Brief information about the project

Name of the project	ИРН AP13067762 «Study of the mismatch-specific thymine-DNA-glycosylase initiated, aberrant base excision repair pathway of complex DNA damage in vitro and in vivo». 0122PK00080
Relevance	In the present project, we propose to study the aberrant BER pathway initiated by human mono-functional DNA glycosylase TDG towards complex DNA damages *in vitro* and *in vivo*. To decipher mechanisms of aberrant repair and its regulation, we will perform *in vitro* reconstitution of DNA repair assay using purified DNA glycosylase and *in vivo* studies on *Escherichia coli* and *Saccharomyces cerevisiae* cells to establish the role of aberrant repair in mutation fixation and characterize the spectrum of mutation induced by mismatch-specific mono-functional DNA glycosylases.
Purpose	To decipher and characterize at the molecular level the mechanisms involved in the aberrant BER pathway initiated by the mismatch-specific Thymine-DNA glycosylase (TDG) *in vitro* and *in vivo* and estimate the physiological role of aberrant BER in DNA damage-induced and spontaneous mutations accumulation in living cells.
Objectives	**Task 1: Construction of the synthetic DNA substrates containing various DNA base modifications and purification of the homogenous human TDG DNA glycosylase;** **First objective,** we will perform detailed biochemical characterization of the human mono-functional DNA glycosylase TDG initiated aberrant BER pathway towards DNA duplexes containing complex DNA damages *in vitro*. For this, we will construct: (i) the synthetic oligonucleotide substrates with varying chemical lesions mimicking a wide panel of oxidative DNA base damage including complex DNA lesions (oxidative DNA base damage, ethenobases, inter-strand DNA crosslinks, G-T and G-G intra-strand DNA crosslinks, UV products, aristolactam adducts) and with varying configurations; and (ii) the recombinant purified human TDG proteins from *E. coli*. We will clone cDNA coding for human TDG and express them in *E. coli*. The purified proteins will be characterized using synthetic DNA substrates. **Task-2: Biochemical studies: *in vitro* reconstitution of the aberrant repair pathways with purified protein and synthetic DNA substrates;** **Second objective,** we propose to reconstitute *in vitro* aberrant DNA repair assay by using purified DNA glycosylase. We will use radiolabeled synthetic DNA substrate and electrophoretic denaturing gel analysis to study detailed molecular mechanism of action of DNA repair enzymes. Before experiments, the activity of each

	protein will be verified using its classic DNA substrate. The efficiency of DNA repair will be measured by analysis of DNA products on denaturing PAGE followed by phosphor-imaging using Typhoon FLA 9500 system. Our French collaborators has a long-time expertise in this type of analysis (40-44). **Task-3: Characterization of TDG initiated aberrant BER in living cells;** **Third objective,** we will characterize cellular responses to genotoxic stress of *S. cerevisiae* and *E. coli* strains over-expressing human mono-functional DNA glycosylase TDG. In this task, we will expose yeast and bacterial cells to various DNA damaging treatments including aristolochic acids (to generate dA-Ali I&II), chloroactealdehyde (to generate ethenobases), methylmethanesulfonate, H2O2, UV light and DNA crosslinking agents. After exposures, we will measure the mutation rates, characterize mutational profiles, compare these to wild type, and control non-treated cells. The differences between mutation rates and patterns will enable us to establish the role of aberrant repair in mutation fixation and characterize the spectrum of mutation induced by mismatch-specific mono-functional DNA glycosylases *in vivo*. In conclusion, using biochemical and genetic approaches we aim to decipher and characterize at molecular level the mechanisms involved in the DNA glycosylase-initiated aberrant BER pathway. Furthermore, identification of critical DNA lesions, that can induce aberrant repair in cells, will provide mechanistic insight into environmental and genetic factors associated with aging and degenerative disorders and therefore help to develop new prevention and therapeutic strategies.
Expected and achieved results	The tasks that are planned to solve in this project are the first attempt in better understanding of the molecular mechanism of Mismatch-specific TDG-glycosylase initiated aberrant excision repair pathway of complex DNA damages *in vitro* and *in vivo*. Obtaining of tasks will provide a unique angle on the problems of mechanisms and treatment of age-related human diseases. Particularly, achieving the following results can be considered as success: i. Construction of the synthetic DNA substrates containing complex DNA lesions; ii. Purification and characterization of the homogenous human TDG glycosylase and its active site mutants; iii. Reconstruction of DNA repair *in vitro* to obtain the biochemical evidences of the aberrant TDG glycosylase-mediated repair of non-damaged DNA strand that leads to mutation fixation;

