

## **Fruit Flies Provide New Insights in Low-Radiation Background Biology at the INFN Underground Gran Sasso National Laboratory (LNGS)**

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## COMMENTARY

# Fruit Flies Provide New Insights in Low-Radiation Background Biology at the INFN Underground Gran Sasso National Laboratory (LNGS)

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Deep underground laboratories (DULs) were originally created to host particle, astroparticle or nuclear physics experiments requiring a low-background environment with vastly reduced levels of cosmic-ray particle interference. More recently, the range of science projects requiring an underground experiment site has greatly expanded, thus leading to the recognition of DULs as truly multidisciplinary science sites that host important studies in several fields, including geology, geophysics, climate and environmental sciences, technology/instrumentation development and biology. So far, underground biology experiments are ongoing or planned in a few of the currently operating DULs. Among these DULs is the Gran Sasso National Laboratory (LNGS), where the majority of radiobiological data have been collected. Here we provide a summary of the current scenario of DULs around the world, as well as the specific features of the LNGS and a summary of the results we obtained so far, together with other findings collected in different underground laboratories. In particular, we focus on the recent results from our studies of *Drosophila melanogaster*, which provide the first evidence of the influence of the radiation environment on life span, fertility and response to genotoxic stress at the organism level. Given the increasing interest in this field and the establishment of new projects, it is possible that in the near future more DULs will serve as sites of radiobiology experiments, thus providing further relevant biological information at extremely low-dose-rate radiation. Underground experiments can be nicely complemented with above-ground studies at increasing dose rate. A systematic study performed in different exposure scenarios provides a potential opportunity to address important radiation protec-

tion questions, such as the dose/dose-rate relationship for cancer and non-cancer risk, the possible existence of dose/dose-rate threshold(s) for different biological systems and/or end points and the possible role of radiation quality in triggering the biological response. © 2018 by Radiation Research Society

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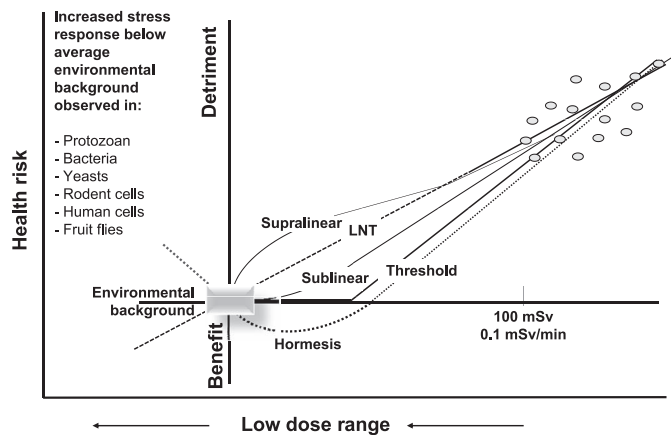
## INTRODUCTION

A limited understanding of the biological effects induced by ionizing radiation at low dose/dose rate continues to be the major challenge in predicting radiation risk to human health. In radiation protection, the dose optimization and limitation procedures are currently founded on the assumption that stochastic risk is directly proportional to dose (*I*). Accordingly, any dose, no matter how small, increases this risk. Moreover, below background no detriment is expected.

The linear no-threshold (LNT) model extrapolates epidemiological data on cancer risk from medium/high doses to those in the low-dose region of interest for radiation protection; however, the existing human exposure cohorts are restricted to specific dose, dose rate and radiation qualities. Epidemiological data below doses of 100 mSv and dose rates of 0.1 mSv/min lack appropriate statistical power. Mechanistic studies are therefore fundamental to complement the epidemiological data.

To date, several lines of radiobiological evidence have challenged the LNT model (Fig. 1). Overall, the scenario currently derived from *in vitro* and *in vivo* studies is complex and difficult to use in radiation protection practice, so the LNT model is still widely used for pragmatic purposes, although no longer considered as a dogma. Experimental studies have predicted dose-response relationships that deviate from linearity in two opposite directions: those pointing to supralinear extrapolation, such as may be expected from bystander effects, hypersensitivity or geno-

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**FIG. 1.** Several lines of radiobiological evidence have challenged the LNT model based on the assumption that stochastic risk is directly proportional to dose. Furthermore, no detriment is expected below background according to LNT. Circles represent epidemiological data; the box represents the variability of environmental radiation background. Examples of average annual background radiation exposure are: 3 mSv/y (U.S.); 2 mSv/y (UK); 7 mSv/y (Finland). Higher radiation level areas are Kerala, India, with up to 45 mSv/y; and Iran (around Ramsar), with up to 131 mSv/y.

mic instability; or those pointing to sublinear extrapolation, such as from adaptive responses, threshold or hormetic effects. This evidence definitely needs to be consolidated and the underlying mechanisms elucidated.

