Report for 2023

Topic 1132, project “Study of the radioprotective properties of the Damage suppressor (Dsup) protein on the model object *D. melanogaster* and human cell culture HEK293”.

The goal of the project is to study the radioprotective properties of a new unique tardigrade protein Damage suppressor (Dsup) and the mechanisms of action of this protein. During 2023, the following tasks were completed:

1. Study of the structural properties of the Dsup protein.

   To conduct experiments to determine the structural properties of the Dsup protein, the *E. coli* cell culture was transformed with the pCold-I-Dsup vector (#90021) obtained from the non-commercial organization Addgene. The protein produced by the culture was purified, concentrated and studied using small-angle X-ray scattering (SAXS) (FLNP JINR), circular dichroism (CD) spectroscopy (MIPT), computer analysis was used to analyze the data, including using neural network algorithms. As a result, it was shown that the Dsup protein belongs to intrinsically disordered proteins (IDP); its shape, size (Rg, Dmax) (Fig. 1), flexibility (Rflex), proportions of various components of the secondary structure were determined; ab initio molecular models were obtained; the behavior of the protein in various physiological and denaturing solutions was described (Fig. 2).
2. Study of the parameters of the Dsup-DNA complex.

   The Dsup protein interacts electrostatically with DNA, forming a complex. A circular double-stranded DNA of about 8000 bp was used as a model. The methods of small-angle X-ray scattering (SAXS) (FLNP JINR), circular dichroism (CD) spectroscopy (MIPT), microscopic thermophoresis (MIPT) were applied to study the Dsup-DNA complex. As a result, some properties of the complex were determined, changes in the structure of the protein and DNA during the formation of the complex were revealed, the Dsup-DNA binding constant (Kd) was determined, and the binding rate was estimated.

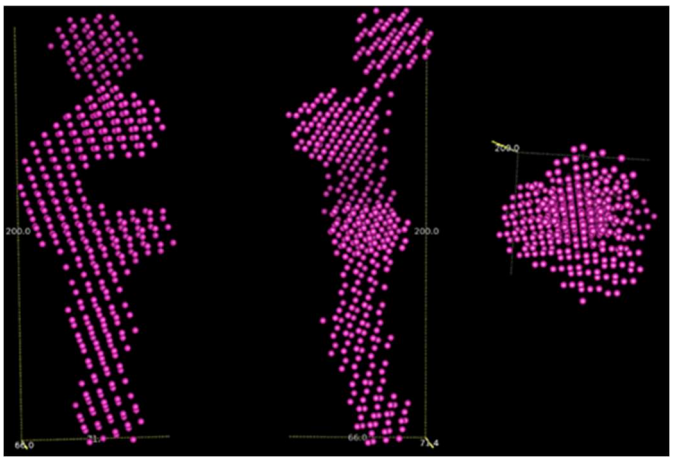

Fig. 1. Model of Dsup protein structure based on experimental data (DAMMIF package).

