

Diverse Mechanisms of Radiation Tolerance: Insights from Flatworms and Tardigrades

Hengzhi Liu

Guangzhou Dublin International College, South China Agricultural University, Guangzhou, China
Gigi_liuhz@outlook.com

Abstract. High levels of ionizing radiation represent a great danger to living cells. These damages can manifest in various ways, the most notable being the substantial harm it causes to biological DNA; in addition, the potential of ionizing radiation to impair cellular function is also of considerable concern. However, there are some organisms which show unusual resilience to harsh environments. Although many studies have been conducted on such organisms, the exact molecular mechanisms of their unusual tolerance are not entirely known, especially those associated with stem cell activity and tissue regeneration. Exploring the principles and evolutionary processes of these radiation-resistant mechanisms is crucial for studying the effects of radiation on living organisms. This review describes three model organisms that use different approaches to show various survival and adaptation patterns to irradiation with a high dose of radiation. These mechanisms include improved DNA repair and antioxidant systems, dispersed proliferation and tissue-specific radiation tolerance. Moreover, possible medical application of these unique mechanisms is considered.

Keywords: Radiation tolerance, DNA damage response, *Macrostomum lignano*, *Tardigrada*, *Stenostomum brevipharyngium*

1. Introduction

1.1. Ionizing radiation

Ionizing radiation refers to particles or electromagnetic waves with enough energy to ionize atoms or molecules by displacing electrons from their orbitals by a process called ionization, caused various damages including DNA double-strand breaks (DSBs), reactive oxygen species (ROS) increase, oxidative DNA damage, autophagy, apoptosis, senescence, cell cycle changes, functional defects and epigenetic modifications. Ionizing radiation can be divided into high doses of irradiation (HDIR) and low doses of irradiation (LDIR), and shows different effects in damages. In addition, research indicates that different stem cell populations exhibit vastly different radio-sensitivities based on their tissue of origin and physiological roles. Hematopoietic stem cells are relatively radioresistant and can repopulate damaged bone marrow following moderate exposure, whereas intestinal stem cells are highly sensitive; doses exceeding 14 Gy in mice lead to gastrointestinal syndrome and death within 7 to 12 days due to the rapid turnover of the small intestinal mucosa [1]. Skin stem cells display an intermediate sensitivity, highlighting a spectrum of tissue maintenance strategies where

some tissues favor the survival of rare stem cells while others prioritize the elimination of genomically compromised cells [1]. The differential effects of HDIR and LDIR on hematopoietic stem cells have been further characterized [2].

Table 1. Comparison of different kinds of damage in hematopoietic stem cells caused by HDIR and LDIR [2]

Damage type	HDIR	LDIR
DNA DSB	Yes [3, 4]	High LET: yes [5, 6] Low LET: no [7, 8]
ROS increase	Yes [3, 9]	Yes [5-8]
Oxidative DNA damage	Yes [3, 9]	Yes (human) [7]
Autophagy	Yes [10]	Yes (mouse) [8]
Apoptosis	Yes [4, 11, 12]	No [7, 8]
Senescence	Yes [13]	Unknown
Cell cycle changes	Yes [11]	No [7, 8]
Functional defects	Immediate [4, 9]	Mouse: immediate + delayed [5, 6, 8] human: delayed [7]
Epigenetic modifications	Yes [14]	High LET: yes [15] Low LET: unknown but probable

The scientific understanding of radiation effects is a constantly evolving field, given the different ways in which it can be expressed, which is mentioned in table 1. At present, effects of HDIR are quite well characterized, for example, in humans, the hematopoietic system which after exposure to irradiation, changes in blood composition might be evidenced with irradiation doses as low as 1 Gy. Deaths start from 2 Gy and are inevitable after 7 Gy in a time period between 4 to 6 weeks owing to hematological failure [2]. In the case of HDIR, it has always been claimed that irradiation causes either linear dose dependence or threshold dependence, where DNA damage plays an important role. However, there have been doubts related to the effects of low dose irradiation (LDIR) with no consensus on what constitutes a low dose; doses lower than 500 mGy, and sometimes even up to 1 Gy, are considered as low doses. Recently, there has been growing apprehension related to the dangers of LDIR (under the threshold which is considered harmful and therefore used for medical imaging, for instance). This comes in view of the increased incidence of leukemia and brain tumors among children with multiple CT scans, where the doses received were from 0 to 50 mGy in bone marrow (BM) and 0 to 350 mGy in the brain [16, 17], which require further research of not only ionizing radiation itself, but also irradiation-resistance creatures, since mammals typically exhibit a lethal dose for 50% of the population (LD50) between 2.5 and 5 Gy, extremophiles such as *Caenorhabditis elegans* and tardigrades can withstand doses ranging from 1000 to 5000 Gy [18] (Figure 1).

