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DSUP Project

How to make the human body resistant to radiation

TISF-2025, Taipei Project.*

Federico Bergo: G. Galilei High School of Science, Alessandria

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ABSTRACT

In radiation treatments and manned interplanetary space travel, radiation is one of the biggest problems. The radiotolerance of cancer cells makes it necessary to apply high doses to surrounding healthy tissues by subjecting the cells to heavy stress. With regard to space travel (which involves a minimum travel time of 6 months) (1,2) the danger concerns cosmic radiation which is capable of inducing genetic mutations that, in turn, can evolve into very serious pathologies, such as cancer, damage to dendrites consequently compromising synapses. The project is aimed at developing a technology that can address these issues and aims to make human DNA radioresistant. This study involves a nucleosome-binding protein called DSUP (Damage Suppressor Protein) unique to the tardigrade Ramazzottius Varieornatus and the subject of its radioresistance. It can theoretically safeguard genetic material damaged by radiation.

**Internship theme at the Pino Torinese Astronomical Observatory and the DISIT-UPO Environmental Molecular Toxicology Laboratory.*

<u>The study molecule:</u> Numerous tests have been carried out through the "in silico" approach geared toward mathematical modeling of its protein structure and complex mechanisms of action simulated through artificial intelligence systems, followed by direct laboratory analysis involving biosynthesis of DSUP by genetically modified bacteria and related tests. Both approaches,

applied synergistically, aim to make it accessible and useful for the protection of human health.

1. INTRODUCTION

The production of recombinant proteins in bacteria is a milestone in modern biotechnology. The ability to express foreign proteins in bacterial systems has revolutionized research, diagnostics and the production of therapeutic proteins. This technique will be applied in this Study in order to obtain the naturally unique protein "DSUP" (Damage Suppressor Protein) and proceed accordingly with laboratory analysis on the purified molecule. Recombinant protein production involves the introduction of foreign genes into a host organism. Bacteria, particularly *Escherichia coli* (E. coli), are used because of their rapid growth, well-characterized genetics and ease of manipulation (Rosano & Ceccarelli, 2014). *Gene* synthesis, or artificial synthesis of DNA sequences, offers an efficient and precise method to obtain optimized genes for recombinant protein expression in specific host systems, introduce desired mutations, and adapt sequences to facilitate protein purification. Such a technology is therefore highly advantageous for our purposes as it will allow the purified protein to be obtained solely from its amino acid sequence, available from NCBI databases, without necessarily having to have the tardigrade.

2. CASE STUDY

The proposed activity involves the study of a therapy capable of protecting human DNA from external damaging agents and making it radioresistant through the implementation of a special gene that carries the information necessary for the synthesis of a protein (DSUP - Damage SUppressor Protein), theoretically capable of safeguarding DNA from radiation and byproducts of radiation-environment interaction such as hydroxyl radicals(3,4,5,6,7) .

My "adventure" in physics began in May 2018 (actually, I started documenting physics topics at the age of about 8) when I presented the prototype of a nuclear fusion reactor I had designed for a science competition for secondary schools in which Prof. Paolo Trivero was on the jury, who, following the award ceremony, provided me with his contact information. Since then he has followed me on this path, supporting me with advanced lectures, contacts with the university world and fundamental advice. I learned a lot of knowledge and strategies and built a subatomic particle detector under his supervision when I was 13 years old. I also got into science popularization by actively participating in several editions of the "European Researchers' Night." From there, I never stopped studying to refine my knowledge on the subject, until I matured the multidisciplinary idea that would later turn into the "DSUP Project."

Some application hypotheses are based on the use of an adeno-associated virus as a vector and an endopeptidase called Imlifidase (IdeS): a protein capable of degrading IgG antibodies (immunoglobulin G). The organism that attracted attention for its radiotolerance capabilities was the tardigrade Ramazzottius Varieornatus(8,9,10,11) , a terrestrial invertebrate belonging to the Phylum Tardigrade of the family Eutardigrada that is extremely tolerant to extreme conditions such as irradiation, chemicals, dehydration and high pressure. This tolerance is believed to be related to the presence of the "Damage Suppressor" protein. DSUP is a unique protein of 42.8

