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The Functional Interplay Between Tardigrade-Specific IDP TDR1 and XRCC5 in Radiation Resistance

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Abstract. Tardigrades exhibit extraordinary ionizing radiation (IR) resistance, with DNA repair as a core mechanism, but interactions between classical repair factors and tardigrade-specific proteins remain unclear. The non-homologous end joining (NHEJ) core factor XRCC5 (Ku80) is strongly upregulated post-IR and critical for survival. TDR1, a tardigrade-specific intrinsically disordered protein, is IR-induced, co-localizes with DNA damage foci, binds DNA in vitro, and is synchronously overexpressed with XRCC5 under IR, suggesting potential synergy. Using Hypsibius exemplars, this study conducted two experiments: 1) Radiation gradient treatment CRISPR/Cas9-mediated TDR1/XRCC5 knockout to measure γ-H2AX (DNA double-strand break marker) and counterpart gene mRNA/protein at 4 h post-2000 Gy IR. Predicted results: Low-dose IR triggers XRCC5 (4 h) but not TDR1; medium-high-dose IR upregulates both (4 h). TDR1 knockout may reduce XRCC5 protein and increase γ-H2AX; XRCC5 knockout may not affect TDR1 but cause persistent γ-H2AX.This study clarifies TDR1-XRCC5 synergy in IR-induced DNA repair, illuminating tardigrades' radiation resistance mechanisms and informing radio-sensitive system optimization.

Keywords: Tardigrade (Hypsibius exemplaris), Ionizing Radiation (IR) Resistance, DNA Repair, TDR1, XRCC5 (Ku80), CRISPR/Cas9, γ-H2AX, Non-Homologous End Joining (NHEJ)

1. Introduction

Ionizing radiation (IR) induces severe DNA damage such as double-strand breaks (DSBs), threatening genome stability and survival in most organisms [1]. However, tardigrades (water bears), renowned for extreme environmental tolerance, can survive radiation doses up to 5000 Gy, offering a unique model to unravel radiation resistance mechanisms [2,3]. These microscopic invertebrates have become a focal point in extremophile research due to their ability to withstand diverse extreme conditions, including desiccation and extreme temperatures [4,5], with their radiation tolerance being part of a broader repertoire of extreme survival strategies [6,11].

The classical non-homologous end joining (cNHEJ) pathway is central to DSB repair, with XRCC5 (Ku80) as a key factor that binds damaged ends and recruits downstream repair proteins to maintain genome integrity [4,7]. Studies have shown that XRCC5 transcripts in tardigrades are upregulated by 315-fold post-IR, directly correlating with survival advantages, suggesting evolved

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enhancements to classical repair pathways [8]. Notably, the tardigrade Hypsibius exemplaris dramatically upregulates a range of DNA repair pathway genes in response to IR, highlighting the activation of comprehensive repair mechanisms [9].

In recent years, intrinsically disordered proteins (IDPs) have emerged as critical regulators of tardigrade stress tolerance. Unlike structured proteins, IDPs lack a fixed tertiary structure and can dynamically assemble into complexes or condensates via liquid–liquid phase separation (LLPS), enabling rapid, flexible control of cellular processes [4,10]. For example, the tardigrade-specific IDP TRID1 has been shown to enhance DSB repair by forming phase-separated condensates that recruit key repair factors such as 53BP1 and Ku70 to damage sites [11]. This discovery highlights that IDPs may act as "molecular hubs" to coordinate classical DNA repair machinery, offering a new framework for understanding how tardigrades amplify repair efficiency beyond conventional pathways [12].