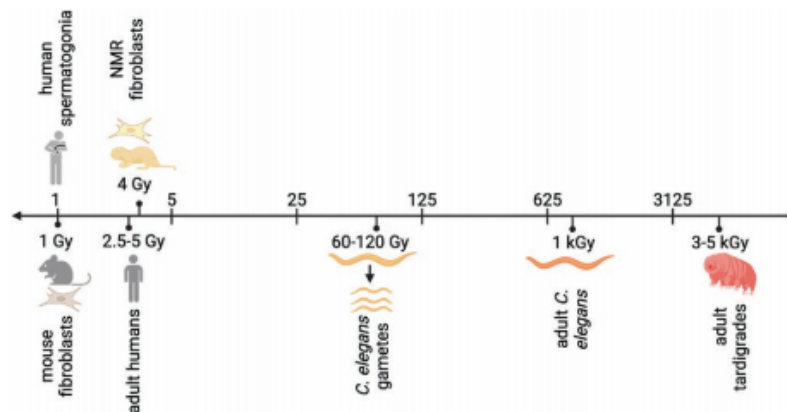


Fig. 1. Variation in radiotolerance between species and cell types is represented in log scale of LD50. More radiotolerant cells and organisms with higher LD50 values are also colored in yellow and red. Created with biorender.com

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1.2. DNA damage response

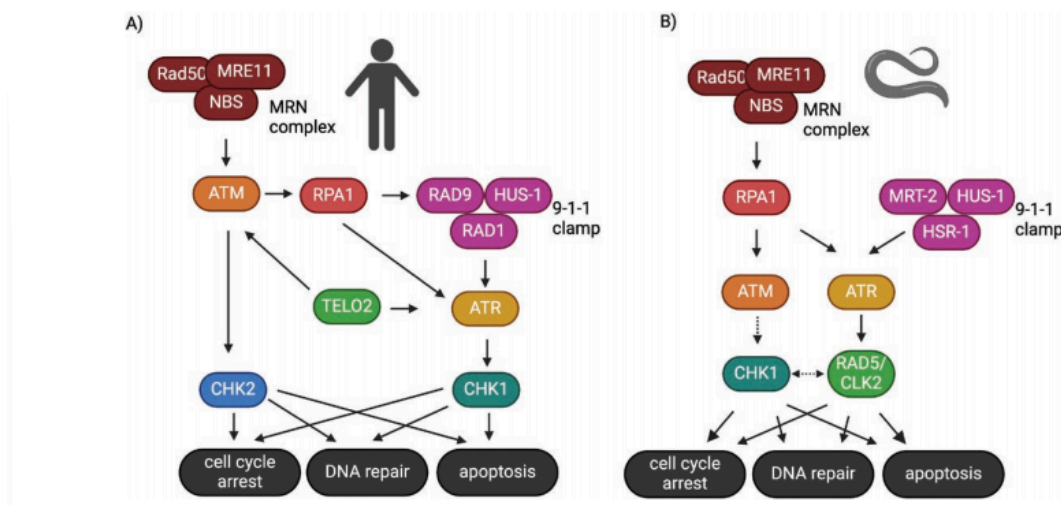


Figure 2. DNA damage response signaling following ionizing radiation in mammals a) and *C. elegans* b). Orthologous proteins are color-coded [18]

