On X-ray Sensitivity in Xenopus Embryogenesis

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Highlight: High doses of electromagnetic radiation pre- and post- fertilization produce different developmental outcomes in Xenopus laevis.

Abstract

We examined the effects of X-ray irradiation on *Xenopus laevis*, focusing on pre- and post-fertilization exposure. We applied X-ray doses of 10, 50, 100, 250, and 500 Gy. 50% of 360 eggs irradiated at 250 Gy failed to fertilize, while fertilized eggs developed normally until the blastula stage. Doses of 10-50 Gy caused developmental anomalies, with significantly lower mortality than doses of 100-500 Gy. Post-fertilization irradiation of 50-100 Gy led to higher lethality than exposure of 10 Gy, although with similar kinds of developmental anomalies to pre-fertilization irradiation. The study highlights how the timing and intensity of exposure critically affect embryo viability, especially during the sensitive stages of fertilization and gastrulation. We establish the necessary and sufficient dosage to further investigate the molecular mechanisms of X-ray damage to both DNA and proteins.

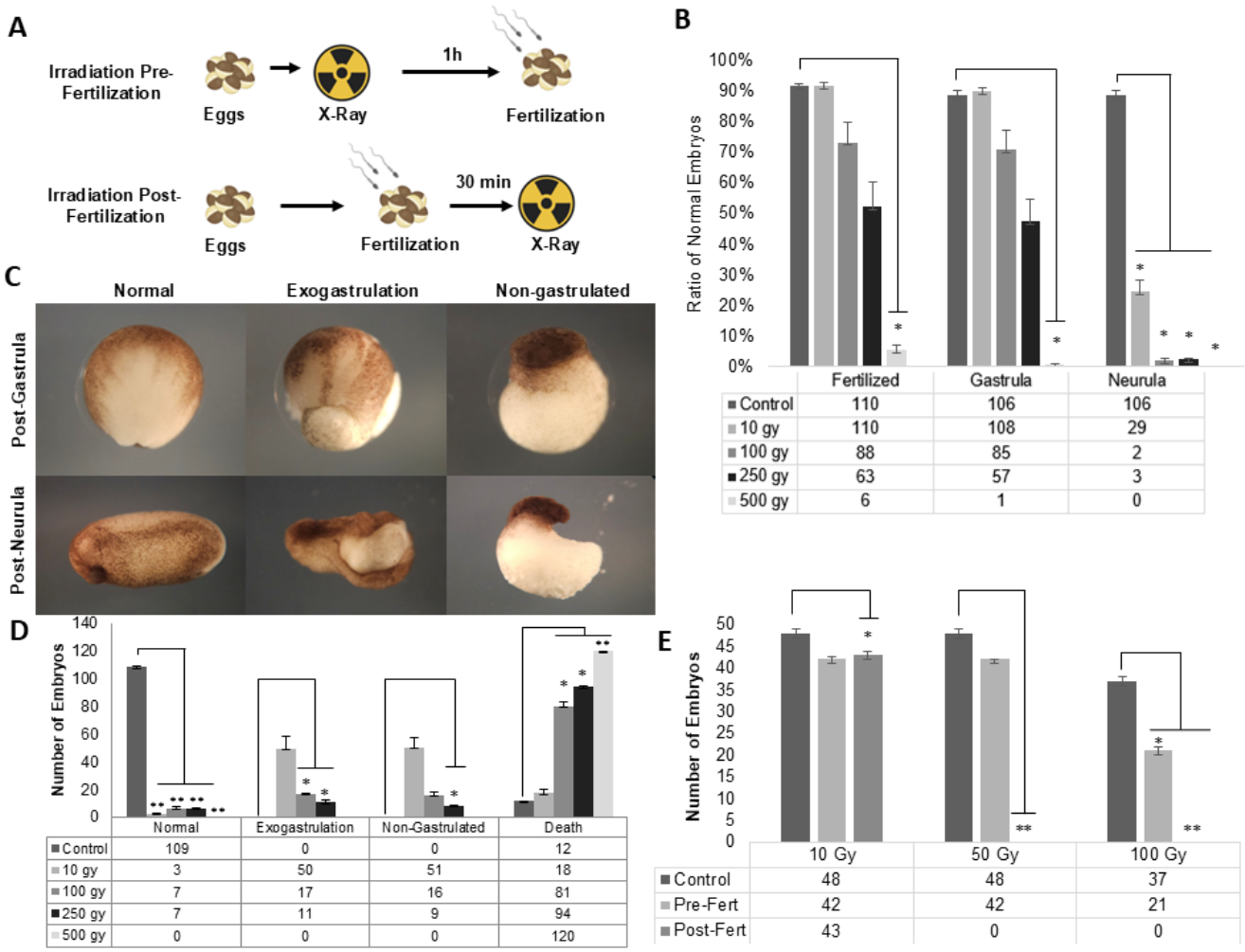

Figure 1. X-Ray Impact on *Xenopus laevis* development pre- and post-fertilization. (A) Two alternative experimental designs: irradiation pre- and post-fertilization. **(B)** Bar plot of the average of normal development of eggs irradiated with 10, 100, 250, and 500 Gy pre-fertilization. P values: Test-T, n=3 of 120 eggs per condition. Conditions significantly decreased from control (*: two-tailed paired t-test $P < 0.05$). **(C)** Sample embryos illustrate the phenotype classes observed during X-ray exposure: normal, exogastrulation, and non-gastrulation. **(D)** Distribution of phenotypes observed in C in different X-ray exposures. P values: Test-T, n=3 of 120 eggs per condition. Conditions significantly decreased from control (*: two-tailed paired t-test $P < 0.05$; **: t-test $P < 0.001$). **(E)** Average survival rate of irradiated eggs after fertilization at 10, 50, and 100 Gy. Each group had 50 initial eggs, and the test was duplicated. P values: Test-T, n=2 of 50 embryos per condition. Conditions significantly decreased from control (*: two-tailed paired t-test $P < 0.05$; **: t-test $P < 0.001$).

Description

