

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

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

The 16S/18S rRNA based-tree of life by Woese et al. (1990, PNAS, 87: 4576-4579) has been treated as the "standard" tree of terrestrial life, although there are many objections. In this tree, all extant terrestrial organisms have common ancestor (the last common universal ancestor: LUCA or Commonote), and are classified into three domains, Bacteria, Archaea, and Eukarya. If all extant terrestrial life has the Commonote, its nature is the next question. In particular, the growing temperature of Commonote (or LUCA) has been interested and discussed. Pace (1991, Cell, 65: 531-533) proposed that the LUCA (or Commonote) was thermophilic. However, there are many objections. However, the discussion on this issue 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, so that they are not proven by the experimental data (e.g. Galtier et al. (1999, Science, 283:220-221)). Recently, as one of powerful tools to evaluate the characteristics of extinct organisms, 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 (e.g. Gaucher et al. (2003, Nature, 425: 285-288)).

To evaluate the growth temperature of ancient organisms, we resurrected amino acid sequences of nucleoside diphosphate kinases (NDKs) of the last archaeal common ancestor (LACA) and the last bacterial common ancestor (LBCA) with the maximum likelihood method for tree reconstruction by using NDK amino acid sequences of extant archaea and bacteria. 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 °C. Since there is strong correlation between the  $T_m$  of NDKs and optimal growth temperature of their host organisms, both LACA and LBCA are suggested to be hyperthermophiles. Errors of estimation of ancestral sequences and different tree topologies used for resurrection of sequences did not affect seriously on the thermal stabilities of resurrected NDKs of LACA and LBCA. We also estimated the possible NDK sequences carried by the Commonote based on the sequences of resurrected NDKs of LACA and LBCA. The  $T_m$  of the most thermally unstable Commonote's NDK we resurrected was 90 °C (Akanuma et al. 2013, PNAS, 110: 11067-11072). This suggests that the Commonote was thermophilic organism.

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

## Evolution of the Earth's environment: A view from sedimentary alkyl porphyrins

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Alkyl porphyrins are derivatives of chlorophylls that are formed in the surface of the Earth by photosynthesizers. Structural changes associated with the diagenetic processes have been intensively studied during the last half a century. Now we know that some alkyl porphyrins are derived only from specific chlorophylls that are originated from a specific type of photosynthesizers. Together with carbon and nitrogen isotopic compositions, such structural information provides a profound insight on the critical evaluation of the surface water environment in the geological past. In this presentation, I will review the diagenetic alteration of chlorophyll structures and review the current evidence.

Keywords: Porphyrins, Sediment, Earth's surface environment

## The close correlation between environmental change and evolution of metazoans: Genome duplication and rapid adaptation

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The Neoproterozoic to Cambrian is one of the most exciting periods when Metazoa first appeared and quickly evolved. The origin and early evolution of Metazoa are very attractive firstly because the events suddenly happened after very long calmness, over 2000 m.y. since the emergence of eukaryotes, and proceeded very quickly, and secondly because appearance of new phylum was limited to this period (Cambrian explosion). Recent paleontology, biomarker study and molecular biology suggested early origin, especially of sponges and cnidarians, and cryptic evolution of the metazoans (e.g. Maloof et al., 2010; Love et al., 2010; Peterson et al., 2008; Sperling et al., 2010, Erwin et al., 2011). On the other hand, recent comprehensive study of multi-elemental and multi-isotopic chemostratigraphies of drill core samples in Three Gorges, Tianping and Beidoushan areas revealed that redox condition and bioessential element contents of seawater such as P, Ca,  $\text{NO}_3^-$ , Fe, Mn, Mo, and Sr drastically changed from the Neoproterozoic to the Early Cambrian. Sr isotope values display positive excursions at ca. 580, 570-550 and 540 Ma, indicating repeated high continental influxes at those times. P contents of carbonate minerals were very high until ca. 550 Ma, and then decreased, suggesting the seawater was enriched in phosphorus before 550 Ma and then depleted due to oxidation of seawater and deposition of phosphorite. High nitrogen isotope values of organic matter and high Ca isotope values of carbonate rocks indicate that seawater was depleted in  $\text{NO}_3^-$  and Ca contents until ca. 550 Ma, and then increased. Mo isotopes of black shale, and Fe and Mn contents and REE patterns of carbonate rocks indicate that seawater became more oxic since ca. 550 Ma. In addition, the Mo contents of black shale increased in the Late Ediacaran and Early Cambrian, indicating Mo content of seawater increased due to the oxidation of seawater. On the other hand, iron and manganese contents of carbonate rocks decreased, suggesting that iron and manganese contents of seawater decreased because of the oxidation of seawater.

