

Elongation of tandem repetitive DNA by the DNA polymerase of the hyperthermophilic archaeon *Thermococcus litoralis* at a hairpin-coil transitional state: a model of amplification of a primordial simple DNA sequence

Ogata N., Miura T.

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高度好熱性古細菌 *Thermococcus litoralis* の DNA ポリメラーゼによるヘアピン-コイル遷移状態での反復 DNA の伸長：始原単純 DNA 配列の増幅モデル

[Abstract]

DNA is replicated by DNA polymerase semiconservatively in many organisms. Accordingly, the replicated DNA does not become larger than the original DNA (template DNA), implying that replicative synthesis by DNA polymerase alone cannot explain the diversification of primordial simple DNA. We demonstrate that a single-stranded tandem repetitive oligodeoxyribonucleic acid (oligoDNA) composed of a palindromic or quasi-palindromic motif sequence and 25-50% GC content is elongated *in vitro* to more than 20 000 bases at 70-74°C by the DNA polymerase of the hyperthermophilic archaeon *Thermococcus litoralis* without a bimolecular primer-template complex. The efficiency of elongation decreased when the palindromic structure of the oligoDNA was destroyed or when the GC content of the oligoDNA was outside the range of 25-50%. The thermal melting transition profile of the oligoDNA, as observed by ultraviolet spectroscopy, exhibited a biphasic curve, reflecting a duplex-hairpin transition at 31-40°C and a hairpin-coil transition at 70-77°C. The optimal reaction temperature for the elongation, for instance, of oligoDNA (AGATATCT)₆ (72°C) was very close to its hairpin-coil transition melting temperature (70.4°C), but was markedly higher than the temperature at which duplex oligoDNA can exist stably (< 35.9°C). These results suggest that a hairpin-based "intramolecular primer-template structure" is formed transiently in the oligoDNA, and it is elongated by the DNA polymerase to long DNA through repeated cycles of folding and melting of the hairpin structure. We discuss the implication of this phenomenon, "hairpin elongation", from the standpoint of potential amplification of simple DNA sequences during the evolution of the genome.

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