The DNA damage response varies significantly among different organisms, which is also one of the reasons for the great differences in their radiation tolerance. On the other hand, DNA double-strand breaks (DSBs) are among the most serious damages to cell viability in the spectrum of radiation-induced damages [18]. For dealing with this serious damage, there have been developed DNA damage response (DDR) pathways (Figure 2). The core of this pathway includes MRN complex that recognizes DSB and brings essential kinases such as ATM and ATR. The output of this signal pathway may include the results such as cell cycle arrest for repair mechanisms or apoptosis regulation in case of irreparable damage [18]. The radiation tolerance varies not only from species to species but also varies according to tissue-specific radiation sensitivity that was historically

characterized by Bergonié and Tribondeau rules where it was stated that tissues with high proliferative potential and low degree of differentiation have high degree of radio-sensitivity [18]. One testicular dose of about 1 Gy can cause sterilization in humans, while *Caenorhabditis* will keep 50% of its fertility after exposure to 60-120 Gy [18]. In mammals, normal stem cells should always balance between tissue homeostasis and genomic stability [1]. For example, HSCs in the bone marrow show high degree of resistance while intestinal stem cells are highly sensitive because of fast proliferation [1]. Furthermore, the biological effects of radiation do not exhibit strict linearity --- as the graph mentioned before, it can be seen that the responses induced by low-dose ionizing radiation (LDIR) in HSCs differ from those of high doses, and ROS-mediated oxidative DNA damage also plays a complex role in the loss of self-renewal potential [2]. Understanding these diverse DDR strategies is crucial for comprehending the evolutionary adaptations underlying the extreme radio-resistance observed in model organisms.

1.3. From basic mechanisms to translational potential

The extraordinary radiation tolerance seen in model systems can lead to advances in human science, especially in the disciplines of oncology, regeneration science, and space science. The study of stem cell responses to ionizing radiation is necessary in order to combat the harmful effects of radiotherapy and radiation exposure.

In addition, the study of damage induced by low-dose LET of radiation is becoming more and more important because of its application in protecting astronauts who are exposed to heavy ion radiation in space missions. At the same time, the application of these basic mechanisms in human treatments might require new ideas other than radioprotective pharmacology.

Currently, through studying the radioresistance strategies of model organisms with high resistance to radiation, scientists have designed several methods with possible applications in therapy. For example, studies have revealed that the application of pulsed direct current stimulation (pDCS) is able to repair the DNA damage caused by ionizing radiation in flatworm tissues and normalizes the cell cycle. This bioelectric stimulus triggers transcriptional activation after just fifteen minutes, whereby new stem cell and calcium signaling markers are activated, thus enabling the repair proteins to function through homologous recombination and non-homologous end joining processes. Importantly, pDCS is capable of rescuing flatworms from lethal irradiation. These results suggest that there are possibilities of using bioelectric stimuli in repairing DNA damages in human tissues. In addition, advanced pDCS methods have managed to achieve DNA repair and cell cycle progression in irradiated flatworm tissues [19].

2. Main

2.1. Model organisms for studying radiation tolerance

This review mentions three model organisms, which can be divided into two categories: flatworms and tardigrades. They are famous for their remarkable regenerative abilities and the capacity to withstand doses of ionising radiation that would be lethal to vertebrates. However, the mechanisms involved are different. For example, the marine flatworm *Macrostomum lignano* is noted for being a robust model in studies of ageing, genetics and physiology. It has a strong stem cell system that allows it to recover from intense gamma radiation without catastrophic tissue failure (Figure 3) [16, 20].

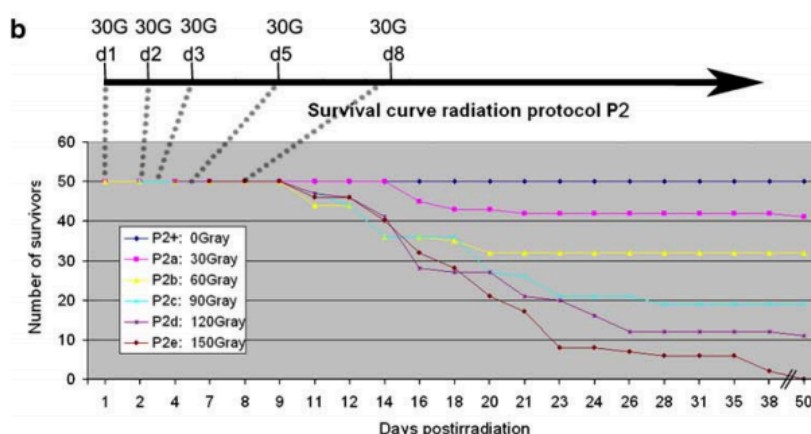


Figure 3. Effect of various fractionated γ -ray exposures on the survival rate of *Macrostomum lignano*. Survival curves following fractionated radiation setup protocols [16]