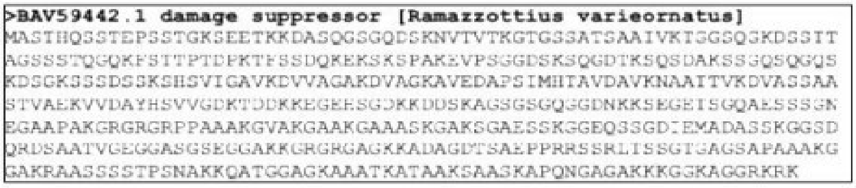
kDa that binds chromatin and has also conferred protection to heterologous systems, such as human, drosophila, and plant cells. Recently, it has been shown that it can create genomic instability in neurons(12) and thus must be modified or at least epigenetically regulated to achieve differential expression. The molecular functions of DSUP and its direct interactions with the physical and chemical agents against which it provides protection are virtually unknown. Its three-dimensional structure is unknown, which severely limits further studies.

Preliminary investigations have shown that the most convenient technique for studying the protein under investigation is the computational approach, aimed at reducing the variables involved by performing a theoretical analysis of the system, to be confirmed experimentally later in the laboratory. Laboratory(8,13,14) **.** A hybrid approach, known as quantum/molecular chemistry (QM/MM), can be used to overcome some of the challenges associated with the complexity of biological systems. In this approach, only the most critical or active regions are treated quantum, while the rest of the system is treated classically.

3. EXPERIMENTS AND RESULTS

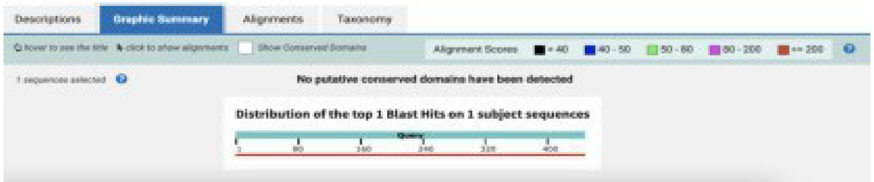
DSUP sequence database search

The damage suppressor (DSUP) protein of Ramazzottius Varieornatus has been identified in the National Center for Biotechnology Information (NCBI) protein database. The primary structure, i.e., amino acid sequence, is presented below:



```
>BAV59442.1 damage suppressor [Ramazzottius varieornatus]
MASTHQSSTEPSSTGKSEETKKDASQGSGQDSKNVTVTKGTGSSATSAAIVKTGGSQGKDSSIT
AGSSSTQGQKFSTTPTDPKTFSSDQKEKSKSPAKEVPSGGDSKSQGDTKSQSDAKSSGQSQGQS
KDSGKSSSDSSKSHSVIGAVKDVVAGAKDVAGKAVEDAPSIMHTAVDAVKNAAITVKDVASSAA
STVAEKVVDAYHSVVGDKTDDKKEGEHSGDKKDDSKAGSGSGQGGDNKKSEGETSGQAESSSGN
EGAAPAKGRGRGRPPAAAKGVAKGAAKGAAASKGAKSGAESSKGGEQSSGDIEMADASSKGGSD
QRDSAATVGEGGASGSEGGAKKGRGRGAGKKADAGDTSAEPPRRSSRLTSSGTGAGSAPAAAKG
GAKRAASSSSTPSNAKKQATGGAGKAAATKATAAKSAASKAPQNGAGAKKKGGKAGGRKRK
```

Using BLAST software, which identifies regions of similarity between biological sequences, I demonstrated that DSUP is a unique protein in nature, with no candidate orthologs in other organisms (Figure 1).

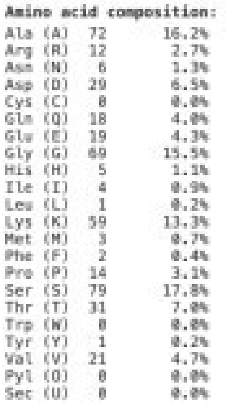


No putative conserved domains have been detected

Distribution of the top 1 Blast Hits on 1 subject sequences

Fig 1:Blast results for the DSUP protein of R.Varieornatus.