Comparison of the geochemical evidence with biostratigraphy suggests that the emergence of Metazoan in the Early Ediacaran was caused under the relatively less oxic and P-rich condition, whereas their diversification occurred under oxic,  $\text{NO}_3^-$  and Ca-rich condition. Especially, the transition from phosphorus-rich to  $\text{NO}_3^-$ -rich seawater possibly increased Redfield ratio, and contributed to diversification of more actively mobile multicellular animals. In addition, the comparison of geochemical and paleontological evidence indicates that the biological evolution occurred just after the environmental changes, especially the timing of increase in nutrients, allowing a new insight of biological evolution of multicellular animals. The quick response of biological evolution to the environments suggests that the fundamental innovation for biological functions was already established long before the environmental changes. The quick adaptation implies that early metazoans or a common ancestor have genomes for the functions before they acquired the functions, indicating genome duplication plays important role on the early evolution of metazoans.

Keywords: Biological evolution, paleoenvironmental change, Ediacaran, Nutrients of seawater, Evolution of Metazoa and Cambrian explosion, Genome duplication

## Reconstruction of the gene sets for the developmental signaling ligands in ancestral protostome animals

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Recently, a draft genome sequence of the pearl oyster *Pinctada fucata* was reported, enabling to infer a possible evolutionary scenario of the gene sets that are important for body plan formation in protostomes including both lophotrochozoans and ecdysozoans. We report the results of phylogenetic character mapping carried out for the gene families that encode developmental signaling ligands (Fgf, Hedgehog, PDGF/VEGF, TGF- $\beta$ , and Wnt families) to reconstruct possible copy numbers of signaling molecule-coding genes for hypothetical ancestral protostomes. Our reconstruction suggests that *P. fucata* retains the ancestral protostome gene complement, providing further justifications for the use of this taxon as a model organism for developmental genomics research.

Keywords: paleogenomics, metazoan evolution, evo-devo, signaling ligand genes, Cambrian explosion, lophotrochozoans

## Reconstruction of paleo genomic information of metazoan based on a microsynteny analysis

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Since a variety of metazoan genome decoding, the conservation of gene order on the DNA (synteny) is recognized as a common event in the metazoan. For example, Putnam reported the some amounts of signatures of macrosynteny between human and sea anemone (*Nematostella vectensis*) (Putnam et al 2007). Subsequently, Irimia reported their hypothesis that two adjacent genes that shared their 5' cis region (head to head) may restraint their translocation. Because of this situation, I'm trying to use these syntenic constraint for reconstructing the ancient genome. To the start of such reconstruction analyses, I'm compared among the genome of Hemichordate, Sea urchin and Amphioxus. These analyses revealed their possible common developmental mechanisms kept since their common ancestor.

Keywords: metazoan, genome, microsyntey, reconstruction

## A close relationship between global oceanic environmental changes and seafloor mineral deposition during the Phanerozoic

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Metal deposition on seafloor is strongly controlled by marine redox conditions. Fe-Mn and Mn oxide deposits are formed under oxygenated oceans. In striking contrast, Cu and Zn-bearing sulfide deposits are stable under anoxic oceans. Seafloor mineral deposits in turn are good indicators to redox conditions or redox changes of modern and ancient oceans.

There are numerous strata-bound ore deposits in the Japanese accretionary complexes. These deposits are mainly divided into three types; umber (Fe-Mn), Mn-rich, and volcanogenic massive sulfide (VMS; Besshi-type). The Mn-rich deposits are further divided into two subtypes that are associated with greenstone and NOT associated. Ages of these deposits provide us important constraints for a secular change of marine redox conditions over the past ~360 Myr. Depositional ages of umber and Mn deposits were previously determined by microfossils including radiolarians and conodonts. On the other hand, ages of the Besshi-type deposits are determined by Re-Os method (Nozaki et al., 2013). Oxide ore deposits such as umbers and Mn deposits were very likely precipitated in the modern-style oxygenated deep-sea. In contrast, Mn carbonate and VMS deposits were precipitated in the stagnant, O<sub>2</sub>-deficient deep-sea during the Triassic and Jurassic periods. Seafloor mineral deposition closely related to global oceanic environmental changes may give us a hint for exploring the causes of mass extinction, and further for elucidating the evolution of life.