Experiments performed in deep underground laboratories (DULs) have further challenged the LNT model. These locations, shielded from cosmic radiation, represent the ideal scenario for extremely low-dose/dose-rate investigations. So far, the biological response below the average environmental radiation background has been investigated in protozoan (2, 3), bacteria (4, 5), yeasts (6) and mammalian cells of rodent (7, 8) and human origin (4, 9). The overall message derived from these studies is that environmental radiation is necessary to trigger mechanisms that increase the ability to respond to stress.

Recently, we obtained the first evidence of a differential response below and above ground at the organism level using the fruit fly *Drosophila melanogaster*. We have recently reported a comparative data set on lifespan, fertility and response to genotoxic stress in different *Drosophila* strains that were raised in parallel at the Gran Sasso National Laboratory (LNGS) and in the reference laboratory at L'Aquila University (both in L'Aquila, Italy) (10). Our study has shown that the permanence in a strongly reduced radiation environment can indeed affect *Drosophila* development and, depending on the genetic profile, may affect viability for several generations even when flies are moved back to the reference radiation environment. Of further relevance is that changes in *Drosophila* growth and development are observed as soon as after 2 weeks of permanence underground, giving suggestion for possible mechanisms involved.

The evidence that radiation doses/dose rates comparable to the average radiation background influence cell and tissue homeostasis is not so surprising (11). The evolution of living species over approximately 4 billion years in the presence of a variable radiation environment has eventually led to the integration of this daily stimulus into the normal biochemical and physiological cellular processes. Deep underground biology experiments have thus far shown compelling evidence that cell physiology and metabolism respond to relatively small variations in radiation background, providing some insights into how life has adapted and evolved.

### DEEP UNDERGROUND LABORATORIES AROUND THE WORLD: THE MOST EFFECTIVE WAY TO MINIMIZE COSMIC-RAY BACKGROUND

Originally, DULs were created to host particle, astroparticle or nuclear physics experiments requiring a low-background environment with vastly reduced levels of cosmic ray particle interference (e.g., the search for proton decay, the neutrinoless double beta decay, the search for the existence of magnetic monopoles, etc.). More recently, the range of science projects seeking an underground experiment site has greatly expanded, and now DULs are truly multidisciplinary science sites hosting important studies in fields such as geology, geophysics, climate and environmental sciences, technology/instrumentation development and biology.

So far, underground biology experiments are ongoing or planned in a few of the DULs (Table 1). However, it is possible that in the near future other DULs will serve as sites for biology experiments. In 2015, a meeting was organized in Canfranc, Spain, with the goal of establishing a common path for underground laboratories in “deep life” studies. European, American and Canadian researchers participated and shared their experience (<https://indico.cern.ch/event/436589/>). The increasing interest in this field and the establishment of new projects, such as the REPAIR Project at SNOLAB (36, 37), will have a significant effect on both basic knowledge and applied research.

For these studies, it is mandatory to characterize in detail the environmental conditions, particularly in terms of the dose contributions to the biological model systems coming from the different components of the radiation field.

Exploiting DUL characteristics is the most effective way to minimize the cosmic-ray background flux. Thanks to the rock overburden, such contribution can actually be reduced by several orders of magnitude (at least by a factor of  $10^3$ ). Table 1 lists the facilities that, to the best of our knowledge, are currently operating or under construction, along with their most relevant radiation field characteristics.