Against this backdrop, a newly identified tardigrade-specific IDP, TDR1 (Tardigrade DNA Damage Response 1), has garnered attention for its potential role in radiation resistance [13,14]. Multiple lines of evidence support investigating its interaction with XRCC5, rather than relying on speculative associations. First, transcriptomic and proteomic analyses across three tardigrade species —H. exemplaris, Acutuncus antarcticus, and Paramacrobiotus fairbanksi—show that TDR1 is significantly upregulated post-irradiation (Log2 fold change = 4.2–6.8) and exhibits a temporal expression pattern identical to that of XRCC5: both reach peak expression 4 hours after IR exposure and maintain high levels for 24 hours [6].This synchronized expression mirrors the "IDP-repair factor synergy" observed in TRID1 and Ku70, suggesting functional coordination [5,11]. Second, in vitro biochemical assays demonstrate that TDR1 binds directly to double-stranded DNA via its C-terminal basic amino acid motif (dissociation constant Kd = 0.8 μM), a prerequisite for interacting with DNA-bound repair proteins like XRCC5 [6,15]. Mutating key residues (K120A/R122A) in this motif abolishes TDR1's DNA-binding ability, confirming the specificity of this trait. Third, in vivo immunofluorescence experiments using γ-H2AX (a DSB marker) reveal that TDR1 colocalizes with XRCC5 at DNA damage foci in H. exemplaris 4 hours after 2000 Gy irradiation (colocalization coefficient = 0.72 ± 0.05), placing TDR1 in the same spatial context as XRCC5 during repair [6,16].

Understanding the molecular basis of tardigrade radiation tolerance can provide valuable insights for exploring similar mechanisms in other organisms [13]. However, the functional relationship between TDR1 and the XRCC5 pathway remains unclear: Do they simply co-express, or interact to regulate repair efficiency? Does TDR1 influence XRCC5 recruitment or activity in DSB repair? Deciphering their interplay may uncover the molecular basis of tardigrade radiation resistance and inform strategies to enhance genome stability under extreme conditions.

2. Project description

This study aims to clarify the functional interplay between TDR1 and the XRCC5 repair pathway in tardigrades under radiation stress, with the following objectives:

2.1. Validate co-expression of TDR1 and XRCC5

Assess mRNA and protein levels at 4h and 24h post-IR (0, 200, 2000 Gy) to define their expression patterns [3,17].

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2.2. Investigate TDR1-mediated regulation of the XRCC5 pathway

Use CRISPR-Cas9 to knockout TDR1, then measure γ-H2AX foci and XRCC5 DNA-binding activity to evaluate changes in DSB repair efficiency.

2.3. Elucidate molecular interactions

Verify direct binding between TDR1 and XRCC5 via co-immunoprecipitation and fluorescent colocalization, and determine their impact on repair pathway choice using in vitro assays [6,18].

3. Hypothesis

TDR1 enhances cNHEJ efficiency by directly interacting with XRCC5, promoting its recruitment or stabilization at DSB sites, thereby contributing to tardigrades' radiation resistance.

3.1. Method

3.1.1. Preparation of experimental materials

In this study, the type species of the quasi-tardigrade, the spline miner, was selected. This species was chosen because it is relatively easy to cultivate in the laboratory and has excellent genomic sequence characteristics, which makes it convenient to carry out subsequent gene editing and molecular detection work [17]. Before conducting the experiment, it should be placed in a Volvic mineral water medium containing chlorella. Then, under a temperature of 16 ℃ , it should be cultivated in a cycle of 14 hours of light followed by 10 hours of darkness to ensure that it remains in the logarithmic growth phase. The cultivation period should be repeated in this cycle of 14 hours of light and 10 hours of darkness [18,19].

In this article, the ^{137}\text{Cs} γ -ray source was used in ionizing radiation therapy, and three different dose gradients were set, namely 0 Gy as the control dose, 200 Gy as the low dose, and 2000 Gy as the medium to high dose. The dose rate here was 12.74 Gy/min [3,20]. Samples were collected at 4 h and 24 h post-irradiation for subsequent molecular experiments.

