Animal physiology: How do tardigrades survive intense radiation?

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Summary

Tardigrades survive levels of radiation that would kill most animal life. A new paper unveils more of their tricks, including a pigment that can protect cells from radiation damage and a protein that can concentrate DNA repair machinery at sites of DNA damage.

Main text

There's an irradiator device in my research building that has a tiny chip of Cesium-137 in it. If a human climbed in — and they can't, thankfully, because the device fits nothing bigger than a cup — they would be dead after just a 2–3-minute dose. But our tardigrades can survive more than 24 hours in it1. How can certain animals survive conditions that should kill most life? My lab reported recently that tardigrades can withstand ionizing radiation by upregulating expression of DNA repair genes to remarkable levels2, and near the same time, another group published their own similar data3. In addition, they identified a tardigrade-specific gene that's induced by irradiation and encodes a protein that can coat DNA and protect it from damage3. Now a third shoe has dropped — a recent paper in Science reporting similar results and identifying some new mechanisms by which tardigrades can survive irradiation4. Perhaps we shouldn't be surprised by more shoes. Tardigrades have eight legs, after all.

Tardigrades are famed for surviving a variety of extreme conditions that should damage or destroy what living organisms are made of5. For example, the gamma rays produced as Cesium-137 decays will damage DNA, causing single strand breaks, double strand breaks, and oxidation of bases6. Many tardigrade species also survive desiccation, which should damage DNA5,7. How certain animals can survive conditions that are generally incompatible with life is a fascinating issue, and research in this area has potential practical value toward protecting many fragile biomedical materials.

Lingqiang Zhang's group and their colleagues collected their own tardigrades in China instead of using the same cultures as other labs because, as they reported in 2020, national laws and regulations presented obstacles to importing tardigrade cultures8.

Among the tardigrades they found was one species that resembled the lab model Hypsibius exemplaris (Figure 1). In the new paper4, Zhang and colleagues reported that this species is instead a close relative of H. exemplaris and a newly described species that they named H. henanensis for the location where it was collected — from moss in a scenic, mountainous area of old-growth forest in Henan Province.

To begin making use of the new species, the authors sequenced and assembled its genome. By comparing Hi-C-based assemblies of both Hypsibius species' genomes4,9, they found well-conserved gene orders on each of the chromosomes, with some apparent inversions, and with two small chromosomes in H. henanensis matching separate parts of the largest chromosome of H. exemplaris. The authors noted that this conservation of gene order indicated that the two species were closely related. Multiple groups have identified protectants in H. exemplaris, many of which are tardigrade-specific proteins with no recognizable sequence motifs5,10,11,12,13. Having genomes and experimental tools available in two closely related Hypsibius species may be invaluable in the long term for recognizing key, conserved features of novel proteins in tardigrades.

Zhang and colleagues4 then identified genes upregulated in response to irradiation in H. henanensis, and they investigated the functions of some of these genes. Among the genes they focused on was one encoding a DOPA (dihydroxyphenylalanine) dioxygenase that contributes to the synthesis of betalains, a class of yellow-orange pigments. Expressing an active form of this enzyme in cultured human cells offered protection against irradiation: it lowered the level of reactive oxygen species and improved the cells' survival after irradiation. Expressing the enzyme or just adding the pigments to cultured cells also mitigated radiation-induced DNA fragmentation4.

The authors also showed in vivo roles for the DOPA dioxygenase in H. henanensis. They could detect the newly synthesized yellow-orange pigments by mass spectrometry, but they didn't report whether the tardigrades became visibly pigmented after irradiation4. They also found that soaking the tardigrades in siRNAs targeting DOPA dioxygenase cut in half the degree to which its mRNA was upregulated upon irradiation and made the tardigrades susceptible to doses of radiation that they'd normally survive4. These results define an in vivo role for DOPA dioxygenase in tardigrades' ability to survive extreme doses of radiation, and they show that the enzyme and the pigments it produces can promote radiation tolerance in cells from other organisms.

Another gene upregulated in H. henanensis upon irradiation encodes a tardigrade-specific disordered protein with a prion-like domain, a protein that Zhang and colleagues named TRID1 (tardigrade-specific radiation-induced disordered protein 1)4. TRID1 was necessary and sufficient to enhance radiation tolerance: modestly lowering TRID1 mRNA level in the

tardigrades, by soaking in siRNA, compromised the animals' ability to survive irradiation, and expressing the protein in mammalian cells offered the cells some protection against radiation4.

