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Differences in substrate specificity of C5-substituted cytidine derivatives by DNA polymerases from hyper thermophilic bacteria and archaea

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The RNA world hypothesis postulates that RNA played the roles of information carrier and catalysts in an early stage of origins of life, and that the DNA-RNA-protein world developed in the later stage from the ancestral RNA-based system. Pyrimidine bases of RNA are uridine (U) and cytosine (C), on the other hand, thymidien (T) and C are used for DNA. The C-5 position of C and U is un-substituted, whereas the C5 of T is substituted with a methyl group. Some DNA has 5-methyl substituted cytosine, and 5-metrhyl C plays an important role in regulation of expression of the DNA. 5-Methylation of C in the DNA takes place after DNA synthesis by DNA polymerase, but not direct incorporation of 5-methyl cytosine nucleotide. Miller *et. al.* hypothesized that the C-5 position of U was reacted with formaldehyde giving 5-hydroxymethyl U, followed by reaction with various nucleophiles forming various C5-substituted U. They also suggest that the C5-substituted U could play important roles in the transition sate from RNA world to DNA world. Why 5-substituted methyl group is present in U(T), but not in C as a substrate nucleotide for DNA synthesis? It is also believed that the last common ancestor life was originated in the hydrothermal environment, and that hyper-thermophilic bacteria and archaea are old-type lives that are close to the last common ancestor. Recently, we reported that that substrate specificity of dUTP and several C5-substituted dUTPs for the DNA polymerases differs greatly depending on the type of the C5-substituent group and on the kinds of DNA polymerases from different families, A-family DNA polymerases from hyper thermophilic bacteria, B-family and D-family polymerases from hyper-thermophilic archaea. Here, we present whether DNA polymerases from hyper-thermophilic bacteria and archaea can accept d5-MeCTP or other various C5-substituted dCTPs as a substrate, giving the corresponding DNA by PCR. The difference in the substrate specificity of the DNA polymerases between bacteria and archaea for the evolution of genetic system will be described briefly.