Inside DULs, the neutron flux provides insight into the concentrations of the radioactive sources and the composition of the surrounding rocks. The gamma background field is essentially of terrestrial origin, and depends on nuclear

**TABLE 1**  
**DUL Facilities Currently Operating and/or under Construction around the World**

DULs	Location	Muon flux ( $\text{cm}^{-2} \text{s}^{-1}$ )	Neutron flux ( $\text{cm}^{-2} \text{s}^{-1}$ )	Radon ( $\text{Bq m}^{-3}$ )	Reference
Yangyang (Korea) (tunnel)	700 m earth overburden (2,000 m water equivalent)	$2.7 * 10^{-7}$	$6.7 * 10^{-5}$	40–80	(12, 13)
Gran Sasso (Italy) (tunnel)	1,400 m rock overburden (3,800 m water equivalent)	$3 * 10^{-8}$	$3.78 * 10^{-6}$	50–120	(14)
Modane (France) (tunnel)	1,700 m rock overburden (4,800 m water equivalent)	$4.7 * 10^{-9}$	$5.6 * 10^{-6}$	15	(14)
ANDES (Argentina) (tunnel)	1,750 m rock overburden (4,800 m water equivalent)	$\sim 10^{-9}$			(15, 16)
BNO (Russia) (tunnel)	1,800 m rock overburden (4,900 m water equivalent)	$3.03 * 10^{-9}$	$1.4 * 10^{-6}$ (E > 1 MeV)	40	(17, 14)
Jinping (China) (tunnel)	2,400 m rock overburden (6,700 m water equivalent)	$2 * 10^{-10}$	$2.69 * 10^{-5}$	34–133	(18, 19)
SUL (Ukraine) (mine)	430 m depth (1,000 m water equivalent)	$1.7 * 10^{-6}$	$2.7 * 10^{-6}$	33	(20)
WIPP (U.S.) (mine)	650 m below-ground (1,600 m water equivalent)	$4.77 * 10^{-7}$	$< 6 * 10^{-8}$ (thermal)	7	(4, 21, 22)
Soudan (U.S.) (mine)	700 m rock overburden (2,500 m water equivalent)	$2 * 10^{-7}$	$7 * 10^{-7}$ (thermal)	300–700	(14, 21)
Canfranc (Spain) (mine)	850 m rock overburden (2,500 m water equivalent)	$2-4 * 10^{-7}$	$(2-4) * 10^{-6}$	50 – 80	(14, 23, 24)
Kamioka (Japan) (mine)	1,000 m underground (2,700 m water equivalent)	$3 * 10^{-7}$	$1.98 * 10^{-5}$ (thermal)	30	(14, 25)
SUPL (Australia) (mine)	1,025 m below ground (2,900 m water equivalent)	$\sim 10^{-8}$	$9 * 10^{-6}$		(26)
Boulby (UK) (mine)	1,100 m depth (2,800 m water equivalent)	$4.5 * 10^{-8}$	$1.7 * 10^{-6}$ (E > 0.5 MeV)	2.4	(14, 27)
INO (India) (mine)	1,300 m rock overburden (3,500 m water equivalent)	$3.7 * 10^{-8}$	$2.76 * 10^{-6}$		(14, 28, 29)
CUPP (Finland) (mine)	1,440 m depth (4,000 m water equivalent)	$1.1 * 10^{-8}$	$6 * 10^{-6}$	10–148	(13, 30, 31, 32)
SURF (U.S.) (mine)	1,480 m rock overburden (4,300 m water equivalent)	$4.4 * 10^{-9}$	$(1.7-8.1) * 10^{-6}$ (thermal)	310	(33, 34, 21, 35)
SNOLAB (Canada) (mine)	2,000 m rock overburden (6,000 m water equivalent)	$3 * 10^{-10}$	$9.3 * 10^{-6}$	120	(14, 13)

*Note.* Neutron and muon fluxes as well as radon concentration, where known, are reported.

decays in the rocks and in the atmosphere from daughter products of  $^{222}\text{Rn}$  and  $^{220}\text{Rn}$ . Accordingly, the gamma flux is primarily a function of the local geology and does not depend directly on depth. Typical values are in the range of  $0.13-2.5 \text{ cm}^{-2} \text{ s}^{-1}$  (26, 38–43). In some cases, the gamma-ray background is similar or even higher than in the surface laboratory due to the radioactivity of the rocks and the concrete, which covers the experimental halls. If necessary, using a suitable shielding, it is possible to reduce the gamma flux, particularly for the lower energy component.

