻害剤 inhibitors of Wnt response-1 (IWR-1) と促進剤6-bromo-mumoindirubin-3’-oxime (BIO)を多様な濃度で使用して機能阻害・促進実験を行い、L. stagnalisの発生過程を観察した。その結果供与、L. stagnalisの発生において、Wntシグナルが阻害され活性化されないと、貝殻形成は正常に起こるものの中に組織の形成が正常に開始されないことが観察された。また、抑制にWntシグナルが促進され活性化されると、殻は正常にまかず、平巻になることが示明した。さらに必要以上に活性化されすぎてしまうと分化がうまくできず、殻を含めた全体の組織の形成が正常に起こらなかった。以上のことから、Wntは発生そのものに大きく関与している可能性が高いと考えられる。特に貝殻形成については、ベリジャー前に促進させた場合に観察できた貝殻形態の違いから、殻が巻く速度と成長していく速度に対してWntシグナルの活性度が重要である可能性が示唆される。