Non-programmed transcriptional slippage: comparative analysis of bacterial (*Escherichia coli*) and bacteriophage (T7) RNA polymerases

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Among many observed processes that contribute to abnormalities in the transmission of genetic information, the least-described so far is the non-programmed transcriptional slippage. This process is associated with a deficit in fidelity of mRNA synthesis consistent with the DNA sequence exhibited by RNA polymerases, which catalyse the incorporation of subsequent nucleotides into the growing mRNA chain, under conditions of local destabilization of hydrogen bonds of the RNA:DNA hybrid, moving it forward or backward relative to the DNA, causing nucleotide insertion or deletion errors in mRNA. This phenomenon has its phenotypic consequences, observed as the formation of a heterogeneous pool of mRNA in cells, including restoration of the wild-type protein phenotype when the mutated genes are expressed. In this study, we analysed for the first time the transcriptional slippage ability of bacterial and phage RNA polymerases in *Escherichia coli* cells on both poly(A) and poly(T) homopolymer sequences shorter than 9 nucleotides.

Comparative analysis of the transcriptional slippage ability of bacterial (*Escherichia coli*) and bacteriophage (T7) RNA polymerases by employing the *mboIIM2* methyltransferase model gene and its deletion variant *mboIIM2ΔA356* showed a several dozen difference in the level of expression of the *mboIIM2ΔA56* gene in favour to T7 RNA polymerase. This effect was the result of the lack of correction properties, the lack of influence of GreAB anti-pause proteins and the lack of coupling between transcription and translation processes resulting in transcription errors in the case of T7 RNA polymerase, as opposed to bacterial polymerase. It has been observed that InDel epimutations in the case of *E. coli* RNA polymerase, which is susceptible to the effect of transcription polarity, causes a smaller phenotypic effect that would arise from the analysis of InDel mRNA polymorphism, in contrast to T7 phage polymerase. The results obtained through the use of a series of reporter genes based on green fluorescence protein (GFP) in fusion with poly(A/T) homopolymer sequences confirmed that this process is dependent on the type and length of the poly(A) or poly(T) homopolymer sequences and on the type of nucleotides located both upstream and downstream of homopolymeric sequence, which have a significant impact on the frequency of transcriptional slippage process. The slippage induction threshold for insertion for RNAP T7 is 3-nuclotide A/T repeats, while a significant level of single-deletion detection was observed with 4-5 nt poly (A/T) sequences long. Bacterial polymerase requires for slippage induction by insertion or deletion of at least 7 T-nucleotides in homopolymeric sequence length. In both cases, propensity to single

nucleotide insertions is observed. So, the most likely type of slippage for RNAP T7 is nucleotide insertion in poly(A) and deletion for poly(T) sequences, unlike *E. coli* RNAP which prefers poly(T) sequences in both cases.