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

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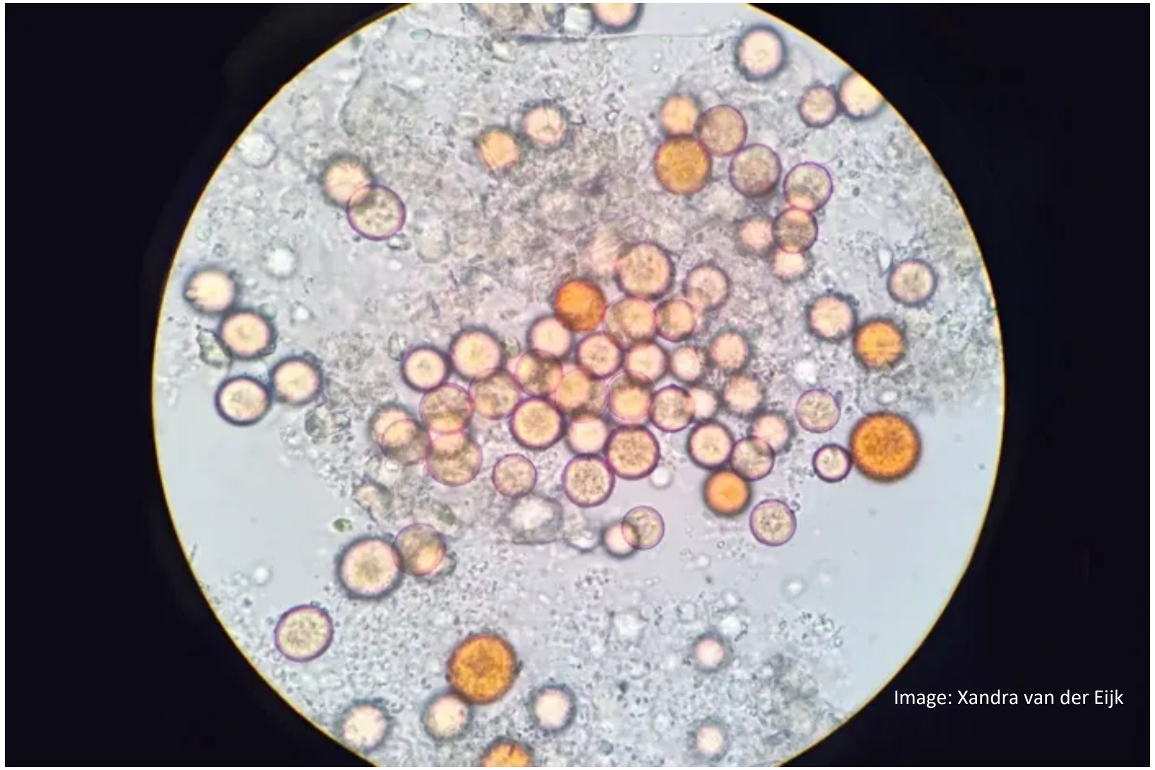
Biomedical and Radiation-Genetic Studies Using Different Types of Ionizing Radiation

Extremophilic organisms adapted to extreme environmental conditions (high / low temperature, high / low pressure, pH <3, pH> 9, high levels of ionizing radiation, salinity, etc.)



Tardigrada

Found in all biomes from the Arctic to the Antarctic, on mountain peaks, in deep-sea springs and cold mud springs in the west of Greenland.

Tardigrades have a well-developed nervous system, with the brain, muscle, digestive and other systems, consisting of differentiated tissues.

https://gfycat.com/fr/energeticcontentirishredandwhitesetter

Tardigrada belong to the group of the most radiation-resistant animals on Earth, able to survive after exposure to both rare and dense ionizing radiation

Resistance to γ-radiation for some species of organisms

Organism	LD_{50} or other available data	Author
Homo sapiens	$LD_{50/30d}$ =2.5–4.5 Gy	Bolus (2001)
Mouse	$LD_{50/30d}$ = 4.5 Gy	Bolus (2001)
Gold fish	$LD_{50/30d}$ = 8 Gy	Bolus (2001)
Cockroach	$LD_{50/30d}$ = 50 Gy	Bolus (2001)
Drosophila melanogaster (Insecta)	$LD_{50/3}$ = 1238–1339 Gy	Parashar et al. (2008)
Deinococcus radiodurans (Bacteria)	LD_{50} = 10000 Gy	Makarova et al. (2001)
Rotifers	No effect on survival up to 1120 Gy	Gladyshev and Meselson (2008)
Tardigrades	LD_{50} = 1270–5000 Gy	Hashimoto and Kunieda (2017)

Tardigrades - a model organism for studying the influence of space conditions on living organisms

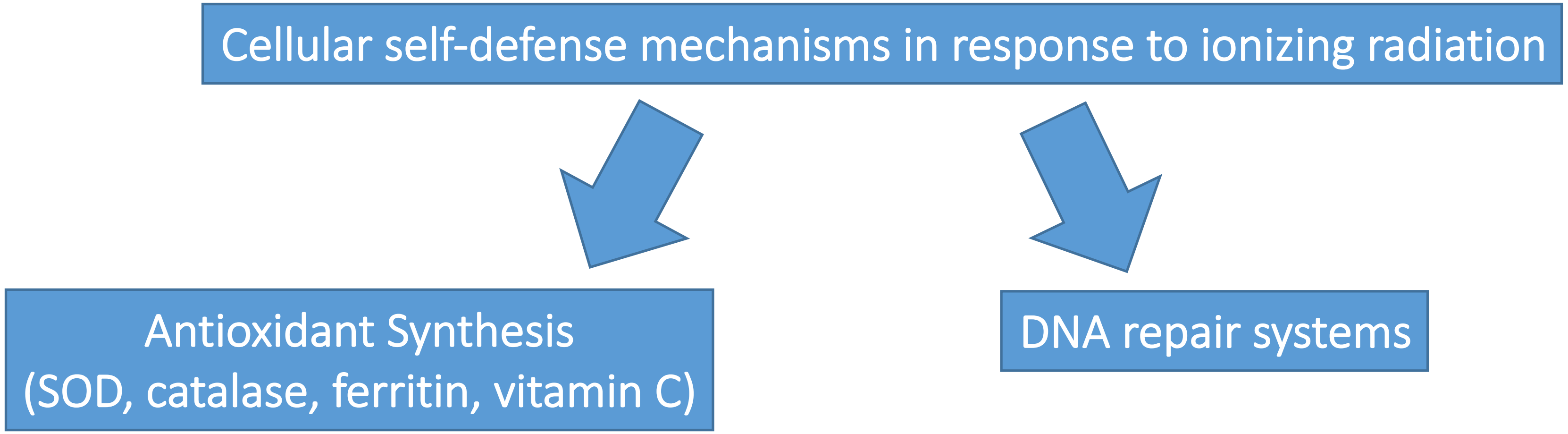
FOTON-M3 mission

TARDIS (Jönsson et al., 2008), RoTaRad (Persson et al., 2011), TARSE (Rebecchi et al., 2011, 2009)

TARDIS (*Tardigrades* in Space) tardigrades 10 days were in conditions of space vacuum (10^{-6} Pa), exposure to cosmic radiation (100 mGy) and UV radiation.

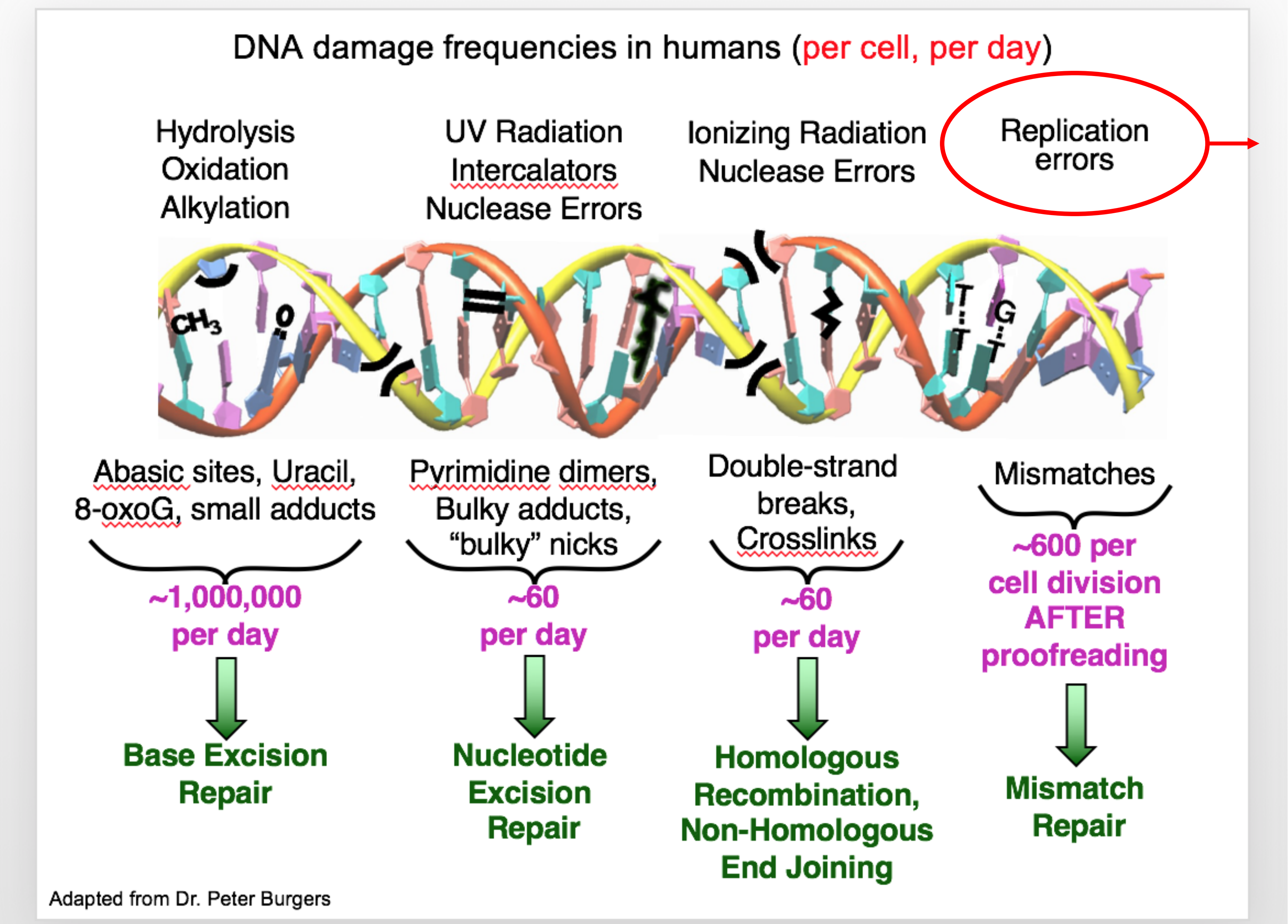
Exposure to vacuum and cosmic radiation did not have a effect on survival. (Jönsson et al., 2016, 2008).

One of the most important targets damaged by radiation is DNA, which contains almost all the genetic information of the body and is necessary for the survival of cells.



