

Radiation damage to DNA-protein complexes

During the working stay in CBM CNRS, Viktorie Stisova continued to study the specific complex formed by DNA bearing the estrogen response element specific sequence and estrogen receptor protein. The previously performed experiments showed that the high concentration of the scavenging compounds in the binding buffer of the complex disables observing the DNA breakage pattern. Therefore, the different composition of the binding buffer without BSA and Tris and lower concentration of glycerol has been searched using the method of native retardation polyacrylamide gel electrophoresis. When the condition has been set up, the method of sequencing polyacrylamide gel electrophoresis has been used to determine the possible footprint of the protein caused by gamma radiation of ¹³⁷Cs.

Furthermore, the condition for formation of the complex in the presence of both estrogens and anti-estrogens (estradiol and tamoxifen respectively) were established. For the complex formed in presence of estradiol, the set of experiments showing the stability of the complex under irradiation, and the formation of the complex between irradiated DNA and non-irradiated protein, or non-irradiated DNA and irradiated protein was performed.

Viktorie Štísová also learned during the STSM a new experimental technique allowing to study the damage of proteins upon irradiation. The methods of SDS-PAGE has been used to follow the fragmentation of estrogen receptor protein after irradiation by increasing doses of low LET radiation.

The new obtained results of experiments are presented during 2005 at two international conferences. The first was 14th Symposium on Microdosimetry (Venezia, Italy, 13th – 18th November 2005), where V. Štísová presented oral lecture „Radiation damage to specific complexes of DNA with proteins: Estrogen response element DNA – estrogen receptor.“ (V. Štísová, S. Goffinot, M. Spothem-Maurizot, M. Davídková). At the moment of the conference, a manuscript of the paper with the same title as presented lecture has been submitted for publication in the special issue of Radiation Protection Dosimetry journal. The second public presentation of the work performed in CBM CNRS will take place at XXVIIth Days of radiation protection (Liptovský Ján, Slovensko, 28th November – 2th December 2005). V. Štísová will present a lecture: „Radiation damage to specific complexes of estrogen response element DNA with estrogen receptor protein.“ (V. Štísová, S. Goffinont, M. Spothem-Maurizot, M. Davídková).

The mentioned experiments have been performed using the biological material (DNA oligonucleotide, estradiol, tamoxifen, estrogen receptor protein, and other chemicals) purchased from the grant 1P05OC085 of the Ministry of Education, Youth and Sports of the Czech Republic, which is national financial support of the COST P9 activities, grant 10-85093 of the Czech Technical University, and grant 202/05/H031 of the Grant Agency CR. The existing equipment, material and know-how of experimental techniques in host laboratory have been used.

For this STSM V. Štísová replaced M. Davídková, who could not accomplish the awarded STSM for health reasons. The dates of the mission were slightly modified (17.10.-5.11. from the originally 15.10.-4.11.), because V. Štísová returned to the home laboratory in Prague for taking chemical compounds (estrogen receptor protein and DNA oligomers) necessary for following experimental campaign.