For all the DULs, the dose/dose-rate contribution due to directly ionizing cosmic rays can be considered negligible compared to that at the Earth's surface, being reduced by a factor between  $10^4$  and  $10^7$ . The dose/dose-rate contribution due to neutrons is also extremely low, being reduced by a factor between  $10^2$  and  $10^4$  with respect to that at the Earth's surface.

One further contribution to the overall dose/dose rate can come from radon decay products. This contribution depends on the radon concentration, which should be reduced as much as possible inside the DULs, and also on the

biological system (cultured cells or organisms having different respiratory systems).

Terrestrial gamma rays contribute significantly to the dose/dose rate inside the DULs. As previously mentioned, this contribution can be reduced by some order of magnitude using shielding.

In addition to radiation, other environmental parameters need to be evaluated and monitored to completely characterize the environment where biological experiments are performed, in particular, pressure, temperature and humidity, which can affect the biological response.

#### THE LNGS UNDERGROUND LABORATORY: AN IDEAL SITE FOR BELOW-BACKGROUND RADIATION BIOLOGY EXPERIMENTS

The Apennine mountain range runs along Italy's highway A24 from the central Adriatic coast towards Rome. There, under its highest peak of Gran Sasso, is a gate to the 10-km-long tunnel that leads to the LNGS. Lying under 1,400 m of rock, it offers an efficient shelter from cosmic-ray noise.

LNGS is one of the world's largest underground research centers, consisting of three huge halls, each approximately 20 m wide, 18 m high and 100 m long, and bypass tunnels, for a total volume of 180,000 m<sup>3</sup>. Access to experimental halls is horizontal and is made easier by the highway tunnel. Inside the Gran Sasso Mountain, the natural temperature is of approximately 6–7°C and the relative humidity is approximately 100% (<https://bit.ly/2IKd76X>). To ensure proper working conditions, halls are equipped with all technical and safety equipment and plants necessary for the experimental activities. In particular, the experimental halls are waterproofed, heated and ventilated at a rate of 35,000 m<sup>3</sup> of air per hour from outside to maintain low concentrations of radon, which is pumped outside the LNGS.

At LNGS the flux of cosmic rays is reduced by approximately 6 orders of magnitude; moreover, given that dolomite rocks are poor in both thorium and uranium content, the neutron flux is 1,000 times reduced with respect to external environment, (44, 45). In addition, given that the mountain is of sedimentary origin, the natural occurrence of  $\gamma$  and  $\mu$  radiation is minimal and further reduced by the low-activity concrete lining of the laboratory walls.

The PULEX cell culture facility is located in one of the bypass tunnels. It was set up for *in vitro* experiments in extremely low-radiation background in the context of a close collaboration among the Istituto Nazionale di Fisica Nucleare (INFN), Istituto Superiore di Sanità (ISS) and Centro Fermi. In the PULEX facility, the radon concentration is kept at a level comparable to that of above ground by an efficient, dedicated ventilation system that pumps air from the outside. Moreover, PULEX hosts two CO<sub>2</sub> incubators, one of which is shielded with 5 cm of iron (Fe) to further reduce the gamma component of the radiation spectrum (65).

An animal housing facility was recently built next to PULEX. This new facility, named COSMIC SILENCE, is provided with temperature and light control systems, as well as an independent ventilation system. This facility is currently used to maintain and analyze different *Drosophila melanogaster* lines to investigate the effects of reduced background radiation at the organism level. However, the COSMIC SILENCE facility is also designed to host other model organisms with a greater biological complexity.

Above ground, a reference cell culture laboratory has been set up in the LNGS Chemistry building, which is located outside the tunnel. Moreover, the close proximity of L'Aquila University, and the relative short distance to ISS and "La Sapienza" University of Rome (approximately 1 h by car) makes it possible to exploit additional well-equipped sites as reference laboratories for both cells and organisms.

Inside the LNGS underground facilities, as well as in the reference laboratories, dosimetric measurements are routinely performed. In particular, thermoluminescent dosimeters are used to monitor gamma rays of any origin. Furthermore, the presence of AlphaGUARD equipment

allows for continuous monitoring of the environmental radon concentration, as well as pressure, temperature and humidity. Additional information is reported elsewhere (8).