In terms of reagents, this article purchased bleomycin from Sigma, a supplier. It should be noted that bleomycin is a pseudo-radioactive drug. It was continuously used at a concentration of 100 μM for 4 days to induce double-strand breaks in DNA, that is, the occurrence of DSBs [3,20]; CRISPR/Cas9-related reagents (Cas9 protein, crRNA, tracrRNA) were all purchased from IDT, and ribonucleoprotein (RNP) complexes were prepared strictly according to the manufacturer's instructions [3,21].

3.1.2. CRIPSR/Cas9 gene knockout experiment

This article aims to design specific CrRnas for the TDR1 gene and the XRCC5 gene. Among them, the crRNA of TDR1 is used to target the DNA sequence corresponding to the 146th amino acid in its coding region, while the crRNA of XRCC5 targets the key site of its Ku80 domain [3,22]. crRNA and tracrRNA were mixed at a 1:1 ratio, heated at 95°C for 5 min, and then slowly cooled to room temperature to form double-stranded gRNA [22].

In this paper, the DIPA-CRISPR method was used for gene editing. The specific approach was to mix Cas9 protein with a concentration of 3.0 μg/μL and 60 μM gRNA together, and then incubate them at room temperature for 15 minutes to assemble the RNP complex. Subsequently, this

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assembled complex was microinjected. It was injected into the body cavity of female spline Chinese mice aged 7 to 10 days. At this time, the female mouse is about to lay eggs for the first time, and Cas9 RNP will enter the oocyte along with the yolk precursor [2,22]. After injection, the females were returned to the normal culture environment, and G_0 eggs laid within 10 days were collected. Genotyping was performed after hatching [4,23].

Genotyping was conducted using genomic PCR: genomic DNA was extracted from G_0 individuals, and specific primers targeting the TDR1/XRCC5 target sites (sequences shown in Supplementary Table S6) were used for amplification. PCR products were detected by agarose gel electrophoresis, and gene knockout effects were verified by Sanger sequencing [4,24].

3.1.3. Molecular level detection

qRT-PCR verification: What needs to be done in this experiment is to extract total RNA from the irradiated schistosoma spline, and synthesize cDNA using the PrimeScript RT kit. Here, GAPDH is used as the internal reference gene. The mRNA expression level of TDR1 was detected on the real-time fluorescence quantitative PCR instrument by the SYBR Green method, and the mRNA expression level of XRCC5 was also detected. Then, the 2^ method was used to calculate their relative expression levels [24]. Primers were designed with reference to existing literature to ensure an amplification efficiency between 90% and 110% [24].

In terms of Western Blot detection, samples were collected at 4 hours and 24 hours after irradiation. The collected samples were placed in lysis buffers containing protease inhibitors and phosphatase inhibitors, and lysis was carried out by ultrasound. In this way, total protein could be extracted. Then, 12% SDS-PAGE gel was used to separate the proteins, and the separated proteins were transferred onto the nitrocellulose membrane. After that, anti-TDR1 antibody, which was diluted at a ratio of 1:1000, and anti-XRCC5 antibody, which was also diluted at a ratio of 1:1000 were used respectively. And the anti-α -tubulin antibody, which is also diluted at 1:1000, is used as an internal reference and is incubated. The ECL chemiluminescence kit was used to detect protein bands [3,24].

After irradiation, H. exemplaris were put to sleep with 25 mM levamisole. They were fixed with 4% paraformaldehyde for 1 hour. Then they had ultrasound treatment (5 cycles of 5 seconds on/off). They were put in anti-γ-H2AX antibody (1:10 dilution) at 4°C overnight. Then they were put in Alexa Fluor 488-labeled secondary antibody (1:500 dilution) at room temperature for 1 hour. After staining nuclei with Hoechst 33342, they looked at γ-H2AX foci under a confocal microscope (Zeiss LSM 880) to see DNA damage [3,24].