Another flatworm mentioned in this review, *Stenostomum brevipharyngium*, as a member of the early-branching flatworms within Catennulida, it challenges conventional understanding of stem cell biology. Despite lacking canonical neoblasts, this organism still has the ability for whole-body regeneration and asexual reproduction, suggesting that cells capable of division may be distributed across multiple differentiated somatic tissues rather than confined to a single stem cell lineage [17]. The last animal that would be mentioned in this review, tardigrades, are like the gold standard for surviving extreme radiation. Species like *Hypsibius dujardini* can handle over 5000 Gy of ionizing radiation, much higher than what mammals can tolerate [18, 21]. After a lot of research, scientists found that this extreme resistance probably comes from their special molecular adaptations, including tardigrade-specific DNA repair proteins and their own DNA repair systems [21].

By studying these diverse model organisms and deeply analyzing the evolution of their radiation tolerance mechanisms, these creatures provide valuable tools to understand how life can persist under extreme radiation stress, providing a rich comparative framework for basic biology research and practical applications.

2.2. *Macrostomum lignano*

The marine flatworm *Macrostomum lignano* has emerged as an important model organism for studying tissue regeneration and radiation recovery (Figure 4), first described in 2005 [20]. *Macrostomum* encompass significant species diversity, providing a critical taxonomic context for their use as model systems in detailed phylogenetic and morphological analyses [22]. Furthermore, it exhibits unique recovery capabilities under intense gamma radiation: studies have shown that proliferating cells in irradiated tissues can be effectively eliminated without immediately killing differentiated tissues, these cells are almost undetectable in the first week. However, cell proliferation and the expression of key stem cell markers like macpiwi, macboule, and macactin would recover within a month [16]. This flatworm can withstand a cumulative radiation dose of up to 150 Gy, but it's worth noting that a single dose of 210 Gy could be fatal [16]. In the stem cell recovery mechanism of *Macrostomum*, regenerative stem cells are initially confined to the lateral cavity and do not cross the midline, indicating that the stem cell niche is spatially highly regulated in its reorganization [16]. From the figure shown below, it can be seen that BrdU as well as actin expression remains unaffected for up to two weeks post-irradiation, whereas a single gamma-ray exposure in triclads typically results in the complete and permanent loss of the stem-cell system [16]

(Figure 5). Even exposing to 60 Gy show only slightly reduced proliferation after one hour, showing the robustness of this system [16].

