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Effects of chronic gamma irradiation: a multigenerational study using *Caenorhabditis elegans*

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1. Introduction

Emission of ionizing radiations into the environment is a natural and ubiquitous phenomenon, which may be enhanced by human activities. The environmental risk assessment of chronic exposure to ionizing radiation is currently hampered by the lack of knowledge, and hence, is often based on extrapolation from data obtained from acute exposure [1]. Indeed, most of ecotoxicological studies on ionizing radiation have focused only on partial lifecycles of organisms and on one generation, whereas it is known that pollutants acting on both survival and reproduction could also lead to phenotypic changes occurring across generations [2]. The free-living nematode *Caenorhabditis elegans* is a relevant biological model for studying multigenerational effects because of its short lifecycle, small size, and high fecundity, making this organism easy to culture under laboratory conditions. The aim of this study is to better evaluate the disturbances related to chronic exposure to ionizing radiation and their possible consequences on the population.

2. Materials and methods

The wild-type N2 strain of *C. elegans* was used in this study. Gravid worms were randomly selected from the stock population at $t = 0$. After 1 h, the adults were removed and the released fertilized embryos were considered to be age-synchronized; these embryos represented the first generation (F0). After 4 days of exposure, the adult worms from F0 were transferred onto a new experimental unit, replaced in irradiator, and left for 1 h for embryo collection and preparation of the next generation (F1), as previously described for F0. The same method was again repeated to obtain the third generation (F2) and recovery generations (F1', F2'). 10 replicates were used for control of each generation and 7 replicates were used for each dose rates of each generation.

Reproduction and body length were observed daily. To monitor brood size, the worms were transferred into individual Petri dishes every day since the onset of spawning. For the measurement of body length, the nematodes were photographed using a stereomicroscope. The pictures were then analyzed with the software ImageJ® and a micrometer scale measure. Growth parameters (initial length, maximal length, and growth rate) were obtained thanks to a Gompertz model.

External gamma irradiation was provided by a ¹³⁷Cs source (1.55 GBq), and seven dose rates (excluding the control) of 6.6, 8.1, 10.5, 13.7, 19.4, 28.1, and 42.7 mGy/h were tested. The Figure 1 presents the experimental design.

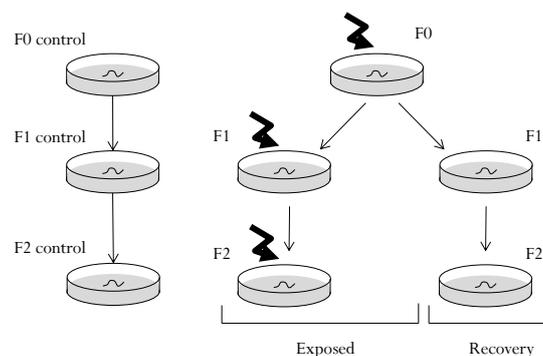


Figure 1: Exposure scenarios: F1 and F2 were continuously exposed to the same dose rate as F0 whereas F1' and F2' were placed to recovery

3. Results and discussion

Whatever the generation and the exposure statuses (exposed or recovery), no significant difference was observed in the growth parameters (growth rate, minimum, and maximum length) between the control and tested dose rates.

3.1. Decrease in the reproductive ability across generations

As presented in Figure 2A, a significant decrease was found in the cumulated number of larvae between the first and the third generation following exposure to the highest dose rate (42.7 mGy/h). The effects on reproduction noted between these two generations could be explained by the genetic effects resulting from complex events occurring in previous generations.

3.2. Difference between F1 and F1': transgenerational effects of parental irradiation?

Figure 2B showed that the non-exposed generation (F1') laid out less number of eggs than the exposed generation (F1). These differences could be explained by the transmission of the biological effects of parental radiation exposure to the non-exposed progeny through the germline.

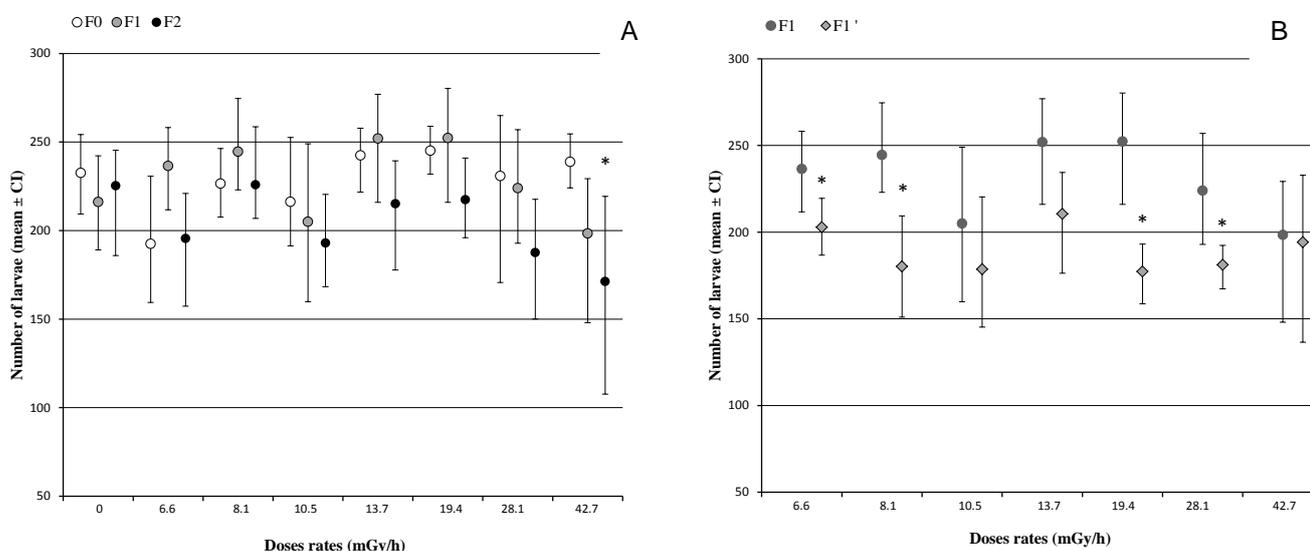


Figure 2: (A) Cumulated number of larvae in the successive generations exposed to ionizing radiations (F0, F1, and F2). Asterisk indicates significant differences between F0 and F2 generations (p -value < 0.05). (B) Cumulated number of larvae in F1 and F1'. Dose rates for F1' correspond to parental exposure dose rates. Asterisks indicate significant difference between F1 and F1' (p -value < 0.05).

4. Conclusions

Reproduction (cumulated number of larvae) was observed to be the most sensitive endpoint affected by multigenerational exposure to ionizing radiation. We found a significant decrease in the egg laying capacity between the first and third generations exposed to the highest dose rate (42.7 mGy/h). The results obtained revealed transgenerational effects from parental exposure in the second generation (F1'). Using these results on reproduction, molecular and cellular effects of chronic exposure to ionizing radiations on germline are examined to better understand the mechanisms underlying the observed effects.

5. References

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