Fifth International Workshop for the Design of the ANDES Underground Laboratory
Towards the ANDES laboratory flag-ship and further experiments
Buenos Aires, Argentina
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The underground biology at the INFN Gran Sasso National Laboratory

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Underground laboratories represent a unique opportunity for investigating the response of bilogical systems to very low radiation doses

Relevant scenario for both basic and applied science

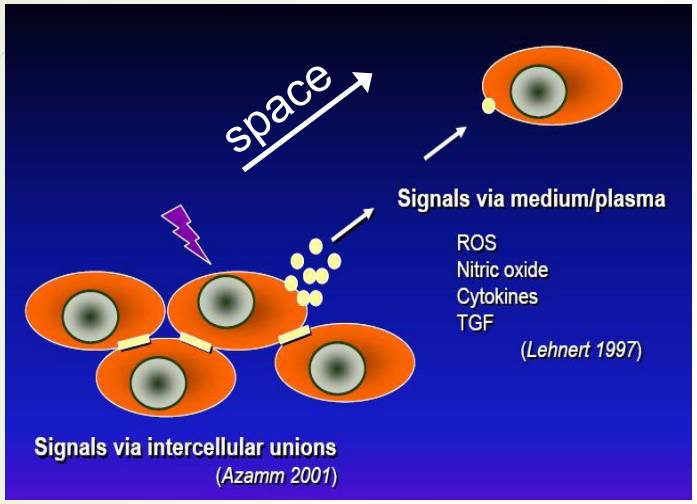
- **All living organisms have to cope with the natural level of radioactivity on the Earth as well as with cosmic rays**. Natural variations of background radiation likely played a critical role **during the evolution** and contributed to the **development of still poorly characterized defense mechanisms to minimize genotoxic damage**

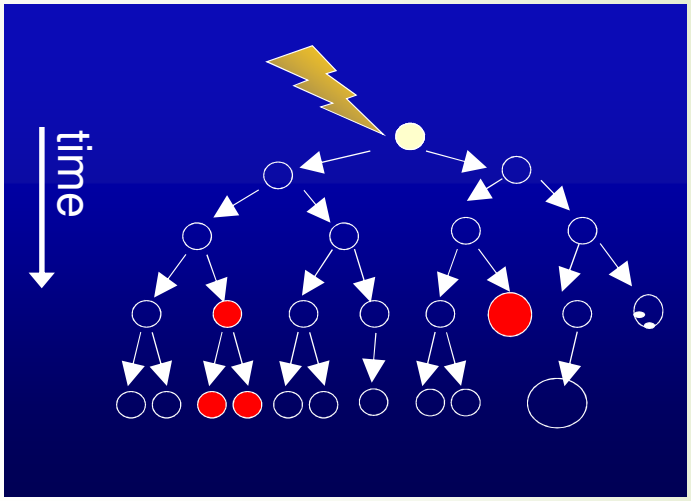
- **The basic assumption in radiation protection is that stochastic risk is directly proportional to dose.** Underground laboratories give the **opportunity to test the linear no-threshold** (LNT) **model** for which **below the averave natural environmental background no detriment is expected**

At low doses effects responsible for non linear responses become particularly relevant