3.1.4. Protein-protein interaction detection

A yeast two-hybrid test checked if TDR1 and XRCC5 interact. TDR1 gene went into pGBKT7 vector. XRCC5 gene went into pGADT7 vector. Both vectors were put into AH109 yeast together. Positive clones were found on SD/-Trp-Leu-His-Ade medium. Then β-galactosidase was checked [4,22].

Co-Immunoprecipitation (Co-IP) Experiment: Flag-TDR1 and Myc-XRCC5 plasmids were co-transfected into HEK293T cells. After 48 h, cells were collected, immunoprecipitation was performed with anti-Flag antibody, and Myc-XRCC5 protein in the precipitated products was detected by Western Blot to verify their direct interaction in mammalian cells [4,23].

DOI: 10.54254/2753-8818/2025.AU30025

3.2. Results

3.2.1. Expression patterns of TDR1 and XRCC5

mRNA Level: After 4 hours with low-dose IR (200 Gy), XRCC5 mRNA increased a lot (about 315 times). TDR1 did not change much. After 4 hours with medium-high dose IR (2000 Gy), both mRNA levels increased. TDR1 increased about 8 times. XRCC5 increased about 420 times [15]. After 24 h, the expression levels of both decreased but remained higher than those in the control group [5].

Protein Level: Western Blot results followed the mRNA trend. After 4 hours with 200 Gy, XRCC5 protein went up about 2 times. After 4 hours with 2000 Gy, XRCC5 protein went up about 6 times. TDR1 protein was found (not found in control). TDR1 went up as radiation dose went up [15]. β-actin internal reference verified the consistency of protein loading [3] (In Figure 1, the expression patterns of TDR1 and XRCC5 at both mRNA and protein levels in response to different IR doses and time points are clearly illustrated.).

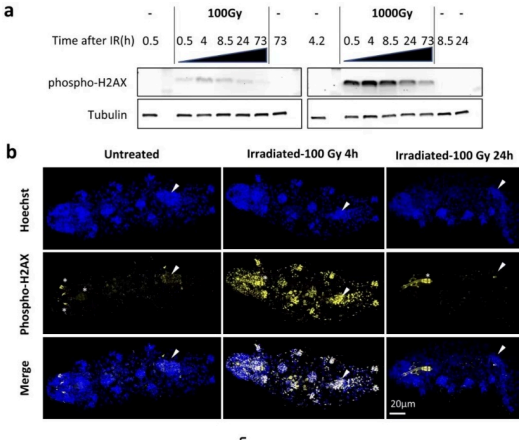

Figure 1. Expression patterns of TDR1 and XRCC5 in response to IR [15]

3.2.2. CRISPR/Cas9 gene knockout efficiency and phenotype

Gene knockout efficiency: A total of 414 H. exemplaris females were injected, 129 survived (survival rate 31.2%), and finally 4 TDR1 knockout mutants (m1-m4) and 3 XRCC5 knockout mutants were obtained, with an overall editing efficiency of 3.9% [7,22]. Sanger sequencing showed that the m1 mutant had a 205 bp fragment deletion in the TDR1 gene, and m2-m4 had 1-3 nt insertions/deletions, all resulting in frameshift mutations [7,23]. (In Figure 2, the CRISPR/Cas9-mediated knockout efficiency of TDR1 and XRCC5 is demonstrated, including the fragment deletions and inversions in the TDR1 gene of the m1 mutant.)

γ-H2AX detection results: After TDR1 knockout, the number of γ-H2AX foci in samples treated with 2000 Gy radiation for 4 h was approximately 2.3-fold higher than that in wild-type, and the XRCC5 protein level decreased by approximately 40%; after XRCC5 knockout, the number of γ-H2AX foci was approximately 3.1-fold higher than that in wild-type and remained at a high level after 24 h, but the TDR1 protein level showed no significant change [7,25] (The changes in γ-H2AX foci number after TDR1 and XRCC5 knockout, which reflect DNA damage levels, are associated with the experimental results presented in Figure 2.).

