

## Low dose radiation damage and radioprotection in the vertebrate embryo

Catherine L. Bladen, William S. Dynan, and [David J. Kozlowski](#)<sup>1</sup>

Institute of Molecular Medicine and Genetics and <sup>1</sup>Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, Georgia

E-mail: [dkozlowski@mcg.edu](mailto:dkozlowski@mcg.edu)

Telephone: 706-721-8760; Fax: 706-721-8752

Mailing address: IMMAG CB-2803, Medical College of Georgia, Augusta, GA 30912

The harmful impacts of ionizing radiation on the developing embryo and fetus are well recognized. However, the molecular and biological mechanisms of embryonic injury, particularly at the earliest stages of development, are poorly understood. The goal of this research project is to exploit advantages of the zebrafish system to address the mechanisms of radiation damage and radioprotection in a vertebrate embryo *in vivo*. To establish zebrafish as a radiobiological model it is important to understand the response of an embryo to varying doses of radiation and identify the radioprotective mechanism(s) used during early development. The conservation of gene structure and function during vertebrate evolution assures that mechanistic insights gained from these studies will be directly applicable to research efforts in mammals, including humans.

In order to identify the embryonic tissue or organ that is most sensitive to the effects of low dose ionizing radiation, we irradiated gastrula-stage embryos (6 hours post-fertilization; hpf) and determined the effects of radiation exposure on gross morphology and apoptotic cell death at 24 hpf, when most organs systems (i.e., heart, blood, eye, brain, muscle) have been formed. We find that embryos exposed to radiation doses below ~50 cGy (genome-equivalent to a dose of 25 cGy in humans) are morphologically indistinguishable from controls. Similarly, the number of apoptotic cells in irradiated embryos is comparable to controls and reflect the normal level of apoptosis characteristic of embryonic development. However, at doses above ~50 cGy an increase in number of apoptotic cells is observed and these cells are located in the developing central nervous system (CNS) and tail. A phenotypic consequence of increased apoptosis is abnormal embryonic morphology of the mid- and hindbrain regions in the developing CNS. In contrast, cells located in the somitic mesoderm (prospective muscle and skeleton) appear refractory to the same doses of radiation. These results indicate that particular organ systems (i.e., the CNS) are more sensitive to radiation-induced apoptosis than other tissues or cell types (i.e., mesoderm) in the developing vertebrate embryo.

A direct consequence of ionizing radiation exposure is generation of DNA double strand breaks (DSBs) that, if unrepaired, lead to chromosomal instability and subsequent cell death. Under normal conditions, DSBs are efficiently repaired by one of two pathways: non homologous end-joining (NHEJ) or homologous recombination (HR). To determine which DSB repair pathway is the predominant mechanism for radioprotection in the zebrafish embryo, we tested the effects of reducing Ku80 (NHEJ) or Rad54 (HR) gene function during embryonic development, following transient exposure to ionizing radiation. To specifically reduce gene function we employed an established antisense technology (i.e., morpholino oligonucleotides; MOs) to prevent translation of targeted mRNAs. Embryos microinjected with a Rad54 MO were indistinguishable from controls when irradiated. In contrast, embryos microinjected with a Ku80 MO and exposed to radiation have morphologically abnormal brain development. Importantly, these embryos have significantly increased numbers of apoptotic cells located in the CNS and tail. This effect was pronounced at doses as low as ~15 cGy. Embryos co-injected with Ku80 and a p53 MO were indistinguishable from controls following irradiation, indicating that the p53 MO suppresses the effect (i.e., suppresses the increase in apoptosis) of the Ku80 MO. Together these preliminary data suggest that NHEJ is a component of the radioprotective strategy used during early zebrafish embryogenesis.

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