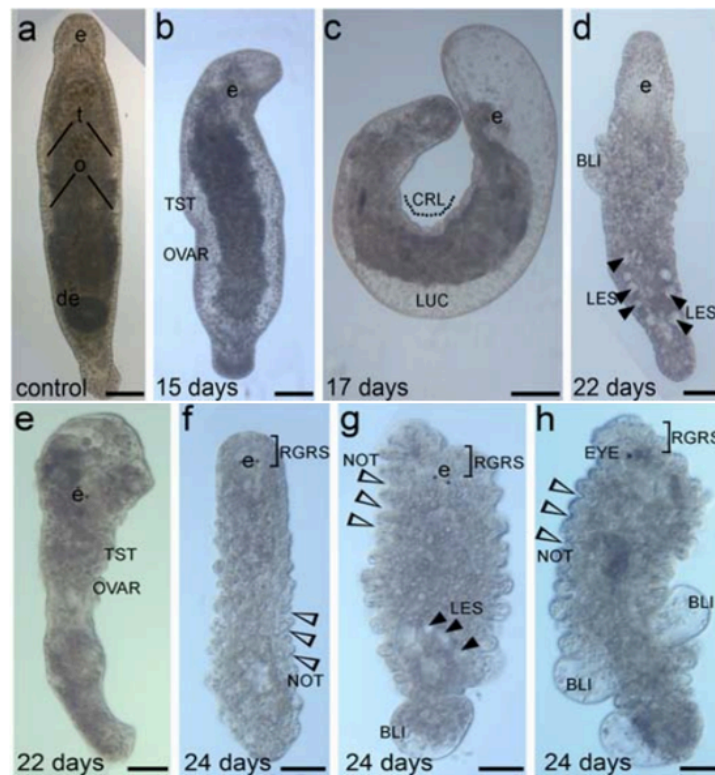


Figure 4. Effect of lethal radiation exposure (210 Gy, Protocol 3b) on morphology (e: eyes, de: developing egg, t: testes, o: ovaries) [16]

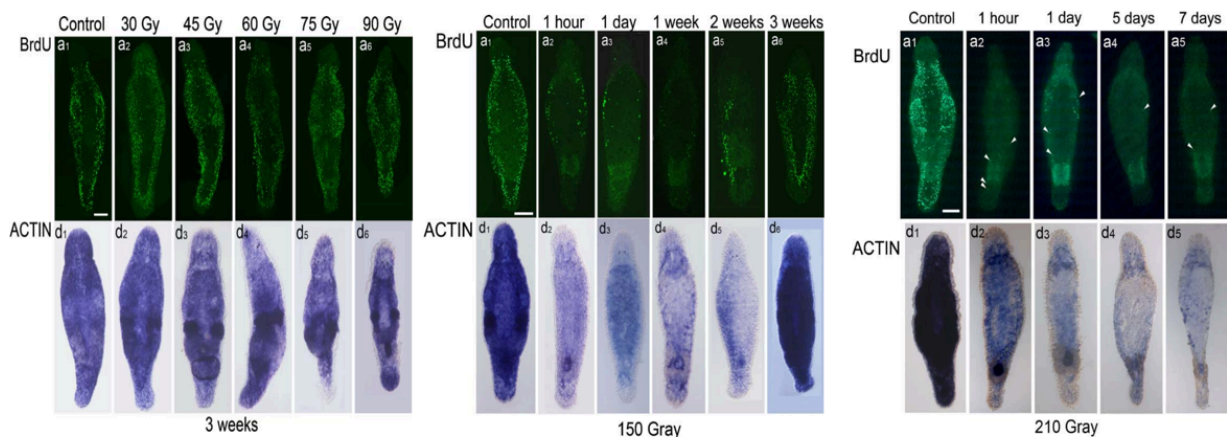


Figure 5. Cell proliferation, macactin expression dynamics of *Macrostomum*, following fractionated irradiation for up to 3 weeks, 150 Gy, and the situation when lasting absence of cell proliferation, macatin under 150 Gy and 210 Gy [16]

In addition, *Macrostomum* also possesses a complex cellular defence mechanism. For example, the organism's multidrug resistance (MDR) transport proteins, particularly the ABCC subtype, can monitor cell viability through Calcein AM staining, linking stress responses to physiological

behavior [20]. The unique combination of radiation resistance, spatial control of stem cells, and stress signaling in *Macrostomum* provide us with a lot of knowledge about tissue homeostasis. This is highly beneficial for translation to human stem cell therapy, where we can use this information to increase regenerative ability and maintain the stem cell niche after DNA damage.

2.3. Tardigrades

Tardigrades, especially *Hypsibius dujardini*, can survive doses ranging from 1000 to 5000 Gy (Figure 2) [18, 21]. The molecular basis of this extraordinary tolerance has been a subject of intensive study, and one of the most important discoveries is the identification of Tardigrade DNA Repair Protein 1 (TDR1), a novel gene unique to tardigrades that plays a central role in radiation protection [21]. The general function of this protein is: upon exposure to ionizing radiation, TDR1 translocates into the cell nucleus and binds directly to DNA through a conserved positive region. At high concentrations, TDR1 forms concentration-dependent aggregates, which may physically shield DNA or facilitate the assembly of repair complexes. (Figure 6) [21].

