Photoaffinity Labeling as an Approach for Analysis of DNA Replicative Machinery

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Photoaffinity labeling has become one of the most efficient approaches for studying the structure of enzyme active sites and supramolecular DNA structures like DNA replication, repair and transcription complexes. Base-substituted photoreactive dNTP and NTP derivatives carrying photoreactive arylazido groups have been synthesized and characterized (Wlassoff W.A. et al, 1995). It was shown that these dNTP (NTP) derivatives are efficient substrates of various DNA(RNA) polymerases (Doronin S.V. et al, 1994, Lavrik O.I. et al, 1996, Scherbik N.V. et al, 1997). Substrate properties of dNTP analogs permit their introduction into the definite or several positions of synthesized DNA by the action of DNA polymerases. UV-irradiation by near UV-light with the following structural analysis of crosslinking products opens the possibility to analyze interaction of substrates with DNA polymerases as well as to investigate the loading on DNA the other factors of DNA replication. Base-substituted dNTP analogs containing pyrene group have been synthesized and used to make highly selective affinity labeling of DNA polymerases by switching of primer photocrosslinking by dNTP photosensitizer bound to the active sites. These approaches were applied to study interaction with substrate DNA of human DNA polymerase α•primase, DNA polymerase beta and replication protein A (Lavrik O.I. et al, 1998). The mechanism of interaction of these enzymes and replication factors with DNA has been studied *in vitro* and *in vivo* on the chromatin level.

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