Nozaki, T., Y. Kato, K. Suzuki (2013) Late Jurassic ocean anoxic event: evidence from voluminous sulphide deposition and preservation in the Panthalassa. *Scientific Reports*, 3: 1889; doi:10.1038/srep01889.

Keywords: oceanic environmental change, seafloor mineral deposit, Japanese accretionary complexes, marine redox condition, Phanerozoic

## Global paleogeography and life evolution: 2. Mesozoic

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The Mesozoic witnessed the Pangean breakup. Since the Triassic, the southern half of Gondwana successively rifted/separated, kicking out numbers of continents northward to form Laurasia, i.e., the northern half of Pangea, ca. 200 Ma. Multiple collisions among the Russian platform, Kazakhstan, Siberia, N. China, S. China, Indochina, Tarim, and other minor continental blocks were completed mostly in the Triassic or in the Early Jurassic at the latest. Gondwana has started to be fragmented immediately after its birth at 540Ma, except the collision of Laurentia at 430 Ma. The apparent supercontinent Pangea formed when Laurasia came in shape by 200 Ma. Its disassembly began first by the opening of the central Atlantic domain induced by the eastward moving of Africa for ca. a few thousands of km. The birth of South Atlantic Ocean was delayed until ca. 120Ma, whereas the opening of Northern Atlantic already started. The separation of S. America from Africa occurred ca. 120Ma. There was a pulse period of Pacific superplume ca. 120-85Ma when the production rate of MORB was 150-300 % higher than the rest of the Mesozoic. Numbers of huge oceanic plateaus were formed in the Pacific domain, including the Caribbean plateau. The birth of Indian Ocean occurred at ca. 100-120Ma by the separation of India from Gondwana. It is composed of 4 distinct oceanic lithospheres (separated by NS-trending major transform faults) behaved uniquely. The sea-level was kept relatively high according to such Mesozoic global tectonics; warm period without global glaciation but with oceanic anoxia and remarkable production of oil, gas, and coal. The climate was generally dryer than the Cenozoic, with higher production of evaporites. The mammalian diversification was triggered by the ca. 120Ma separation of the final bridge among Africa, S. America, and Laurasia. The appearance of the fox monkey in Madagascar, and of new-world monkeies in S. America, was likely connected to Indian migration and narrow arc bridge to S. America.

Keywords: paleogeography, Mesozoic, supercontinent, Pangea, Atlantic Ocean, evolution

## Recent results of foraminiferal calcification

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Foraminifera, marine unicellular organism, have been thought as one of the major carbonate producer in ocean. Their calcareous tests are commonly 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 calcareous test itself is interested by many foraminifer scientists. The knowledge about the cytological process on carbonate precipitation has been described for couples of decade using by many legacy technology. Cellular regulations of ions uptake into calcareous tests from seawater are of great interest for broad fields of earth science. Our recent studies showed the potential to understanding the biomineralization of foraminifera by the application of fluorescent indicators. Recently, we visualize the spatial distributions of cytological calcium and pH in living cell at same time under several pH conditions (7.5-8.1). Observed results show that foraminifera controls very detailed timing of pH variation and concentration of calcium at any stage of chamber formation dynamically even ambient pH are varied. These observations results will help to consider how the geochemical compositions arranging on the foraminiferal test, sensitivity of pH proxy of boron and others.

## Left-right reversal in unicellular eukaryotes, planktonic foraminifera

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Aquatic unicellular organisms are little motile and passively disperse in general. Holoplankton, which spend their entire life-cycle floating in the water column, are likely carried by water flow and exposed to diverse conditions of environment. Their morphology may vary over wide distribution ranges by phenotypic plasticity or allelic variation. Among these organisms, planktonic foraminifera are an excellent system to examine diversity and evolution in cellular responses to the environment because of two reasons: (1) occurrence in every ocean and (2) visible asymmetry in coiled shell. Both left- and right-coiled forms are often found within single morphospecies. Their coiling direction has traditionally been thought to change phenotypically depending on environmental factors, especially water temperature, based on coil-morph distributions but without statistical evidence. Molecular phylogenetic studies have revealed that morphospecies often contain multiple cryptic species. The arguments on the role of temperature for coil reversal most probably confused cryptic species into single taxa. In the present study, we examined the dependence of morph frequency on temperature by focusing on populations that are dimorphic for coiling direction and occur across wide ranges of temperature. *Globorotalia truncatulinoides* includes five genetically isolated species, and each of them is dimorphic for coiling direction. The statistically meaningful regression analysis was possible in three species that are distributed in global ranges. The results showed that morph frequency does not depend on water temperature in warm or cold seasons or on the annual mean temperature. Moreover, the geographic patterns of frequency variation among water masses in these species suggest that gene flow affects morph frequency. The majority exhibits the same coiling direction among populations that inhabit water masses connected by ocean circulation system. In contrast, morph frequency greatly varies between unconnected water masses regardless of climatic conditions. The present results, therefore, reject temperature-dependence of coiling direction and suggest the presence of genetic basis for coiling direction in planktonic foraminifera. Our study provides a base to explore the evolution on left-right asymmetry in unicellular eukaryotes.