Using the ProtParam software package available from Expasy (https://web.expasy.org/cgi-bin/protparam/protparam), it was possible to deduce that the DSUP protein is rich in positively charged amino acid residues, such as arginine (Arg), lysine (Lys) and histidine (His). In addition, a protein with a dominant composition of alanine (Ala), glycine (Gly) and serine (Ser) might have interesting properties, although its functionality and structure depend on various factors such as sequence, folding, and interactions with other molecules. DSUP might have unique structural and functional characteristics. The predominance of Ala and Gly might suggest a high degree of flexibility or disorder, potentially involved in binding interactions or structural roles where flexibility is advantageous. In addition, the presence of Lys indicates the possibility of interactions with other molecules, particularly negatively charged molecules such as DNA. The presence of serine and threonine (Thr) suggests potential involvement in post-translational modifications or specific binding interactions due to its hydroxyl group due to the presence of hydroxyl (-OH) groups in their side chains that can form hydrogen bonds with other molecules. In the context of DNA interactions, serine and threonine are highly capable of forming bonds, contributing to the stability of the DNA-protein complex rather than providing specificity for particular base sequences.



```
Amino acid composition:
Ala (A)  72        16.2%
Arg (R)  12         2.7%
Asn (N)   6         1.3%
Asp (D)  29         6.5%
Cys (C)   0         0.0%
Gln (Q)  18         4.0%
Glu (E)  19         4.3%
Gly (G)  69        15.5%
His (H)   5         1.1%
Ile (I)   4         0.9%
Leu (L)   1         0.2%
Lys (K)  59        13.3%
Met (M)   3         0.7%
Phe (F)   2         0.4%
Pro (P)  14         3.1%
Ser (S)  79        17.8%
Thr (T)  31         7.0%
Trp (W)   0         0.0%
Tyr (Y)   1         0.2%
Val (V)  21         4.7%
Pyl (O)   0         0.0%
Sec (U)   0         0.0%
```

Figure 2. Amino acid composition of DSUP.

However, without knowing the structure and context in which this protein functions, it is difficult to determine its exact properties or functions because of its high dependence on context, environment and interactions with other molecules. Therefore, the 3D structure of DSUP was deduced using the alfafold algorithm2(15) . This implementation produces 5 structures for each protein, ranked from best to worst based on their average pLDDT for monomers (Table 1).

Table 1. Statistics of Alfafold2; Ranking of the model Mean pLDDT pTM

1	49.03	0.28
2	46.63	0.24
3	45.62	0.28
4	45.02	0.20
5	43.85	0.23

The pLDDT measures the confidence of the predicted protein structure. The overall value is less than 50, rather low, but high predictive scores (88-95) were observed in the region spanning amino acids 170-203, corresponding to the central portion of the protein, where two- or single-alpha helix domains could be assigned. In contrast, the remaining portion of the protein appears to adopt a random spiral conformation, indicative of structural unpredictability perhaps attributed to its high degree of flexibility. Predictions with low pTMs, in fact, typically indicate stochastically assigned residue patterns that show negligible or no correlation with the putative native structure. Alternatively, such predictions may suggest the presence of intrinsically disordered proteins.

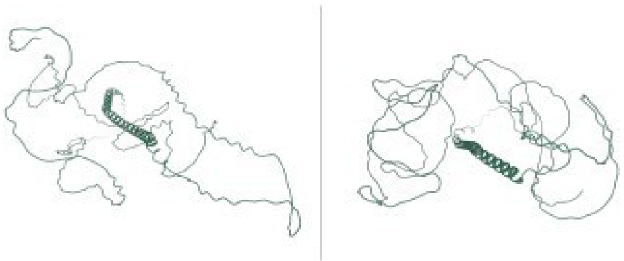


Figure 3. Structure of the DSUP. Shown are two alternative structures (No. 1 and No. 3) showing a central groove supported by a

Alpha-helix structure.

To infer the interaction with the nucleosome, molecular docking was carried out using the DSUP No.1 structure and the 1AOI crystal structure of the central nucleosome particle at 2.8 A. The 1AOI is a complex composed of histones (H3,H4,H2A,H2B) and a 146-bp-long DNA fragment (https://www.rcsb.org/structure/1aoi). **VinaDock**(16) , a generative diffusion model for computer simulation of protein interactions, was used for this purpose (Figure 4). The simulation showed multiple possible significant interactions.

Figure 4. DSUP/nucleosome interaction. Computer modeled docking analysis showing the interaction of DSUP with the 1AOI nucleosome complex.

