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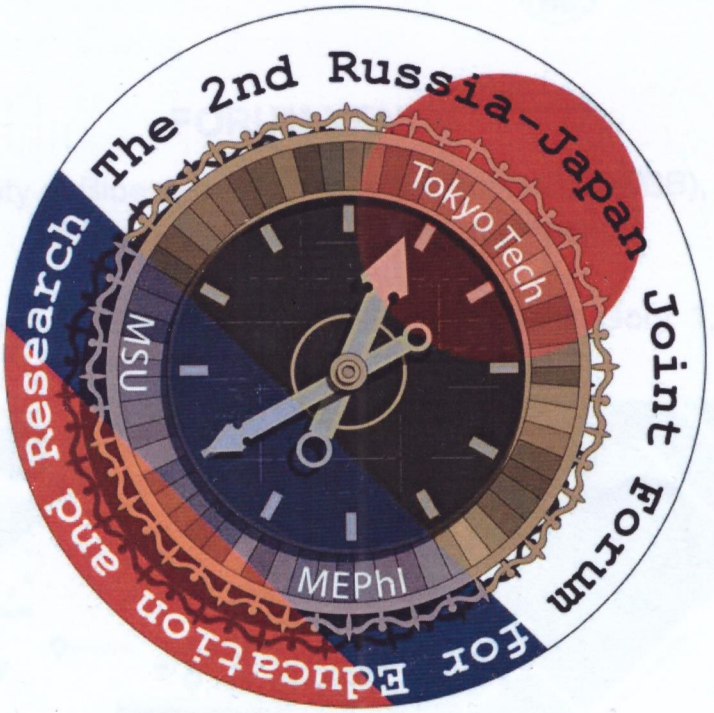

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ГОД ЯПОНИИ
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Investigation of the interactions of BspD6I endonuclease and MutL protein with molecular partners using crosslinking and mass spectrometric approaches

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The crosslinking reaction involving cysteine residues was performed in order to elucidate the possible contacts of nicking endonuclease (NE) BspD6Iwith DNA. This enzyme recognizes a specific pentanucleotide sequence in DNA and cuts only one strand nearby the recognition site. No crystal data for the complex of NE BspD6I with DNA is available to date. To perform the crosslinking reaction, we used DNA with disulfide group in 2'-positionof nucleoside that reacted only with cysteine residues. We obtained DNA-protein conjugates in the case of wild type NE BspD6I and its variant NE BspD6I(C11S/C160S). The received data allow suggesting that cysteine residues of C-terminal catalytic domain are necessary not only for effective DNA hydrolysis but also for DNA binding.

DNA mismatch repair (MMR) system was another object of the study. First, MutS protein binds to the mismatch. At the next step the complex formation of MutS with other MMR protein – MutL – occurs. The existence of the ternary complex DNA-MutS-MutL activates DNA repair. This multistage process is thoroughly studied for *E. coli* MMR system. Taking into account the conformation variability and dynamic nature of the MMR proteins themselves and in the complex with DNA, one of the ways to study the interaction of compounds of this complex is their covalent fixation.Earlier, the crystal structure of *E. coli* MutS-MutL site-specifically crosslinked complex was obtained (Groothuizen *et al.*, eLife, 2015). Nevertheless, this crystal structure did not contain DNA molecule and therefore did not represent the native "sliding clamp" stage to the full extent. We suggested to study the contacts between MutL protein and MutS preliminarily covalently linked to DNA. The protocol of obtaining MutS (N497C)-DNA conjugate with a high yield and in its purification preserving the catalytic activity was developed (Monakhova *et al.*, Journal of Chromatography, 2015). For further comprehensive study of MutS-MutL contacts, the crosslinking reaction between the MutL cysteine and amino groups of MutS was performed using N-β-maleimidopropyl-oxysuccinimide ester in the presence of DNA. The crosslinked biomolecules were analyzed by MALDI mass spectrometric method. One of the obtained conjugates was shown to contain peptides from both MutS and MutL proteins. Thus, for the first time we demonstrated close proximity of MutL cysteine residue in position 131 and some lysine residue from MutS during the MMR process.

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