X-rays primarily interact with human and animal cells through the ionizing radiation they emit, which can lead to various effects depending on the dose and duration of exposure. High ionization levels can remove tightly bound electrons from atoms, creating ions. This process can result in DNA breakage, impair the cellular repair mechanisms, and ultimately cause cell death (Saini and Gurung, 2025; Huang and Zhou, 2021). In addition to DNA damage, X-rays can directly or indirectly affect proteins, which are critical for cellular structure and function. Ionizing radiation induces protein damage through direct ionization and ROS-mediated oxidation (e.g., hydroxyl radicals), leading to carbonylation, fragmentation, and cross-linking of amino acid side chains (Hall & Giaccia, 2018; Dalle-Donne et al., 2006). This disrupts enzymatic activity, signal transduction pathways, and structural integrity, exacerbating cellular dysfunction. Accumulated damage overwhelms proteostasis systems (e.g., ubiquitin-proteasome, chaperones), triggering ER stress, misfolded aggregates, and apoptosis (Malhotra & Kaufman, 2007). Carbonylated proteins serve as oxidative stress biomarkers linked to neurodegeneration, aging, and radiation-associated pathologies (Stadtman & Levine, 2003; Azzam et al., 2012). Gametes and zygotes are particularly radiosensitive compared to somatic cells due to radiation-induced DNA damage, interference with the cell cycle, and differentiation during these phases. This presents a convenient model to induce and study protein damage and aggregation in a controlled way.

Amphibians, including *Xenopus laevis*, have been utilized as models to study the effects of a wide range of radiation doses during embryonic development, pre-metamorphosis, and adult stages (Hamilton, 1967; Ijiri, 1979; Landreth and Dunaway, 1974; Anderson et al., 1997; Dong et al., 2010; Stark et al., 2015; Carotenuto et al., 2016). These studies generally indicated lethal doses at various stages of the amphibian life cycle. Research by Hamilton in the late 1960s demonstrated that haploid *Xenopus* embryos are more sensitive to X-ray exposure than their diploid counterparts (Hamilton, 1967). Hamilton attributed this difference in X-ray dose response to genetic factors related to chromosome number. However, in his study, he raises the possibility that DNA damage alone may not fully account for the observed results, suggesting that protein damage could also play a significant role.

While these studies have highlighted how amphibians respond to radiation, further investigation is needed to understand their resistance to extreme doses. We decided to investigate the effects of pre- and post-fertilization radiation on the survival and embryonic development of *Xenopus laevis*. We applied various X-ray doses: 10, 100, 250, and 500 Gy to the *Xenopus* eggs, and then fertilized them 1h after irradiation to observe the effects (Fig. 1A).