	iv. Construction of genetically modified bacterial and yeast cell lines over-expressing human mono-functional DNA glycosylase TDG; v. Characterization of cellular responses to genotoxic stress of modified bacterial and yeast cells. vi. Characterization of mutation spectra induced by aberrant repair of complex DNA damage in living cells.
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List of publications with links to them	**1.Taipakova S.,** Kuanbay A., Saint-Pierre C. , GasparuttoD., Baiken Y., Groisman R., Ishchenko A.A., Saparbaev M., Bissenbaev A.K., The *Arabidopsis thaliana* Poly(ADPRibose) Polymerases 1 and 2 Modify DNA by ADP-Ribosylating Terminal Phosphate Residues//Frontiers in Cell and Developmental Biology. –2020. –Vol.8. 606596. Citation-3, Q1, IF-5.18, Процентиль: 21, SJR-2.572 DOI: 10.3389/fcell.2020.606596 2. Baiken, Y., Kanayeva, D. **Taipakova, S.** Groisman, R. Ishchenko, A.A. Begimbetova, D. Matkarimov, B. Saparbaev, M. Role of Base Excision Repair Pathway in the Processing of Complex DNA Damage Generated by Oxidative Stress and Anticancer Drugs // Frontiers in Cell and Developmental Biology. – 2021. –Vol. 8. 617884. Citation-3, Q1, IF-5.18, Процентиль: 21, SJR-2.572 DOI: 10.3389/fcell.2020.617884 (Соавтор) 3.**Taipakova S.M.,** Smekenov I.T., Saparbaev M.K., Bissenbaev A.K. Characterization of *Aspergillus niger* endo-β-1,4-glucanase ENG1 secreted from *Saccharomyces cerevisiae* using two different expression vectors// Genet.Mol.Res. -2015. -Vol.14, №2. -P.6439-6452. Citation-2, Q2 (SJR-1.1), Procentile-16%. DOI:10.4238/2015.June.11.20 4. Akishev Zh., **Taipakova S.,** Joldybayeva B., Zutterling C., Smekenov I., Ishchenko A., Zharkov D., Bissenbaev A., Saparbaev M. The major *Arabidopsis thaliana* apurinic-apyrimidinic endonuclease, ARP is involved in the plant nucleotide incision repair pathway// DNA repair. -2016. -Vol.48. -P.30-42. Citation-10, Q1(SJR-2.22), Procentile-71%. DOI:10.1016/j.dnarep.2016.10.009

	5. Bazlekowa-Karaban M., Prorok P., Baconnais S., **Taipakova S.,** Akishev Z., Zembrzuska D., Popov A.V., Endutkin A.V., Groisman R., Ishchenko A.A., Matkarimov B.T., Bissenbaev A., et al. Mechanism of stimulation of DNA binding of the transcription factors by human apurinic/apyrimidinic endonuclease 1, APE1// DNA repair. –2019. –Vol.82. - №102698. Citation-1, Q1(SJR-2.22), Procentile-71%. https://doi.org/10.1016/j.dnarep.2019.102698.
Patents	**Taipakova S.M.,** Smekenov I.T., Bissenbaev A.K. Patent of the Republic of Kazakhstan for a useful model "Integrative plasmid vector for gene expression in yeast", registration No. 2017/ 0230.2.