Enzymatic synthesis of random sequences of RNA and RNA analogues by DNA polymerase theta mutants for the generation of aptamer libraries

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ABSTRACT

Nucleic acid aptamers, especially RNA, exhibit valuable advantages compared to protein therapeutics in terms of size, affinity and specificity. However, the synthesis of libraries of large random RNAs is still difficult and expensive. The engineering of polymerases able to directly generate these libraries has the potential to replace the chemical synthesis approach. Here we start with a DNA polymerase that already displays a significant template-free nucleotidyltransferase activity, human DNA polymerase theta, and we mutate it based on the knowledge of its three-dimensional structure as well as previous mutational studies on members of the same polA family. One mutant exhibited a high tolerance towards ribonucleotides (NTPs) and displayed an efficient ribonucleotidyltransferase activity that resulted in the assembly of long RNA polymers. HPLC analysis and RNA sequencing of the products were used to quantify the incorporation of the four NTPs as a function of initial NTP concentrations and established the randomness of each generated nucleic acid sequence. The same mutant revealed a propensity to accept other modified nucleotides and to extend them in long fragments. Hence, this mutant can deliver random natural and modified RNA polymers libraries ready to use for SELEX, with custom lengths and balanced or unbalanced ratios.