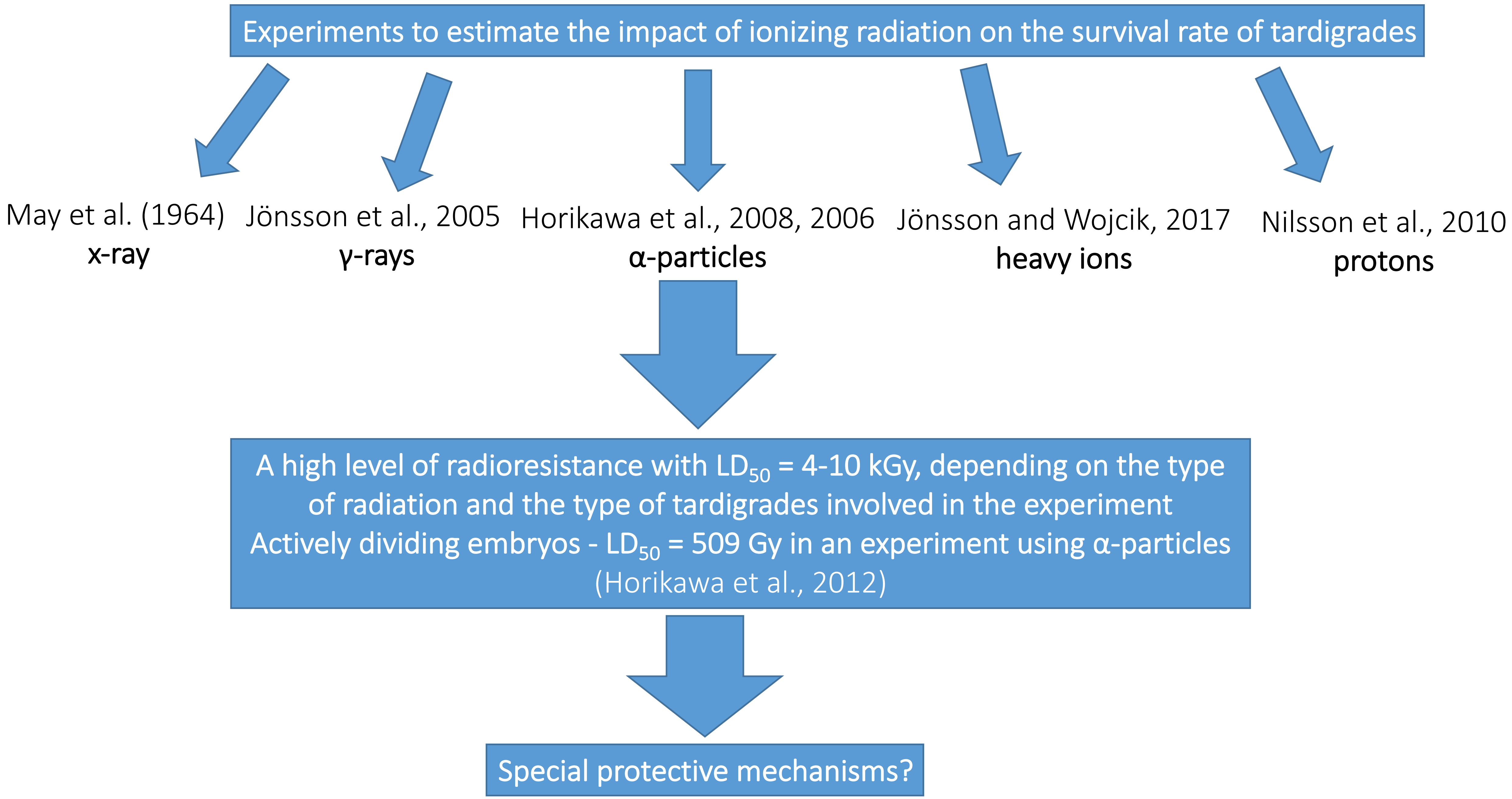
$$2O_2^{\cdot -} + 2H^+ \xrightarrow{(SOD)} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$



DNA-polymerase makes mistakes when copying DNA with a probability of 10^{-4}

After work of all repair mechanisms the frequency of abnormal nucleotides is 1 * 10^9 => in our body there are no 2 cells with exactly the same genome

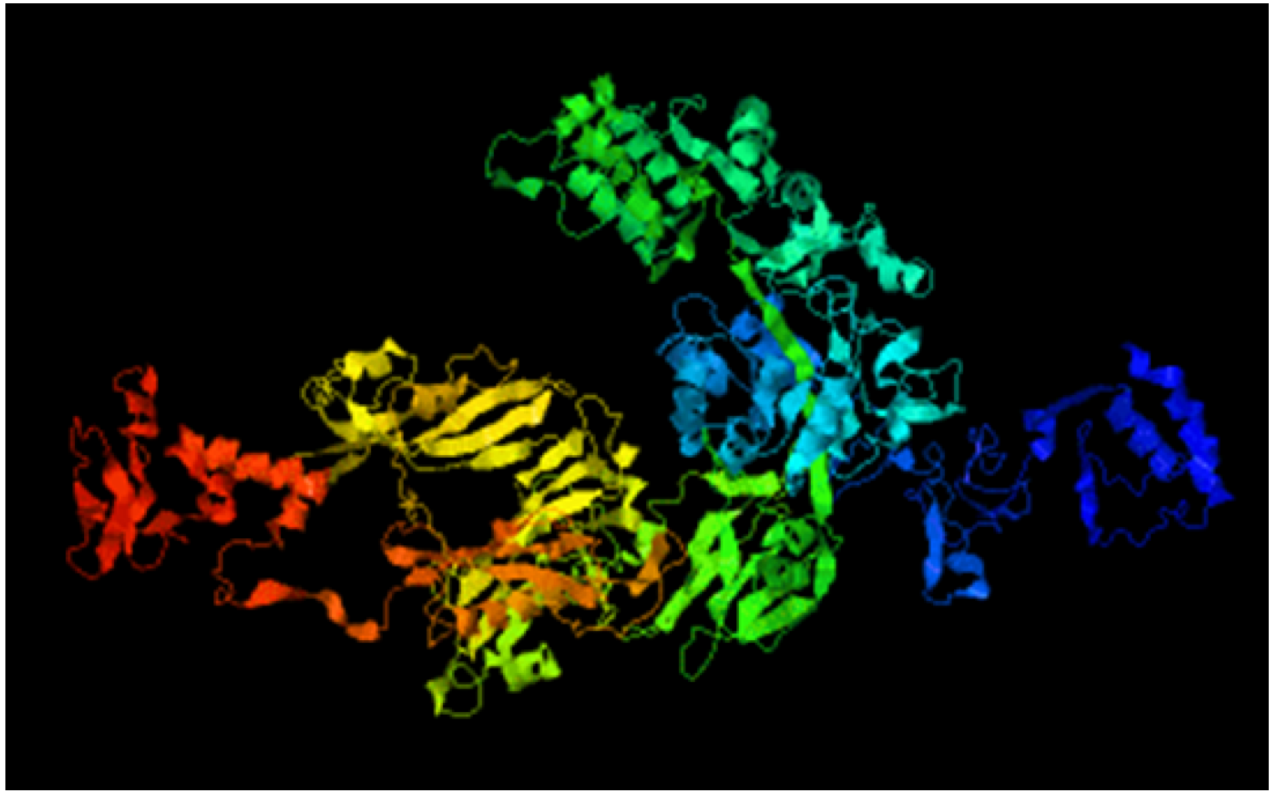

Experiments to estimate the impact of ionizing radiation on the survival rate of tardigrades
May et al. (1964)
x-ray
Jönsson et al., 2005
γ-rays
Horikawa et al., 2008, 2006
α-particles
Jönsson and Wojcik, 2017
heavy ions
Nilsson et al., 2010
protons
A high level of radioresistance with LD_{50} = 4-10 kGy, depending on the type of radiation and the type of tardigrades involved in the experiment
Actively dividing embryos - LD_{50} = 509 Gy in an experiment using α-particles (Horikawa et al., 2012)
Special protective mechanisms?

Molecular mechanisms of tardigrade radioresistance

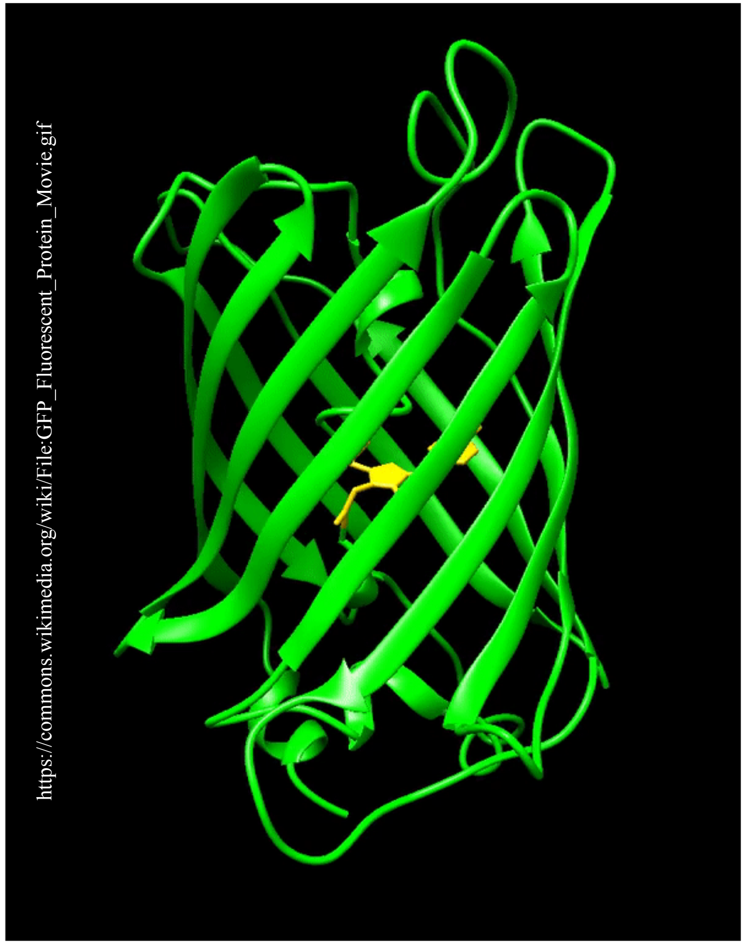
In 2016, the genome *Ramazzottius varieornatus* - one of the most radioresistant species of tardigrades - was sequenced (Hashimoto et al., 2016)

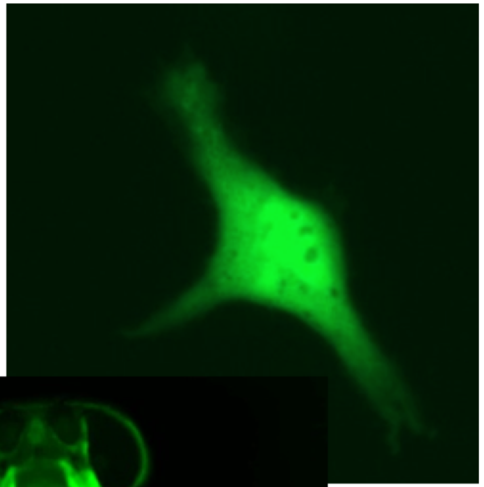
After analyzing the data and comparing the proteins of *R. varieornatus* with all the known proteins of other organisms, the unique protein was discovered - Damage suppressor (Dsup), which is present only in tardigrades.

