Design of RNA polymerases recognizing synthetic nucleotides

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Nowadays, advances in gene modification and viral therapy have led to the development of various viral vectors, capable to replicate and express therapeutic proteins in specific tissues and even tumor cells. However, viral therapy still has some limitations due to the viral nature of genetic material delivery. First of all, the existing limitations are associated with the risk of uncontrolled viral replication and premature expression of therapeutic proteins. We have proposed a system that will include a reporter gene, carrying artificial nucleotides, and a mutant T7 DNA-dependent RNA polymerase capable of reading artificial nucleotides in genes and transcribing them into natural mRNA. Such a genetic construction will not be able to replicate, and the genes in it will not be transcribed by cellular DNA and RNA polymerases. Thus, its existence will be completely controlled. To choose positions for site-directed mutagenesis, we carried out molecular modeling, in particular, docking of DNA templates containing artificial nucleotides in the active site of T7 RNA polymerase. The ligand was a sequence consisting of natural and unnatural nucleotides. Having used molecular docking, we obtained the affinity of the oligonucleotide ligand with T7 RNA polymerases carrying various amino acid substitutions. Selected candidates were further tested in the proposed new method based on a double coupled (coupled2) cell-free transcription-translation system, in which we determine the activity of mutant T7 RNA polymerases by the luminescence of synthesized nanoluciferase (Nluc). As a result, we were able to evaluate the efficiency of transcription of unnatural templates with different mutants of T7 RNA polymerases. We have shown the high sensitivity and simplicity of the developed method, and also demonstrated the ability of the studied mutants of T7 RNA polymerase to recognize UBPs.
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