Disordered proteins sometimes phase-separate in cells via multivalent interactions14,15. The authors showed that TRID1 could form phase-separated droplets in buffer or when expressed in mammalian tissue cultured cells, and that droplet formation depended on TRID1's prion-like domain4. After irradiation of the cultured cells, TRID1 accumulated at damaged DNA sites, and served to locally concentrate some DNA repair proteins such as Ku70, which contributes to DNA repair by non-homologous end-joining in diverse organisms, and the p53-binding protein 53BP1, which also promotes non-homologous end-joining4. TRID1 therefore appears to promote DNA repair at least in part by concentrating the factors involved in non-homologous end-joining DNA repair at sites of damage.

Taken together with the earlier studies2,3, the TRID1 results reveal a two-step mechanism — so far unique to tardigrades — that contributes to their survival of DNA-damaging conditions: non-homologous end-joining gene expression is strongly upregulated after irradiation, as much as about 100 to 300-fold, and the protein products of these genes are additionally enriched at sites of DNA damage in phase-separated TRID1 droplets that are also induced by radiation — concentrating the repair machinery where it's needed2,3,4.

Tardigrades appear to have a diverse set of tricks for surviving extremes, including another protein that can coat DNA and help protect it from damage10. Labs are continually finding new protectants — for various cellular compartments and for specific classes of molecules — that are thought to help the animals survive other extreme conditions5,12,13. Understanding these protectants' potency outside of tardigrades has been of interest, given their potential practical uses for guarding fragile biomedical materials. But demonstrating in vivo roles for protectants has been a challenge. The recent development of transgenic and CRISPR techniques for tardigrades16,17,18, along with RNAi by injected double-stranded RNAs19 or by soaking siRNAs as in the recent paper4, promises fruitful routes toward understanding in vivo roles for protectants and toward dissecting their mechanisms of action.

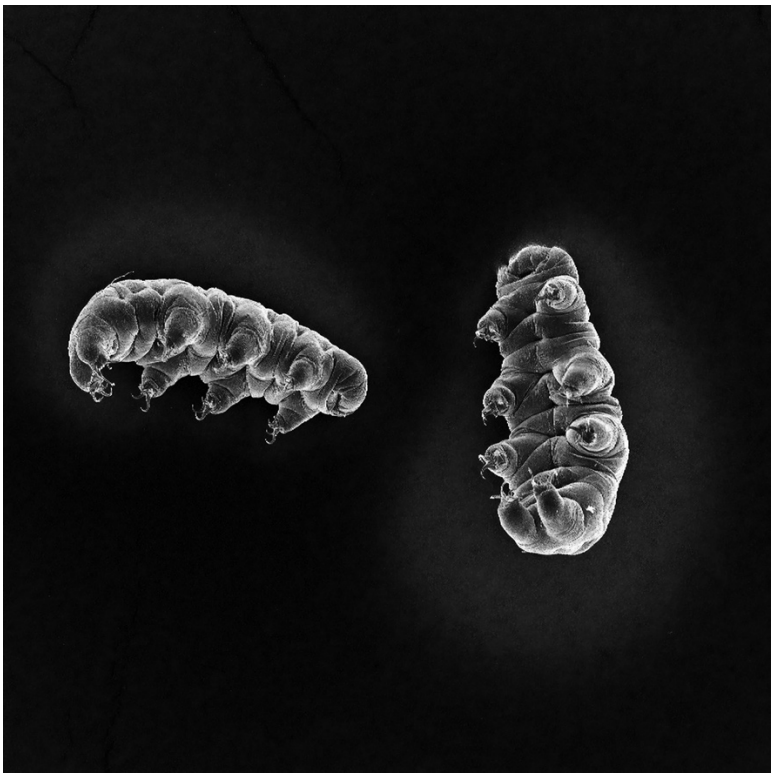

Figure 1. Two tardigrades (scanning electron microscopy of Hypsibius exemplaris). Credit: B. Goldstein and V. Madden.