### IN VITRO RADIOBIOLOGY AT LNGS

Since 1995, following the brilliant intuition of L. Satta, the unique features of LNGS have been exploited to address the effects of reduced background radiation on the homeostasis of different cell lines. The pioneering study on *Saccharomyces cerevisiae* has indeed revealed that budding yeast previously grown at LNGS for 1 week (120 generations) elicited defects in DNA repair upon exposure to radiomimetic agents (6). A few years later, the LNGS hosted a large set of experiments on rodent and human cells that were cultured for several months (to reach the same number of generations as the yeasts) at the PULEX facility in a low-radiation environment (LRE), as well as in an external reference radiation environment (RRE). By constantly checking for a variety of biological end points, these *in vitro* experiments revealed that cells grown at the LNGS PULEX facility displayed an impaired activity of antioxidant enzymes, accumulated frequent radiation-induced mutations and elicited a reduced efficiency at scavenging reactive oxygen species (ROS) with respect to same cell lines maintained in parallel in reference laboratories, in the presence of natural radiation background (7–9). The results of these experiments clearly indicated that environmental radiation contributes to the development of defense mechanisms at the cellular level (Table 2). Data collected from different underground laboratories (2–5) or using a shielding approach (46–50), showed similar findings, undoubtedly proving that the absence of natural levels of environmental radiation significantly affects the physiology of both prokaryotic and eukaryotic cells. However, whether deprivation of environmental radiation also influences the development and physiology of a multicellular organism remained largely unaddressed until recently.

### THE FLYINGLOW PROJECT

A few years ago, in the framework of a collaboration between INFN, ISS, Rome University and Centro Fermi, we launched the FLYINGLOW project, a research program to determine whether the LNGS underground environment could affect different life parameters associated with *Drosophila melanogaster*, a well-established model organism. The recently reported results (10) indicated that the reduction of environmental radiation affects developmental parameters of *Drosophila*, providing evidence for the first time of the influence of radiation background in a complex organism (Table 2).

We chose the *Drosophila* as our preferred model system because it is considered to be a valuable model for the analysis of the biological consequences of radiation exposure (51–55), as well as one of the most effective models

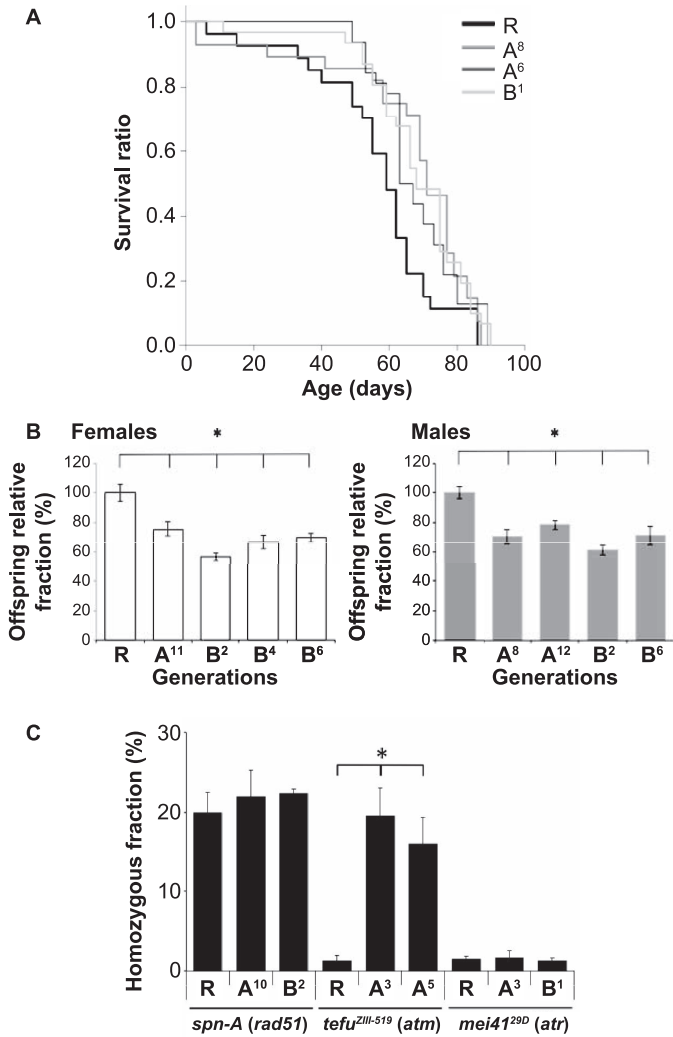
**TABLE 2**  
**Summary of LNGS Results**