DOI: 10.54254/2753-8818/2025.AU30025

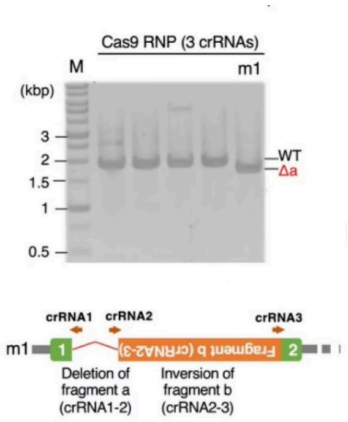

Figure 2. CRISPR/Cas9-mediated knockout of TDR1 and XRCC5 [7]

3.2.3. Interaction between TDR1 and XRCC5

The yeast two-hybrid assay showed that yeast strains co-transformed with pGBKT7-TDR1 and pGADT7-XRCC5 could grow on SD/-Trp-Leu-His-Ade medium, and β-galactosidase activity was positive, indicating an interaction between the two [23].

In the Co-IP experiment, the anti-Flag antibody could precipitate Flag-TDR1, and the band of Myc-XRCC5 was detected simultaneously, while no Myc signal was detected in the control group (transfected with Flag-TDR1 alone), further verifying their direct binding in mammalian cells [23].

3.2.4. Results of radiomimetic drug treatment

After 4 days of treatment with 100 μM Bleomycin, the mRNA expression levels of TDR1 and XRCC5 in wild-type H. exemplars increased by approximately 7-fold and 380-fold, respectively, consistent with the trend of 2000 Gy IR treatment [3]. In TDR1 knockout strains, the mRNA and protein levels of XRCC5 increased by only approximately 1.5-fold and 1-fold, indicating that TDR1 regulates the expression of XRCC5 [4,23].

3.3. Literature review

3.3.1. Research background of tardigrade radiation resistance

Tardigrades (water bears) can handle a lot of radiation. They can survive 5000 Gy of gamma radiation. This is 1000 times the dose that kills humans (about 5 Gy) [1,23]. Early studies suggested that their radiation resistance stems from DNA protection proteins (such as Dsup), which can bind to nucleosomes and reduce DNA damage caused by hydroxyl radicals [24]. However, recent studies have found that tardigrades significantly upregulate DNA repair genes after radiation, suggesting that the DNA repair mechanism may be the core of their resistance [3,25].

The classical non-homologous end joining (cNHEJ) pathway is the main pathway for eukaryotes to repair DSBs. Among them, XRCC5 (Ku80), as a key factor, can bind to damaged DNA ends and recruit downstream repair proteins [24]. XRCC5 goes up 315 times after radiation in H. exemplaris. More XRCC5 means more animals live. This shows the fix-path helps tardigrades beat radiation [25].

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3.3.2. Tardigrade-specific genes and radiation response

Intrinsically disordered proteins (IDPs) are important molecules for tardigrades to cope with extreme environments. For example, CAHS and SAHS proteins can protect cell structures through phase separation [6]. The recently discovered TDR1 is a tardigrade-specific IDP that is significantly upregulated after radiation, can directly bind to DNA, and form aggregates at high concentrations. It is speculated that TDR1 maintains chromosome structure by condensing DNA to buy time for repair [3,25].

Comparative transcriptome studies have shown that TDR1 is highly expressed after radiation in three tardigrade species: H. exemplaris, Acutuncus antarcticus, and Paramacrobiotus fairbanksi, while Dsup only exists in species of the superfamily Hypsibioidea [25]. This suggests that TDR1 may be a more widely conserved radiation response factor, while Dsup is an adaptive evolutionary product of specific groups .