References

1
E. Beltrán-Pardo, K.I. Jönsson, M. Harms-Ringdahl, S. Haghdoost, A. Wojcik
Tolerance to gamma radiation in the tardigrade Hypsibius dujardini from embryo to adult correlate inversely with cellular proliferation
PLoS One, 10 (2015), Article e0133658

2
C.M. Clark-Hachtel, J.D. Hibshman, T. De Buysscher, E.R. Stair, L.M. Hicks, B. Goldstein
The tardigrade Hypsibius exemplaris dramatically upregulates DNA repair pathway genes in response to ionizing radiation
Curr. Biol., 34 (2024), pp. 1819-1830.e6

3
M. Anoud, E. Delagoutte, Q. Helleu, A. Brion, E. Duvernois-Berthet, M. As, X. Marques, K. Lamribet, C. Senamaud-Beaufort, L. Jourdren, et al.
Comparative transcriptomics reveal a novel tardigrade-specific DNA-binding protein induced in response to ionizing radiation
eLife, 13 (2024), p. RP92621, 10.7554/eLife.92621

4
L. Li, Z. Ge, S. Liu, K. Zheng, Y. Li, K. Chen, Y. Fu, X. Lei, Z. Cui, Y. Wang, et al.
Multi-omics landscape and molecular basis of radiation tolerance in a tardigrade
Science, 386 (2024), Article eadl0799

5
K. Arakawa
Examples of extreme survival: Tardigrade genomics and molecular anhydrobiology
Annu. Rev. Anim. Biosci., 10 (2022), pp. 17-37

6
J.F. Ward
DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability
Prog. Nucleic Acid Res. Mol. Biol., 35 (1988), pp. 95-125

7
S. Asada, M. Takano, I. Shibasaki
Deoxyribonucleic acid strand breaks during drying of Escherichia coli on a hydorohobic filter membrane
Appl. Environ. Microbiol., 37 (1979), pp. 266-273

8

J. Chen, L. Li, Y. Guo, R. Bi, P. Shen, L. Wang, Y. Dong, L. Zhang
Establishment of Hypsibius dujardini laboratory culturing system (杜氏高生熊虫实验室培养体系的建立)
Mil. Med. Sci., 44 (2020), pp. 137-141

9
C. Hoencamp, O. Dudchenko, A.M.O. Elbatsh, S. Brahmachari, J.A. Raaijmakers, T. van Schaik, Á. Sedeño Cacciatore, V.G. Contessoto, R.G.H.P. van Heesbeen, B. van den Broek, et al.
3D genomics across the tree of life reveals condensin II as a determinant of architecture type
Science, 372 (2021), pp. 984-989

10
T. Hashimoto, T. Kunieda
DNA protection protein, a novel mechanism of radiation tolerance: Lessons from tardigrades
Life, 7 (2017), p. 26

11
J.D. Hibshman, J.S. Clegg, B. Goldstein
Mechanisms of desiccation tolerance: Themes and variations in brine shrimp, roundworms, and tardigrades
Front. Physiol., 11 (2020), Article 592016

12
C. Hesgrove, T.C. Boothby
The biology of tardigrade disordered proteins in extreme stress tolerance
Cell Commun. Signal., 18 (2020), p. 178

13
B. Goldstein
Tardigrades and their emergence as model organisms
B. Goldstein, M. Srivastava (Eds.), Emerging Model Systems in Developmental Biology
Current Topics in Developmental Biology, Academic Press, Cambridge, MA (2022), pp. 173-198

14
W. Borcherds, A. Bremer, M.B. Borgia, T. Mittag
How do intrinsically disordered protein regions encode a driving force for liquid-liquid phase separation?
Curr. Opin. Struct. Biol., 67 (2021), pp. 41-50
View PDF

15
E.W. Martin, A.S. Holehouse
Intrinsically disordered protein regions and phase separation: sequence determinants of assembly or lack thereof
Emerg. Top. Life Sci., 4 (2020), pp. 307-329

16
S. Tanaka, K. Aoki, K. Arakawa
In vivo expression vector derived from anhydrobiotic tardigrade genome enables live imaging in Eutardigrada
Proc. Natl. Acad. Sci. USA, 120 (2023), Article e2216739120

17
K. Kondo, A. Tanaka, T. Kunieda
Single-step generation of homozygous knockout/knock-in individuals in an extremotolerant parthenogenetic tardigrade using DIPA-CRISPR
PLoS Genet., 20 (2024), Article e1011298

18
H. Kumagai, K. Kondo, T. Kunieda
Application of CRISPR/Cas9 system and the preferred no-indel end-joining repair in tardigrades
Biochem. Biophys. Res. Commun., 623 (2022), pp. 196-201

19
J.R. Tenlen, S. McCaskill, B. Goldstein
RNA interference can be used to disrupt gene function in tardigrades
Dev. Genes Evol., 223 (2013), pp. 171-181