In search of the mechanism of the DSUP

Obtaining the recombinant DSUP protein in E. coli is a significant step toward understanding its function and mode of action. A nucleosome-binding protein such as DSUP has the potential to protect DNA from ionizing radiation and other oxidative insults through several mechanisms: structural protection, facilitation of DNA repair, radical scapitoylation, histone modifications, and transcriptional regulation. Despite the paucity of data available in the literature, computer simulations have shown a high likewood of electrostatic interactions between DSUP and DNA(13) and there is growing evidence that DSUP is a protein that binds the nucleosomes(4,17) . Structural protection through nucleosome binding thus seems the most likely function of DSUP. Nucleosome binding may help to compact and organize DNA into chromatin structures. The tight packing of DNA within nucleosomes may provide physical protection by shielding DNA from direct exposure to ionizing radiation. To test this hypothesis, it is necessary to purify the DSUP protein to homogeneity and conduct further functional and structural studies. To this end, gene synthesis of a modified DSUP DNA sequence LC050827.1(18) was designed so that DSUP can be cloned in frame with the 5´leader of the bacteriophage T7 *10 gene* in the bacterial expression plasmid vector pET-20b(+). This recombinant *E. coli* expression system enables highly efficient transcription and translation of the recombinant DSUP protein under the control of an inducible promoter responsible for IPTG.

EXPRESSION

Fifty ml of BL21-Pro competent cells containing the TEV protease expression vector pRK603 were transformed with 100 ng of the DSUP expression vector pET20b(+) , laid on an agar plate containing ampicillin and kanamycin and incubated at 37°C overnight. The colonies were then placed in LB medium and grown to OD-600 nm 0.5, optimal conditions for the addition of IPTG at 30° (TEV protease working temp.in E.Coli). The treated culture was separated into two labeled Erlenmeyer flasks to which anhydrotetracycline was added in the first, leaving the second unaltered. After 2 hours of growth, the cells were centrifuged, lysed in an appropriate buffer using a VCX600 sonicator and treated with ?-mercaptoethanol i n order to obtain samples for SDS.Page. I loaded the buffers of each culture into the electrophoresis apparatus in adjacent lanes to allow easy evaluation of solubility and molecular weight and stained the proteins in the gel with GelCode Blue reagent. After obtaining confirmation regarding mini-induction, I transferred the drug-resistant cultures obtained from transformation into 4 l of LB medium and incubated according to the same directions until OD-600 nm of 0.5 was reached, added the inducer IPTG, pelleted and suspended in a suitable buffer of sodium phosphate, sodium chloride and imidazole containing complete protease inhibitor tablets without EDTA. Following this, the suspension was lysed using the APV-1000 homogenizer, centrifuged and filtered through a polyetherosulfone membrane suitable for removing residual particulate matter before chromatography. His6-TEV protease is added to the supernatant overnight by monitoring by SDS-PAGE, then diluted with phosphate buffer in order to collect the unbound fractions (flow through) passing through the Ni-NTA column. After checking the purity by SDS-PAGE I concentrated the protein sample about tenfold and applied the sample to a Hiprep 26/60 Sephacryl S-100 HR column equilibrated with gel filtration buffer, prepared 0.5-1 ml aliquots frozen with liquid nitrogen to proceed with the verification of the structural protection of DNA provided by nucleosome-binding proteins, which consists of assessing the susceptibility of DNA to ionizing radiation damage in the presence and absence of the protein.

NB: The various tests are carried out at the "Laboratory of Environmental Molecular Toxicology" of DISIT UPO in Alexandria, under the supervision of Prof. Francesco Dondero.

The following tasks were planned: First, prepare nucleosomes from a eukaryotic cell line (e.g., human HEK 293 cell line, ATCC CRL-1573). Alternatively, you can use a synthetic nucleosome and divide them into several samples, including control samples and expose both sets of samples to ionizing radiation under controlled conditions. After irradiation, assess the extent of nucleosomal DNA damage using:

Comet assay (single cell gel electrophoresis): to detect DNA strand breaks by measuring the extent of fragmented DNA migration in an agarose gel which reflects the level of damage to the double helix. DNA double-strand break (DSB) assay: DSBs can be detected by techniques such as immunofluorescence staining for γ-H2AX, a histone variant phosphorylated in