Predicted three-dimensional structure model of Dsup (I-TASSER modelling)



Green fluorescent protein, GFP is widely used in molecular biology as a fluorescent label to study the expression of cellular proteins (395/498 nm)

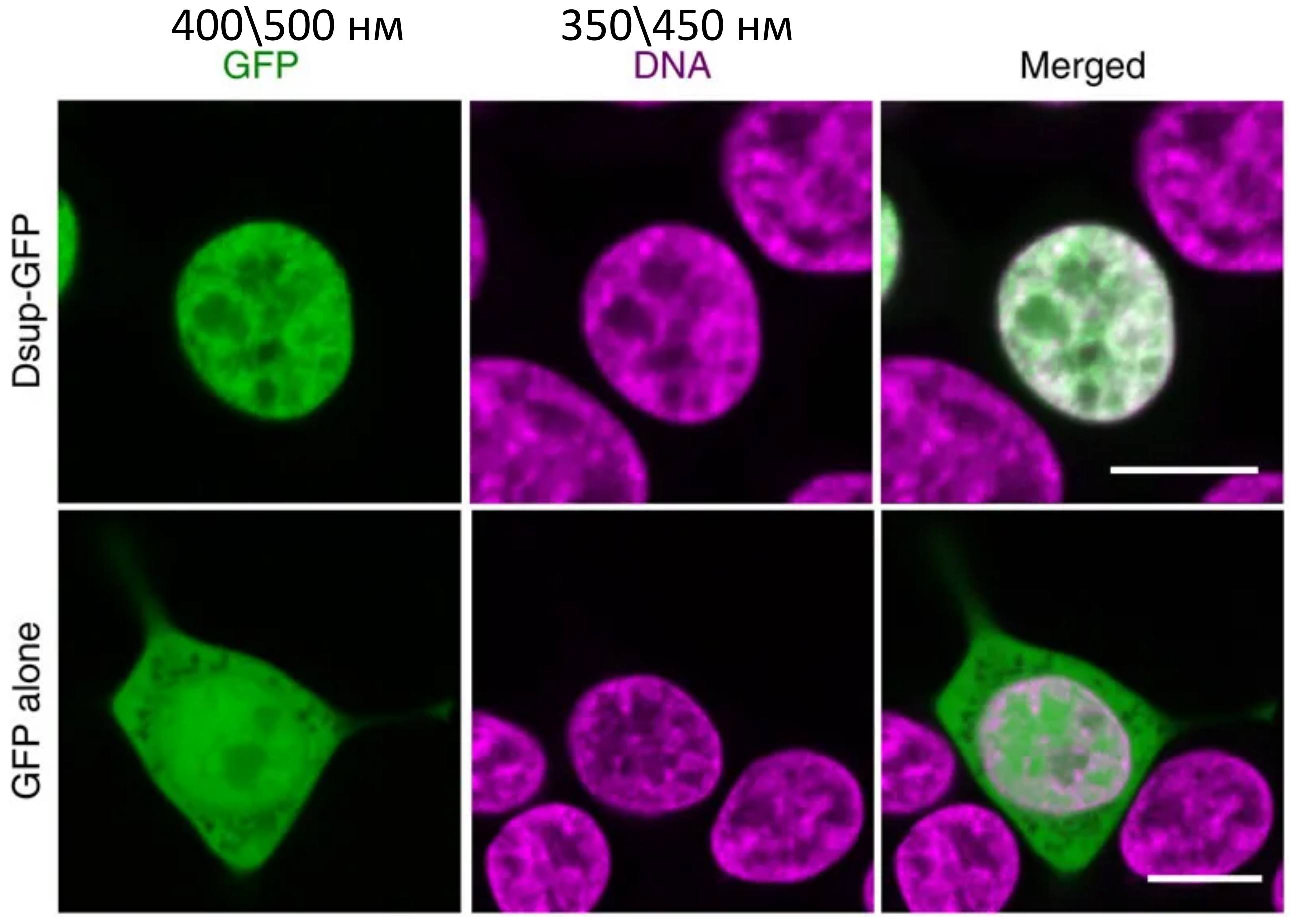


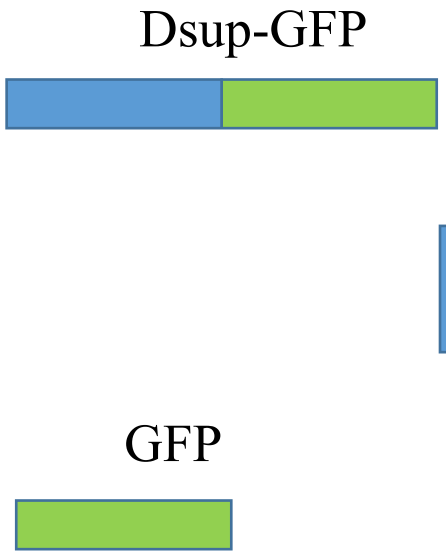


HEK293T

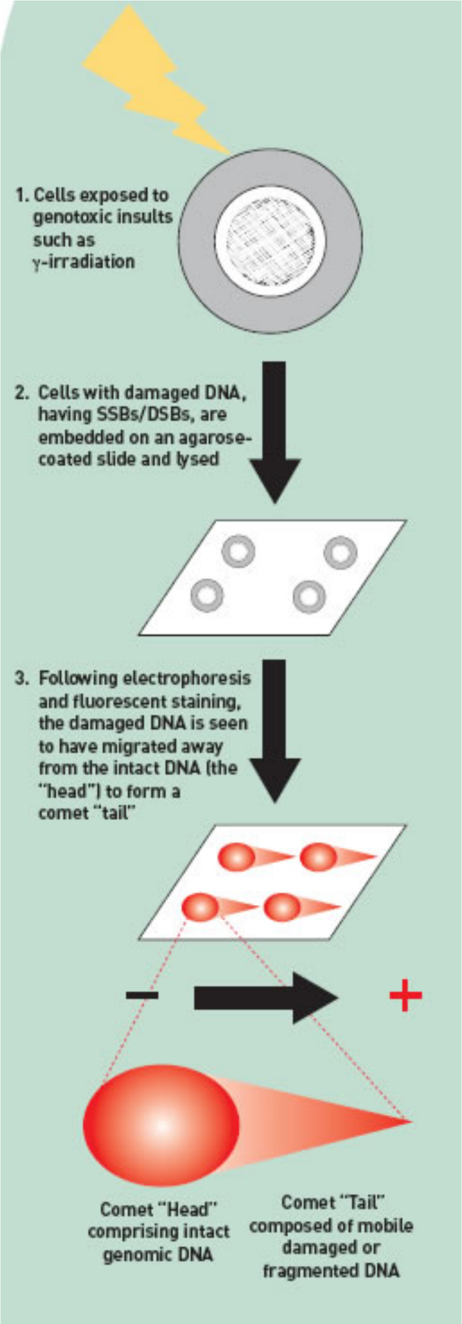
D.melanogaster

Hashimoto, T., Horikawa, D., Saito, Y. et al. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat Commun 7, 12808 (2016).

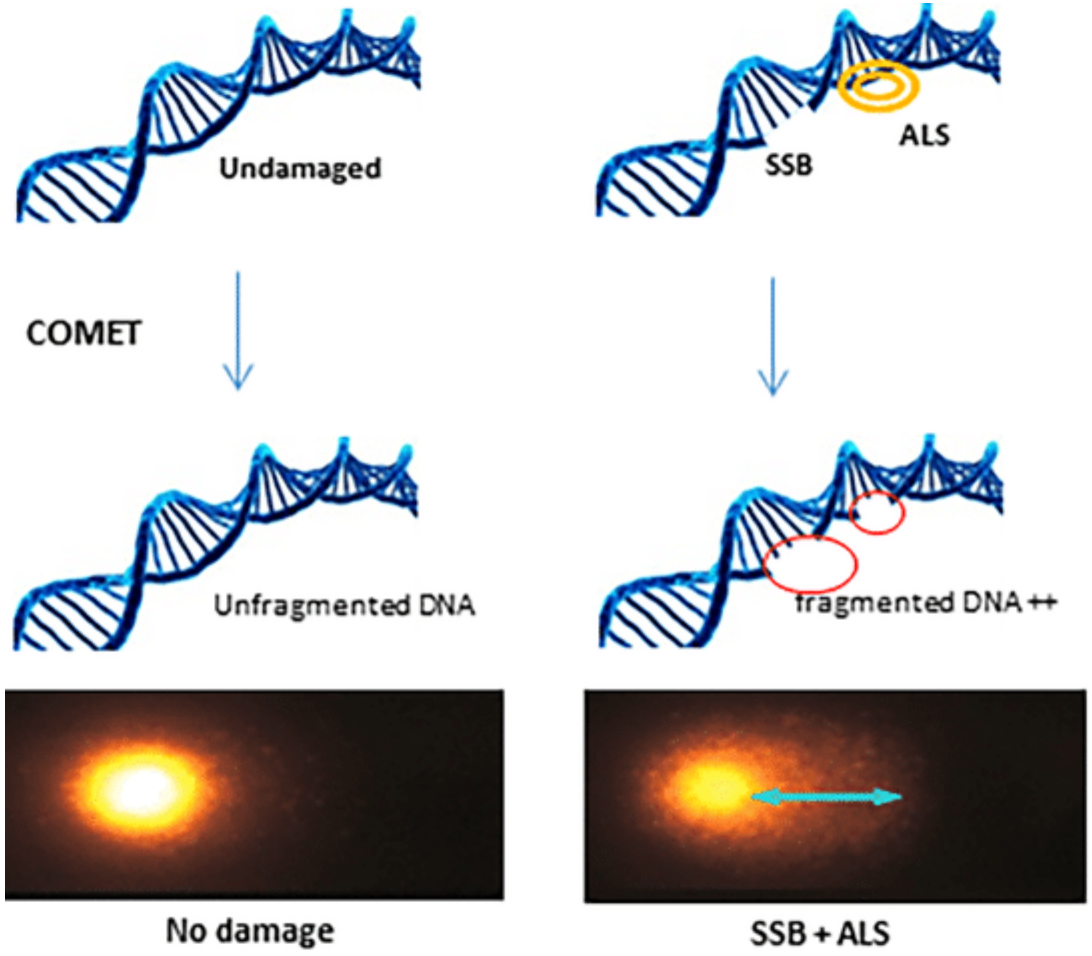


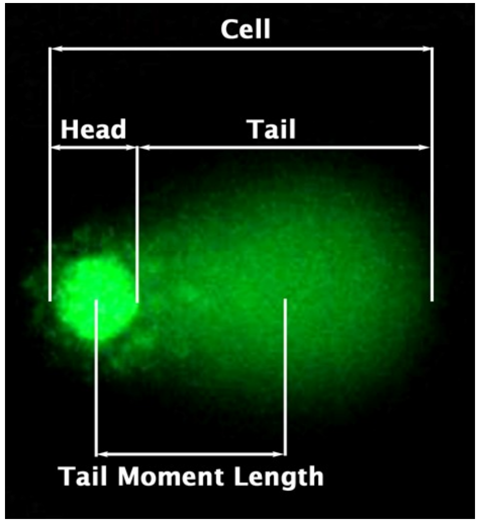


Dsup is colocalized with nuclear DNA, i.e. works in the nucleus https://topac.com/comet_electrophoresis.html

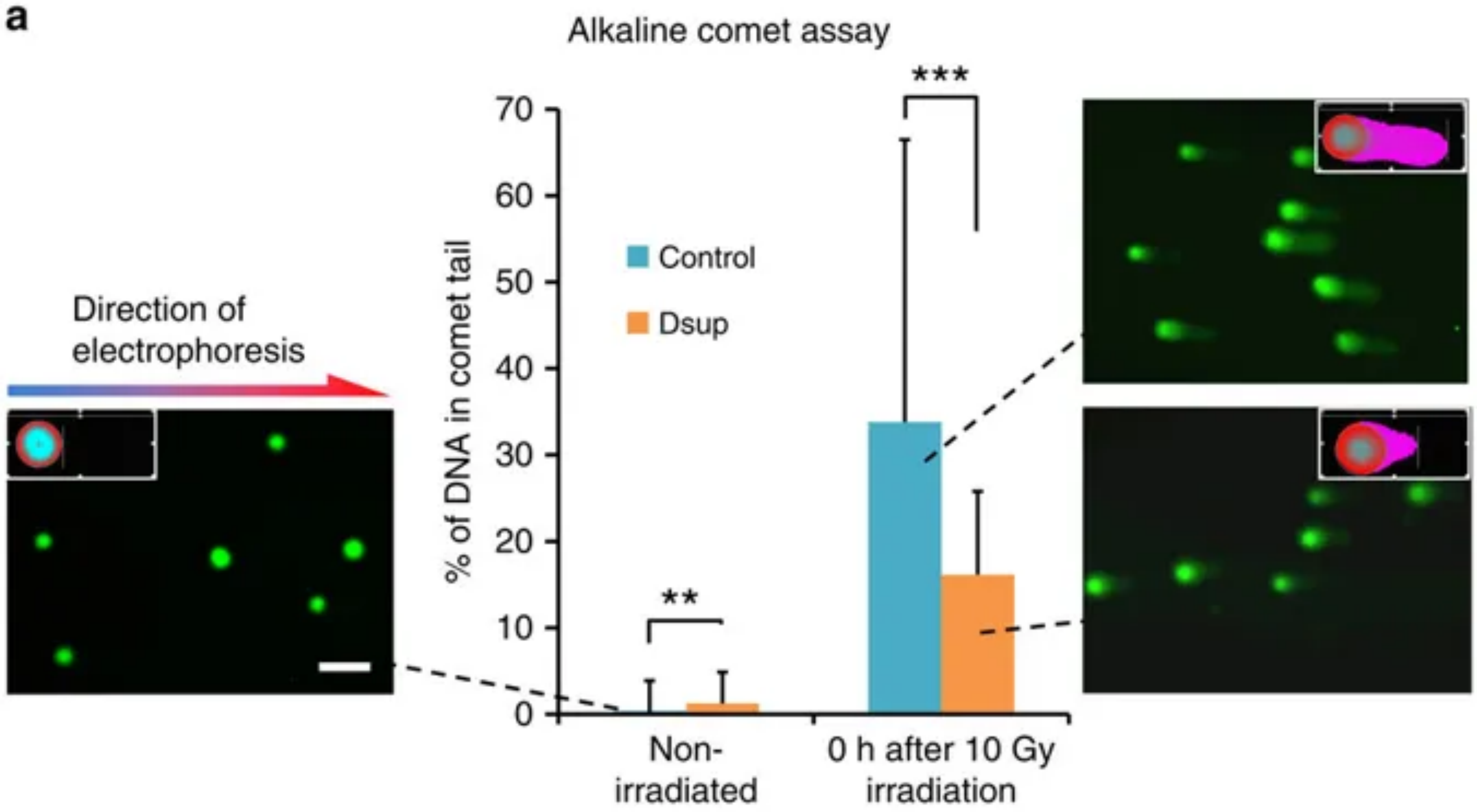


Metral E, Bechetoille N, Demarne F, Damour O, Rachidi W (2018) Keratinocyte stem cells are more resistant to UVA radiation than their direct progeny. PLoS ONE 13(9): e0203863. https://doi.org/10.1371/journal.pone.0203863





https://www.abcam.com/comet-assay-kit-3-well-slides-ab238544.html#description_images_1

