Title of Project 1 (2 students): “Comparative PCR-assay of the intragenic DNA lesions induced by γ-rays and fission neutrons in Drosophila melanogaster”

2. Project Leaders:
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3. Project Summary:
3.1. Goal of Project:
The goal of the Project is to perform the “gene walking” using the PCR-assay to detect the quality and frequency patterns of neutron-induced mutational DNA lesions in comparison to γ-ray-induced ones for different Drosophila genes.

3.2. Background and Topicality of Project:
As it has been considered earlier [1], neutrons are one of the major type of a high-LET ionizing radiation with which in peacetime the humanity is consistently dealing with both on the Earth and in the Space as well. In the first case, a certain levels of occupational exposures to neutrons are almost ubiquitous for atomic energy workers outside the nuclear reactors, on the one hand, and for hospital personnel in the course of radiotherapy of cancer by neutrons, on the other hand. In the space, the humanity is exposed to neutrons which come from the cosmic (galactic and solar) radiation as a primary or secondary particles acting in the course of travels in supersonic aircrafts or during the manned space flights.

The expansion of exposure to neutrons observed to-day demands a better knowledge of fundamental molecular mechanisms and salient features of the mutagenic action of neutrons in comparison to sparsely ionizing radiation. A better understanding of these issue will also improve the assessment of the potential genetic risks resulting from exposure to neutrons the mode of energy deposition of which is vastly different from that of electromagnetic radiation and heavy ions as well [2].

A large body of experimental radiobiological data are known to show that fast neutrons are more effective than sparsely ionizing radiation in mutation induction and chromosomal aberrations in mammalian somatic cells in vitro or in vivo (for example, see [3]) although no wide molecular analysis of mutations detected was performed so far. While, when the fine genetic (complementation) analysis was used to distinguish between point changes and gross intragenic deletions induced by fission neutrons in Drosophila germ cells the relative genetic effectiveness (RGE) of neutrons in
comparison to γ-rays of $^{60}$Co found to fall about to 0.44 for point changes but not for gross deletions and chromosome exchanges as well [4] showing that a volume of “sensitive target” (DNA for point changes and chromatin for chromosome rearrangements, respectively) as well as the quality of initial damage determined by the quantity of energy deposition in this volume play essential role in determination of genetic endpoints.

It is not clear, however, so far if there are any qualitative difference at the DNA level between γ-ray- or neutron-induced point changes and what are the mode of intragenic distribution of these changes for genes that differ from each other by the physical size, exsone-intron structure and positioning on the chromosome map.

For study these issues in a whole organism, the classical genetic subject – the fruit fly Drosophila melanogaster – constitutes a very attractive system. Practically the complete spectrum of the heritable genetic alterations induced by radiation can be detected after treatment of male germ cells. Moreover, several loci can be used and studied as target genes simultaneously. Using this genetic system, a large set of γ-ray- and neutron-induced mutations at the 5 different loci were obtained and each heritable mutation was studied sequentially by conventional genetic (tests on allelism, complementation, recombination) and cytological (on the polytene chromosomes) analyses [5,6] to detect a share of mutations with point intragenic alterations among all mutations scored for each locus. As a result, a large series of point mutants for black ($b$) and cinnabar ($cn$) loci (among others) were obtained after exposure of wild-type Drosophila sperms to γ-rays or monoenergetic 0.85 MeV fission neutrons. Both genes are located on the autosome 2 at its different areas (a middle of 2L arm and pericentromeric region of 2R arm for $b$ and $cn$, respectively). The $b^+$ gene encodes for a glutamate decarboxylase and has a size of 2692 bp (3 exons and 2 introns). The $b$ mutants have black body and wings. The $cn^+$ mutants have eye color bright red instead of dark red in wild-type flies. The $cn^+$ is the structural gene for kynurenine hydroxylase and has a size of 2410 bp (3 exons and 2 introns).

To detect presumably DNA alterations in γ-ray- and neutron-induced $b$ and $cn$ point mutants, the polymerase chain reaction (PCR) is best suitable as relatively inexpensive, highly specific and efficient express-method which permits in vitro enzymatically to synthesize (to amplify) defined wild-type and mutant sequences of DNA. The resulting products (amplicons) are electrophoresed on an agarose gel and visualized with ethidium bromide staining.

For precise location of induced lesions on the gene map, it is necessary to amplify the sequence of the gene as a whole. Because one PCR permits to synthesize up to 2000 bp of the template DNA as maximum the gene map is usually subdivided into several overlapping regions for which the specific pair of primers are matched. These procedures for the $b$ and $cn$ genes were
done and the sequences of each gene were subdivided into 3 and 4 overlapping fragments, respectively.

3.3. Research Programme:
Taking into account the results of our earlier experiments, the PCR-assay of quality and frequency patterns of intragenic DNA alterations should include the following research steps:
(i) conversance with general guidelines for PCR (basic protocols and optimization strategies) as well as with specific PCR protocols adapted to analysis of gene sequences under study;
(ii) isolation of wild-type and mutant genomic DNA as template;
(iii) performance of PCR-assay (ten PCR per day for each student);
(iv) performance of gel-electrophorese for visualization and documentation of the resulting products of PCR;
(v) assessment of quality and frequency patterns of DNA alterations observed for the gene under study after action of neutrons or $\gamma$-rays.

3.4. Expected Results:
The new results expected are following:
(i) for the first time to identify the quantity and intragenic distribution of neutron-induced DNA alterations in comparison with $\gamma$-ray-induced ones;
(ii) to compare the mutation induction patterns for two genes of different sizes and exon-intron structure and for two different quality radiations after action on the male germ cells.

References: