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Robustness of the thermophilicity of ancient organisms

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All of the modern organisms are thought to have evolved from a single common ancestor named Commonote (1). In order to investigate the environmental temperatures of the ancient organisms, we resurrected amino acid sequences of ancestral nucleoside diphosphate kinases (NDKs) that might be hosted by the last common ancestors of Archaea and of Bacteria. The enzyme catalyzes the transfer of a phosphate from a nucleoside triphosphate to a nucleoside diphosphate.The ancestor of NDK family is thought to have been possessed by the ancient organisms because most extant cells, from bacteria to human, contain the gene(s) that encode a member of this family of proteins. More importantly, the denaturation temperature of a NDK correlates well with the optimal growth temperature of its host. Therefore, we can estimate the environmental temperature of the ancient organisms by reconstruction ancestral NDK's amino acid sequences and characterizing their thermal stabilities. In our previous study (2), the ancestral amino acid sequences of NDK were inferred from two phylogenetic trees with different topologies using a maximum likelihood program. The sequences were then reconstructed and characterized. From thermal denaturation experiments of the reconstructed enzymes, we estimated that the common ancestors of Archaea and of Bacteria lived at 81-97 ℃ and 80-94 ℃, respectively. The Commonote was also likely to be a (hyper)thermophile that lived at a temperature above 75 ℃. However, a criticism for our conclusion is that the ancestral sequences have been inferred with an assumption that the amino acid composition has been constant through evolutionary time. Gouy and coworkers (3) predicted the amino acid sequences of ancestral proteins using a Bayesian method that does not assume the constant evolutionary process through time. They estimated the optimal environmental temperature of the ancestral organisms from the amino acid composition of seven amino acid types: leucine, isoleucine, valine, tyrosine, tryptophan, arginine, and glutamate. Based on the analysis, they suggested that the archaeal and bacterial ancestors were thermophilic but the Commonote was not a thermophilic organism. In this study, we re-inferred ancestral NDK sequences using the same Bayesian program. The ancestral NDK sequences were inferred from two phylogenetic trees. One tree was built without constraints and the other with the constraint that Archaea and Bacteria each represent a monophyletic group. The gene encoding the ancestral NDK sequences were synthesized, expressed in *Escherichia coli*, and then the ancestral NDKs were purified. Thermal denaturation measurements showed that the newly inferred ancestral NDK sequences are also extremely thermally stable. Therefore, our conclusion of the (hyper)thermophilic ancestry is robust even if the ancestral amino acid sequences were inferred without the assumption that the amino acid composition has been constant over evolution.

(1) Yamagishi et al. In Thermophiles: the keys to molecular evolution and the origin of life? (1998), pp287-295.
(2) Akanuma et al. Proc. Natl. Acad. Sci. USA (2013)110, 11067-11072
(3) Boussau et al. Nature (2008)456, 942-947

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