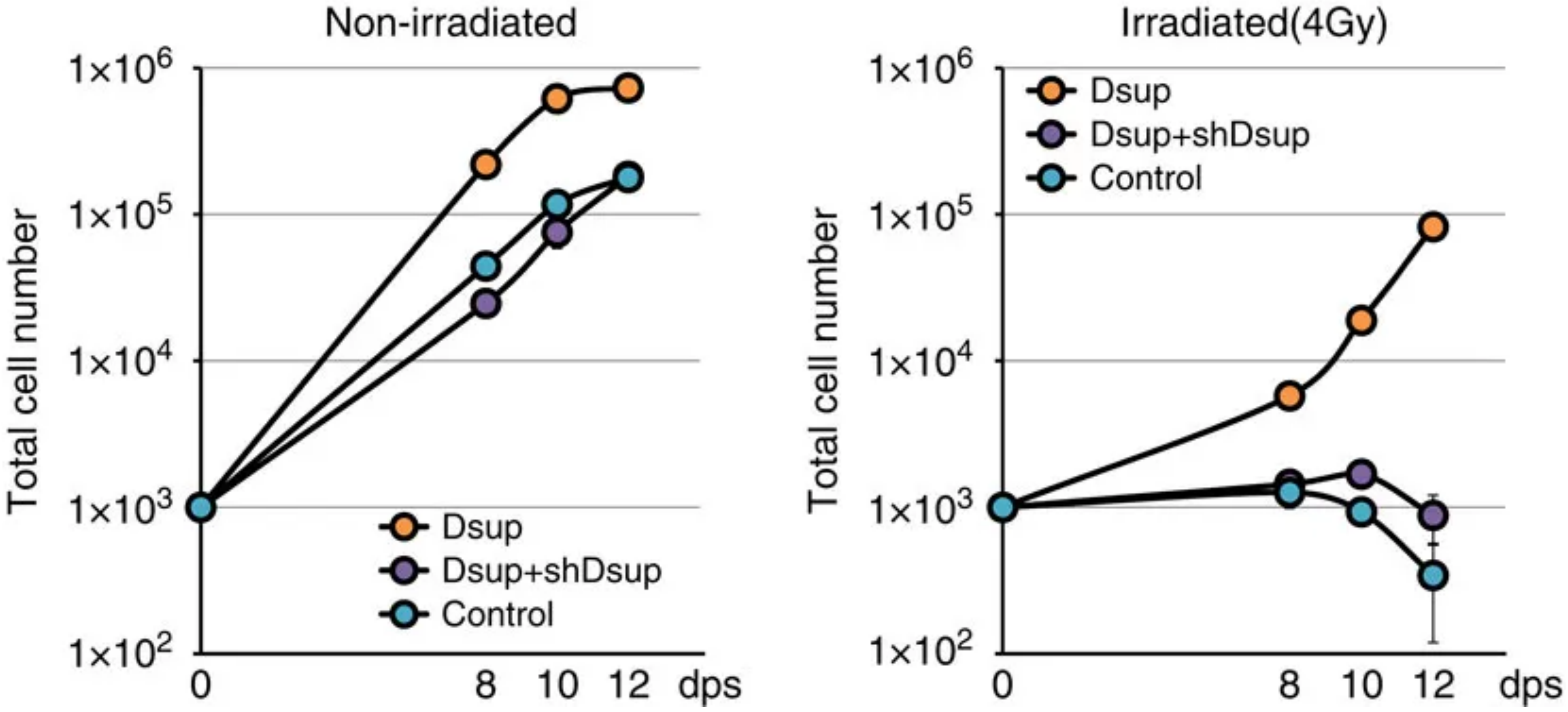


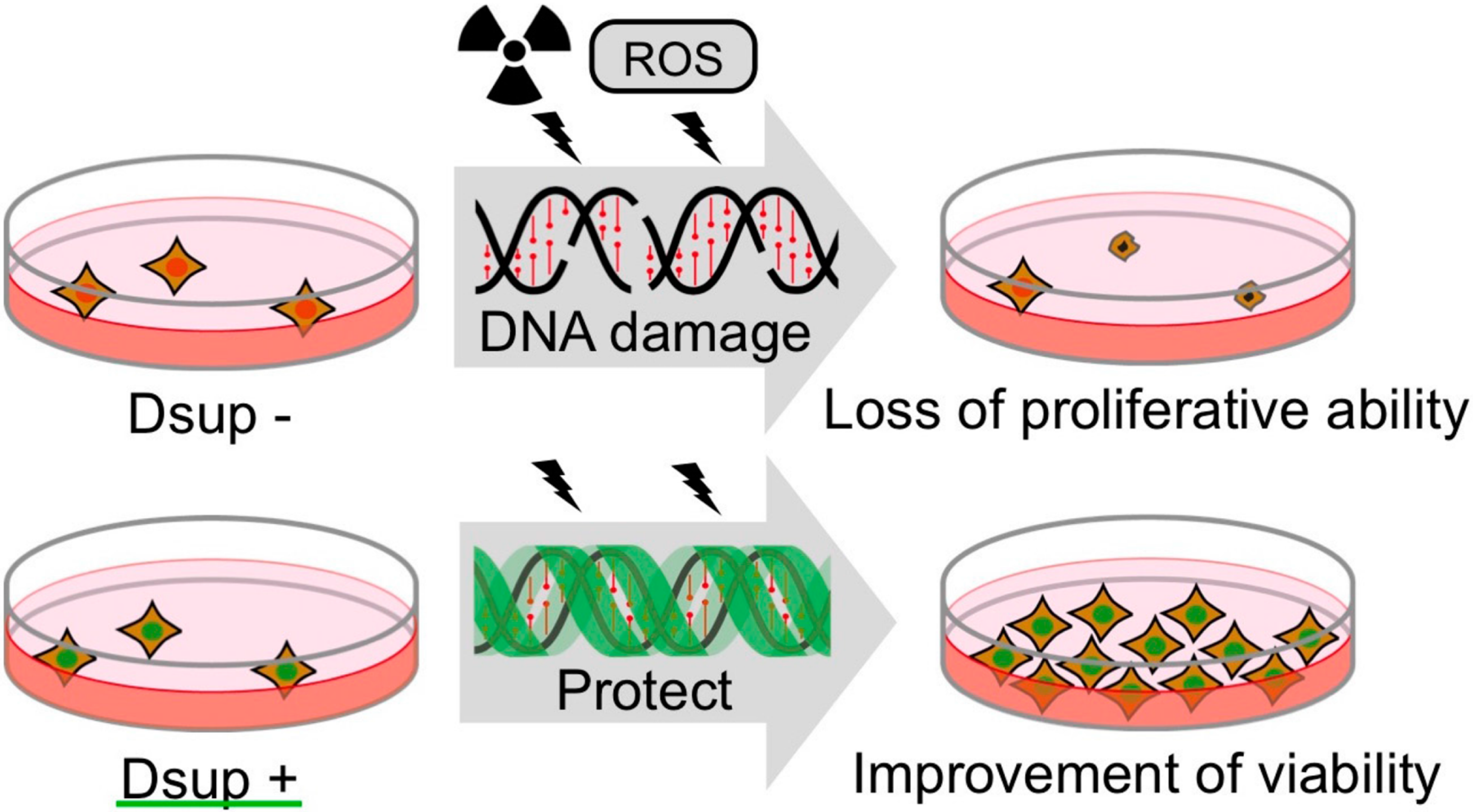
Dsup reduces fragmented DNA in human cell culture after exposure to radiation

Hashimoto, T., Horikawa, D., Saito, Y. et al. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat Commun 7, 12808 (2016).

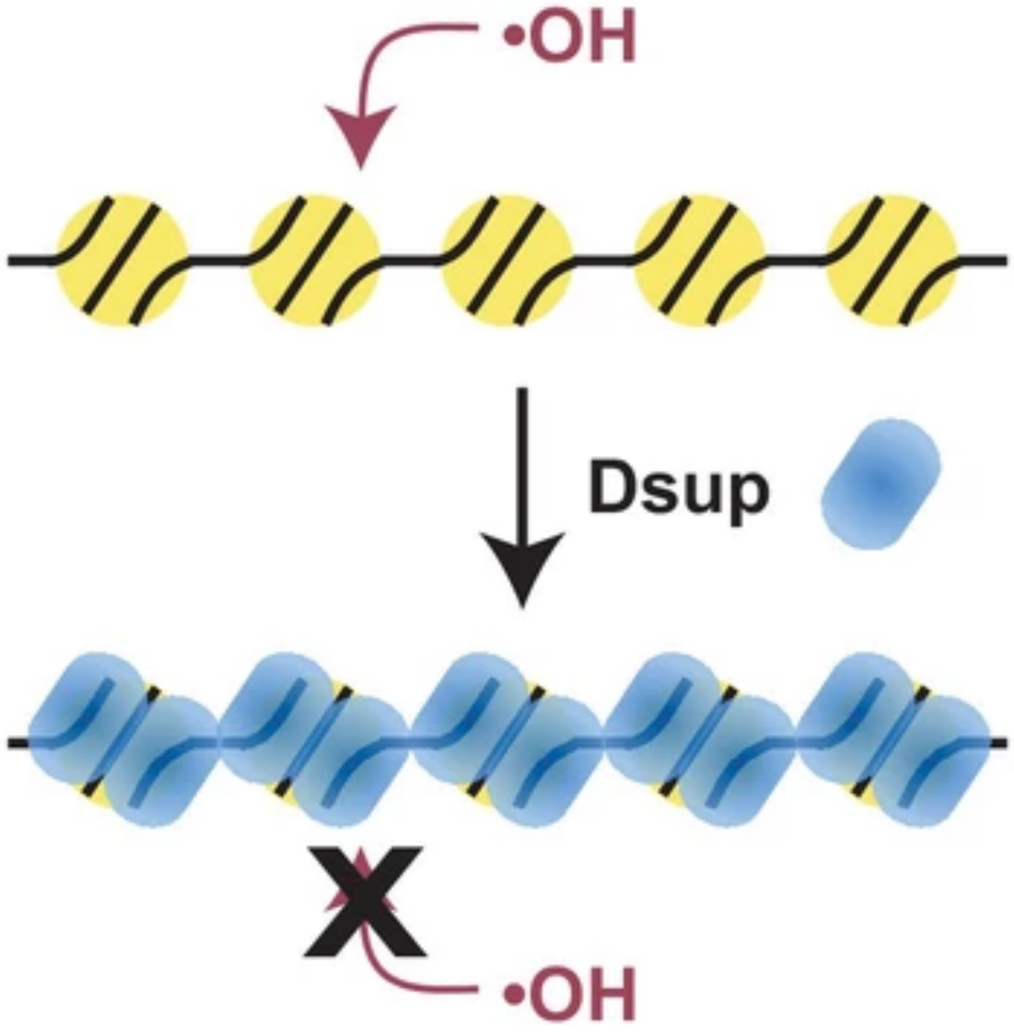
Hashimoto, T., Horikawa, D., Saito, Y. et al. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat Commun 7, 12808 (2016).