Keywords: left-right asymmetry, coiling direction, cryptic species, unicellular eukaryote

## Were marine microplankton in the Japan Sea geographically isolated during the Last Glacial Maximum?

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The Japan Sea is connected by three straights (the Soya, Tsugaru, and Tsushima Straights) to the Sea of Okhotsk and Pacific Ocean with the shallow sill depth (140 m). During the last glacial maximum (LGM: 23-19 kilo years before present), the sea-level was decreased at least 120 m lower than today and the Japan Sea was almost isolated from surrounding seas. It is possible that such geographic isolation reduced and/or impeded gene flow of marine organisms between the Japan Sea and surrounding seas. Previous phylogeographic studies of coastal vertebrates (only whose larval stage is planktonic) have actually suggested that the Japan Sea was closed during the LGM. However, there is no phylogeographic study with marine microplankton, which inhabit the water column throughout their entire life cycle.

Radiolaria, the major marine planktonic protists, are passively transported in the pelagic ocean. Their geographic distribution would be easily affected by geographic changes through geological time. Moreover, their siliceous shells have been preserved in marine sediments and form a good fossil record. The sensitivity to geographic changes and well-preserved fossil record of Radiolaria could allow us to elucidate a past geographic isolation of marine microplankton. *Larcopyle buetschlii*, a morphospecies of radiolarians analyzed in the present study, is found in the surface waters in the Pacific Ocean, whereas it has a characteristic distribution vertically ranged from the surface to deep layers in the Japan Sea. In addition, its fossil specimens are continuously observed in the Japan Sea before the LGM. Therefore, *L. buetschlii* could be a good model to study a link between geographic isolation during the LGM and reproductive isolation of marine microplankton.

Heterogeneity of internal transcribed spacer regions of ribosomal DNA (ITS1 and ITS2) is observed in many eukaryotes (e.g., vertebrates, dinoflagellates, and diatoms). The ITS1 and ITS2 regions are spliced out during the maturing process of ribosome, causing a nucleotide substitution rate higher than ribosomal DNA coding regions. Nevertheless, the ITS1 and ITS2 sequences are functionally important for their splicing, because the premature transcript composed of 18S, 28S, 5.8S rRNA, ITS1, and ITS2 is folded into a secondary structure followed by the self-splicing of ITS1 and ITS2. Based on the secondary structures of ITS1 and ITS2 sequences, compensatory base changes (CBCs: base changes occurring on both sides of a double-stranded portion) and hemi-CBCs (HCBCs: base changes occurring on one side of a double-stranded portion) are often observed among closely related species. The correlation between CBCs and HCBCs in the ITS2 sequences likely reflects sexual compatibility among individuals of a closely related species. Thus, the CBCs/HCBCs correlation is a useful marker to infer whether geographically isolated populations are reproducible.

We demonstrated that there is heterogeneity of the ITS2 sequences within an individual of *L. buetschlii* and that all individuals of *L. buetschlii* collected from the surface to deep layers in the Japan Sea do not have a significant difference in the CBCs/HCBCs of the ITS-2 sequences. Furthermore, the CBCs/HCBCs of the ITS-2 sequences do not show a significant difference between individuals of the Japan Sea and Pacific Ocean. These findings suggest that *L. buetschlii* in the Japan Sea and Pacific Ocean likely forms a reproducible single population. Thus, the geological isolation during the LGM is unlikely effective for the reproductive isolation of this radiolarian species.