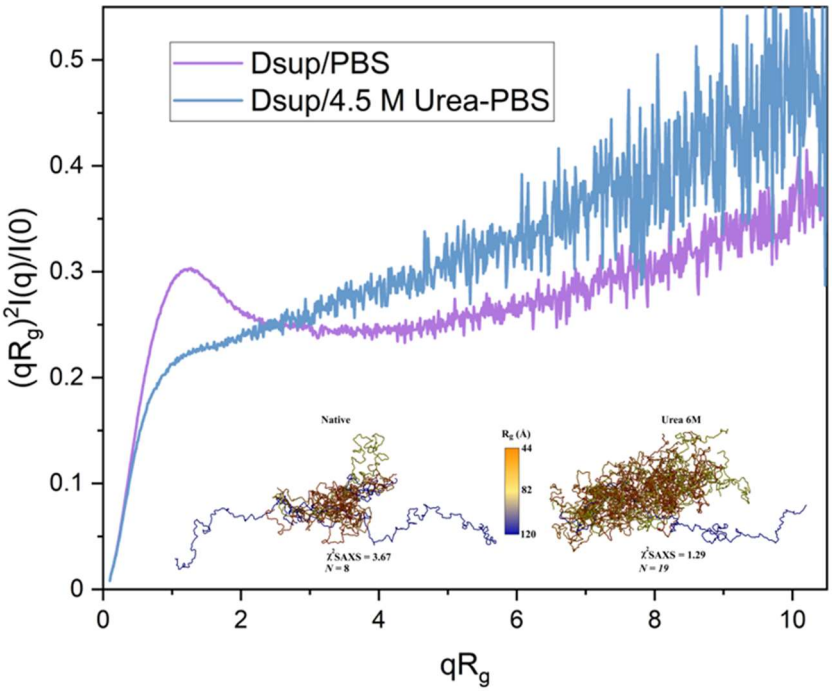

Fig. 2. The effect of the denaturing agent (4.5 M urea) on the structural parameters of the Dsup protein. The small-angle scattering data are presented as a Kratky plot and demonstrate the transition of the protein from the natural disordered to the denatured state.

3. Comet assay of *D. melanogaster* hemocytes

   To assess the effect of the Dsup protein on the degree of damage to nuclear DNA by reactive oxygen species, a comet assay of *D. melanogaster* hemocytes was carried out during exposure to hydrogen peroxide. A significant decrease ($p<0.001$) in the level of DNA damage by reactive oxygen species in Dsup-expressing hemocytes (~17%) was observed confirming the protective properties of the Dsup protein (Fig. 3).

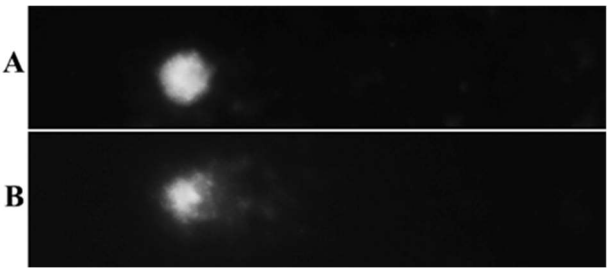

Fig. 3. A – undamaged nucleus. B - damaged nucleus with fragmented DNA in comet tail.

List of publications and conferences where results of the project were presented:

Publications:

1. The tardigrade Dsup (damage suppressor) protein enhances radioresistance and oxidative stress tolerance in *D.melanogaster* and acts as an unspecific repressor of transcription. Mikhail Zarubin , Talyana Azorskaya, Olga Kuldoshina, Sergey Alekseev, Semen Mitrofanov, Elena Kravchenko*. iScience (IF - 6.107, Q1). Accepted for publication

2. Unique Radioprotective Damage Suppressor protein (Dsup): Comparative Sequence Analysis M. Zarubin, O. Kuldoshina, E. Kravchenko. Particles and Nuclei, Letters (PEPAN Letters) (IF – 0.565, Q4), 2022, 19, № 3(242). p. 212

International conferences:

1. M. Zarubin, E. Kravchenko. Radioprotective DNA-binding damage suppressor protein (Dsup) affects the functioning of neural system in *D. melanogaster*, 46th Congress of The Federation of European Biochemical Societies (FEBS). Lisbon, Portugal. 07.09.22-07.14.22

2. Zarubin M., Murugova T., Ivankov O., Ryzhykau Y., Soloviov D., Popov A., Kravchenko E. The insight into structural properties of unique tardigrade radioprotective damage suppressor protein (Dsup). Bioinformatics of Genome Regulation and Structure/Systems Biology" – BGRS/SB-2022, Institute of Cytology and Genetics of SB RAS, Novosibirsk, Russia, 07.04.22-07.08.22

Popular science activities:

1. Lecture at 2022 science festival "NAUKA 0+" "Ionizing radiation protection with unique tardigrade protein" by Kirill Tarasov.

2. RIA News podcast "We will all die. But not sure" - What does ionizing radiation do to DNA molecules and how does molecular genetics reveal that. Mikhail Zarubin and Kirill Tarasov. (https://ria.ru/20230112/genetiki-1844187060.html)