Deep Underground Biology: Past, Present, and Future

Michel R. Lapointe, Ph.D.

SNOLAB Future Projects Workshop

April, 2025

Overview

- Summary of current sub-NBR findings
  - Separated by biological systems
- Interpretation of the data
- Challenges in translating results
- Next Step: Global deep underground biology collaborative project

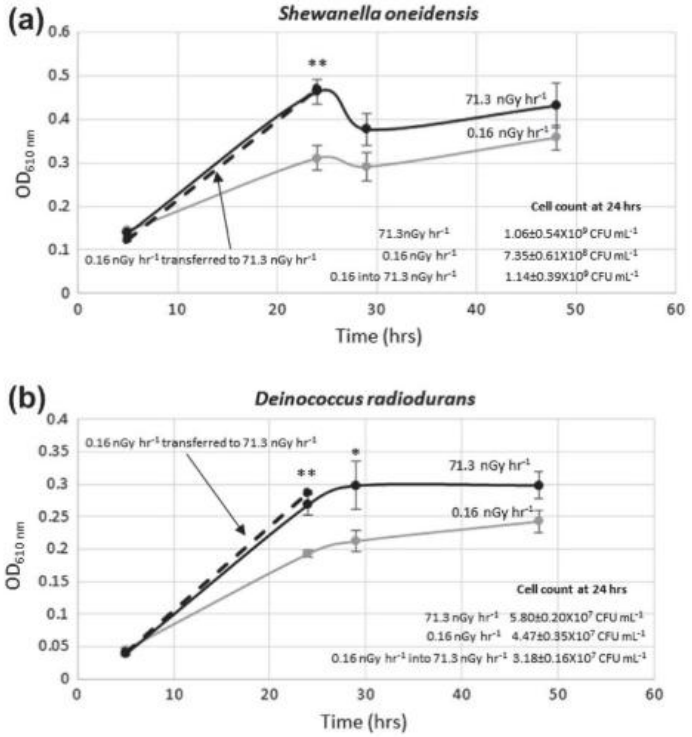
Logo design generated by AI (ChatGPT/DALL-E, OpenAI, 2025).

Summary of Current Sub-NBR Findings

Organism	Shielding	Dosimetry				Results	Reference
		Shielded	Control	Reduction	Duration		
Prokaryotes							
Cyanobacteria (Synechococcus lividus)	Lead	0.27 mGy/yr	1.49 mGy/yr	6 fold	35 days	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of thorium nitrate (1.52 mGy/yr)	Conter 1983, Planel 1987 [70,71]
	Lead	0.27 mGy/yr	1.49 mGy/yr	6 fold	21 days	· Reduction in growth rate with shielding of media only	Conter 1987 [70]
Bacteria (Deinococcus radiodurans)	WIPP[a]	0.018 mGy/yr	0.27 mGy/yr	15 fold	48 hours	· Reduction in growth rate with shielding	Smith 2011 [72]
	WIPP	0.0014 mGy/yr	0.62 mGy/yr	445 fold (79 fold)*	48 hours	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of KCl (0.62 mGy/yr) · Increased expression of HSP genes (dnaK) with shielding · Expression levels down regulated when background radiation reintroduced	Castillo 2015 [64]
	WIPP	0.008 mGy/yr	0.63 mGy/yr	79 fold	48 hours	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of KCl (0.62 mGy/yr) · Increased expression of DNA repair genes (lexA) and HSP genes (dnaK) with shielding · Decreased expression of oxidative stress genes (dps) and glucose metabolism genes (gapdH) with shielding · Expression levels return to normal when background radiation reintroduced	Castillo 2017 [73]
	WIPP	0.008 mGy/yr	0.63 mGy/yr	79 fold	72 hours	· Upregulation of membrane transport proteins with shielding · Downregulation of protein transport, protein folding, and protein hydrolysis with shielding	Castillo 2021 [66]
Bacteria (Shewanella oneidensis)	WIPP	0.0014 mGy/yr	0.62 mGy/yr	445 fold*	48 hours	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of KCl (0.62 mGy/yr) · Increased expression of oxidative stress genes (katB), DNA repair genes (recA) and putative efflux pump (SOA0154) with shielding · Expression levels down regulated when background radiation reintroduced	Castillo 2015 [64]
	WIPP	0.008 mGy/yr	0.63 mGy/yr	79 fold	48 hours	· Increased expression of oxidative stress genes (katB, oxyR), DNA repair genes (recA, lexA), HSP genes (dnaK), and putative efflux pump (SOA0154) with shielding · Expression levels return to normal when background radiation reintroduced	Castillo 2017 [73]
	WIPP	0.008 mGy/yr	0.63 mGy/yr	79 fold	24 hours	· Reduced expression of ribosomal protein and tRNA genes during early exponential growth with shielding · Increased expression of membrane transport, oxidative phosphorylation, amino acid synthesis genes and reduced expression of ribosomal protein, protein folding genes during late exponential growth with shielding.	Castillo 2018 [65]

Prokaryotes

10 publications in total, dating back to 1983, as recent as 2021. Primary findings in relation to sub-NBR exposure are decreased growth rates, increased heat shock protein expression, DNA damage response protein expression, decreased glucose metabolism genes, dysregulation of protein transport and membrane transport proteins.