Dsup increases survival in human cell culture after x-ray exposure

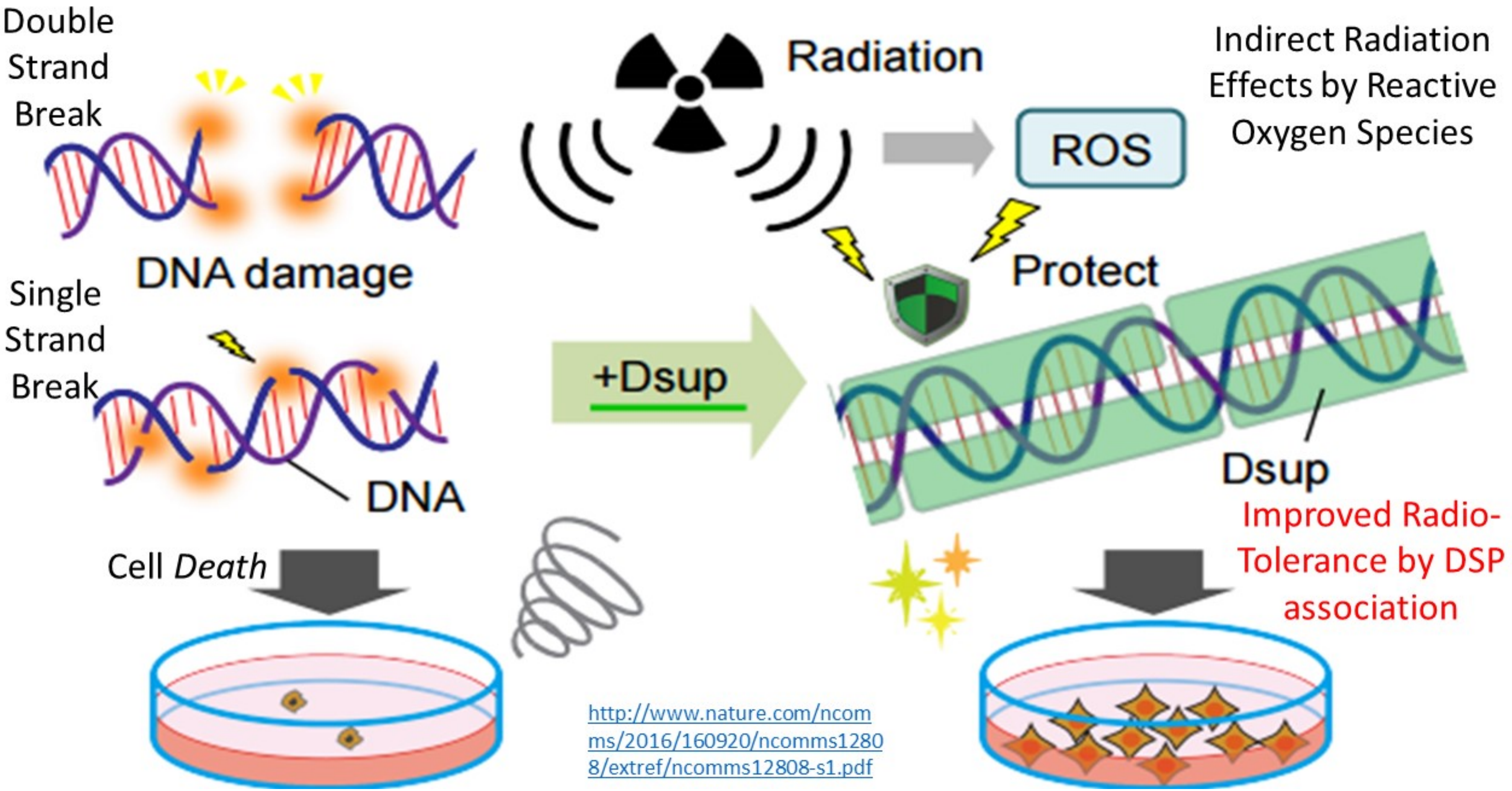



ROS
DNA damage
Dsup -
Loss of proliferative ability
Protect
Dsup +
Improvement of viability

The tardigrade damage suppressor protein binds to nucleosomes and protects DNA from hydroxyl radicals. Elife. 2019. Chavez C, Cruz-Becerra G, Fei J, Kassavetis GA, Kadonaga JT.



DNA Protection by Dsup Protein from Radiation Damage



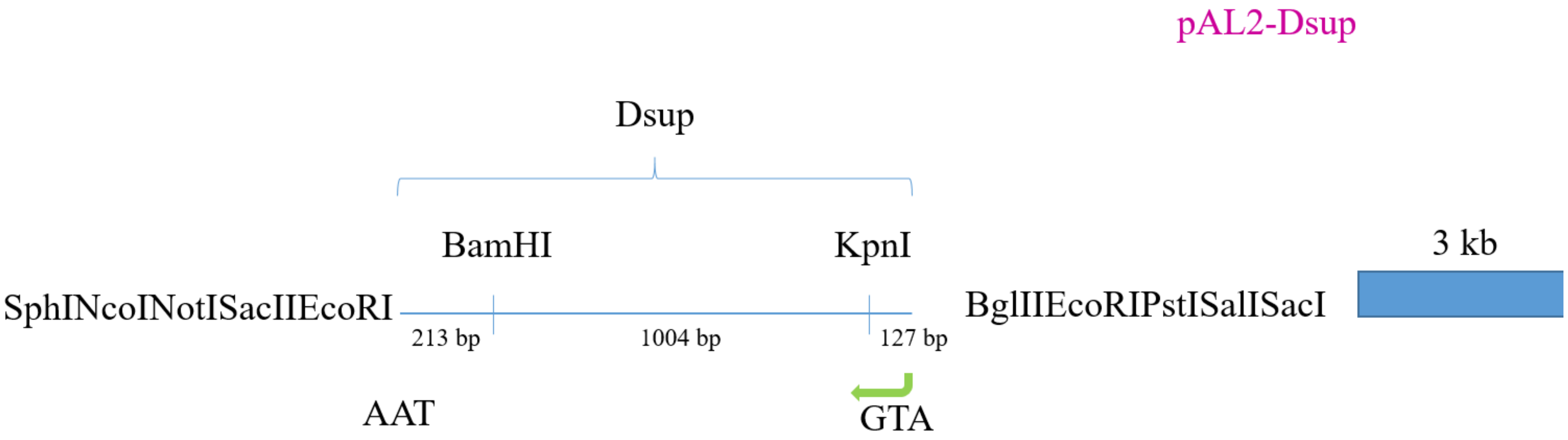
http://www.nature.com/ncomms/2016/160920/ncomms12808/extref/ncomms12808-s1.pdf

The main goals of the project are:

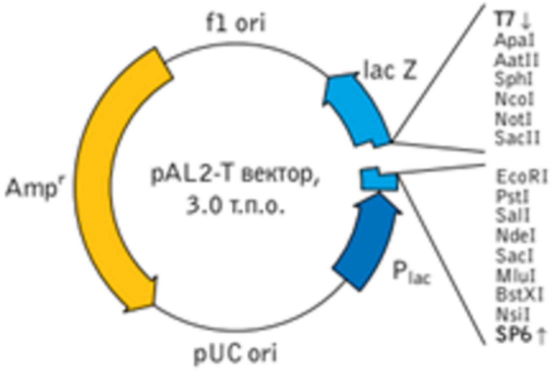
- study of the mechanisms of Dsup protein action
- assessment of the prospects for using Dsup to increase the radioresistance of multicellular complex organisms

Project objectives

1. Optimization and synthesis of DNA sequence encoding a Dsup protein

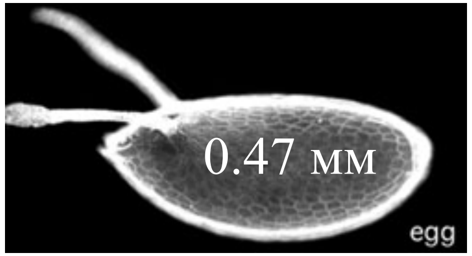


The source of the Dsup protein is the 1338 bp DNA sequence encoding the Dsup protein (LC050827.1), that was optimized for the frequency of occurrence of synonymous codons in the *D. melanogaster* genome for a stable high level of synthesis of this protein.

