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

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

From the phylogenetic tree based on the 16S/18S rRNAs, Woese et al. (1990, PNAS, 87: 4576?4579) classified extant terrestrial life into three domains, Archaea, Bacteria, and Eukarya. In this tree, all extant terrestrial organisms have common ancestor (the last common universal ancestor: LUCA or Commonote). If all extant terrestrial life has the Commonote, the next question is what Commonate's nature was. The growing temperature of Commonote (or LUCA) has been interested and discussed. The LUCA (or Commonote) was poposed to be thermophilic by Pace (1991, Cell, 65: 531?533). However, there are many objections. The discussion on this issue, however, 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. Therefore, they are not proven by the experimental data (e.g. Galtier et al. (1999, Science, 283:220?221), Boussau et al. (2008, Nature, 456:942-945), Groussin et al. (2013, Biol. Lett., 9: 21130608)). Recently, 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, as one of powerful tools to evaluate the characteristics of extinct organisms (e,g. Gaucher et al. (2003, Nature, 425: 285?288)).

There is strong correlation between the T_m of nucleoside diphosphate kinase (NDK) and optimal growth temperature of its host organism. Therefore, T_m of resurrected ancestral NDKs can be used to evaluate the growth temperature of the ancient organisms. We resurrected amino acid sequences of NDKs of the last archaeal common ancestor (LACA) and the last bacterial common ancestor (LBCA). Then, 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 ℃. Therefore, both LACA and LBCA are suggested to be hyperthermophiles. We also estimated the possible Commonote NDK sequences based on the comparison of sequences of resurrected NDKs of LACA and LBCA. The Tm of the most thermally unstable Commonote's NDK we resurrected was 90 ℃ (Akanuma et al. 2013, PNAS, 110: 11067?11072). This suggests that the Commonote was thermophilic organism.

Boussau et al. (2008) suggested that the Commonote was psychrophiles and/or mesophiles. Groussin et al. (2013) suggested that the Commonote was mesophiles and/or moderate thermophiles. Their conclusions were based on the molecular phylogenetic analyses under the condition where amino acid composition is permitted to change during evolution. We resurrected amino acid sequences of NDKs of LACA, LBCA, and Commonote with same method used by Boussau et al. (2008), and then the ancestor NDKs were expressed, purified, and measured temperature-dependence of their denaturation. The T_m of these ancestor NDKs, LACA NDK, LBCA NDK, and Commonote NDK, were higher than 100 ℃, so that LACA, LBCA, and Commonote were estimated that to have been thermophiles or hyperthermphiles.

These results are experimental support on the thermophilic or hyperthermophilic nature of LACA, LBCA, and Commonote. In addition, we will discuss cellular pH of ancestral organisms based on the pH dependence of enzymatic activity of resurrected NDKs.

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