Bystander effect

The target for radiation damage is greater than the initial irradiated volume

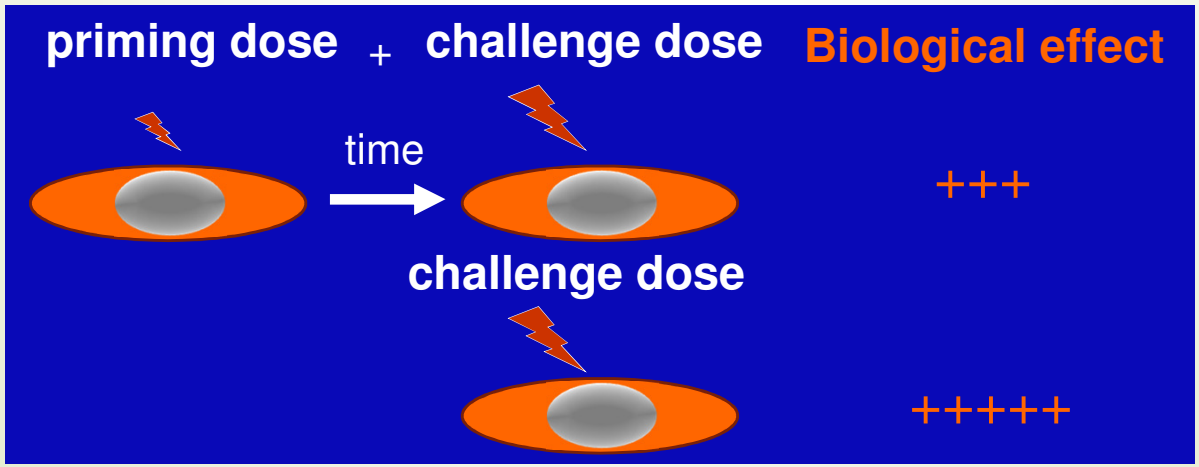




Genomic instability

Damage occurs in the progeny of irradiated or bystander cells

Adaptive response

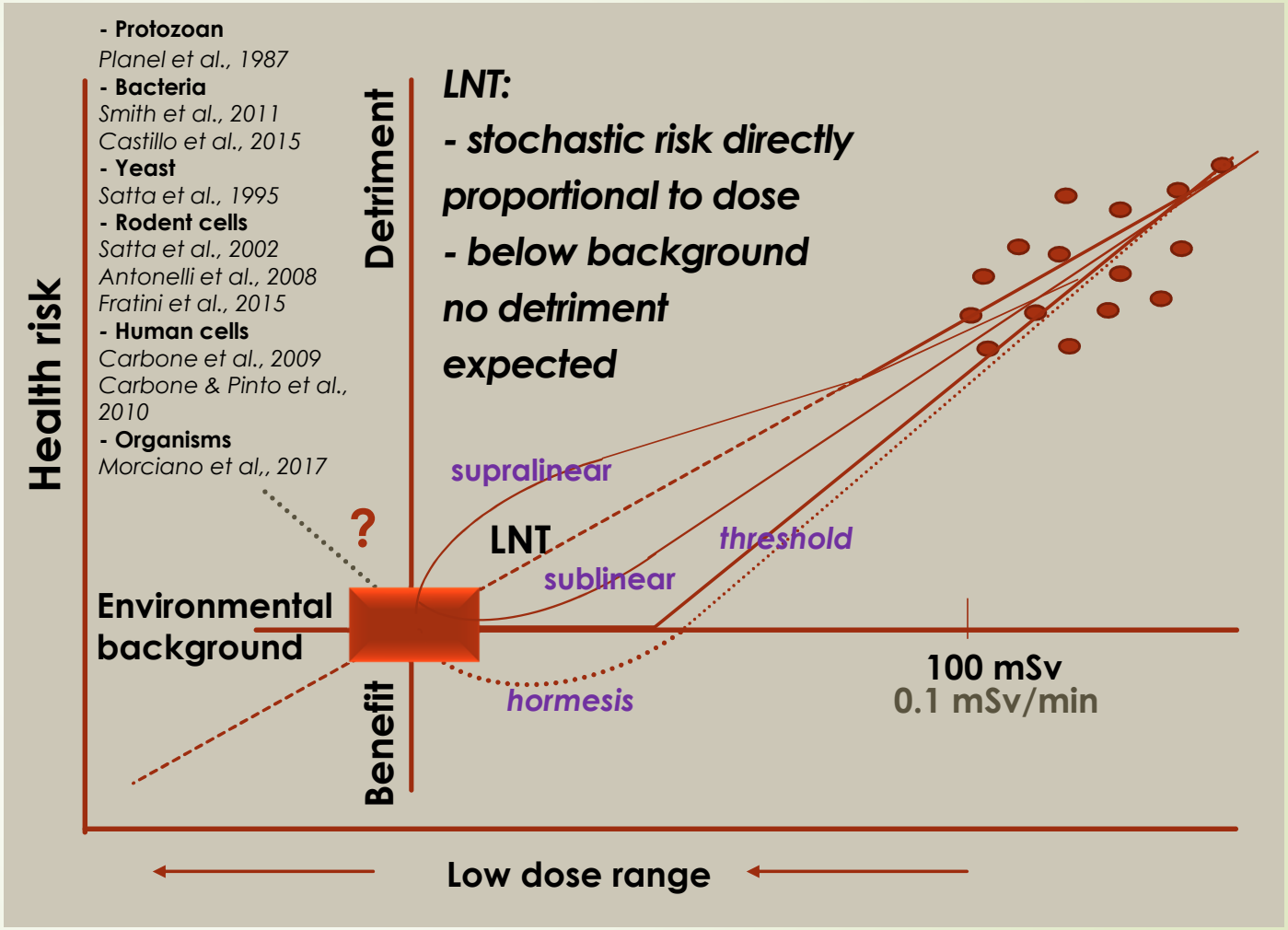


Low doses trigger response mechanisms rendering subsequent treatment with higher doses less effective

Several lines of radiobiological evidence have challenged the LNT

Experimental studies have predicted dose-response relationships which deviate from linearity in two opposite directions: those pointing to **sub-linear extrapolation**, such as from **adaptive response, threshold or hormetic effects** or to **supra-linear extrapolation**, such as may be expected from **bystander effects and genomic instability**

Moreover, **increased stress response** have been observed **below the average environmental background**



average annual background radiation exposure: 3 mSv/y (USA); 2 mSv/y (UK), 7mSv/y (Fi)
higher radiation level areas: Iran (around Ramsar: from 10 to 260 mSv/y); Guarapari Brazil; Kerala, India; Yangjiang, China

At **doses comparable to the environmental background**, there is **little effect** which **disappears in the noise of everyday adverse health effects**

The **design** of an experiment **is a delicate and challenging work**

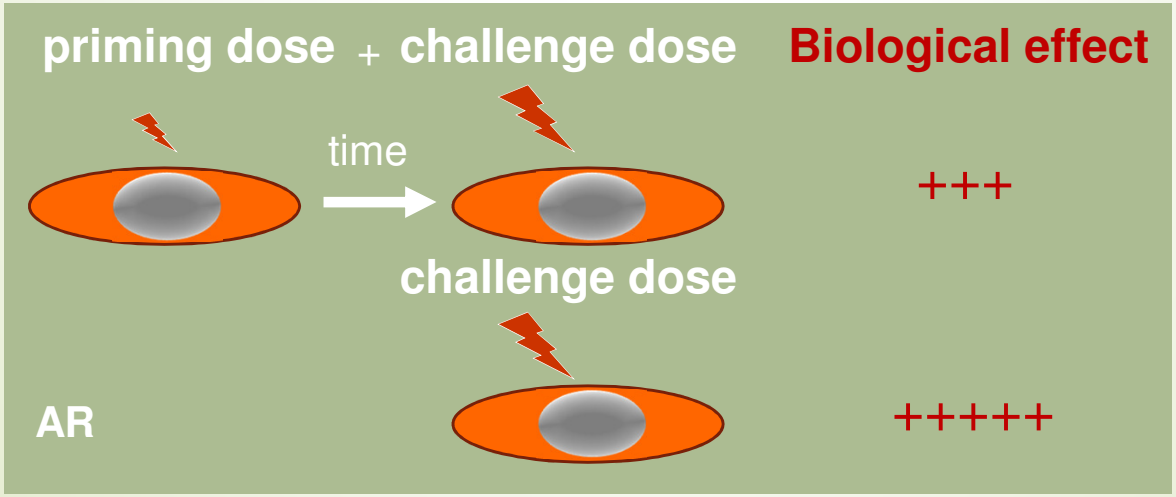
*All the **parameters (biological and physical)** that may influence the response of a biological system **have to be considered** and **possible differences minimized as possible** in the attempt **to make the level of radiation the "sole" difference** between control and treatment*

Among the most important ones:

- **Temperature** (use of incubators or temperature control systems)
- **Pressure** (monitoring)
- **Air quality** (use of filters)
- **Radon** (ventilation systerms and monitoring)
- **Cell culture medium and reagents/food for organisms** (purchase and use of the same products for the entire dutation of the experiment)
- **Environmental radiation** (dosimetric measurements of some components of the radiation spectrum during the experiments)

A **possible strategy** to asses the modulation of the biological response induced by very low doses is **to challenge the biological system with higher doses** of a genotoxic agent (radiation, drugs, …). In this way is possible to amplify possible differences

Adaptive Response (AR)-like scheme: chronic priming dose represented by the environmental radiation

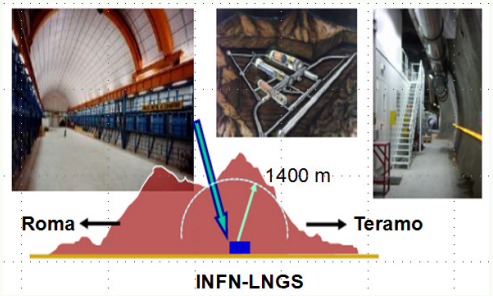


Experimental approach

SET UP OF PARALLEL EXPERIMENTS UNDER DIFFERENT RADIATION ENVIRONMENTS

Low Radiation Environnment (LRE)

LNGS INFN (underground lab)



ISS / UnivAQ (external labs)

Reference Radiation Environment(s) (RRE)

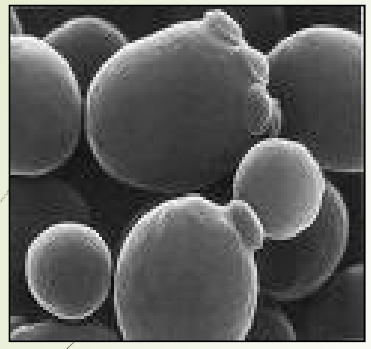
external LNGS

From the LNGS web site:

- The average 1400 m rock coverage gives a reduction factor of one million in the cosmic ray flux; moreover, the neutron flux is thousand times less than on the surface, thanks to the smallness of the Uranium and Thorium content of the dolomite rocks of the mountain
- *The mission of the Laboratory is to host experiments that require a low background environment in the field of astroparticle physics and nuclear astrophysics and other disciplines that can profit of its characteristics and of its infrastructures*

(http://www.lngs.infn.it/lngs_infn/index.htm?mainRecord=http://www.lngs.infn.it/lngs_infn/contents/lngs_en/public/about/)

The pioniering work of Satta et al.



