

古代タンパク質の復元に基づく全生物の最後の共通祖先の生育環境の復元 Estimation of the environmental condition at the early evolutionary periods by resurrection of ancient proteins

横堀 伸一^{1*}; 別所 瑞萌¹; 笹本 峻弘¹; 中島 慶樹¹; 赤沼 哲史¹; 山岸 明彦¹
YOKOBORI, Shin-ichi^{1*}; BESSHO, Mizumo¹; SASAMOTO, Takahiro¹; NAKAJIMA, Yoshiki¹;
AKANUMA, Satoshi¹; YAMAGISHI, Akihiko¹

¹ 東京薬科大学・生命科学部・応用生命科学科

¹Dept. Applied Life Sci., Sch. Life Sci., Tokyo Univ. Pharm. Life Sci.

地球上の生命の起源と進化を理解する上で、初期の生命の生育環境を明らかにすることは重要である。しかし、現生生物の祖先となる生物の生育環境を推測することは、地球上の生命の初期進化に関する地質記録は極めて限られており、容易ではない。

Woese 等 (1990, PNAS, 87: 4576-4579) が作製した 16S/18S rRNA に基づく系統樹によれば、現生生物は 3 つのドメイン、古細菌 Archaea、真正細菌 Bacteria、真核生物 Eukarya、に分かれ、共通祖先をもつ。異論はあるが、それぞれ単系統群である古細菌と真核生物は姉妹群であり、全生物の最後の共通祖先 (LUCA。我々は Commonote と呼んでいる) の位置は、真正細菌と古細菌+真核生物の間であると考えられる。全生物の共通祖先が存在したとすると、次の疑問はその共通祖先がどのような生物であったかである。「全生物の共通祖先は超好熱菌であった」という仮説が Pace (1991, Cell, 65: 531-533) によって提案されたが、その解釈に対する反論も多かった。しかしながら、これらの議論のほとんどは、分子系統解析により全生物の共通祖先の核酸の G+C 含量やアミノ酸組成を推定し、そこから生育温度を推論したものであり、実験的に検証されたものではない (例えば Galtier et al. (1999, Science, 283:220-221)、Boussau et al. (2008, Nature, 456:942-945)、Groussin et al. (2013, Biol. Lett., 9: 20130608))。しかし、近年、分子系統解析による祖先蛋白質のアミノ酸配列の推定と、その配列をコードする祖先型遺伝子の実験的な復元が、過去の生物の性質を理解するために行われるようになって来た (例えば Gaucher et al. (2003, Nature, 425: 285-288))。

ヌクレオシド二リン酸キナーゼ (NDK) は、至適生育温度が異なる様々な微生物の NDK の変性温度が至適生育温度と強い相関を持つ。そのため、祖先配列の推定から復元した NDK の変性温度から、その NDK を持った過去の生物の生育温度環境を推定することができる。そこで我々は、古細菌共通祖先生物 (LACA) と真正細菌共通祖先生物 (LBCA) の持っていたと考えられる NDK のアミノ酸配列を推定し、遺伝子工学的手法により復元した祖先 NDK 遺伝子が大腸菌内で発現し、祖先 NDK の精製と熱変性測定をおこなった。復元した LACA NDK、LBCA NDK は、どちらも変性中点温度が 100 °C を超える高い耐熱性を有していた。よって、LACA と LBCA はそれぞれ超好熱菌であったと推定された。また、LACA NDK と LBCA NDK の配列はよく似ており、Commonote の NDK も同様なアミノ酸配列を持っていたことが期待されたことから、LACA NDK と LBCA NDK の配列から Commonote NDK の配列を作製した。その変性温度は 90 °C 以上であり、この NDK を持った全生物の共通祖先 (Commonote) は 75 °C 以上に生息する好熱菌であったと考えられた (Akanuma et al. 2013, PNAS, 110: 11067-11072)。

また、LUCA/Commonote が好冷菌ないしは常温菌であったと推定した Boussau 等 (2008) や常温菌ないしは中等度好熱菌であったと推定した Groussin 等 (2013) の推定の根拠の一つは、進化の過程でのアミノ酸組成の変化を許容する分子系統樹推定法を用いて推定した Commonote の蛋白質のアミノ酸組成であった。我々は、同様の分子系統樹推定法を用いて新たに LACA NDK、LBCA NDK、そして Commonote NDK のアミノ酸配列を推定し、遺伝子工学的手法により復元した祖先 NDK 遺伝子が大腸菌内で発現、精製を行った。これらの LACA NDK、LBCA NDK、ならびに Commonote NDK の変性中点温度はいずれも 100 °C 以上であり、LACA、LBCA、Commonote はいずれも、好熱菌ないしは超好熱菌であったと推定された。

