Приложение 2

**Questionnaire**

for the extraordinary session of the PAC for Condensed Matter Physics for the assessment of related JINR projects

**Project: Study of the radioprotective properties of the Damage Suppressor (Dsup) protein on a model organism *D. melanogaster* and human cell culture HEK293T**

**The leader of the Project E.V. Kravchenko**

 **PART A: Achievements**

1.   Contributions of the JINR group:

- the JINR group performs all the stated tasks of the project and bears full responsibility

2.   Publications:

- since the project started only 3 months ago, there are no publications yet, but the group has recent publications on similar topics demonstrating the group's ability to the making of world-class research.

1. **Q1:** Transcriptome analysis of *Drosophila melanogaster* laboratory strains of different geographical origin after long-term laboratory maintenance. **Mikhail Zarubin, Alena Yakhnenko, Elena Kravchenko**✉. 2020. Ecology and Evolution. https://doi.org/10.1002/ece3.6410

2. **Q1:** First transcriptome profiling of *D. melanogaster* after development in a deep underground low radiation background laboratory. **Mikhail Zarubin**, Albert Gangapshev, Yuri Gavriljuk, Vladimir Kazalov and **Elena Kravchenko**✉. 2021. PLoS ONE (In press)

3. **Q1:** The Oxidation-Induced Autofluorescence Hypothesis: Red Edge Excitation and Implications for Metabolic Imaging. Semenov Alexey N., Yakimov Boris P., Rubekina Anna A., Gorin Dmitry A., Drachev Vladimir P., **Zarubin Mikhail P**., Velikanov Alexander N., Lademann Juergen, Fadeev Victor V., Priezzhev Alexander V., Darvin Maxim E., Shirshin Evgeny A. Molecules. 2020. 10.3390/molecules25081863

3.   PhD theses:

- no

4.   Talks:

- EMBO|EMBL Symposium 2021: Friend or Foe: Transcription and RNA Meet DNA Replication and Repair, EMBO|EMBL, Heidelberg, Germany. “Impact of DNA-binding Damage suppressor protein (Dsup) on *D. melanogaster* transcriptome before and after gamma irradiation”. Zarubin Mikhail, Kravchenko Elena (European Molecular Biology Organization (EMBO)).

**PART B: Plans and requests**

5.   Plans

- We are planning examination of the radioprotective properties of the new Damage suppressor (Dsup) protein on a model object *D. melanogaster* and human cell cultures and studying the mechanisms of Dsup protein action. Dsup protein is a new protein discovered in 2016 in the extremophilic organism *Ramazzottius varieornatus* - one of the most radioresistant species of multicellular organisms. The generation of *D. melanogaster* lines and human cell cultures expressing this protein will make it possible to assess the possibility of increasing their radioresistance during irradiation with different types of ionizing radiation. Also the wide range of methods applicable to these model organisms will allow to begin studying the effects of Dsup on the molecular level and the level of whole organism. Additionally the study of the mechanisms of action of the Dsup protein will contribute to understanding the fundamental laws of chromatin organization in the nucleus and its influence on the regulation of gene expression.

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| Work stages | Work сontent |
| 2021  | Conducting irradiation sessions of *D. melanogaster* lines expressing Dsup and initial lines in 3-5 repeats with γ-rays at the MT-25 accelerator in FLNR JINR and with protons at phasatron DLNP JINR at doses of 500 and 1000 Gy with an estimation of survival rate.Conducting experiments to evaluate the effect of Dsup protein on the life span of *D.melanogaster* under normal conditions compared to the initial lines and locomotion tests. Creation of a molecular genetic construct for expression of the GFP-Dsup fusion protein in *D. melanogaster*. Transcriptomic analysis of the response of *D. melanogaster* lines containing Dsup and the initial line under normal conditions and after exposure to ionizing radiation |
| 2022  | Generation of HEK293T cell line stably expressing the fusion protein GFP-Dsup. Carrying on experiments to assess the effect of the Dsup protein on the radioresistance of this cell line with the construction of dose - response curves (protons). Transcriptome analysis of HEK293T cell line stably expressing the fusion protein GFP-Dsup and the original HEK293T line under normal conditions and after exposure to ionizing radiation. Evaluation of the distribution of GFP-Dsup fusion protein on *D. melanogaster* polytene chromosomes by immunofluorescence analysis. Creation of an expression vector for the production of Dsup protein in *E. coli* cells, isolation and purification of Dsup protein for structural studies by SAXS, SANS (FLNP JINR) and RSA techniques (MIPT). |

6.   Group size, composition and budget

- JINR personnel involved in the project: А.Е. Ivanova engineer 1FTE (DLNP JINR), E.S. Klimenko engineer 0,5FTE (DLNP JINR), E.V. Kravchenko (DLNP JINR) PI 1FTE, O.A. Kuldoshina (DLNP JINR) researcher 1FTE, A.V. Rzyanina (DLNP JINR) researcher 0,5 FTE, A.S. Yakhnenko (DLNP JINR) PhD student 1FTE, M.P. Zarubin (DLNP JINR) PhD student 1FTE.

The total number of people in the project: 8

- The requested project budget for 2021-2022 years is 120 kUSD

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| № | TASKS | Total value | 2021  | 2022  |
|  | Direct costs for the project |  |  |  |
| 1 | Materials (kUSD) | 70 | 40 | 30 |
| 2 | Equipment (kUSD) | 40 | 40 | - |
| 3 | Travel resources (kUSD) | 10 | 3 | 7 |
|  | Total direct cost: | 120 |  |  |