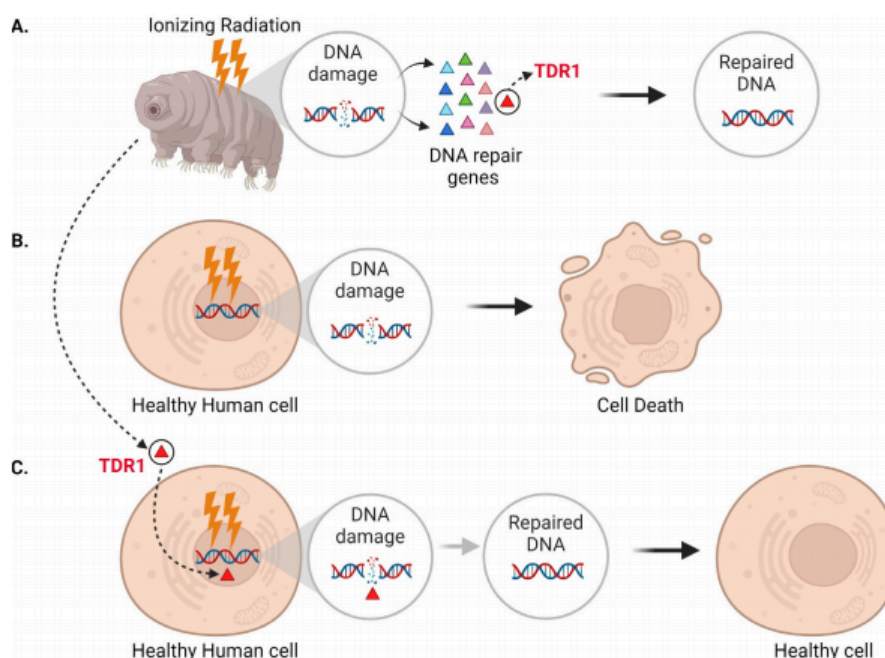


Figure 6. Expressing the tardigrade protein TDR1 in human cells increases DNA damage repair [21]

Furthermore, studies have found that when the TDR1 gene is introduced into healthy human cells, it provides measurable radiation protection, which may indicate its potential as a universal radioprotective factor [21]. In addition to this novel protein, tardigrades also exhibit a broad upregulation of their endogenous DNA repair mechanisms following radiation exposure. Genomic analysis indicates factors that may contribute to its extreme resilience, suggesting the presence of multi-layered defence strategies [18, 21]. Through the combined action of multiple genes associated with DNA repair, tardigrades can employ multi-layered defence strategies, which include enhancing the ability to repair double-strand breaks and mitigating oxidative stress, with oxidative stress being the primary lethal consequence of ionizing radiation [18].

The TDR1 protein and the processes involved in DNA repair offer an exciting avenue for the development of both radioprotectants and gene therapy. Additionally, information on how tardigrades manage to provide adequate DNA protection without impairing normal cell functions

could be employed in protecting stem cells during the radiotherapy process in cancer patients. The combined strategy of protection and repair adopted by tardigrades also makes them stand out from other model organisms through evolutionary advances developed in response to harsh selection pressure. Investigations of the genes of tardigrades and how they work are likely to produce unique ways of increasing human resistance to radiation.

2.4. *Stenostomum brevipharyngium*

Early-branching chain flatworms *Stenostomum brevipharyngium* challenge fundamental assumptions about stem cell biology and regenerative mechanisms (Figure 7). Unlike flatworms that rely on specialised populations of pluripotent stem cells—the neoblasts, *Stenostomum*, despite lacking conventional neoblasts, still possesses whole-body regeneration and asexual reproduction (fission) capabilities [17]. This unique feature may indicate that the flatworm's stem cell system is much more diverse than previously understood. In *Stenostomum*, cell division is not limited to a single specialised cell type; instead, a population of cells with multicellular differentiation potential is distributed across differentiated somatic tissues and organs [17]. This decentralized diffusion method contrasts with the centralized new neoblast system of triclads cells and raises important questions about the evolutionary origins of regenerative capacity (Figure 8).

