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Interference of PNA Binding to the Non-Template Strand with Transcription Supports the General Model for Transcription Blockage by R-loop Formation

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Transcription blockage can strongly affect various DNA and RNA transactions (reviewed in Hanawalt & Spivak, 2008; Belotserkovskii, Mirkin, & Hanawalt, 2013). Thus, it is of interest to study the various factors that can cause transcription blockage and to elucidate mechanisms of their action. Peptide Nucleic Acids (PNAs) are artificial DNA mimics with superior nucleic acid binding capabilities. The effect of PNA binding to the (GAA/CTT)_n sequence within the transcribed DNA region upon T7 RNA polymerase transcription was studied *in vitro*. In the case of the PNA binding to the template strand, the blockage signals concentrated primarily in the narrow area close to the upstream flank of the PNA-bound sequence, consistent with the blockage being caused by RNA polymerase “bumping” into the PNA/DNA hybrid (Belotserkovskii, Liu, & Hanawalt, 2009). In contrast, for PNA binding to the non-template strand, a characteristic pattern of blockage signals was observed, extending downstream from the PNA binding site (Belotserkovskii and Hanawalt, 2014), similar to that produced by G-rich homopurine-homopyrimidine sequences (Belotserkovskii et al, 2010, 2013). This striking similarity between transcription blockage patterns caused by two seemingly unrelated factors suggests a common mechanism of blockage. This common mechanism likely involves R-loop formation, which is facilitated both by PNA binding to the non-template strand and by G-rich homopurine-homopyrimidine sequences, due to sequestration of the non-template strand or due to formation of an extra-stable RNA/DNA hybrid, respectively. We suggest that there is a general mechanism of transcription blockage by R-loop formation, which presumably involves destabilization of the transcription complex, making it more prone to spontaneous pausing or termination.

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