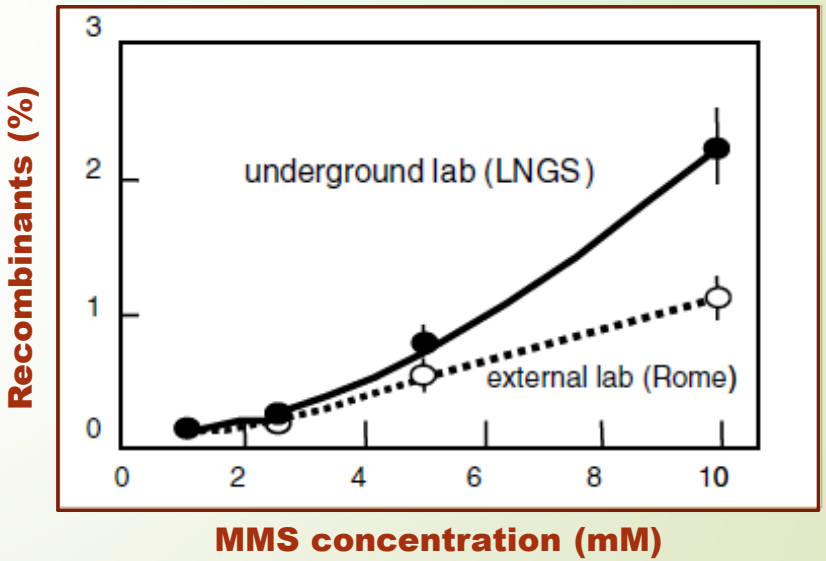
Reference Laboratory: Institute of Genetics, "La Sapienza" University, Rome

Cell line (yeasts): Saccaromyces cerevisiae

Culture time: 1week (120 generations)

Genotoxic agent: Methyl methan sulphonate (MMS), radiomimetic compound

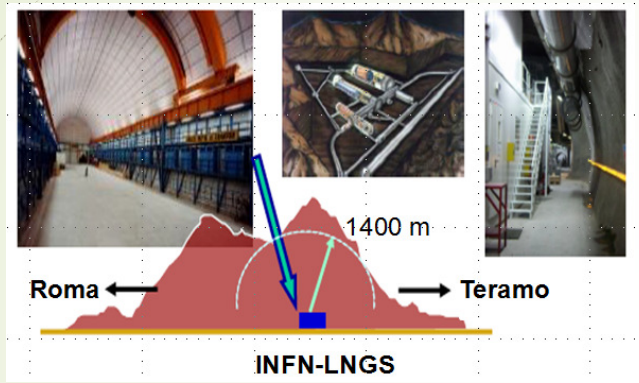
Results: Higher frequency of recombination in yeast cells grown underground LNGS, respect to those grown at La Sapienza University (Rome)



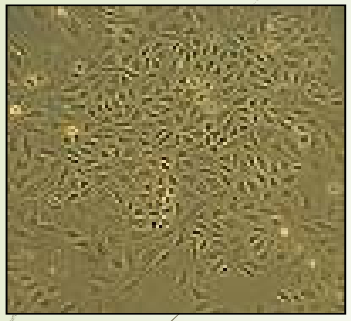
Satta et al., Mut Res, 1995

Since 1995, from yeasts to mamalian cells ….

The PULEX underground cell culture facility



The PULEX experiment



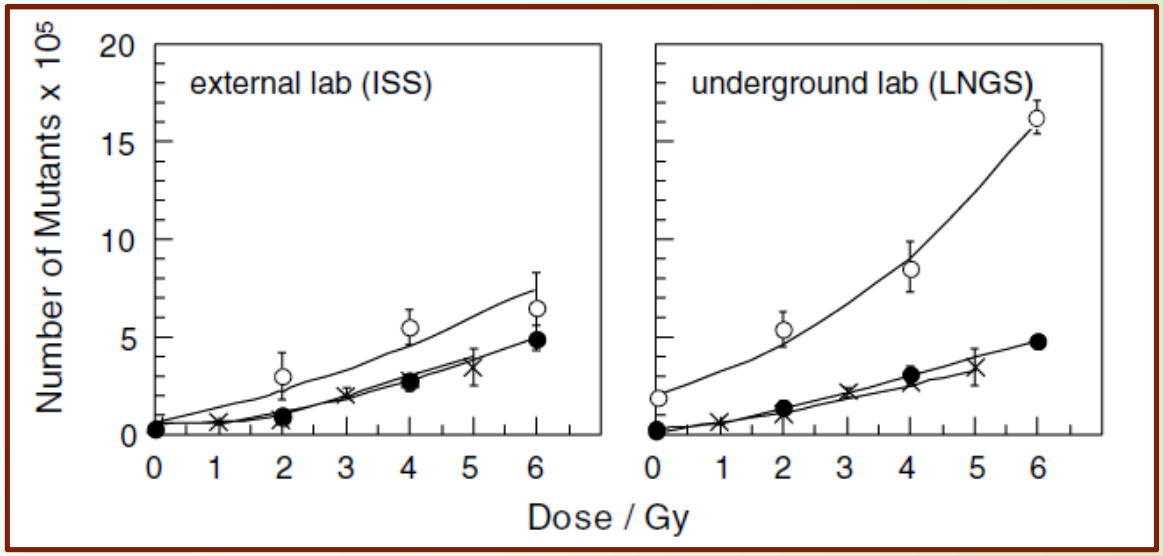
Reference Laboratory: Instituto Superiore di Sanità, Rome

Mammalian cell line (rodent): V79 Chinese hamster lung fibroblasts

Culture time: 3 and 9 months

Genotoxic agent: X-rays

Results: Higher mutation frequency in cells grown in reduced radiation environment

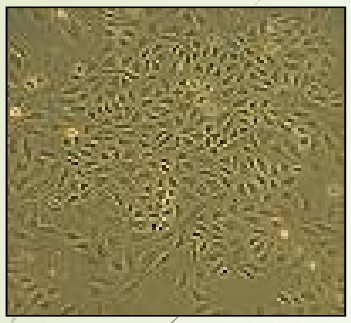


Black symbols: 3 months; White symbols: 9 months

Satta et al., Rad Environ Biophys, 2002; Antonelli et al., Il Nuovo Cimento, 2008

