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Resurrection of ancestral genes to infer the ancient environment temperatures

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The ancient global environment is a topic that has interested many scientists. One debate regarding the ancient environment concerns the growth temperature of ancient organisms. A number of theoretical studies have argued the growth temperature of ancient organisms, but these studies remained inferential due to the lack of empirical testing. Therefore, we developed an experimental way to assess the growth temperatures of ancient organisms using an inferred amino acid sequence of a protein postulated to exist in the last universal common ancestor. Because extant genes are evolutionary descendants of ancient genes, information on ancient genes is embedded in the sequences of extant genes. Therefore, ancestral sequences of a particular protein can be inferred by comparing extant homologous protein sequences. In our experimental method, inferred ancestral residues were introduced into several extant proteins and then the thermal stabilities of the resulting mutant proteins were examined. The mutant proteins, each of which contains one or a few inferred ancestral residues, showed the trend toward enhanced thermal stability when compared to the respective wild-type protein. Because the thermal stabilities of proteins often reflect the living temperatures of host organisms, our results have supported the hyperthermophilic common ancestry hypothesis.

To further improve our knowledge of ancient living systems and of the ancient global environment where early life evolved, the ancestral sequence reconstruction method was used to predict, synthesize, and characterize the complete ancestral sequences of B subunit of DNA gyrase (GyrB) and of nucleoside diphosphate kinase (NDK). The ancestral GyrB sequence was inferred from the sequences of extant DNA gyrases and type-VI DNA topoisomerases as the member of outgroup. Genes encoding the inferred sequence and its isolated N-terminal ATPase domain were PCR constructed and expressed in *Escherichia coli*. The structural properties and thermal stability of ancestral full-length GyrB are similar to those of the extant thermophilic DNA gyrase from *Thermus thermophilus*. The thermal stability of the ancestral ATPase domain is also similar to that of the *T. thermophilus* ATPase domain. Moreover, the ancestral ATPase domain has significant catalytic activity. The fact that the thermal stabilities of the ancestral GyrB and its ATPase domain are comparable to those of the extant thermophilic proteins further supports the idea that the ancient organism lived at high temperatures.

Ancestral NDK sequences were also inferred by the phylogenetic method. For NDKs, the denaturation temperatures of the proteins are roughly correlated with the optimum growth temperatures of the host cells. The genes encoding the inferred amino acid sequences were reconstructed by a PCR-mediated gene synthesis method. The ancestral genes were expressed in *E. coli* and the resurrected proteins purified. The purified ancestral NDKs are catalytically active. Temperature-induced unfolding experiments showed that the ancestral NDKs are significantly stable even around 100^{o}C. The results are again compatible with the hyperthermophilic common ancestry. Thus, our empirical reconstruction of ancestral genes provides experimental evidences that strongly support the hypothesis that ancient organisms lived in thermophilic environments.

Keywords: phylogenetic tree, ancestral gene resurrection, last universal common ancestor, ancient environment temperature