

EXPERIMENTAL TEST: IF THE COMMON ANCESTOR WAS A HYPERTHERMOPHILE?

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Based on the general phylogenetic tree inferred from small subunit ribosomal RNA genes, hyper-thermophilic common ancestor hypothesis has been proposed by several groups (1-4). However, the hypothesis has been criticized from several point of views (5-7).

We have recently developed experimental test for verifying the hypothesis (8). We have inferred phylogenetic tree of the enzymes 3-isopropylmalate dehydrogenase and isocitrate dehydrogenase. These two enzymes have been evolved from the common ancestral enzyme before the separation of Archaea and Bacteria. Based on the phylogenetic tree, amino acid sequence of the common ancestral enzyme has been inferred. The sequence of the ancestral enzyme was compared with 3-isopropylmalate dehydrogenase of a thermophilic archaeon *Sulfolobus tokodaii*. The archaeal enzyme retained significant portion of the sequence of the common ancestral enzyme. However some residues were different from the counter parts of the common ancestral enzyme. Mutant enzymes of isopropylmalate dehydrogenase having the residues of the common ancestral enzyme were made by site directed mutagenesis. The mutant enzymes were expressed in *E. coli*, purified, and thermal stability was estimated. Five mutant enzymes among the seven mutants tested showed higher thermal stability than the original isopropylmalate dehydrogenase of *Sulfolobus tokodaii*. The results support the hyper-thermophilic common ancestor hypothesis (8).

We are going to present the results of other experiments using isopropylmalate dehydrogenase from *Thermus thermophilus* and isocitrate dehydrogenase from *Caldococcus noboribetetus* as starting materials.

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