The PULEX experiment - 2

Reference Laboratory: LNGS external lab

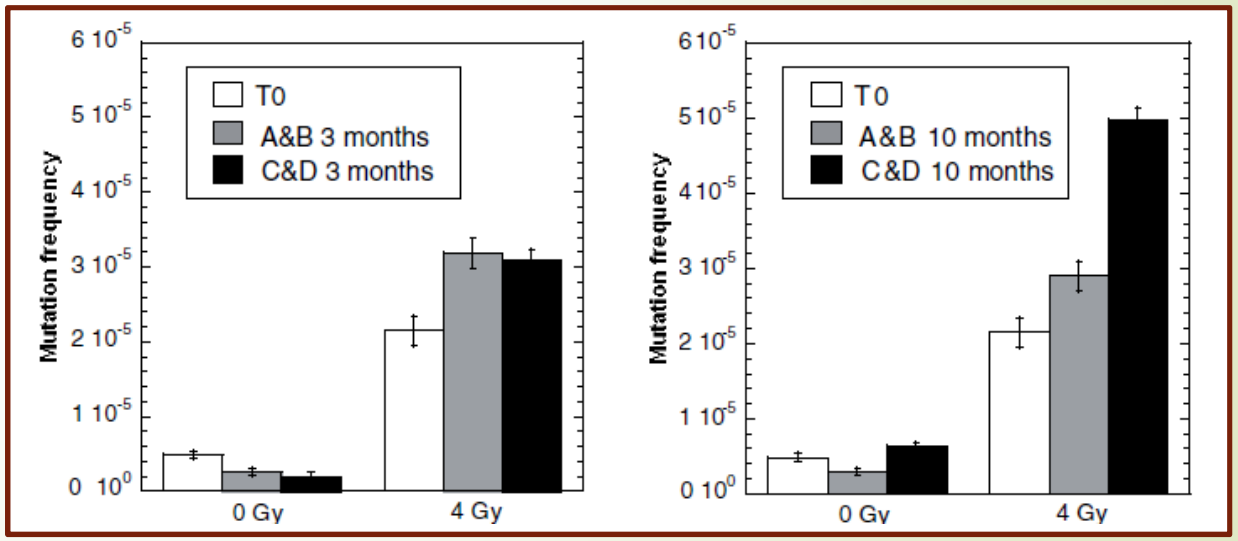


Mammalian cell line (rodent): V79 Chinese hamster lung fibroblasts

Culture time: 3 and 10 months

Genotoxic agent: X-rays

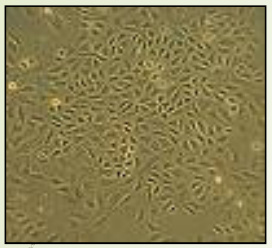
Results: At 10 months higher mutation frequency in cells grown in reduced radiation environment



A&B: external cultures; C&D: underground cultures

Satta et al., Rad Environ Biophys, 2002; Antonelli et al., Il Nuovo Cimento, 2008

The PULEX experiment - 3



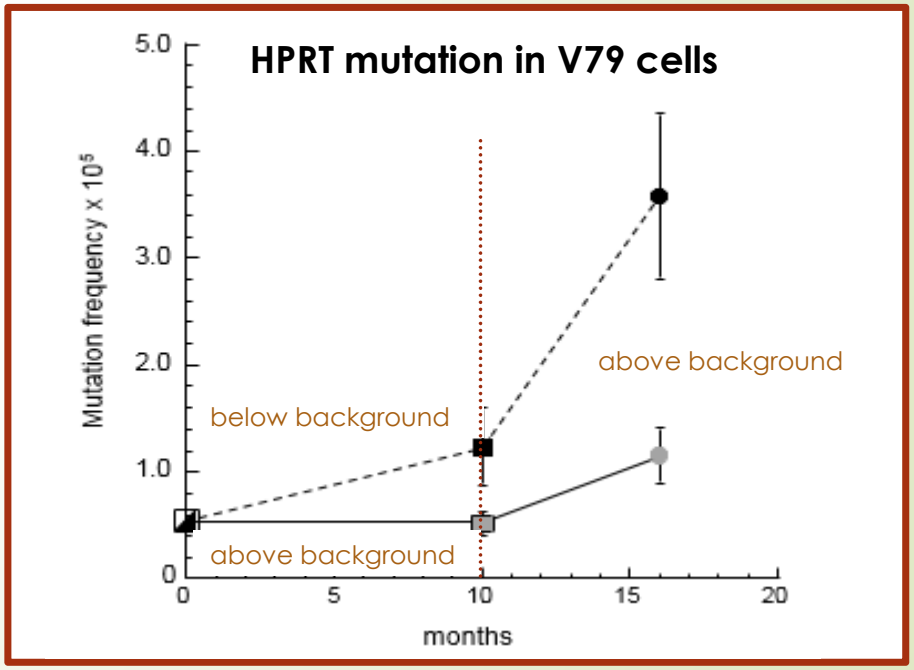
Reference Laboratory: LNGS external lab

Mammalian cell line (rodent): V79 Chinese hamster lung fibroblasts

Culture time: 10 + 6 months above background

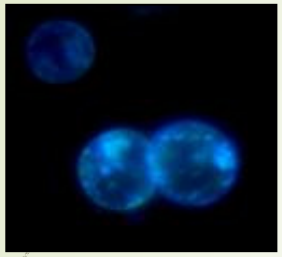
Results: Higher spontaneus mutation frequency in cells grown in reduced radiation environment. After further 6 months above background the MF of cells kept below background increases to an extent comparable to the one observed after a dose of 2.5 Gy X-rays

Moreover, the antioxidant enzyme activity (GPX) is strongly reduced below background



