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Repair efficiency of biorthogonal DNA modifications in human cells

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Motivation and Aim: Non-natural nucleotides are efficiently used in biotechnology and biomedicine studies to increase the stability of DNA oligonucleotides within the cell or organism. Nevertheless, little is known about the interaction of cellular systems with DNA containing non-natural nucleotides. This study aimed to investigate DNA repair of non-natural nucleotides in human cells.
Methods and Algorithms: The repair of biorthogonal DNA modifications was studied using the reporter system. This reporter system was based on the plasmid, containing the sequence of non-fluorescent variant of EGFP [1]. The modification was introduced in the transcribed strand of the *EGFP* sequence. The repair of modification within the cell would restore the original *EGFP* sequence if not RNA polymerase II would incorporate random ribonucleotide opposite to the lesion leading to EGFP fluorescence. To sum up, the level of EGFP fluorescence was in inverse ratio to DNA repair.
Results: The repair of several non-natural DNA modifications were studied such as the enantiomers of natural nucleotides (βLdN), alkylated derivatives of uracil (5etU, C8AlkU) and alkylated abasic site (1etF). The plasmid containing the modification was transfected with a plasmid containing red fluorescent protein as a transfection efficiency control in HeLa cells. Cells were analyzed 24 h after transfection. It was shown that the DNA repair efficiency of βLdN varies. The pyrimidine L-enantiomers were repaired less efficiently than purine L-nucleotides. The 5etU and C8AlkU predominantly did not undergo repair in human cells. 1etF was repaired as efficiently as a synthetic analogue of an abasic site. 1etF was conjugated with a non-bulky heterocyclic ligand that resembles a DNA base. This click-conjugation of triazine derivative with 1etF was more resistant to repair than 1etF.
Conclusion: Non-natural DNA modifications are important tools for basic research and nanotechnology. Thus, it is necessary to investigate the interaction of the cellular machinery with these modifications. We demonstrated that several non-natural DNA modifications were repaired within the cell.
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References

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