Biological system	End point	Result
<i>Saccharomyces cerevisiae</i> cultured for 1 week (~120 generations) at LRE and RRE (RRE: University of Rome) (6).	Mutation induction	Higher frequencies of both recombinants and aberrants in LRE culture after challenge with MMS.
Chinese hamster V79 cells cultured for up to 9 months (~120 generations) at LRE and RRE (RRE: Istituto Superiore di Sanità, Rome) (7).	Cell growth	No difference at 3 and 9 months of growth between LRE and RRE cultures.
	Apoptosis	LRE culture more sensitive to apoptosis induced by CHX after 3 months of culture.
	Antioxidant enzyme activity	Different modulation of enzymatic activities after 9 months in LRE and RRE cultures.
	Mutation induction ( <i>hprt</i> locus)	Increased basal and $\gamma$ -ray-induced mutation frequency after 9 months in LRE culture.
Chinese hamster V79 cells. Two parallel independent cultures maintained for up to 10 months at LRE and RRE (RRE: external LNGS laboratory) (8).	Antioxidant enzyme activity	Different modulation of enzymatic activities after 10 months in LRE and RRE cultures; differences not reverted after further 6 months of growth of all the cultures at RRE.
	Mutation induction ( <i>hprt</i> locus)	Increased basal mutation frequency after 10 months in RRE cultures; differences not reverted after further 6 months of growth of all the cultures at RRE.
TK6 human lymphoblasts cultured for up to 6 months at LRE and RRE (RRE: Istituto Superiore di Sanità Rome) (9).	Cell growth	No difference at 6 months of growth between LRE and RRE cultures.
	Micronuclei induction	LRE cultures more sensitive to a 2 Gy X-ray dose.
	Antioxidant enzyme activity	Different modulation of enzymatic activities after 6 months in LRE and RRE cultures.
<i>Drosophila melanogaster</i> (RRE: L'Aquila University) (10).	Life span	Increased life span in flies growing at LRE: effect observed after 1 generation and maintained constant for several generations.
	Fertility	Reduced fertility of both male and female adults growing at LRE: effect observed after 1 generation and maintained constant for several generations.
	DNA repair	Positive selection on the survival of mutant <i>atm/tefu</i> homozygous flies (with little ATM protein) at LRE: effect observed even when mutant flies are moved back to RRE.

Notes. LRE = low-radiation environment; RRE = reference radiation environment; MMS = methyl-methanesulfonate; CHX = cycloheximide.

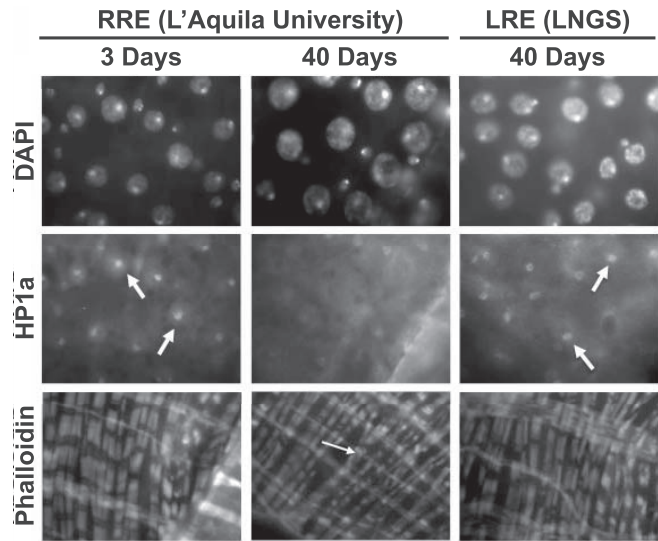
for investigating the function of human disease genes (56, 57). In our initial FLYINGLOW study, we compared different developmental parameters, such as life span, fertility and motility activity, between control flies maintained for different generations at the LNGS (LRE) and at the reference Laboratory at L'Aquila University (RRE), which is very close to LNGS (only 5 km away). We found that the median life span of independent populations, consecutively raised and maintained for several generations at LRE, was significantly increased with respect to that of reference (RRE) wild-type populations, indicating that reduction of natural background radiation alters the survival ratio. Interestingly, the positive effect on lifespan was observed as early as after one generation time (10–15 days) and the extension rate remained constant as well, even after several generations. These results indicated that a short time of permanence underground was sufficient to obtain a significant effect on the biology of a complex organism. Moreover, this finding ruled out the possibility that this effect could be caused by potential LNGS-induced mutations that suppressed normal aging, as they would have required a much longer time to get fixed in the population (Fig. 2A).