Fratini et al., Radiat Environ Biophys 2015

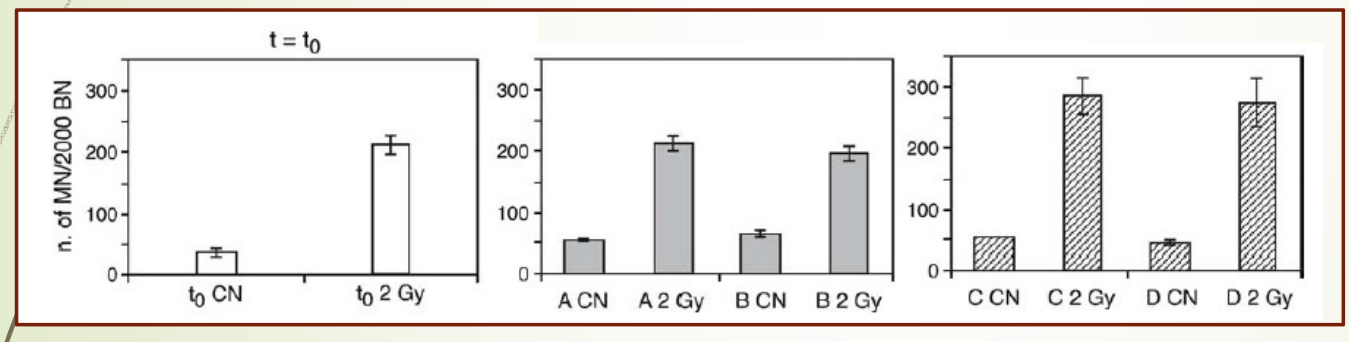
The COSMIC SILENCE experiment



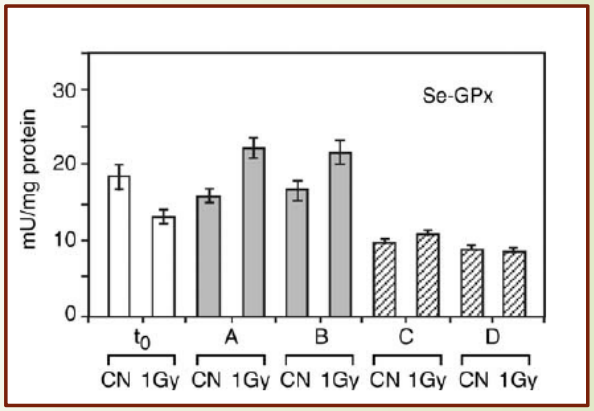
Reference Laboratory: Instituto Superiore di Sanità, Rome

Mammalian cell line (human): TK6 Lymphoblasts

Culture time: 6 months; **Genotoxic agent:** X-rays



A&B: external cultures; C&D: underground cultures



Results: Higher micronuclei induction and reduced capability of ROS scavenging in cells grown in reduced radiation environment

Carbone et al., Rad Environ Biophys, 2009; Carbone & Pinto et al., Il Nuovo Cimento, 2010

Summary of long term experiments on in vitro models

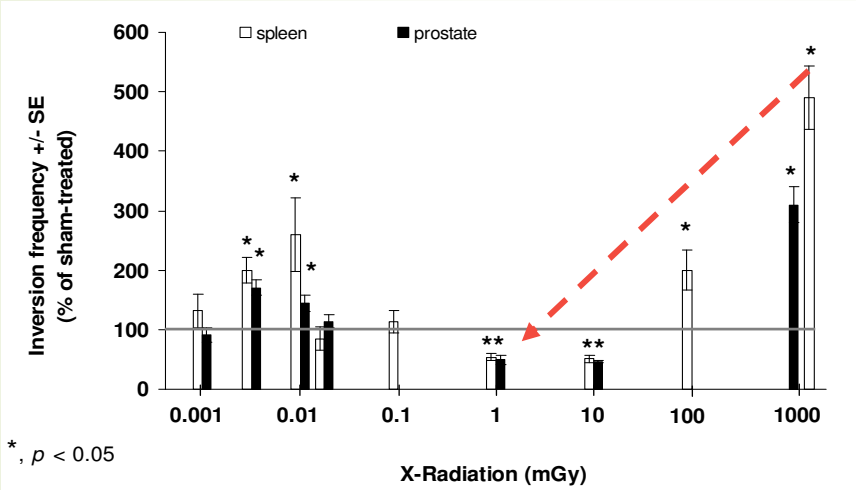
yeasts, rodent and human cells cultured in reduced environmental radiation conditions for several months are:

- **less tolerant to radiation-induced DNA damage**
- **less efficient in scavenging reactive oxygen species**

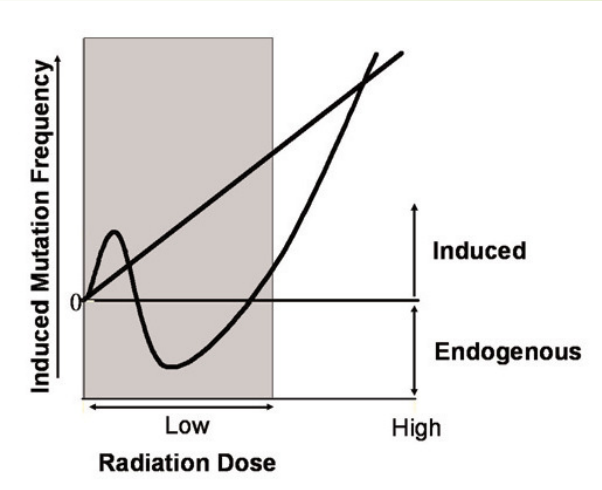
- Moreover, there are suggestions of **epigenetic effects** leading to the maintanance of the below background induced changes, at least for some months

The collaboration with the Flinders University (Adelaide, Australia)

pKZ1 response to acute-dose of ionizing radiations



Zeng et al., 2006



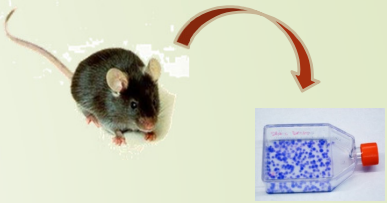
Sykes et al., 2006

Low dose radiation dose-response curve. Inversions were induced in pKZ1 at very low and at high doses of radiation exposure. ***Intermediate doses of radiation caused a decrease below endogenous inversion frequency.*** *The straight line represents the LNT theory.*

What about pkZ1 response after chronic exposures to a very low dose rate ?

Short term experiments on in vitro models

Mammalian cell line (rodent): A11 cells isolated from pKZ1 mouse, kindly donated by Prof. Pam. Sykes



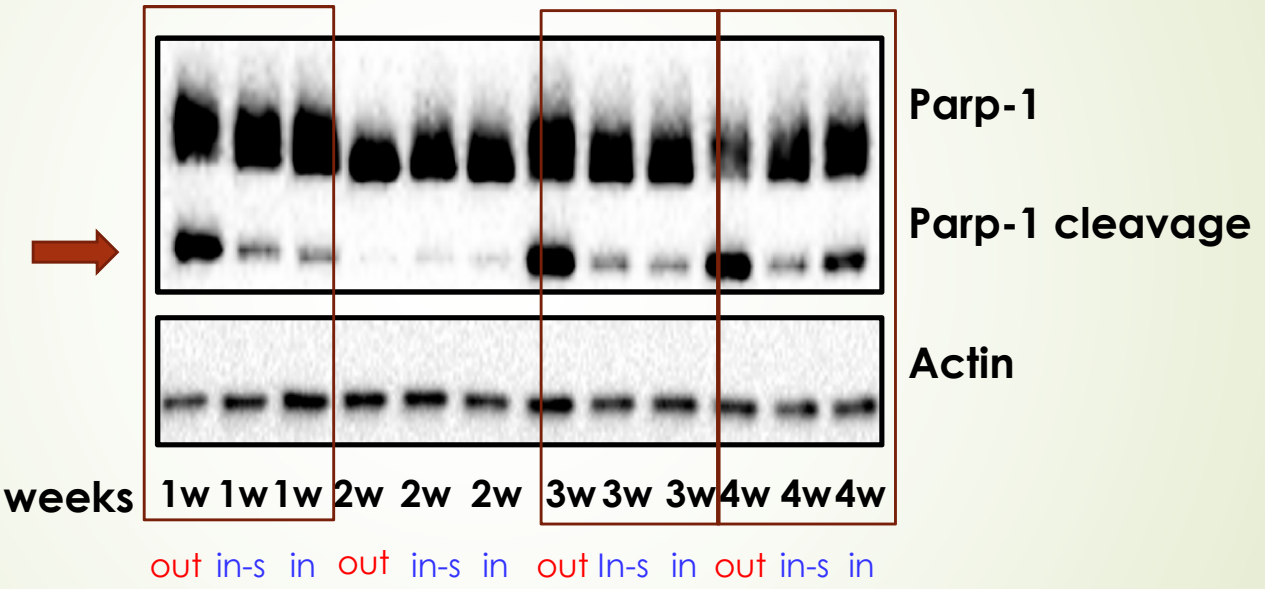
Reference Laboratory: Instituto Superiore di Sanità, Rome