During the initial evaluation, we observed two key effects of radiation: a significant reduction in fertilization rates, particularly at the highest doses of 250 and 500 Gy, where fertilization rates dropped to approximately 50% and 5%, respectively, while the fertilized eggs showed normal development up to the

blastula stage. However, at the onset of gastrulation, we noted several anomalies, including exogastrulation and non-gastrulation, where the irradiated groups showed an average of only 4% of the embryos managed to have normal development. As a result, during neurulation, embryonic deaths increased (Fig. 1B-D). Embryo mortality was also observed mainly in the groups that received 100, 250, and 500 Gy, presenting a mortality rate between 60% and 100% (Fig. 1D). We assessed the distribution of phenotypes throughout embryonic development based on the radiation dose received.

To compare the effects of radiation exposure pre- and post-fertilization, we subjected newly fertilized embryos to radiation doses of 10, 50, and 100 Gy. In contrast to pre-fertilization irradiation, the lethality rate was significantly higher when X-ray occurred after fertilization, especially at 50 and 100 Gy when we observed 100% lethality. Only the embryos exposed to 10 Gy survived (Fig. 1D). Despite this survival, the embryos exhibited the same phenotypic anomalies observed in those subjected to pre-fertilization radiation, exogastrulation, and non-gastrulation (Fig 1C).

This study builds on and further elucidates X-rays' effects, expanding on Hamilton's foundational work (1967). By revisiting this topic, we introduced a pre-fertilization irradiation condition and observed that egg infertility increased proportionally with higher radiation doses. In contrast, eggs exposed to lower radiation levels exhibited a higher rate of phenotypic abnormalities but lower lethality during embryonic development. Our findings align with Hamilton's (1967) observations, showing that pre- and post-fertilization irradiated embryos can survive until the blastula stage without anomalies. However, as gastrulation begins, significant anomalies emerge, often leading to embryonic death. Notably, pre-fertilization irradiation demonstrated greater damage tolerance than post-fertilization conditions. This finding strongly suggests that the new sperm content (proteins and genetic material) counteracts to some degree the egg damage caused by X-ray irradiation from 10 to 250 Gy, which is an extreme dose.

In summary, this study developed a reliable methodology for challenging chromosomes and cellular constituents, such as proteins, through X-ray radiation in *Xenopus* eggs and embryos.

Methods

Animal Preparation. To prepare female animals for ovulation, they were primed with 100 IU of Human Chorionic Gonadotropin (hCG) (Chorulon 5000 IU – Merck A002377) 72 hours before fertilization. The night preceding fertilization, each animal received a booster injection of 750 IU of hCG and was kept in individual containers at 18°C to initiate ovulation.

On the day of fertilization, male testes were surgically extracted according to protocol. Green, Sherril L. The Laboratory Xenopus. 1st Edition. 2010. The

extracted testes were then preserved in a 1X MMR (NaCl 1M, KCl 20 mM, $MgSO_4$ 10 mM, $CaCl_2$ Dihydrate 20 mM, HEPES 50 mM) solution at 4°C.

Egg Collection. Approximately 12 hours following the hCG boost, the females were transferred to containers containing a 1X High Salt Barth solution (NaCl 1.095 M, KCl 0.010 M, $NaHCO_3$ 0.024 M, $MgSO_4$-$7H_2O$ 0.008 M, HEPES 0.100 M, $Ca(NO_3)_2$ – $4H_2O$ 0.003 M, $CaCl_2$-$2H_2O$ 0.004 M). After about 90 minutes, eggs were collected for either fertilization or irradiation.

Fertilization Process. After draining the High Salt Barth solution, eggs were fertilized using macerated testes in 1X MMR. The sperm-egg mixture was homogenized for 4 minutes, followed by adding 0.1X MMR. Cortical rotation occurred within 20 minutes. A 2% cysteine solution (Millipore 1.028838.0100) was added, and embryos were agitated 5 times every 2 minutes, rinsed with 0.1X MMR, and cultured at 18°C.