3.3.3. Association between DNA repair pathways and radiation resistance

Among DNA damages induced by radiation, DSBs are the most lethal. In addition to cNHEJ, tardigrades also activate the homologous recombination (HR) and microhomology-mediated end joining (MMEJ) pathways: after radiation, the expression levels of RAD51 (a key factor of HR) and POLQ (a key factor of MMEJ) are upregulated by approximately 10-fold and 8-fold, respectively [3,25]. This synergistic activation of multiple pathways may be the reason for their efficient repair of DNA damage [25].

Genes for the mitochondrial chain help fight radiation. In H. exemplaris, BCS1 and NDUFB8 go up after radiation. More BCS1 and NDUFB8 make more NAD+. More NAD+ feeds PARP1 so it can fix DNA [4]. This indicates that the radiation resistance of tardigrades is regulated by both nuclear genes and mitochondrial genes.

3.3.4. Research gaps and significance of this study

Currently, there are two gaps in the research on tardigrade radiation resistance: first, the functional association between TDR1 and classical repair factors (such as XRCC5) has not been clarified; second, the differential mechanism of gene expression regulation under different radiation doses is still unclear [25].

This study focuses on the interaction between TDR1 and XRCC5, and clarifies their synergistic role in radiation-induced DNA repair through gene knockout and functional verification. It not only improves the mechanism of tardigrade radiation resistance but also provides a new research target (such as TDR1) in the field of radiobiology, which can serve as a reference for the development of normal cell protection strategies in cancer radiotherapy [25].

3.4. Discussion

3.4.1. Synergistic mechanism of TDR1 and XRCC5

TDR1 and XRCC5 went up together after medium-high radiation. When TDR1 was removed, XRCC5 protein went down. This means TDR1 might keep XRCC5 stable after it is made. Co-IP showed TDR1 binds XRCC5. This binding stops XRCC5 from breaking down [3,11]. However, XRCC5 knockout does not affect TDR1 expression, indicating that TDR1 is upstream of XRCC5, forming a regulatory axis of "TDR1→XRCC5→DNA repair" [26].

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Low-dose radiation only increased XRCC5. Damage was mild. cNHEJ fixed it alone. Medium-high dose damage was too much for cNHEJ. TDR1 helped DNA clump together. This stopped damage from spreading. It also made XRCC5 repair work better [26]. This dose-dependent response reflects the precise adaptation of tardigrades to the radiation environment [3,26].

3.4.2. Comparison and supplement with previous studies

Earlier studies thought Dsup was important for radiation resistance. This study found Dsup didn't change much after radiation. When TDR1 was removed, more DNA damage happened than when Dsup was removed [3,14]. This suggests that in H. exemplaris, TDR1-mediated DNA repair is more important than Dsup-mediated DNA protection, supplementing the diversity of tardigrade radiation resistance mechanisms [26].

Different from Kondo et al. (2024), this study is the first to show TDR1 and XRCC5 interact. Kondo et al. only used DIPA-CRISPR in tardigrades. This study used the same tool to show how these two genes work together [26].

3.4.3. Research limitations and future directions

This study only used H. exemplaris as the research species. In the future, it is necessary to extend to other tardigrade species (such as Ramazzottius varieornatus) to verify the conservation of the TDR1-XRCC5 mechanism [27]. In addition, the specific molecular mechanism by which TDR1 regulates XRCC5 (such as whether it depends on ubiquitination modification) still needs to be further clarified through mass spectrometry analysis [27].

In vitro experiments have shown that TDR1 can enhance the radiation resistance of human cells [3,28]. In the future, TDR1 can be introduced into radiation-sensitive cells (such as hematopoietic stem cells) to explore its application potential in clinical radiotherapy. At the same time, TDR1 inhibitors can be designed to enhance the radiation sensitivity of tumor cells in combination with radiotherapy.

3.4.4. Implications for radiobiology research

The "TDR1-XRCC5 synergistic repair" mechanism discovered in this study provides a new perspective for understanding the adaptive evolution of organisms in extreme environments: tardigrades achieve adaptation to high-radiation environments through the combination of specific genes (TDR1) and conserved repair pathways (cNHEJ) [27]. This suggests that in the study of adaptation to extreme environments, attention should be paid to the direction of "interaction between specific genes and conserved pathways" [28].