The molecular mechanisms underlying the effect on life span are still unclear. However, a comparative analysis of heterochromatin domains in body wall muscle fibers between LRE and RRE in 40-day-old adult flies provided some clues. It has been reported that heterochromatin levels, as revealed by the presence of heterochromatin protein 1 (HP1) foci, in *Drosophila* muscular nuclei, gradually decline with aging and can account for age-related muscle degeneration (sarcopenia) (58). Consistently, we found that HP1 localization at the chromocenter of muscular cells from 40-day-old adult flies kept at RRE, was dramatically reduced with respect to those from young adult flies (Fig. 3). In addition, the phalloidin staining of muscle fibers revealed the presence, although modest, of discontinuous fibers that are normally seen in old flies as a consequence of progressive muscle degeneration. Surprisingly, the number of HP1 foci in muscular nuclei and muscle fiber integrity in 40-day-old LRE flies were almost indistinguishable from those observed in young adult flies (Fig. 3). This finding might present an intriguing notion that LRE could prolong life span by preventing heterochromatin decline, which in turns prevents muscle fragility.



**FIG. 2.** Effects of reduced natural background radiation on *Drosophila melanogaster*. Permanence in reduced background radiation (underground LNGS) extends the lifespan of male adult flies (panel A), reduces fertility of adult flies (panel B) and affects DNA damage response (panel C) (positive selection of *tefu* mutant flies). R = reference flies; Ax and Bx = different populations and generations of LNGS flies. \* $P < 0.01$  (ANOVA test followed by Holm-Sidak test). (Published and modified with permission from: Morciano P, Iorio R, Iovino D, Cipressa F, Esposito G, Porrazzo A, et al. Effects of reduced natural background radiation on *Drosophila melanogaster* growth and development as revealed by the FLYIN-GLOW program. *J Cell Physiol* 2018; 233:23–9).

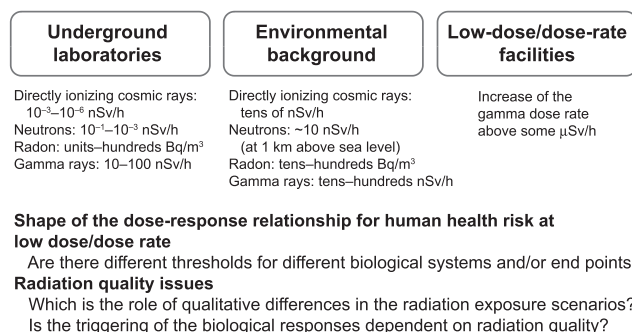
While LRE prolongs the life span, it also strongly limits the reproductive capacity of both male and female flies. Fertility tests that were performed on both wild-type Oregon male and female adults from the same generation time have indeed shown that LNGS background radiation reduces the fertility of both male and female adults by 30% (Fig 2B). Interestingly, as with the effect on lifespan, the fertility reduction was observed in flies as soon as they were raised at the LNGS for two generations, and more importantly the rate of reduced fertility remained almost unchanged along different generations. This latter evidence excluded the possibility that the reduction in fertility was a consequence



**FIG. 3.** Age-dependent loss of heterochromatin is prevented by low-radiation environment (LRE). Gut tissues from young (3-day-old) and old (40-day-old) reference radiation environment (RRE) female flies and from old (40-day-old) low-radiation environment (LRE) female flies were dissected and stained with anti-HP1 to visualize the heterochromatin domains and phalloidin-fluorescein to reveal the longitudinal and circular intestinal body wall muscle fibers. Note the presence of marked HP1-enriched foci in the young RRE gut, which appear more diffuse in the old RRE gut but still prominent in old LRE gut (arrows). The phalloidin staining reveals that muscle fibers in old LRE flies are indistinguishable from that of young RRE flies, whereas, as expected, their morphology appears degenerated in old RRE flies.