Culture time: up to 1 month

End point studied: Cleavage of **PARP-**1, a **key protein in DNA repair** as well as in differentiation, proliferation, and tumor transformation. It has been studied in A11 cells grown for **4 weeks** in **3 different environmental radiation conditions**:

1- Reference Radiation Environment (**RRE**)
2-3 Low Radiation Environment (**LRE**): (i) **in the presence or** (ii) **in the absence of Fe shield**

- PARP-1 cleavage start after 3 days of exponential growth
- At 4 days of culture: LRE cells show a significantly lower level of PARP-1 cleavage than RRE cells
- The presence of Fe shield does not affect the LRE cell response



out = RRE (*ISS*) *in-s* = *LRE* (*with Fe-shield*) *in* = *LRE* (*without Fe-shield*)

1-3 and 4w samples have been collected after 4 days of exponential culture; the 2w samples have been collected after 3 days of exponential culture (no PARP-1 cleavage is expected)

Summary of short term experiments on in vitro models

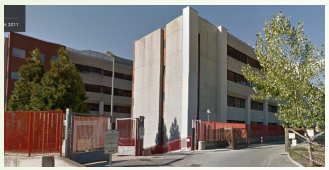
- **Gene expression** experiments have shown **early r**esponse to changes in the radiation environment
- The **gamma component** seems **not significantly influence** the biological response
- The **effect is manifested** only **in the presence of endogenous stress**, possibly related to starvation

***In vitro* biological systems appears to be very good sensors of environmental radiation exposure**

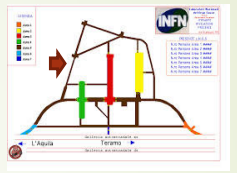
Is this behaviour also present in vivo ?

From in vitro … to in vivo model systems

L'Aquila University/Rome University
Reference Radiation Environment
(RRE lab)




INFN-LNGS
Low Radiation Environment
(LRE lab)

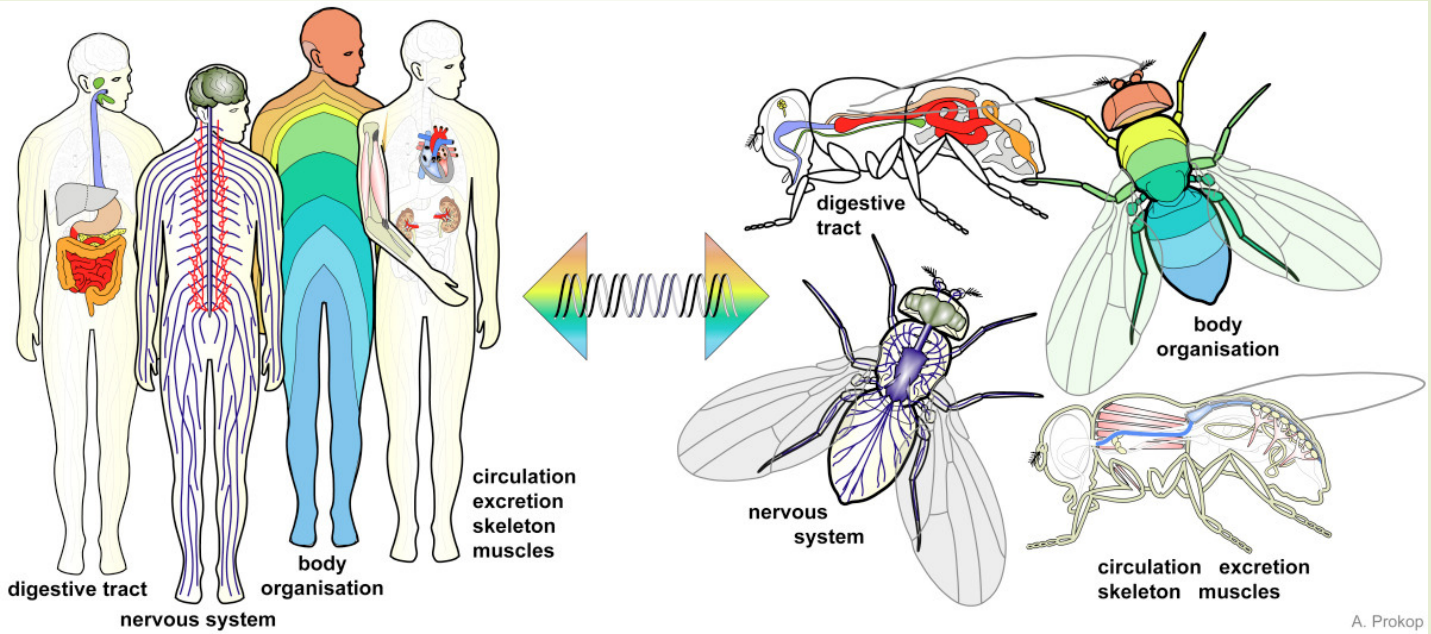



Drosophila melanogaster

pKZ1 mice

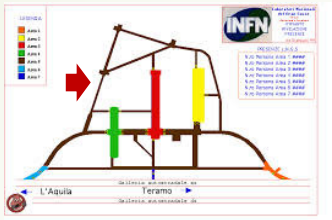
Animal housing and experimental procedures need to be approved by the competent Authorities (ASL, Ethical Committee, Ministero della Salute)

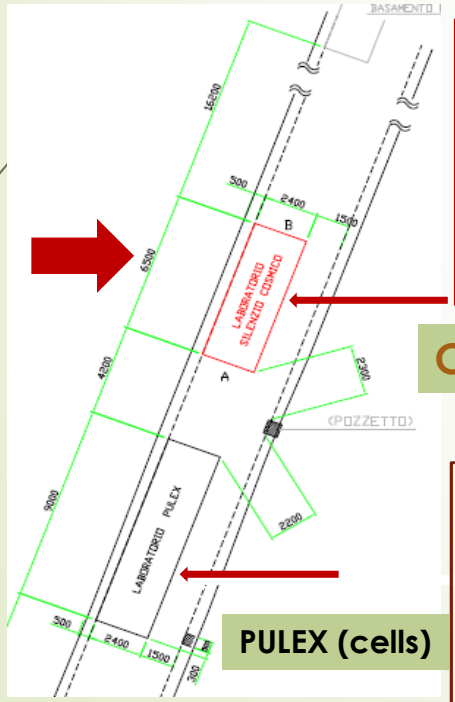
Why the fly?

