Adaptive evolution of marine cyanobacteria to the climate in the Neoproterozoic inferred from ancestral proteins

*Nao Masuda[1], Mariko Harada[2], Sota Yagi[3], Ryutaro Furukawa[4], Akihiko Yamagishi[5], Shin-ichi Yokobori[5]

1. Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 2. Faculty of Life and Environmental Sciences, University of Tsukuba, 3. Center for Biosystems Dynamics Research, RIKEN, 4. Faculty of Human Sciences, Waseda University, 5. Department of Applied Life Sciences, Tokyo University of Pharmacy and Life Sciences

The Neoproterozoic (ca. 1000–542 million years ago) must have been a remarkable period for life on Earth, marked by severe global glaciations, the oxidation of the atmosphere and ocean, and the emergence of diverse large multicellular organisms. Understanding the climatic conditions in the Neoproterozoic and their effect on life is necessary for comprehending the co-evolution of life, biogeochemical cycles, and environments during this period. Based on the $\delta^{18}O$ and $\delta^{30}Si$ compositions of marine cherts, the global seawater temperature was ˜30 ºC in the Neoproterozoic, which is ˜15 ºC warmer than global average temperature today. During the snowball earth events at 730-700 Ma and 665-635 Ma, the global average temperature has dropped to around -40 ºC. However, how life adapted to such warm interglacial and severe glacial periods has been poorly understood.

In the present study, aiming to clarify the temperature adaptation of life to climatic changes, we estimated the optimum growth temperatures of ancestral marine cyanobacteria lived 1.7-0.4 billion years ago based on temperature-dependent stability of experimentally “resurrected” ancestral NDK (nucleoside diphosphate kinase) proteins. NDK is the enzyme that synthesizes a nucleoside triphosphate by adding a phosphate from ATP to a nucleoside diphosphate, which denaturation temperature is known to have a strong correlation with the optimum growth temperature of its host organism. Thus, the optimum growth temperatures of the ancestral cyanobacteria can be estimated by examining the denaturation temperatures of ancestral NDKs.

10 ancestral NDKs from two monophyletic marine groups of cyanobacteria were reconstructed. The ancestral amino acid sequences were statistically estimated by Maximum Likelihood method, based on extant sequences and given two phylogenetic trees. The ancestral NDKs were expressed in *Escherichia coli* and purified to analyze denaturation midpoint temperatures (T_ms). As a result, T_ms of 8 NDKs from an ancestral marine group α-cyanobacteria diverged 1.7-0.5 Ga consistently ranged from ˜65 ºC to ˜70 ºC, which correspond to the optimal growth temperatures of around 33-48 ºC. On the other hand, T_ms of 2 NDKs from another marine group of cyanobacteria were ˜70 ºC (˜400 Ma) and ˜85 ºC (˜800 Ma), which correspond to the optimal growth temperatures of 37-49 ºC and 53-62 ºC, respectively.

The results suggest that the ancestors of marine cyanobacteria adapted to the sea temperatures of interglacial periods and/or the temperatures that are higher than the environments. The difference in the growth temperatures between the two lineages may indicate latitudinal difference of their habitat, or the variation in the way of adaptation between lineages. We could not find any signs of adaptation to the temperature drops during global glaciations including snowball Earths, which suggest that ancestral marine cyanobacteria may have survived the snowball Earths maintaining high/mid-temperature tolerance, with cold tolerance and suppressed growth rates.

Keywords: Cyanobacteria, Ancestral protein reconstruction