of the induction of spontaneous mutations, which could eventually affect fertility progressively as the generation time increases. Although the molecular basis for LRE-induced decrease in fertility is not known, we can speculate that it is related to the general effect of low-radiation background on DNA repair. Indeed, proper DNA repair plays a pivotal role in the complete execution of specific biological processes in *Drosophila*, including male and female meiosis (59, 60). Thus, it is conceivable that both spermatogenesis and oogenesis may be particularly influenced by reduced radiation background. From this perspective, a prolonged lifespan extension is not quite unexpected, since it could reflect the obligate trade-off between survival and reproduction that underlies the widespread “cost of reproduction” (61).

A very peculiar finding emerged when we evaluated the effects of LRE environment on DNA repair. We sought to test whether development and growth of *Drosophila* lines carrying viable mutations in selected DNA repair genes (namely *spnA*, *tefu* and *mei41* encoding Rad51, ATM and ATR, respectively) were indeed affected by LNGS reduced background radiation. As expected, these mutants are semi-lethal (i.e., only rare adults are found) if raised in a RRE. Surprisingly, we found that only in the *tefu* mutant strain the number of adults was much higher (20% vs. 1:3%) than that observed at RRE, indicating that LRE conditions positively selected the survival of flies with little ATM protein (Fig.



**FIG. 4.** Opportunities for a systematic investigation of biological response at increasing dose/dose rate exposure.

2C). This high survival rate was also observed after free recombination of the *tefu*-carrying chromosome, suggesting that this effect was not due to spurious noncomplementing second sites in the genome of this mutant line. Moreover, we observed that the frequency of *tefu* homozygotes also remained high when LRE *tefu* mutant lines were moved and kept at RRE for two more generations, indicating that the LRE-induced positive selection of ATM defective flies is retained in a trans-generational manner. This latter finding is not totally unexpected as, for instance, rodent cells cultured at LRE appeared to keep a memory of their state when moved to RRE (7). Given that trans-generational inheritance is mainly regulated by epigenetics mechanisms (62), it would be interesting to unravel which type of LRE-induced epigenetic change underlies the suppression of lethality of *tefu* homozygotes. An additional question is whether this occurs only for mutations in the ATM encoding gene or if it could be extended to other DNA repair gene products. The possibility of access to a large collection of DNA repair gene mutants and the advantage of exploiting the most recent *Drosophila* molecular tool kits could make this task extremely interesting.

## CONCLUSION

One of the biggest challenges in radiation biology is the exploitation of the unique environments offered by several underground laboratories located around the world with the goal of evaluating the effects of low background radiation on living organisms. Several studies performed on unicellular systems, including bacteria, yeast and mammalian cultured cells, have already shown that cell physiology is indeed influenced at different levels by deprivation of normal radiation background. The determination of whether low-radiation background could affect life and development of multicellular and complex organisms remains a high-priority task in most underground laboratory agendas (4, 11, 36, 63). At the LNGS we have successfully started to address this complex question using *Drosophila melanogaster* as a model system. Our study has provided the first evidence in organisms of how reducing normal environmental radiation could perturb basic biological processes

such as lifespan and fertility and sheds more light on how normal environmental radiation variation had contribute to evolution. We are currently undertaking genome-wide approaches and proteomic analysis to understand the genetic and molecular basis for the LNGS-induced effects on *Drosophila melanogaster*. It would be interesting to extend this analysis further to other model organisms.

For radiation protection purposes, underground experiments can be nicely complemented with above ground studies at increasing dose rate. This can be done by choosing different reference sites above ground and/or using radiation facilities specifically designed for low-dose/dose-rate studies (Fig. 4). Experiments are in progress at the ISS using a dedicated facility (LIBIS), which allows exposure of cells and small organisms to gamma rays in the range  $\sim 2 \mu\text{Gy/h}$ – $\sim 20 \text{mGy/h}$  (64). It is expected that from a systematic study performed in different exposure scenarios, it will be possible to find responses to several open questions. Among these are the shape of the dose/dose-rate relationship for cancer and non-cancer risk, the existence of dose/dose-rate threshold(s) for different biological systems and/or end points and the possible role of radiation quality in triggering the biological response.

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