以上の結果は、古細菌共通祖先生物 (LACA)、真正細菌共通祖先生物 (LBCA) がともに (超) 好熱菌であり、全生物の最後の共通祖先 (LUCA/Commonote) が (超) 好熱菌であったことの実験的な証拠と言える。また、祖先 NDK の酵素活性の pH 依存性を現生生物の NDK と比較することで、祖先生物の細胞内環境の推定も試みているので、その結果についても紹介する。

キーワード: コモノート (全生物の最後の共通祖先), 祖先蛋白質復元, ヌクレオシド二リン酸キナーゼ, 好熱菌
Keywords: Commonote, resurrection of proteins, nucleoside diphosphate kinase, thermophiles

祖先型プロモーター配列推定に基づく大気酸素濃度進化とシアノバクテリア抗酸化酵素遺伝子発現量との関連性の解明
Understanding the relationship between rise of oxygen and gene expression of cyanobacterial antioxidant enzymes

原田 真理子^{1*}; 古川 龍太郎²; 横堀 伸一²; 田近 英一³; 山岸 明彦²
HARADA, Mariko^{1*}; FURUKAWA, Ryutaro²; YOKOBORI, Shin-ichi²; TAJIKA, Eiichi³; YAMAGISHI, Akihiko²

¹ 東京大学大学院理学系研究科, ² 東京薬科大学生命科学部分子生命科学科, ³ 東京大学大学院新領域創成科学研究科
¹The University of Tokyo, ²Tokyo University of Pharmacy and Life Science, ³The University of Tokyo

Though free oxygen (O₂) 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 O₂ 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 O₂ 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 O₂ 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 O₂ 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 O₂ evolution through time. In this presentation, we will first introduce recent models of the atmospheric O₂ evolution through Earth's history, then we will report our progress in resurrecting ancestral promoter sequences of cyanobacterial antioxidant enzymes.

キーワード: 大酸化イベント, 抗酸化酵素, シアノバクテリア
Keywords: rise of oxygen, antioxidant enzymes, cyanobacteria

原生代海洋ユーキシニアのダイナミクスと生物圏への影響 Dynamics of Proterozoic oceanic euxinia and its impact on the biosphere

尾崎 和海^{1*}; 田近 英一²

OZAKI, Kazumi^{1*}; TAJIKA, Eiichi²

¹ 東京大学 大気海洋研究所, ² 東京大学 大学院新領域創成科学研究科

¹AORI, The University of Tokyo, ²Graduate School of Frontier Sciences, The University of Tokyo

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.

キーワード: 原生代, 海洋酸化還元状態, 海洋物質循環モデル, ユーキシニア

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

南中国のエディアカラ紀からカンブリア紀初期における窒素同位体化学層序 Nitrogen isotope chemostratigraphy from the Ediacaran to early Cambrian in South China

小宮 剛^{1*}; 西澤 学²; 土谷 祐貴¹
KOMIYA, Tsuyoshi^{1*}; NISHIZAWA, Manabu²; TSUCHIYA, Yuki¹

¹ 東京大学総合文化研究科、駒場, ² 海洋研究開発機構

¹Department of Astronomy & Earth Sciences, The University of Tokyo, Komaba, ²Japan Agency for Marine-Earth Science and Technology

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.

キーワード: エディアカラ紀, カンブリア爆発, 窒素同位体, 海洋栄養塩, 生物進化, 地球史

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

イメージングと高分解能 U-Th-Pb 年代測定法をくみあわせた炭酸塩の年代学と地球ゲノム計画
Coupling of Imaging Mass Spectrometry and High Resolution U-Th-Pb Geochronology on Carbonates for the Earth Genomics

坂田 周平¹; 平田 岳史^{1*}
SAKATA, Shuhei¹; HIRATA, Takafumi^{1*}

¹ 国立大学法人京都大学
¹ Kyoto University

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 evolutionary 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 ²⁰²Hg, ²⁰⁴Pb, ²⁰⁶Pb, ²⁰⁷Pb, ²⁰⁸Pb and ²³⁸U 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 carbonates. 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

棘皮動物における遺伝子発現の集団間多様性
Genetic variation of gene expression pattern among echinoderm populations.

和田 洋^{1*}
WADA, Hiroshi^{1*}

¹ 筑波大学生命環境系
¹ Graduate School of Life and Environmental Sciences, Univ of Tsukuba

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.

キーワード: 棘皮動物, ウニ, 地域集団, 遺伝子発現
Keywords: Echinoderm, sea urchin, population, gene expression