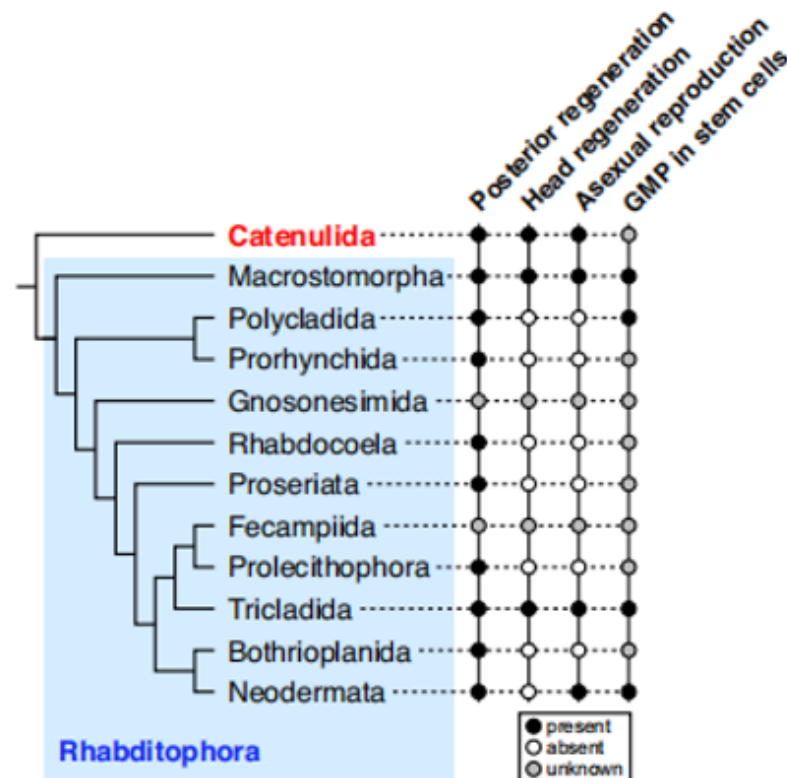


Figure 7. A phylogenetic distribution of regenerative abilities, asexual reproduction, and adult pluripotent stem cells in flatworms [17]

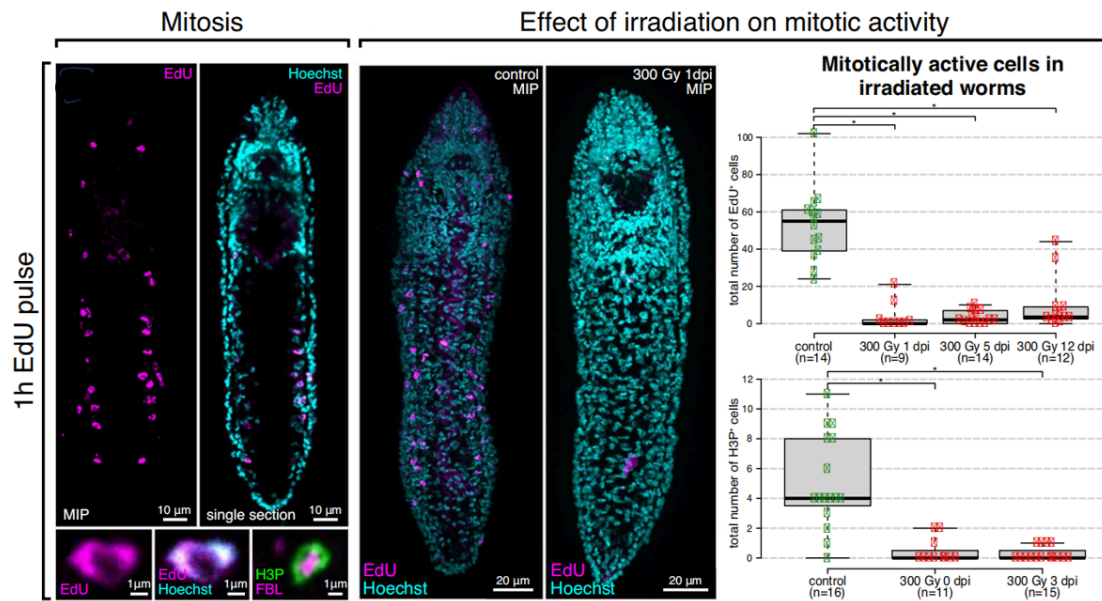


Figure 8. Effects of irradiation on gene expression and cell division in *Stenostomum brevipharyngium* [17]

