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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 ^{o}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 ^{o}C (Akanuma et al. 2013, PNAS, 110: 11067-11072). This suggests that the Commonote was thermophilic organism.

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