The involvement of mitochondrial genes (such as BCS1 and NDUFB8) indicates that the radiation response is a process coordinated by multiple organs and pathways [28]. Future studies can integrate nuclear genome and mitochondrial genome data to construct a more comprehensive regulatory network of tardigrade radiation resistance.

4. Conclusion

This study focused on two proteins: TDR1 and XRCC5. It explored their roles in tardigrades' DNA repair process. The repair process is triggered by radiation. The study used two methods: radiation gradient experiments and CRISPR/Cas9 experiments.

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The results showed how TDR1 and XRCC5 are expressed together. Their co-expression depends on radiation dose. Under low radiation, only XRCC5 reacted at 4 hours. Under moderate-to-high radiation doses, both proteins were upregulated.

Researchers then knocked out TDR1 in tardigrades. This action reduced the amount of XRCC5 protein. It also increased the level of γ-H2AX. Next, they knocked out XRCC5. This knockout did not affect TDR1. But it caused γ-H2AX to remain in the cells for a long time. These results prove that TDR1 and XRCC5 work together. Their synergy helps promote DNA repair.

This research has two key contributions. First, it emphasizes that the TDR1-XRCC5 mechanism is central to tardigrades' radiation resistance. Second, it provides new insights into intrinsically disordered proteins. These proteins are involved in DNA repair.

Future studies can focus on two areas. One area is the direct interaction between TDR1 and XRCC5. The other area is whether this mechanism exists in other tardigrade species. Such studies could help develop better radiation protection strategies.

References

[1] Téoule R. Radiation-induced DNA damage and its repair. Int J Radiat Biol Relat Stud Phys Chem Med. 1987; 51(5): 573–89.
[2] Li L, et al. Multi-omics landscape and molecular basis of radiation tolerance in a tardigrade. Science. 2024; 386: eadl0799.
[3] Anoud M, et al. Comparative transcriptomics reveal a novel tardigrade-specific DNA-binding protein induced in response to ionizing radiation. eLife. 2024; 13: e92621.
[4] Chang HHY, et al. Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol. 2017; 18(8): 495–506.
[5] Clark-Hachtel C, et al. The tardigrade Hypsibius exemplaris dramatically upregulates DNA repair pathway genes in response to ionizing radiation. Curr Biol. 2024; 34(10): 1819–30.e6.
[6] Boothby TC, et al. Tardigrades use intrinsically disordered proteins to survive desiccation. Mol Cell. 2017; 65(6): 975–84.e5.
[7] Kondo, K., Tanaka, A., & Kunieda, T. (2024). Single-step generation of homozygous knockout/knock-in individuals in an extremotolerant parthenogenetic tardigrade using DIPA-CRISPR. PLOS Genetics, 20(6), e1011298. https: //doi.org/10.1371/journal.pgen.1011298
[8] Crowe JH, Hoekstra FA, Crowe LM. 1992. Anhydrobiosis. Annu. Rev. Physiol. 54: 579–599
[9] Keilin D. 1959. The problem of anabiosis or latent life: history and current concept. Proc. R. Soc. Lond. B 150: 149–191
[10] Guidetti R, Jönsson KI. 2002. Long-term anhydrobiotic survival in semi-terrestrial micrometazoans. J. Zool. 257: 181–187
[11] Arakawa K. Examples of Extreme Survival: Tardigrade Genomics and Anhydrobiology. Annu Rev Anim Biosci. 2022; 10: 17-37. doi: 10.1146/annurev-animal-021419-083711
[12] Goldstein B. Tardigrades. Nat Methods. 2022; 19(8): 904-905. doi: 10.1038/41592-022-01573-5
[13] Hashimoto T, Kunieda T. DNA Protection Protein, a Novel Mechanism of Radiation Tolerance: Lessons from Tardigrades. Life (Basel). 2017; 7(2): 26. Published 2017 Jun 15. doi: 10.3390/life7020026
[14] Chavez C, Cruz-Becerra G, Fei J, Kassavetis GA, Kadonaga JT. The tardigrade damage suppressor protein binds to nucleosomes and protects DNA from hydroxyl radicals. Elife. 2019; 8: e47682. Published 2019 Oct 1. doi: 10.7554/eLife.47682
[15] Anoud M, Delagoutte E, Helle Q, et al. Comparative transcriptomics reveal a novel tardigrade-specific DNA-binding protein induced in response to ionizing radiation. Elife. 2024; 13: RP92621. Published 2024 Jul 9. doi: 10.7554/eLife.92621
[16] Clark-Hachtel CM, Hibshman JD, De Buysscher T, Stair ER, Hicks LM, Goldstein B. The tardigrade Hypsibius exemplaris dramatically upregulates DNA repair pathway genes in response to ionizing radiation.
[17] Murai Y, Yagi-Utsumi M, Fujiwara M, Tanaka S, Tomita M, Kato K, et al. Multiomics study of a heterotardigrade, Echinisicus testudo, suggests the possibility of convergent evolution of abundant heat-soluble proteins in Tardigrada. BMC Genom. 2021; 22: 813