Genetic analysis of *Stenostomum* further revealed this divergence: genes associated with germline multipotency programmes (GMP), including *piwi*, *nanos*, *argonaute* and *vasa*, are expressed specifically in the germline rather than in dividing somatic cells [17]. Importantly, irradiation experiments indicate that GMP components are not essential for proliferation and regeneration in this species, as *Stenostomum* retains regenerative capacity even after a 300 Gy ionizing radiation and regeneration analysis showed a lack of typical neoblast markers in proliferating cells [17]. This discovery seems to show that different flatworm populations independently evolved different regeneration mechanisms, rather than inheriting a common ancestral stem cell system.

The unique regenerative mechanism of the *Stenostomum* is of significant importance to regenerative medicine. If regeneration can occur without a dedicated population of pluripotent stem cells, this may indicate that differentiated cells can retain latent regenerative potential, which can be unleashed under suitable conditions, understanding how *Stenostomum* coordinates the proliferation of multiple differentiated tissues may help in researching ways to stimulate the induction of endogenous repair in human tissues. In addition, the organism's extremely high radiation tolerance (surviving 300 Gy) suggests that its distributed cell populations have a robust DNA damage response mechanism. Future research on the molecular identity and regulation of these somatic cells with dividing ability may further discover and refine new ways to enhance tissue regeneration and develop anti-radiation cell therapies.

3. Discussion

After the comparative analysis of *Macrostomum lignano*, tardigrades, and *Stenostomum brevipharyngium*, a remarkable diversity of strategies for surviving and recovering from ionizing radiation has been showed, while they also apply fundamentally different mechanisms. *Macrostomum* appears to rely primarily on a tissue-level strategy in which the stem cell system can be depleted and subsequently restored after fractionated irradiation [16]. Comparing with other

creature, Tardigrades mainly rely on molecular shielding and enhanced DNA repair by the expression of TDR1 protein and upregulated repair machinery, which could reduce damage at the molecular level [21]. Another paradigm is presented by *Stenostomum*, since its regeneration process depends on distributing proliferation among the differentiated cells instead of utilizing a population of stem cells [17].

Despite these advances, many details of these pathways remain unknown. The regulatory networks governing stem cell recovery in *Macrostomum* are not fully explored, particularly the signals that restrict regenerating cells to lateral compartments during recovery [16]. In addition, despite TDR1's ability to protect human cells from damage, the impact of TDR1 on long-term protection and the possibility of being oncogenic requires more studies [21]. The same applies to the identification of division-competent cells in *Stenostomum*, which are still unknown [17]. Furthermore, as what it has been mentioned in the beginning, most studies focus on acute high-dose exposure, while the biological effects of low-dose radiation, as studied in HSCs, may be more relevant to environmental and occupational exposure scenarios [2].

Further research can be conducted on functional genomics and single-cell transcriptomics in the planarian species *Macrostomum* and *Stenostomum* to determine all the genes and pathways responsible for radiation repair and distributed proliferation. Other research areas could be the analysis of whether bioelectrical signaling processes discovered in planarians also exist in *Macrostomum* and even mammals. It would help to understand whether electrical stimulation generally promotes DNA repair [19]. Combining results obtained from extremophiles with those of clinical radiobiology might result in the creation of new therapeutic approaches that will protect normal cells and increase tumor cell sensitivity to radiation. The use of such models in understanding radiation resistance is helpful in gaining knowledge that can be applied when radiation exposure is common practice.

4. Conclusion

Behavior of model organisms not only shows the extent of the diversity of mechanisms of protection against radiation in various organisms but also indicates the diversity of the evolution of radiation tolerance mechanisms among closely related species. Ranging from flatworms found in marine waters to tiny tardigrades, amazing radiation tolerance has been observed through various evolutionary processes. In the case of *Macrostomum*, there is considerable stem cell regeneration; tardigrades use new molecular protection due to TDR1, while *Stenostomum* regenerates without creating new cells.

Future studies of adaptation to such harsh conditions may have implications for regenerative medicine, radiation protection, and stem cell treatments, thus improving our ability to protect and heal tissues that have been exposed to radiation.

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