Irradiation Procedure. Irradiation was carried out using a Rad Source RS 1800 Q X-ray machine. A mandatory 3-hour cooling period between sessions is required in experiments with 500 Gy. Each irradiation session was limited to no more than 20 minutes. Doses of 100, 250, and 500 Gy were administered on shelf 2 of the instrument (9.5 cm from the source), with samples centralized to ensure optimum radiation exposure. A 10 Gy irradiation was conducted on the instrument's floor (21.5 cm from the source), adhering to the same distribution principles. All samples were irradiated in 35 mm Petri dishes (Falcon 353001) containing 50 to 120 eggs or embryos each. Eggs irradiated before fertilization were suspended in a small amount of 1X Barth High Salt solution for preservation. Embryos were irradiated in a 0.1X MMR solution immediately after the cysteine washing step and before the first cleavage stage.

Reagents

Reagent	N° catalog	Available From
Chorulon 5000 IU (hCG)	A002377	Merck
cysteine	1.028838.0100	Millipore
Falcon 35 mm Petri dishes	353001	Corning
NaCl	SX0420-5	Millipore
KCl	7300-500GM	Millipore
$MgSO_4.7H_2O$	230391-500G	Sigma
HEPES	H-400-1	Goldbio
$Ca(NO_3)_2.4H_2O$	A16645.36	Thermoscientific
$CACL_2$	C8106-1KG	Sigma
$CACL_2.2H_2O$	C3881-500G	Sigma

References

- S Saini,P Gurung. 2025. A comprehensive review of sensors of radiation-induced damage, radiation-induced proximal events, and cell death. Immunol Rev 329(1):e13409. PubMed. 10.1111/imr.13409

- R Huang, P-K Zhou. 2021. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. Signal Transduct Target Ther 9;6(1):254. PubMed. 10.1038/s41392-021-00648-7

- Hall, E. J., & Giaccia, A. J. (2018). *Radiobiology for the Radiologist* (8th ed.). Lippincott Williams & Wilkins.*Mechanisms of direct/indirect radiation effects.*

- Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2006). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*, 364(1-2), 23–38.DOI: 10.1016/s0009-8981(03)00003-2

- Malhotra, J. D., & Kaufman, R. J. (2007). The endoplasmic reticulum and the unfolded protein response. *Seminars in Cell & Developmental Biology*, 18(6), 716–731.DOI: 10.1016/j.semcdb.2007.09.003

- Dukan, S., & Nyström, T. (1998). Bacterial senescence: Protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes. *The EMBO Journal*, 17(18), 5269–5279.DOI: 10.1093/emboj/17.18.5269

- Stadtman, E. R., & Levine, R. L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*, 25(3-4), 207–218.DOI: 10.1007/s00726-003-0011-2

- Azzam, E. I., Jay-Gerin, J. P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters*, 327(1-2), 48–60.DOI: 10.1016/j.canlet.2011.12.012

- L Hamilton. 1967. A comparison of the x-ray sensitivity of haploid and diploid zygotes of Xenopus laevis. Radiat Res 30(2):248-60. PubMed.

- KI Ijiri. 1979. X-ray effect on the development of Xenopus laevis embryos--with special reference to primordial germ cells. J Radiat Res 20(1):133-6. PubMed. 10.1269/jrr.20.133

- HF Landreth, PB Dunaway, GE Cosgrove. 1974. Effects of whole-body gamma irradiation on various life stages of the toad, Bufo woodhousei fowleri. Radiat Res 58(3):432-8. PubMed.

- JA Anderson, AL Lewellyn, JL Maller. 1997. Ionizing radiation induces apoptosis and elevates cyclin A1-Cdk2 activity before but not after the midblastula transition in Xenopus. Mol Biol Cell 8(7):1195-206. PubMed. 10.1091/mbc.8.7.1195

- JiaJia Dong, Sean P Mury, Karen E Drahos, Royce K P Zia, Carla V Finkielstein. 2010. Shorter exposures to harder X-rays trigger early apoptotic events in Xenopus laevis embryos. PLoS One 29;5(1):e8970. PubMed. 10.1371/journal.pone.0008970

- K. Stark, D.E. Scott, O. Tsyusko, D.P. Coughlin, T.G. Hinton. 2015. Multi-level effects of low dose rate ionizing radiation on southern toad, Anaxyrus [Bufo] terrestris. PLoS One 30;10(4):e0125327. PubMed. 10.1371/journal.pone.0125327

- R Carotenuto, M Tussellino, P Russo. 2016. Survival fraction and phenotype alterations of Xenopus laevis embryos at 3 Gy, 150 kV X-ray irradiation. Biochem Biophys Res Commun 25;480(4):580-585. PubMed. 10.1016/j.bbrc.2016.10.095