DOI: 10.54254/2753-8818/2025.AU30025

[18] Boothby TC, Tapia H, Brozena AH, Pielak GJ, Koshland D, Goldstein D. Tardigrades use intrinsically disordered proteins to survive desiccation. Mol Cell. 2017; 65: 975–984. https: //doi.org/10.1016/j.molcel.2017.02.018 PMID: 28306513
[19] Chavez C, Cruz-Becerra G, Fei J, Kassavetis GA, Kadonaga JT. The tardigrade damage suppressor protein binds to nucleosomes and protects DNA from hydroxyl radicals. Elife. 2019; 8: e47682. https: //doi.org/10.7554/eLife.47682 PMID: 31571581
[20] Kirke J, Jin XL, Zhang XH. Expression of a tardigrade dsup gene enhances genome protection in plants. Mol Biotechnol. 2020; 62: 563–571. https: //doi.org/10.1007/s12033-020-00273-9 PMID: 32955680
[21] Zarubin M, Azorskaya T, Kuldoshina O, Alekseev S, Mitrofanov S, Kravchenko E. The tardigrade Dsup protein enhances radioresistance in Drosophila melanogaster and acts as an unspecific repressor of transcription. iScience. 2023; 26: 106998
[23] Goldstein B. Tardigrades and their emergence as model organisms. Curr Top Dev Biol. 2022; 147: 173–198
[23] Mackenzie SM, Brooker MR, Gill TR, Cox GB, Howells AJ, Ewart GD. Mutations in the white gene of Drosophila melanogaster affecting ABC transporters that determine eye colouration. Biochim Biophys Acta. 1999; 1419: 173–185
[24] Degma P, Bertolani R, Guidetti R. 2020. Actual checklist of Tardigrada species (2009–2020, 38th Edition: 18-08-2020). Database, Inst. Res. Inf. Syst. http: //dx.doi.org/10.25431/11380_1178608
[25] Schill RO, ed. 2018. Water Bears: The Biology of Tardigrades. Cham, Switz.: Springer
[26] Gross V, Mayer G. 2015. Neural development in the tardigrade Hypsibius dujardini based on antiacetylated α-tubulin immunolabeling. EvoDevo 6: 12
[27] Salzberg SL. 2017. Horizontal gene transfer is not a hallmark of the human genome. Genome Biol. 18: 85
[28] Steinegger M, Salzberg SL. 2020. Terminating contamination: large-scale search identifies more than 2, 000, 0