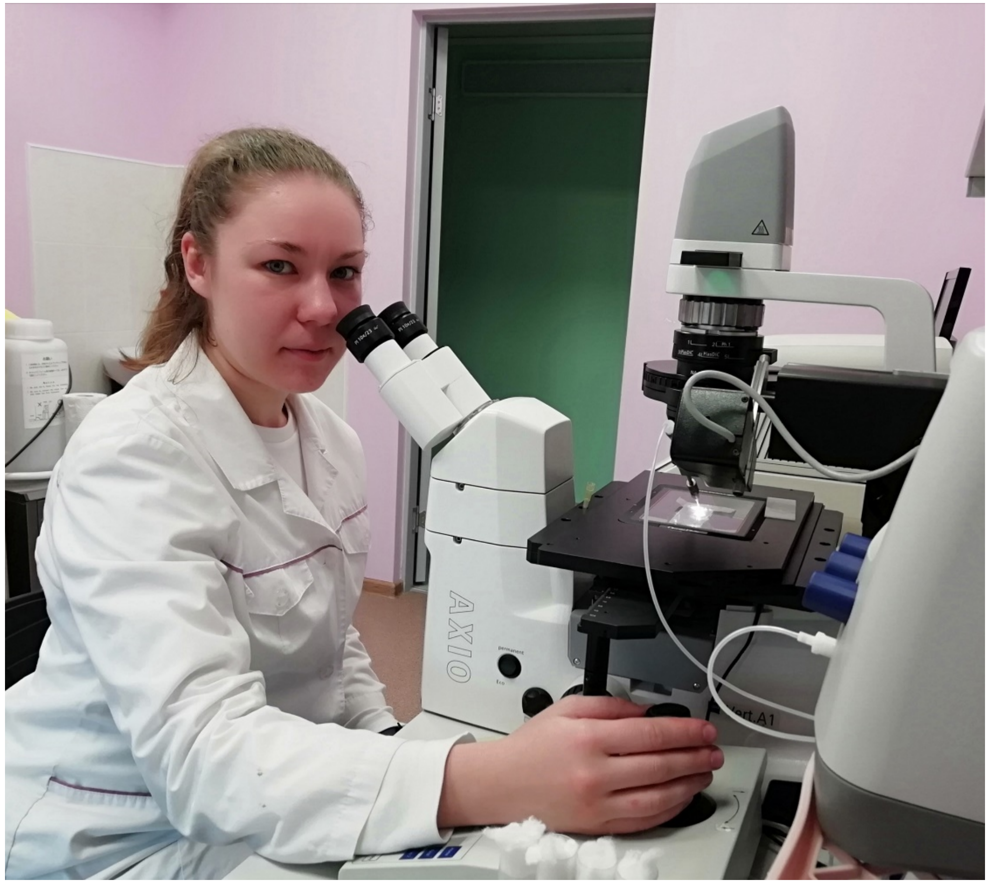


- synthesized
- tested for correct synthesis using sequencing

2. Generation of *D. melanogaster* lines expressing Dsup



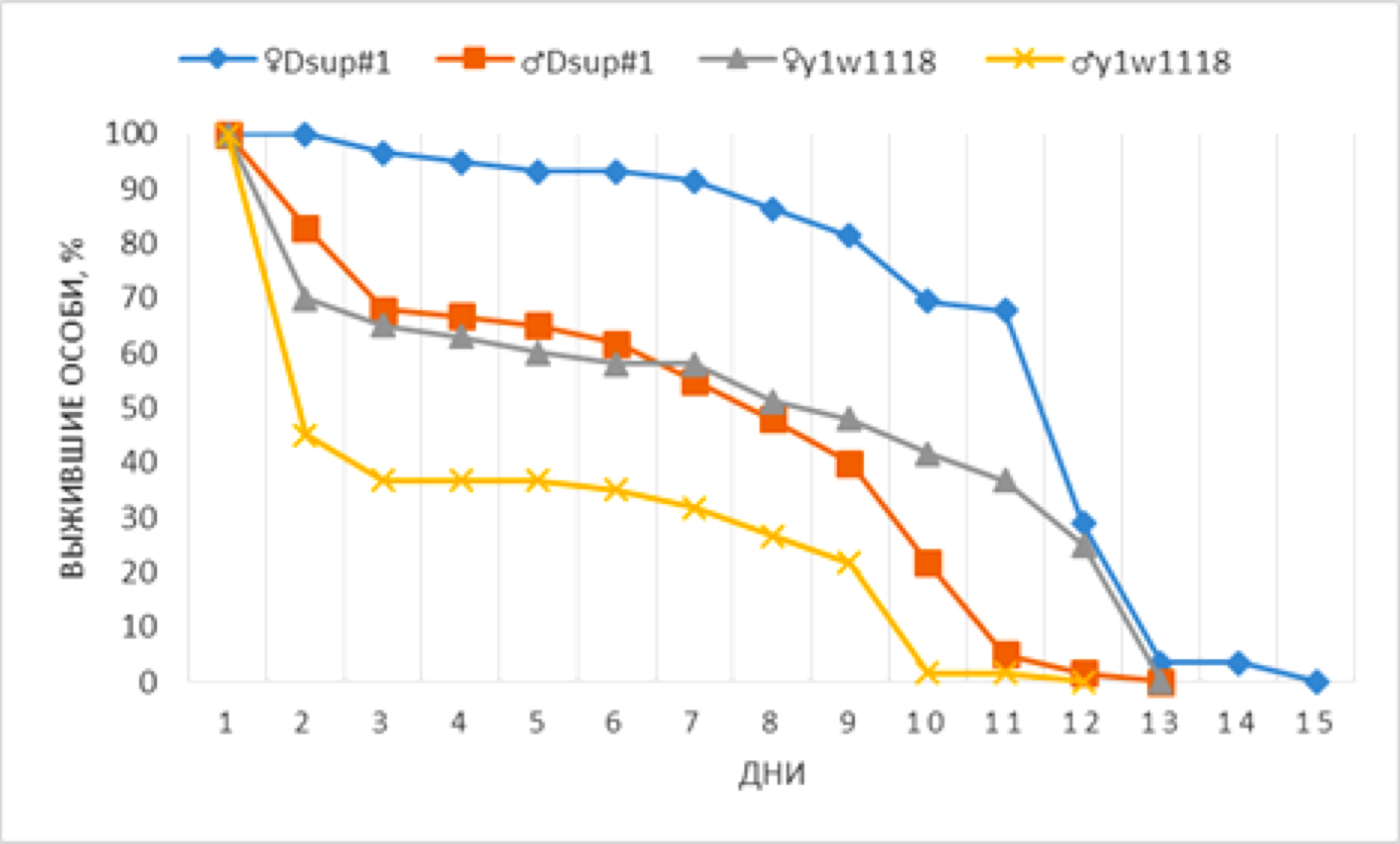
http://biology.mcgill.ca/faculty/nilson/research.html



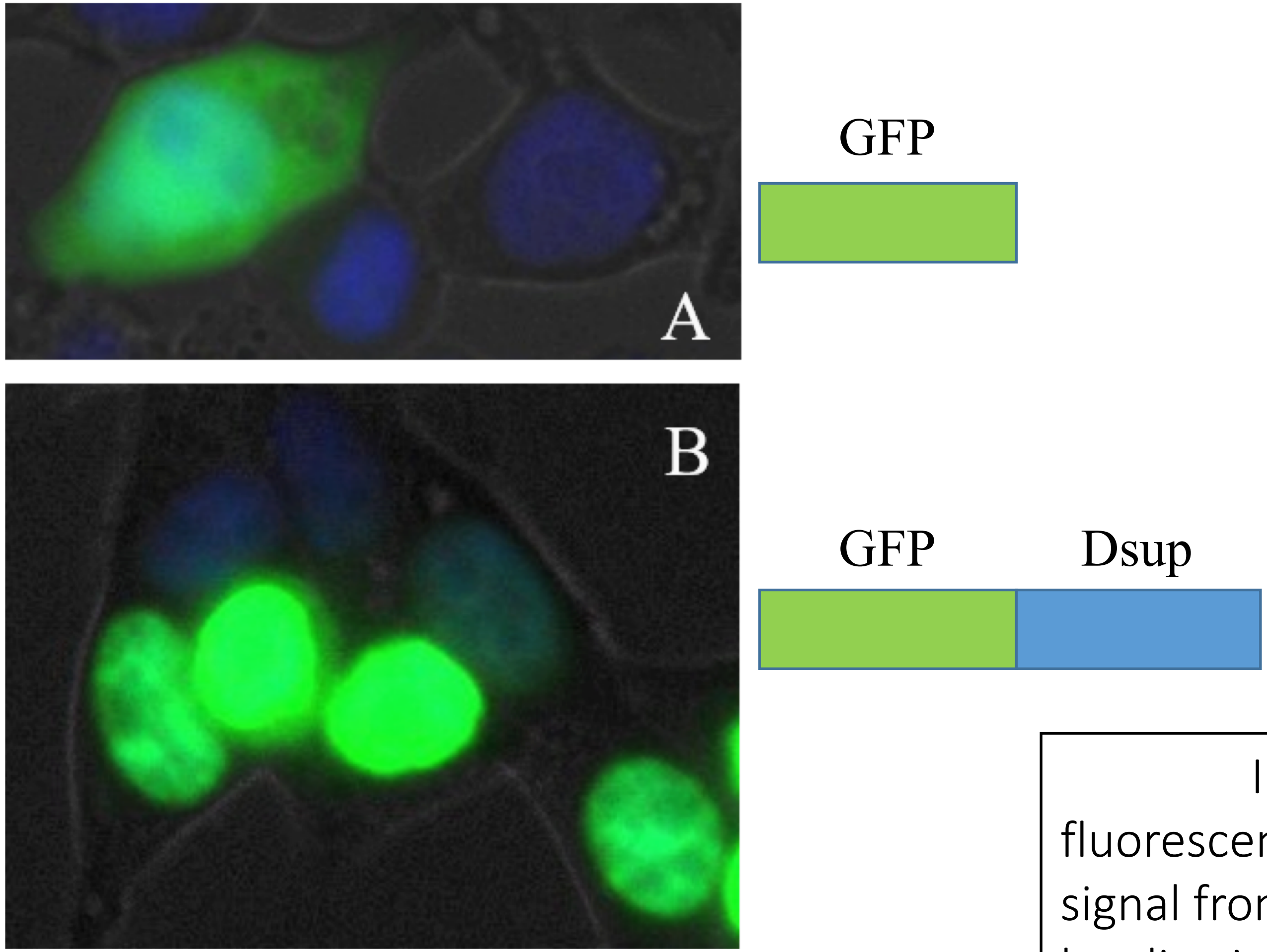
- - 5 independent *D. melanogaster* expressing Dsup strains were obtained

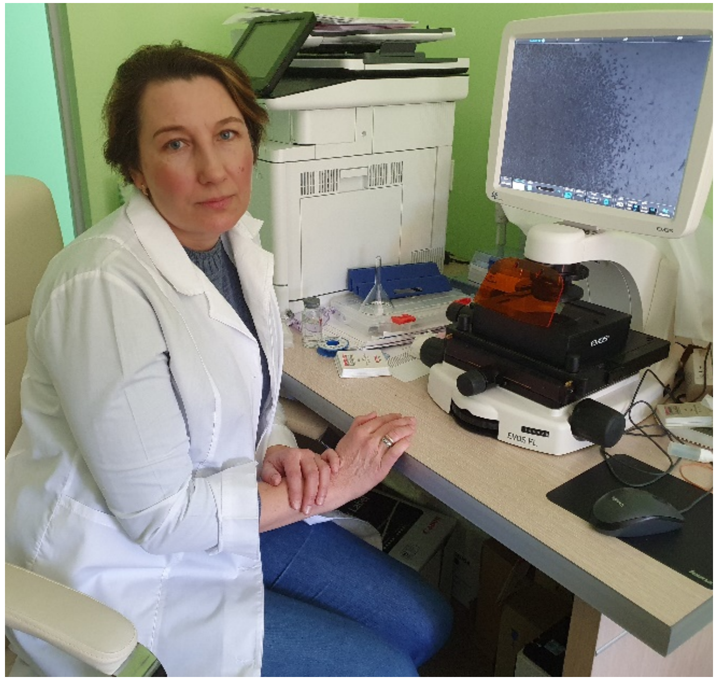
3. Evaluation of the radioresistance of *D. melanogaster* strains stably expressing Dsup

One *D. melanogaster* strain stably expressing Dsup was irradiated with γ-rays at the MT-25 LNR JINR accelerator at a dose of 1000 Gy, which is close to the $LD_{50/3}$



4. Generation of a stable cell line HEK293T expressing fusion protein GFP-Dsup and assessment of the radioresistance of this cell line to various types of ionizing radiation

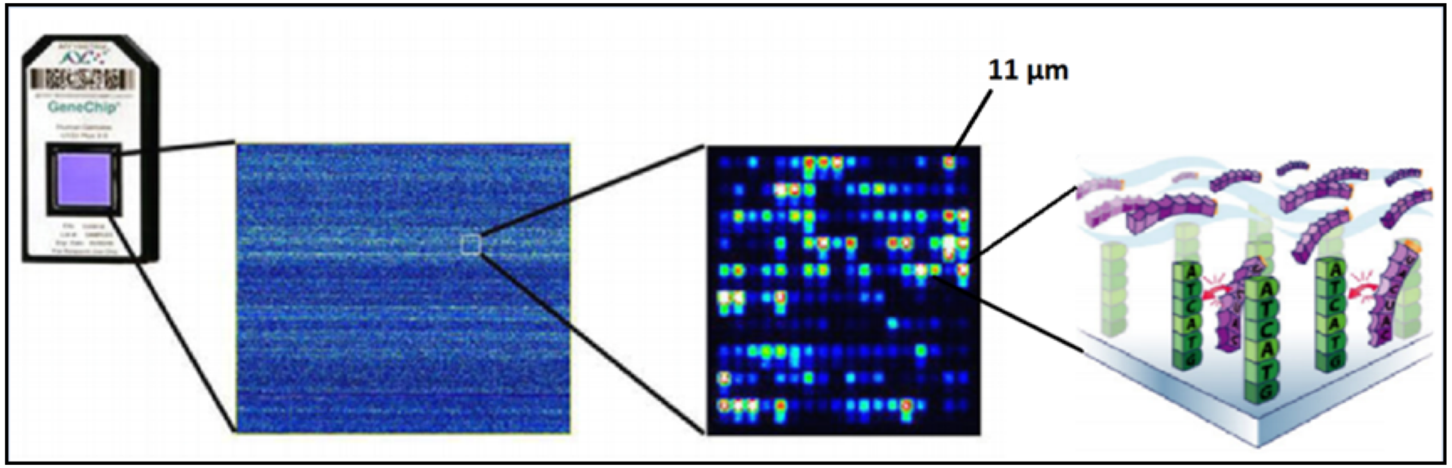


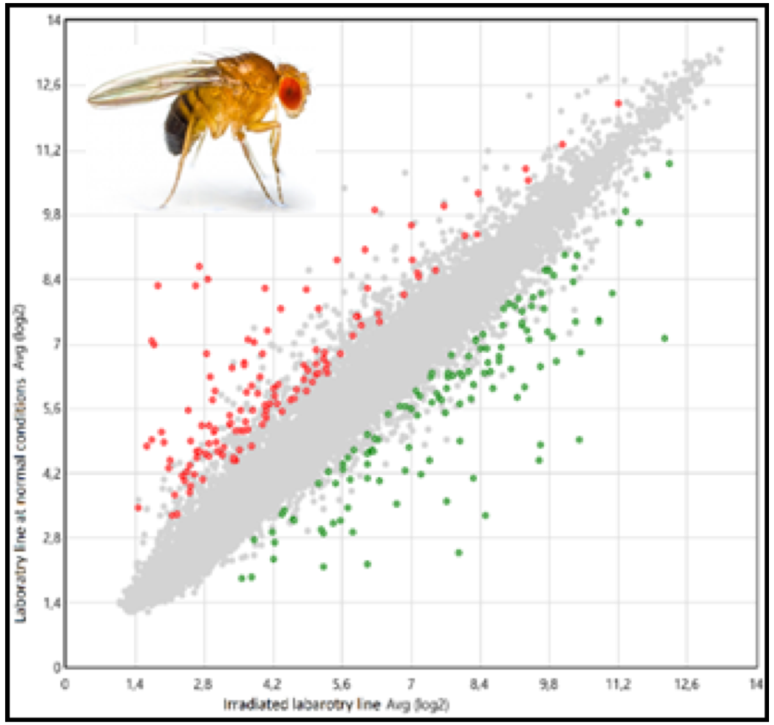


In Fig. B the complete coincidence of the green fluorescent signal from GFP-Dsup and the blue fluorescent signal from DNA located in the nucleus indicates nuclear localization of GFP-Dsup