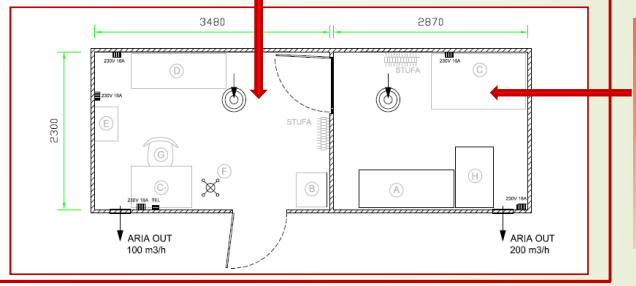


- For almost every organ in humans there is a match in flies, and common genes regulate their development, organisation and function
- About 75% of human disease genes have a recognisable match in fruit flies

COSMIC SILENCE - the new underground animal housing facility

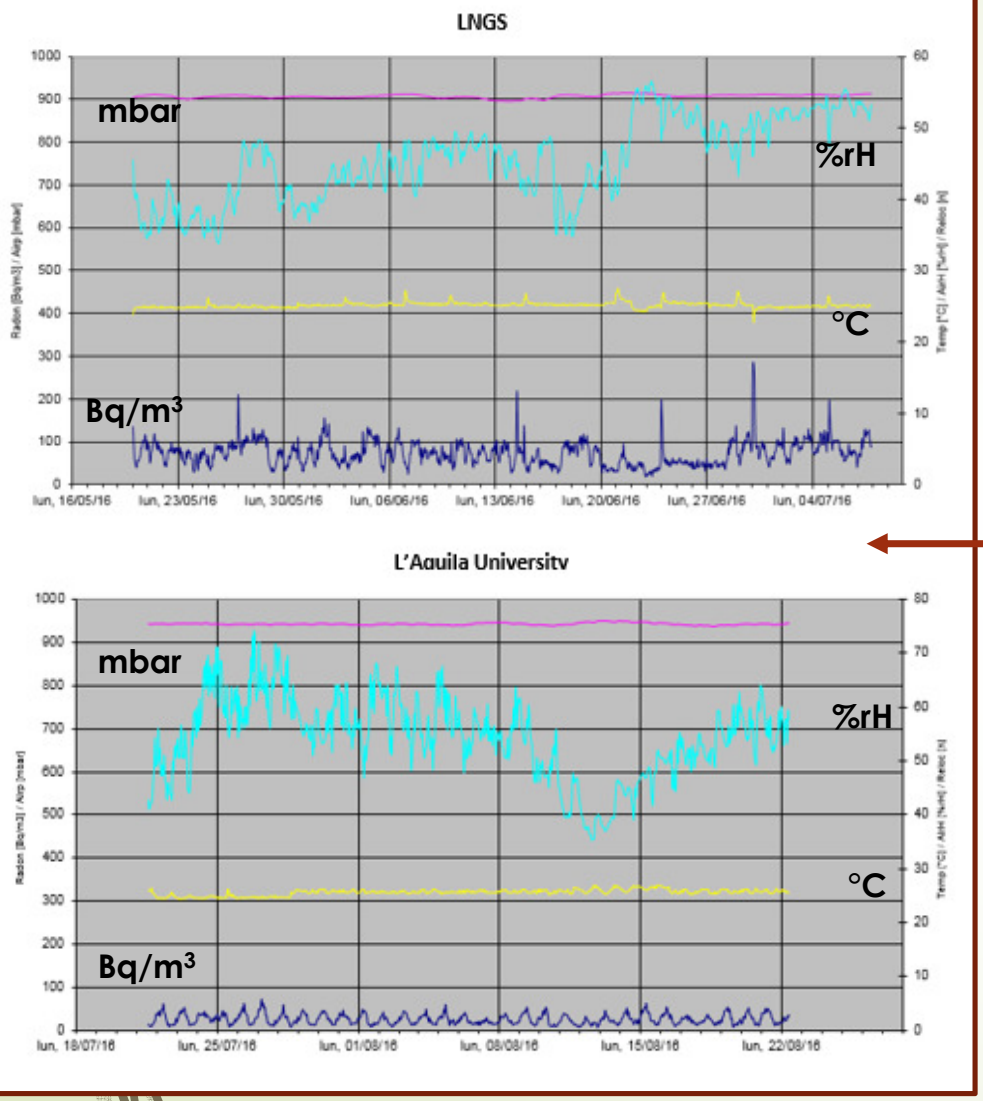






COSMIC SILENCE (animals)

PULEX (cells)



Environmental monitoring using the Alfaguard equipment

During the experiments we carried out **radon monitoring** in both underground and external reference laboratories using the Alfaguard equipment

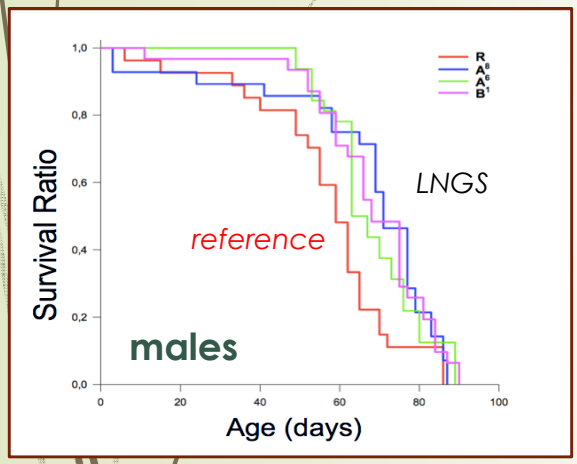
We found radon values in the underground facility higher than those in the reference laboratory. Possible solutions to this problem are under consideration

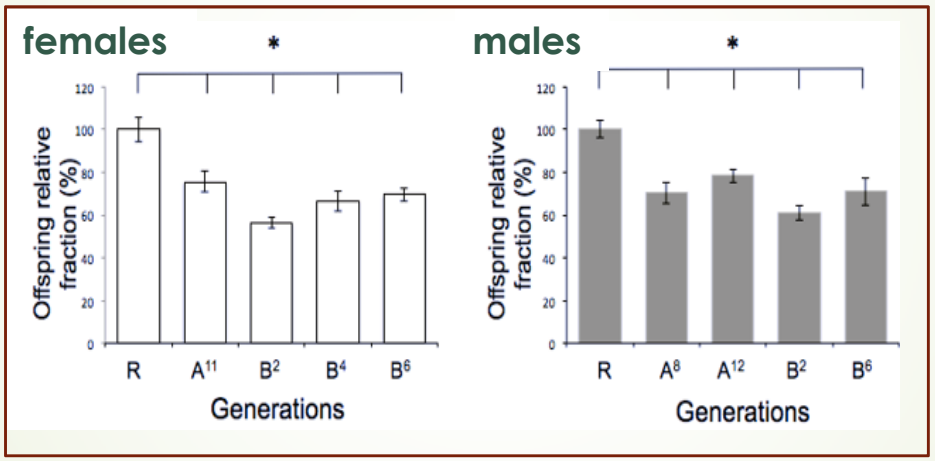
Besides radon, other environmental parameters, namely **temperature, pressure** and **relative humidity** have also been recorded