Keywords: Japan Sea, *Larcopyle buetschlii*, Radiolaria, secondary deep-sea plankton

## Non-coding sequences conserved independently in four different mammalian orders

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Conserved noncoding sequences (CNSs) of vertebrates are considered to be closely linked with protein-coding gene regulatory functions. We examined the abundance and genomic distribution of CNSs in four mammalian orders: primates, rodents, carnivores, and cetartiodactyls. We defined the two thresholds for CNS using conservation level of coding genes; using all the three coding positions and using only first and second codon positions. The abundance of CNSs varied among lineages, with primates and rodents having highest and lowest number of CNSs, respectively, whereas carnivores and cetartiodactyls had intermediate values. These CNSs cover 1.3-5.5% of the mammalian genomes and have signatures of selective constraints that are stronger in more ancestral than the recent ones. Evolution of new CNSs as well as retention of ancestral CNSs contribute to the differences in abundance. The genomic distribution of CNSs is dynamic with higher proportions of rodent and primate CNSs located in the introns compared with carnivores and cetartiodactyls. In fact, 19% of orthologous single-copy CNSs between human and dog are located in different genomic regions.

If CNSs can be considered as candidates of gene expression regulatory sequences, heterogeneity of CNSs among the four mammalian orders may have played an important role in creating the order-specific phenotypes. Fewer CNSs in rodents suggest that rodent diversity is related to lower regulatory conservation. With CNSs shown to cluster around genes involved in nervous systems and the higher number of primate CNSs, our result suggests that CNSs may be involved in the higher complexity of the primate nervous system. This study was published in *Genome Biology and Evolution* (Babarinde and Saitou, 2013; vol. 5:2330-2343).

Keywords: genome, mammals, Primates, Rodents, Artiodactyla, Carnivores

## Development for new hyphenated analytical technologies for paleogenomics research

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Cytometry is the analytical technique, basically applied for quantitative analysis of cells and cell systems. In general, cytometry measures optical properties of cells, and most often uses fluorescence to measure specific antigen molecules, intracellular ions and DNA/RNA. Cells may be live or fixed, depending on the application, and individual cells can often be physically sorted. ? Other optical signals can be measured, including light scatter. The cytometry has blossomed to become the key technique to evaluate the nutritional status or to understand the elemental metabolism for animals. Several advantages can be derived by the cytometry, such as analysis speed, detection sensitivity, the ability to measure many parameters simultaneously, and the ability to sort individual cells (i.e., single cell spectroscopy). Recently, new generation cytometry utilizing the sensitive mass spectrometers (i.e., mass cytometry) was described. With the mass cytometry, further sensitive detection of ions or proteins and higher capability for the multiparameter analysis of individual adherent cells (e.g.,; Benfall et al., *Science*, 2011; Bodenmiller et al., *Nature Biotechnology*, 2012). With the extensive number of information collected from cells or samples through the cytometry, reliable and objective evaluation for the changes in biochemical functions could be achieved. This approach can also be applied to understand the solar system evolution based on the numerous number of age data. In recent ten years, we have demonstrated the unique study approach using the distribution pattern of sample ages based on the series of precise age data collected from large number of samples (i.e., age-cytometry) (e.g., Rino et al., *PEPI*, 2008; Iizuka et al., *Geology*, 2008; Iizuka et al., Iizuka et al., *Chem. Geol.*, 2009; Iizuka et al., *GCA*, 2010). The mass cytometry will become a powerful tool to promote the big-data science for various research fields such as metallomics, medical sciences or the geochemistry. For elemental or isotopic analysis of trace- or ultratrace-elements, plasma ion source mass spectrometry (ICP-MS) has been widely employed because of its high analytical capabilities such as high-elemental sensitivities, minimal sample preparation procedures, high-analysis throughput or user-friendly operations (Bandura et al., *Anal. Chem.*, 2009). With the laser ablation sample introduction technique, distribution of both the elemental and isotopic data for trace- or ultratrace-elements can be successfully derived directly from large-sized solid samples (>10cm). Despite the obvious success in obtaining elemental and isotopic data (age data), it should be noted that stable isotope ratio data for light elements (e.g., C and O) could not be derived by the present LA-ICPMS technique because of serious contribution mass spectrometric interferences on C and O isotopes, which provides key information concerning the physico-chemical conditions for the sample formation. To overcome this, we would like to develop a new analytical technique to measured the C isotopes, at a same time with elemental analysis using the LA-ICPMS technique. Newly developed spectroscopy technique combined to the LA-ICPMS technique can become a major analytical tool to expand the analytical capability for mass cytometry for biochemical samples and geochemical samples through precise, reliable and uniform quality data. The analytical technique develop here will promote the big-data science for various research fields including geochemistry and biochemistry.

Keywords: mass spectrometry, laser ablation, paleogenomics, hyphenated technology, analytical chemistry, geochemistry