DNA damage response. DNA cleavage assays: Assess the integrity of nucleosomal DNA by subjecting it to digestion with enzymes of restriction followed by gel electrophoresis. Cleavage patterns and fragment sizes may indicate the extent of DNA damage. Quantification and comparison: Quantify DNA damage levels in the presence and absence of nucleosome-binding protein by measuring parameters such as comet tail length, γ-H2AX foci intensity, or DNA fragment size. Compare DNA damage levels between control and experimental samples to assess the protective effect of nucleosome-binding protein. By comparing the susceptibility of nucleosomal DNA to ionizing radiation damage in the presence and absence of nucleosome-binding protein, its ability to provide structural protection to DNA can be assessed. These assays provide valuable insights into the protective mechanisms employed by nucleosome-binding proteins to safeguard genomic integrity from radiation-induced DNA damage.

4. DISCUSSION

From the "classical point of view," DNA and proteins are treated as a viscous fluid because of the flexibility of their structure and interactions with their predominantly aqueous surroundings. Coupled then with an additional computational analysis methodology (10,13,14) of the system involving a quantum approach involving the description of electronic states and interactions within biological molecules. For proteins (DSUPs and histones), similar reasoning is followed based on density function theory (DFT), where the total energy of a system is expressed as a function of electron density(6) , from which detailed information is obtained about the electronic structure, interactions in the chemical bonds of amino acid side chains, and molecular vibrations in polypeptides. If DSUP has an enzymatic function, the quantum approach is essential to study its catalytic mechanisms. For example, catalysis enzymatic may involve quantum proton transfer and modulation of the electronic states of the molecules involved in the site active. Given the size of the biomolecules under study(19,20) , we extend this hybrid approach by modeling them, considering DNA in solution as a negatively charged colloid.

__Data representation__:

A problem arises related to the amount of data obtained, the reprocessing of which would be impossible even relying on large supercomputers (e.g., Leonardo Bologna). This analytical approach is complemented with other techniques aimed at reducing the computational reprocessing work, such as analyzing symmetry groups to represent symmetric transformations of proteins and applying fractals (geometric objects showing the same structure at different scales) to protein structure, providing important information about their organization(21,22) . The symmetries identified by the software are then verified through experimental analyses, such as advanced imaging techniques or mutagenesis experiments, X-ray crystallography, nuclear magnetic resonance (NMR), and AFM and STM microscope analyses. All the information obtained, now freed from superfluous and redundant data, can be analyzed by an Artificial Neural Network.

5. FUTURE APPLICATIONS

The versatility of the DSUP protein goes far beyond its role in "epic space adventures." In the field of health(23) emerges as a powerful ally against neurodegenerative diseases due to its link to oxidative stress, promising innovative solutions to counter the progression of such diseases. Its ability to protect DNA during radiation therapy and PET treatments suggests revolutionary applications, offering a molecular shield that preserves patients' health. In addition, DSUP acts as a guardian of genetic heritage, defending DNA from environmental pollutants(24) , opening promising scenarios for safeguarding our health in an ever-changing world.

Research in the laboratory is proceeding through the planning of synchrotron tests on the purified protein and structural analyses by cryo-em microscopy that should take place in the short term.

Microgravity tests on the protein expressed by the Space Rider platform or cubesat mission have also been planned through the INAF OATo.

NB: The lack of substantial funds ant the unexistent support from the High-School have slowed down the progress regarding research.

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With gratitude Alexandria,

June 25, 2024

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【評語】080016

Advantages:

1. Development of radio-resistance strategy is very important for prevention of normal cells damage during treatment of cancers.
2. This is a novel idea.

Comments and suggestions:

1. There are very few experimental results reported in the Experimental and Results sections.
2. Effects of radiation on the bioactive conformation of DSUP could be further examined by CD for detecting ang changes of the secondary conformation of DSUP.
3. They claimed they have expressed DSUP in E. coli and purified the recombinant protein using NiNTA and size-exclusion columns. Then they will verify the structural protection of DNA provided by DUSP. However, no data are shown for the protein purification and the following tasks for DNA protection effect.
4. Even thought DUSP really can protect DNA from radiation damage, how will they apply the protein into human cell nucleus to protect DNA? If they want to knock in the gene, there are many concerns for the human clinical trial.