

Reconstruction and characterization of enzyme of Bacterial ancestor and Archaeal ancestor

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Hyperthermophilic organisms are located at the deepest branches of the phylogenetic tree constructed from 16S rRNA sequences. Based on the finding several authors have proposed that the common ancestor of all living organisms was hyperthermophilic. However these arguments are mere hypothetical inference rather than experimentally confirmed theory. We recently developed an experimental test for the hyperthermophilic common ancestor hypothesis and the early evolution of life. To experimentally test the early evolution of life, we have inferred an ancestral amino acid sequence for the metabolic enzyme, NDK as the model enzyme. We inferred the archaeal and bacterial ancestral (ancient) amino acid sequences of NDK by using a phylogenetic tree. We reconstructed archaeal and bacterial ancestral genes that corresponded to the inferred ancestral amino acid sequences by PCR. The ancestral genes were overexpressed in *E.coli*, ancestral protein products were obtained in soluble states and purified. The thermostabilities and temperature-dependent activity of reconstructed ancestral enzymes were compared with counterparts of extant extreme thermophiles, *Sulfolobus tokodaii* and *Thermus thermophilus*. Temperature-dependent activity of enzymes of archaeal ancestor and bacterial ancestor were also analyzed. These results indicate that bacterial ancestor thrived under the temperature higher than thermophilic environment and archaeal ancestor thrived under hyperthermophilic environment.