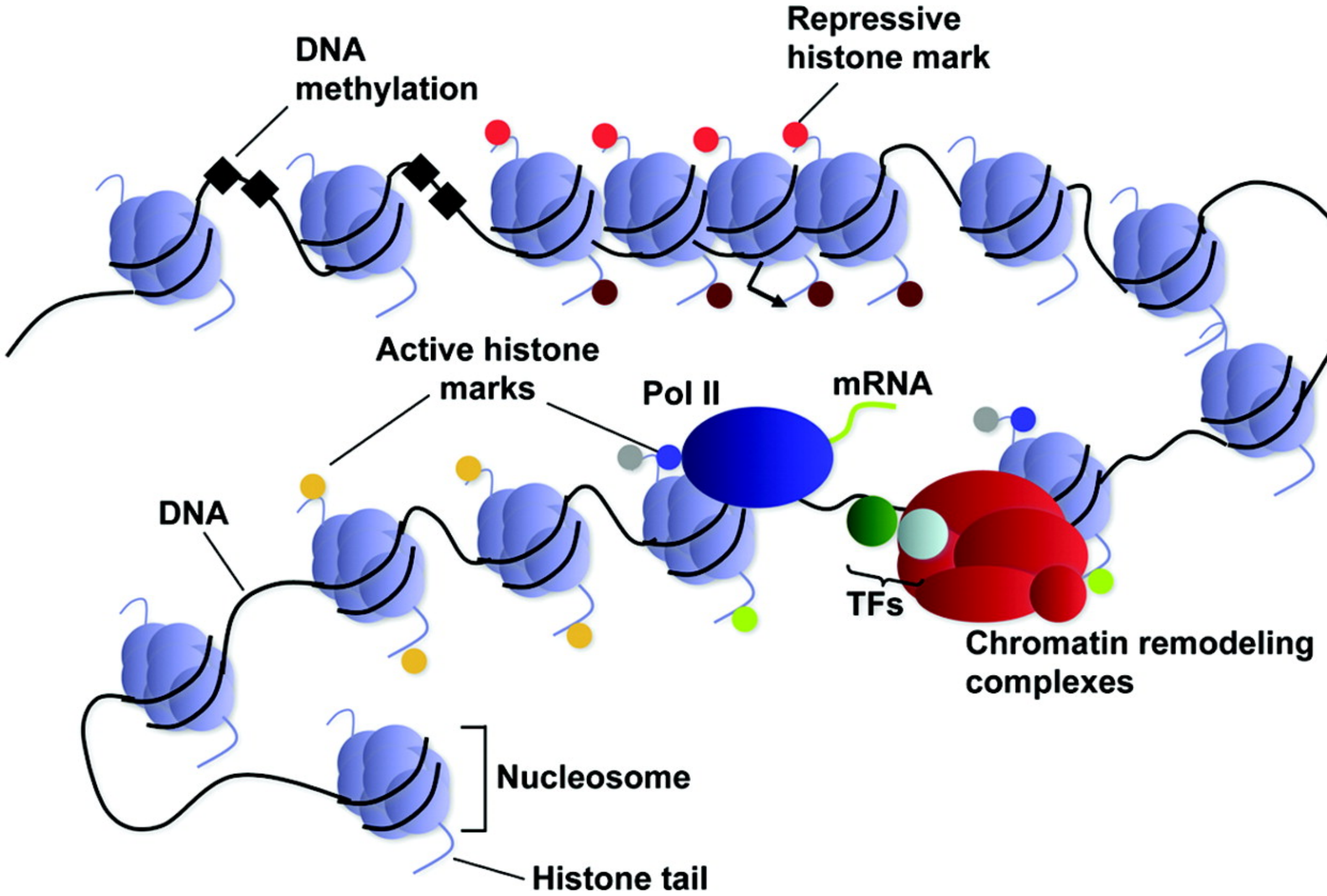
5. Transcriptome analysis of *D.melanogaster* and the HEK293T cell line expressing Dsup protein under normal conditions and after exposure to ionizing radiation

There is no data about what happens at the level of interaction between genes and, as a result, biological processes in the case of the addition of the Dsup protein, which is not characteristic of the Drosophila and human cells and can affect a number of fundamental processes.



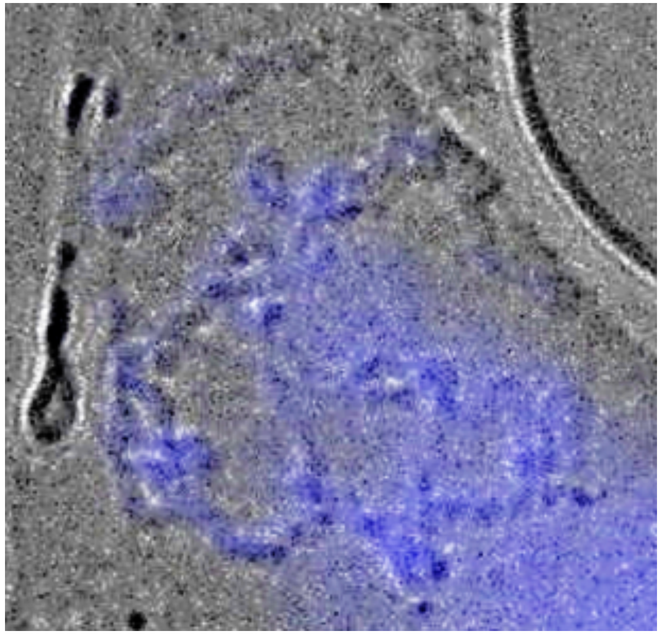


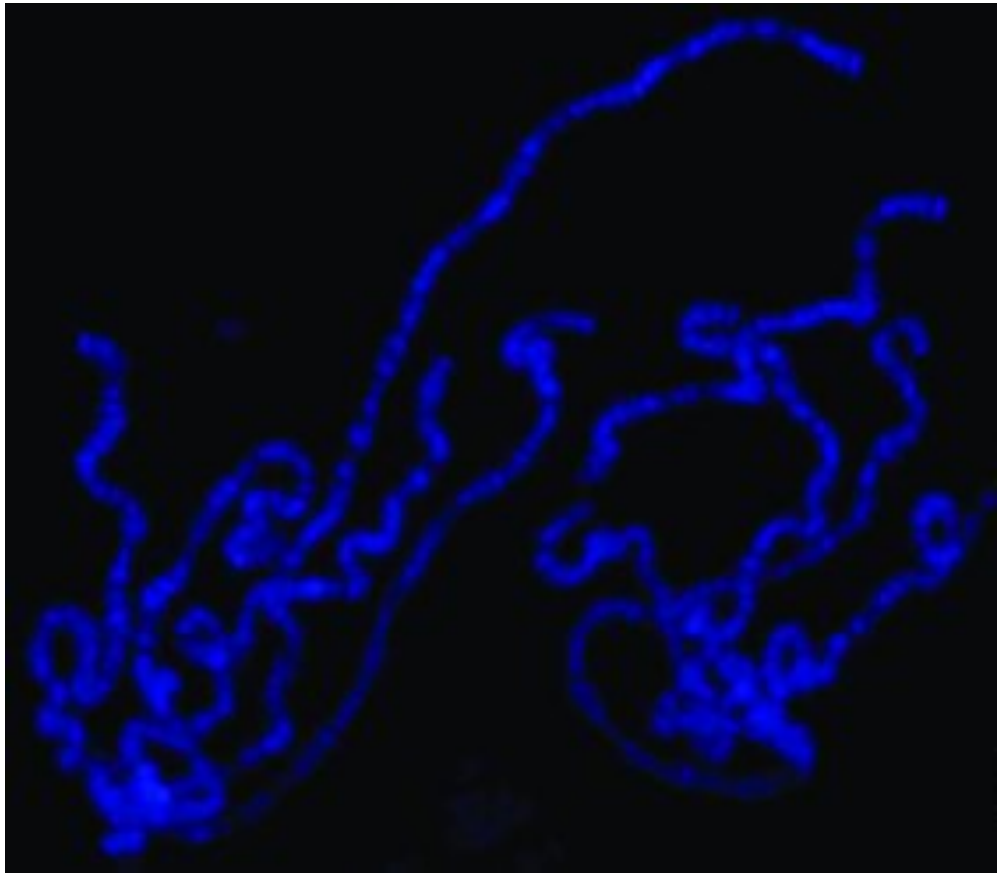
How Dsup protein binding to nucleosomes can affect the regulation of gene expression?



Epigenetic Regulation of the Cardiovascular System
Introduction to a Review Series
Benoit G. Bruneau Circulation ResearchVolume 107, Issue 3, 6 August 2010, Pages 324-326

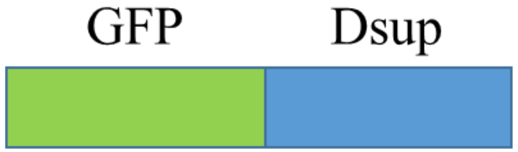
6. Study of GFP-Dsup fusion protein distribution on *D. melanogaster* polytene chromosomes