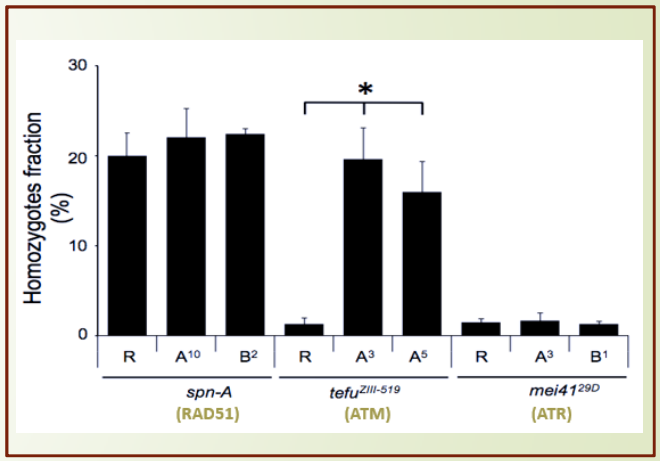
*As an example, in the period **May-August 2016** we registered the **same temperature** in the two experimental sites (25.6 ± 0.6 °C vs 25.2 ± 0.4 °C) and a **slight increase in the average values of pressure** (941.9 ± 2.7 mbar vs 906.7 ± 4.0 mbar) **and relative humidity** (54.1 ± 7.6 % vs 45.5 ± 5.6 %) in the external laboratory. **On the basis of the literature evidence, these differences are not expected to affect the biology of Drosophila.***

Effects of reduced natural background radiation on Drosophila melanogaster

Permanence in reduced background radiation (underground LNGS): **extends the lifespan** of male flies, **reduces fertility** of adult flies and **affects the response to genotoxic stress** (positive selection of *tefu* mutant flies)







Fertility reduction is an **early effect** and remained **unchanged along different generation time**

The positive selection of *tefu* is maintained in mutant lines moved and kept to reference radiation environment for 2 more generations, indicating that it **is retained in a trans-generational manner**

R = reference flies
A^x and B^x = different populations and generations of LNGS flies

Morciano et al., Journal of Cell Physiology 2017

Future plans

These *in vivo* results represent the first data set on the effects of environmental radiation ever obtained from a complex model organism in a underground laboratory and therefore can be considered as a foot hold for deep underground biology of complex multicellular systems

We plan to **further investigate** the **molecular mechanisms** underlying the observed effects **in Drosophila**

In terms of mechanistic interpretation of our results, besides the biological experiments it will be of **crucial importance trying to identify the component(s) of the radiation spectrum triggering the biological response**

Conclusions

The *in vitro* experiments carried out at the LNGS on cultured mammalian cells of rodent and human origin represent the largest evidence on the effects of reduced environmental radiation on eukaryotic cellular systems. Moreover, similar effects have been observed *in vivo*

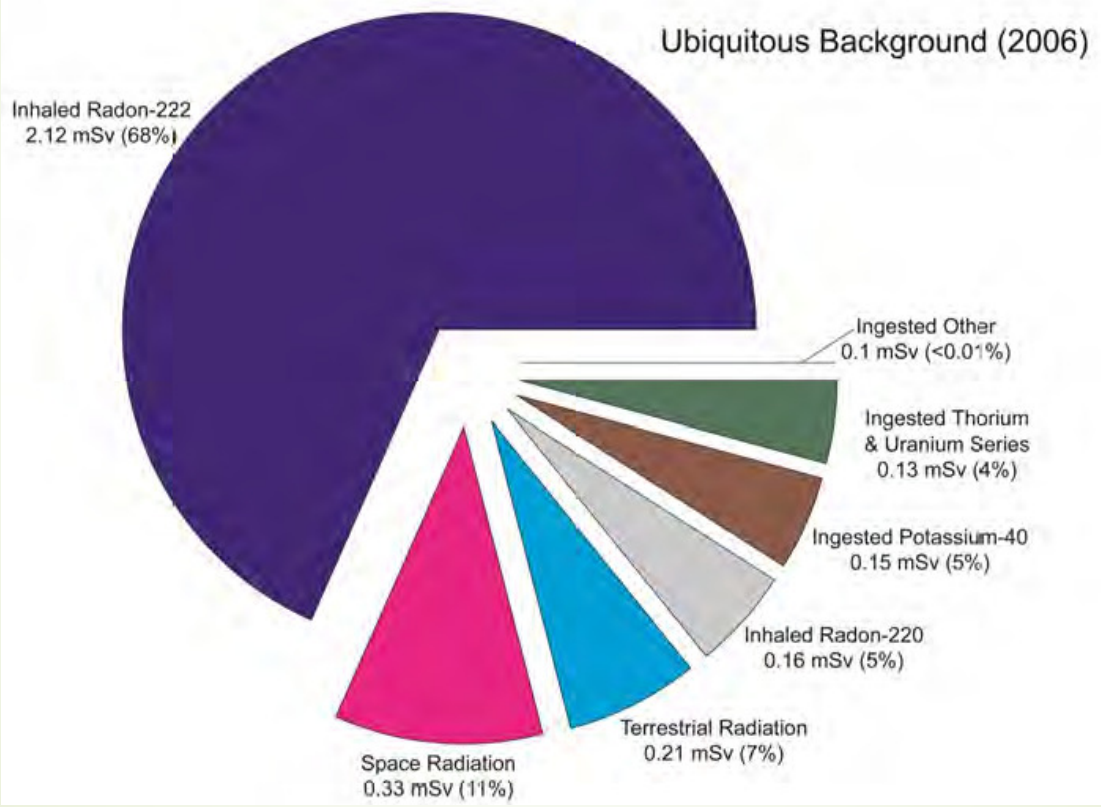
Low background biological research has consistently shown that despite the natural radiation background already being incredibly small, it is nevertheless significant enough for living systems to sense it and respond to it

All this points to the importance at very low doses of cell-cell communication phenomena that are able to produce effects higher than expected on the basis of the single interaction

On behalf of the PULEX-COSMIC SILENCE collaboration

Thank you very much for the attention !

✓ENVIRONMENTAL RADIATION
- constant daily stimulus on Earth
- well preserved defence mechanisms have been developed by all living organisms during phylogenesis



Distribution of the population dose among the various sources of background radiation (from National Council on Radiation Protection and Measurements, http://NCRPonline.org)