DOI 10.18699/SBB-2022-578

Bioorthogonality in the light of cell defense mechanisms: Interaction of non-natural nucleic acids with the base excision DNA repair system

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Key words: non-natural nucleotides, bioorthogonality, synthetic biology, DNA repair, DNA replication, translesion DNA synthesis

Motivation and Aim: The concept of bioorthogonality, which appeared in the field of synthetic biology, refers to any chemical reaction that can occur within living systems without interfering with natural biochemical processes. The modification of nucleic acids, especially DNA, in living cells is tightly controlled by DNA repair and DNA damage response systems. Any non-canonical nucleotide in DNA, with the exception of a very small number of natural modifications, is perceived as damage and removed. Thus, DNA repair, by definition, counteracts the bioorthogonal properties of modified DNA placed inside a living cell. However, although some types of non-natural nucleotides received certain attention, the activity of DNA repair and DNA damage response systems towards modifications used in synthetic biology has not been subject to systematic studies.

Methods and Algorithms: We have investigates the interaction of the base excision repair (BER) system enzymes with several classes of non-natural nucleotide units used in synthetic biology such as nucleotides used for click reactions (C8-alkyne-dU, C8-alkyne-dC, 5-ethynyl-dU, 1-ethynyl-AP site), nucleotides with alternative chirality (β-L-nucleotides of A, C, G, and T), and nucleotides forming modified internucleoside links (triazole linkage). The choice of these types of modifications is mostly guided by their widespread use as tools in synthetic biology or the steadily growing interest in such applications.

Results: We have studied the ability of human DNA glycosylases (and their homologues or functional analogs from *E. coli*) to remove bases from DNA containing these non-natural nucleotides, the ability of human and *E. coli* AP endonucleases to hydrolyze DNA containing them, and the ability of DNA polymerases to include correct and mismatches dNMP opposite to them. Generally, C5-modified pyrimidines were substrates for DNA glycosylases recognizing the products of 5-methylcytosime deamination, and 1-ethynyl-AP site was the substrate for AP endonucleases. The inverted chirality nucleotides were not substrates by themselves but blocked the action of DNA glycosylases that recognize both the damaged base and the opposite base.

Conclusion: The use of nucleic acids containing unnatural nucleotides to control and manipulate living cells is actively developing and will continue to develop in the coming decades. Therefore, the question of the degree of bioorthogonality of such molecules will become more and more urgent. We show that at least some unnatural nucleosides used in cell biology are subject to DNA repair, and the results obtained with such reagents should be interpreted with caution.

Acknowledgements: This work was supported by Russian Science Foundation (21-74-10104 to A.V.E.).