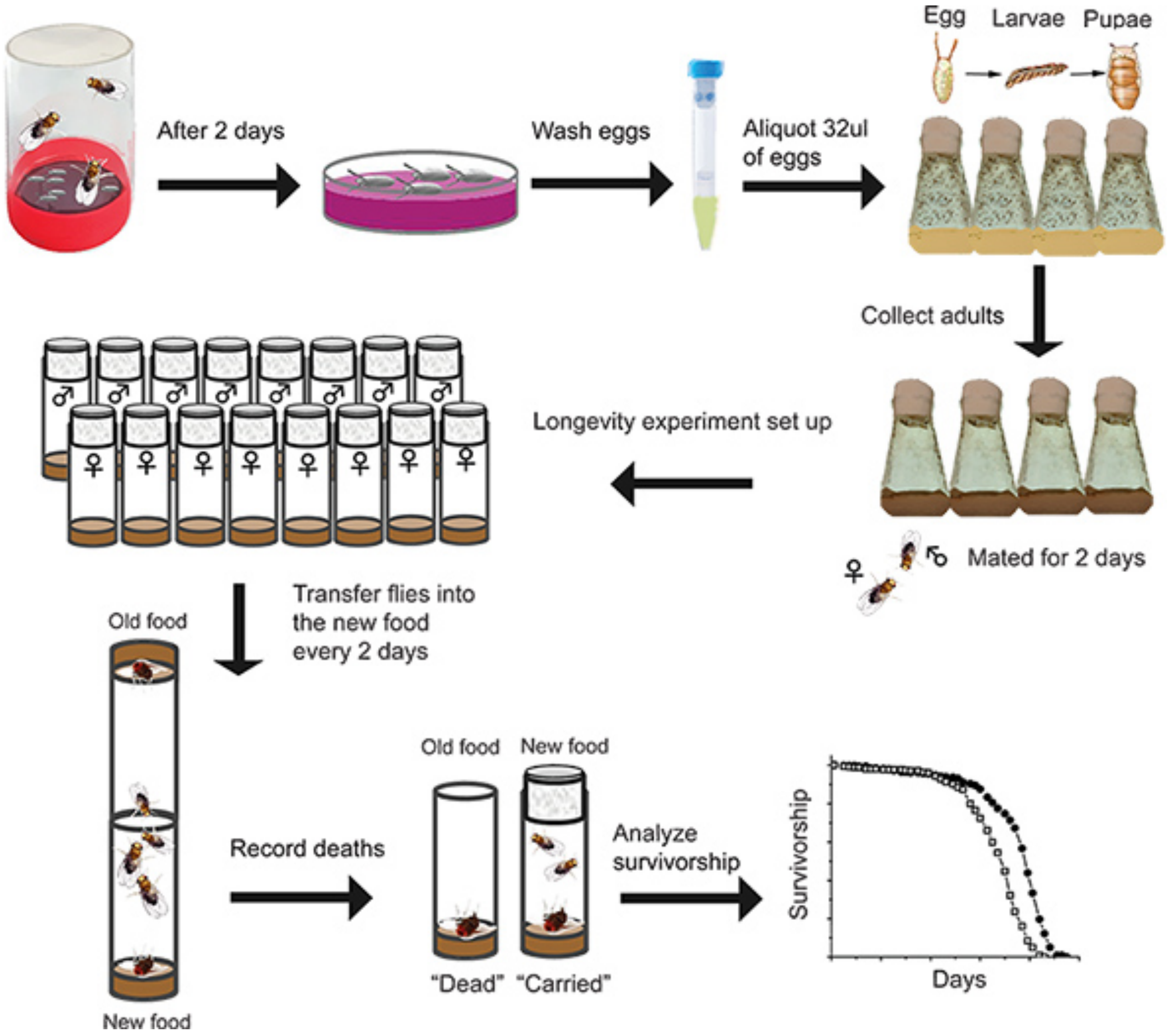
Thiazole Orange as an Alternative to Antibody Binding for Detecting Triple-helical DNA in Heterochromatin of Drosophila and Rhynchosciara December 2017 Journal of Histochemistry and Cytochemistry 66(1):22155417745496 Eduardo GorabPeter L PearsonPeter L Pearson

Is there selectivity in the binding of Dsup to chromosomes?



7. Study of the life span of *D.melanogaster* lines expressing Dsup

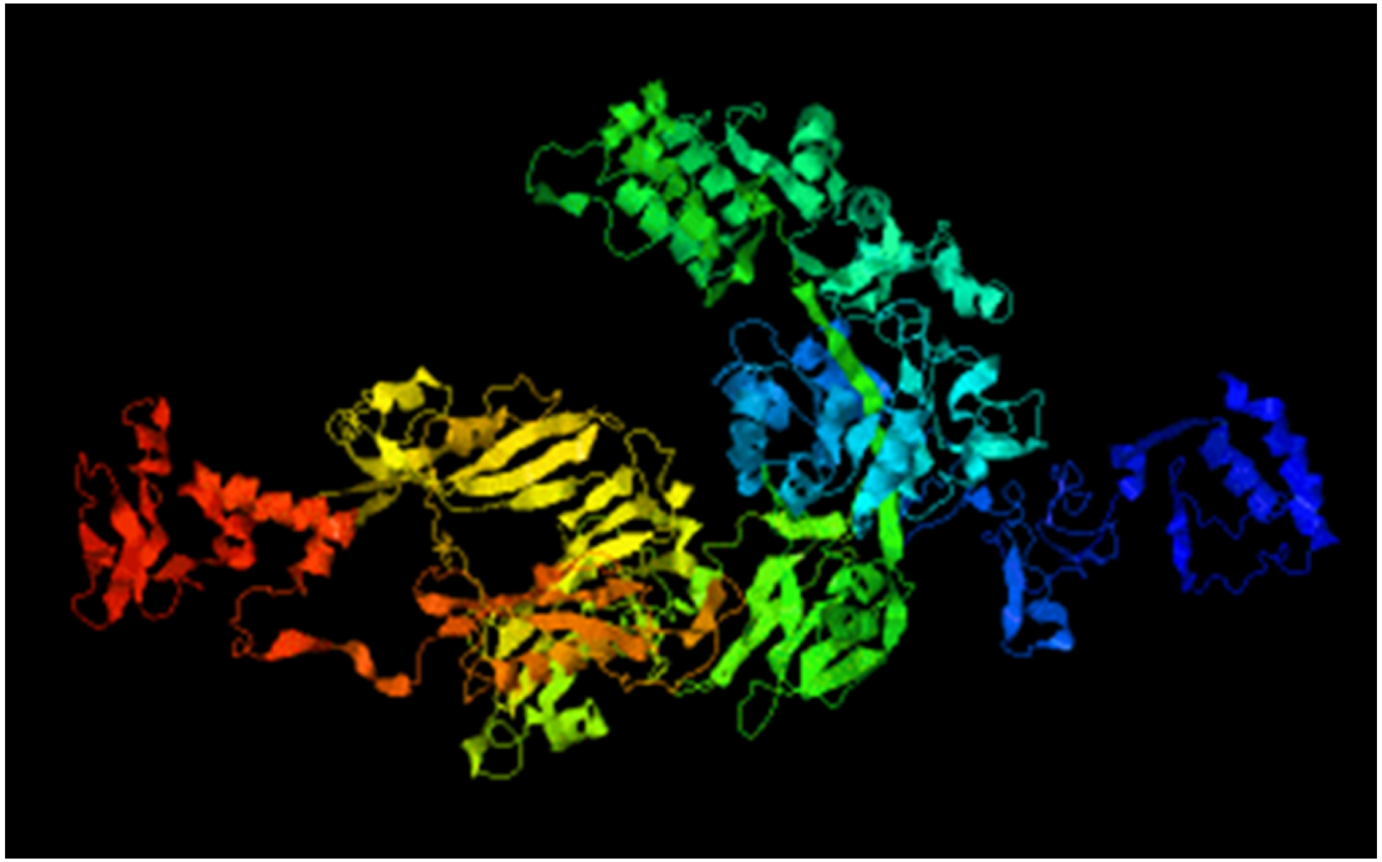
Because of the alleged ability of the Dsup protein to bind to nucleosomes, it is interesting to evaluate the effect of possible changes in chromatin structure on the functioning of the whole organism, for instance – difference in *D.melanogaster* life span between natural and Dsup expressing strains



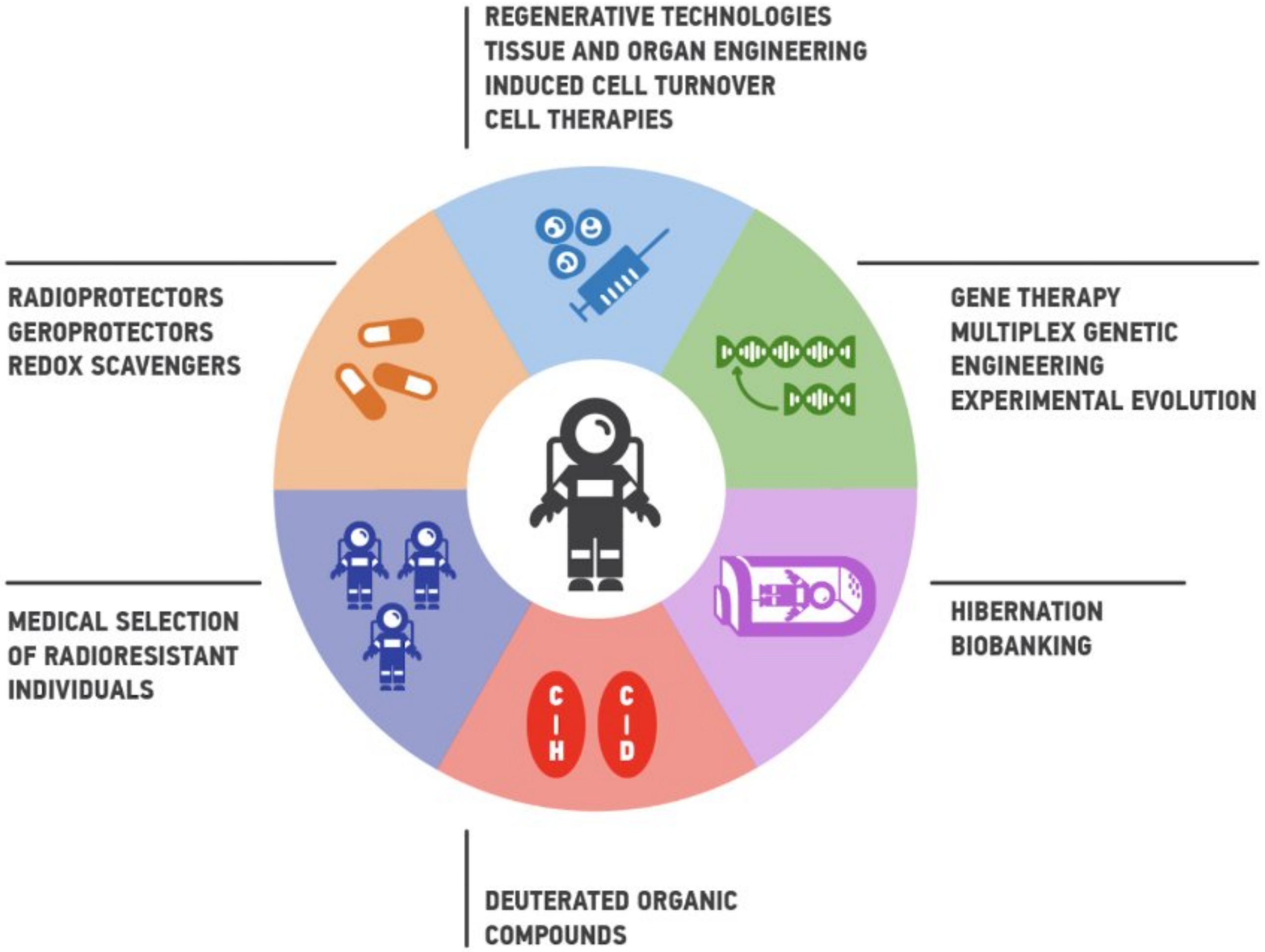
Linford, N. J., Bilgir, C., Ro, J., Pletcher, S. D. Measurement of Lifespan in Drosophila melanogaster. J. Vis. Exp. (71), e50068, (2013).

8. Creation of an expression vector for the production of Dsup protein in *E. coli* cells, extraction and purification of Dsup protein for preliminary crystallization experiments

Since the secondary structure of the Dsup protein has not yet been studied, it is interesting to express the Dsup protein in *E. coli* culture, followed by purification and attempted crystallization or analysis using spectrometric methods. Determination of the secondary structure of the protein will allow to make an assumption about the mechanisms of its binding to DNA and / or other proteins and to simulate various scenarios of its mechanisms of action.



At present, there is no data about multicellular model organisms expressing Dsup protein, therefore, the tasks to be solved during the course of the project are new and important not only for fundamental molecular biology and radiobiology, but also for applied biotechnology, space research, and any other disciplines that require raising the level radioresistance of organisms.



Roadmap to enhance radioresistance for space colonization

Oncotarget. 2018 Feb 12;9(18):14692-14722 Vive la radiorésistance!: converging research in radiobiology and biogerontology to enhance human radioresistance for deep space exploration and colonization.Cortese F, Klokov D, Osipov A, Stefaniak J,, Moskalev A, Schastnaya J, Cantor C, Aliper A, Mamoshina P, Ushakov I, Sapetsky A, Vanhaelen Q, Alchinova I, Karganov M, Kovalchuk O, Wilkins R, Shtemberg A, Moreels M, Baatout S, Izumchenko E, de Magalhães JP, Artemov AV, Costes SV, Beheshti A, Mao XW, Pecaut MJ, Kaminskiy D, Ozerov IV, Scheibye-Knudsen M, Zhavoronkov A.

Project participants

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