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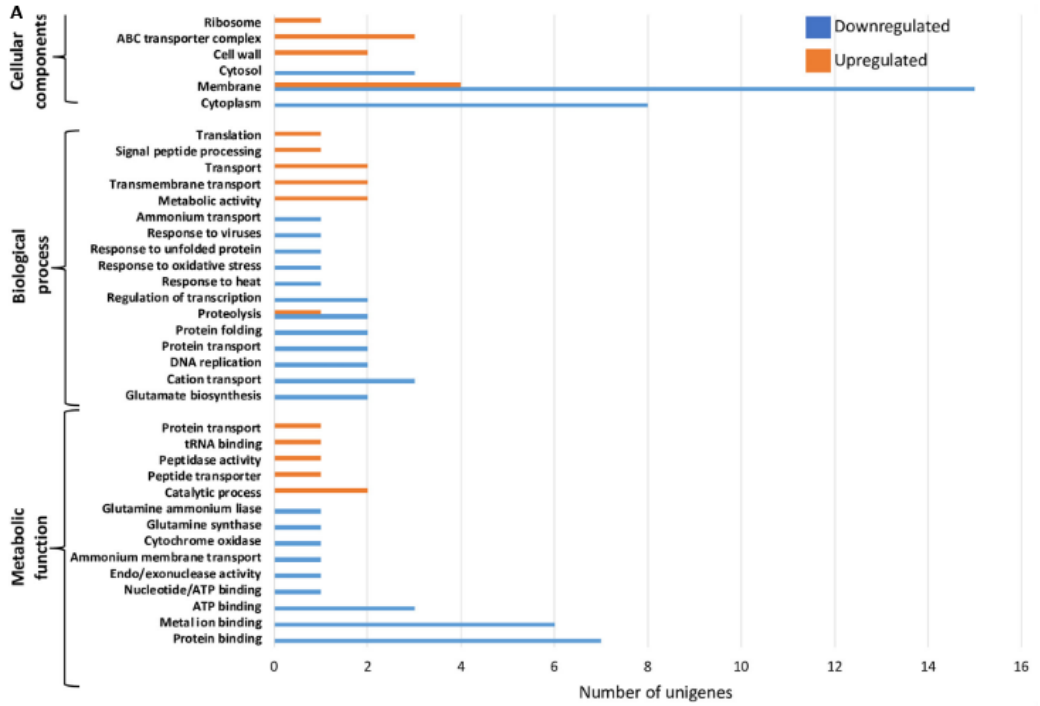
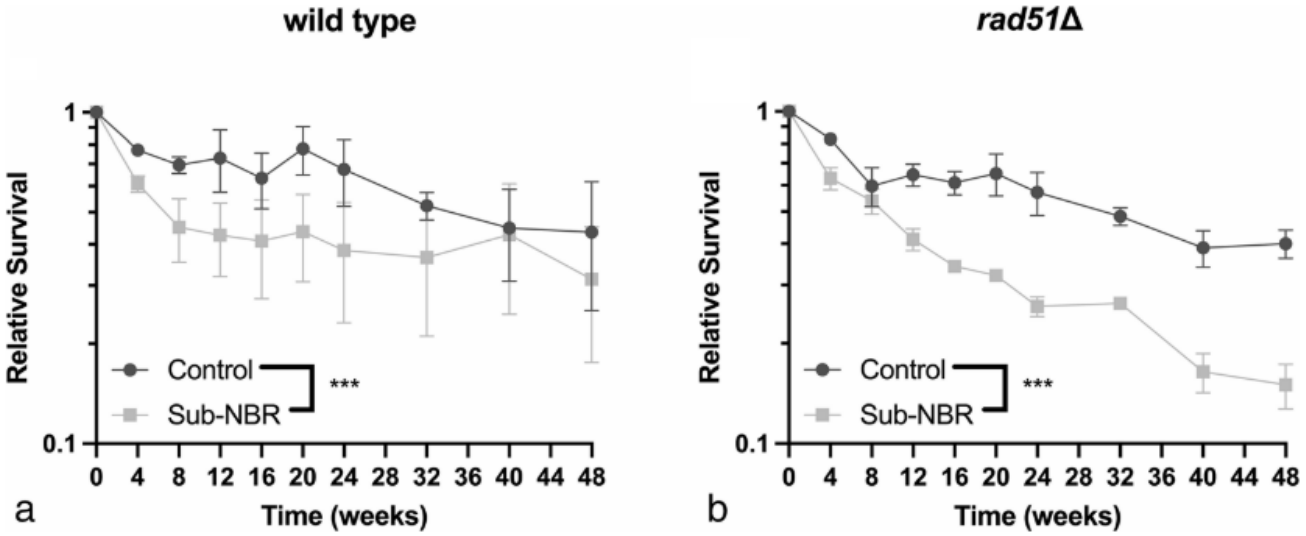


10.3389/fgene.2021.644292

Organism	Shielding	Dosimetry			Duration	Results	Reference
		Shielded	Control	Reduction			
Single cell eukaryotes							
Protozoa (Paramecium tetraurelia)	CNRS[b] and lead	0.1 mGy/yr	1.65 mGy/yr	17 fold	10 days	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of ^{60}Co source	Planel 1976, Planel 1987 [70,74]
	Lead	0.3 mGy/yr	1.75 mGy/yr	6 fold	10 days	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of ^{232}Th source (7 mGy/yr)	Planel 1976, Planel 1987 [70,74]
	Lead	0.12 mGy/yr	0.75 mGy/yr	6 fold	6 days	· Reduction in growth rate with shielding · Reduction greatest post autogamy, when catalase levels are higher	Croute 1980 [75]
	Lead	0.36 mGy/yr	1.25 mGy/yr	3 fold	Lifespan (up to 75 days)	· Increase in total number of fissions with shielding · Fission reduced with introduction of ^{60}Co source (7.51 mGy/yr)	Tixador 1981 [76]
	Iron and paraffin	0.04 mGy/yr	1.03 mGy/yr	26 fold	55 days	· Reduction in growth rate with shielding · Growth reduction occurs only after prolonged growth (> 35 days) · Growth rate returns to baseline with introduction of ^{137}Cs source (0.87 mGy/yr)	Kawanishi 2012 [62]
Protozoa (Tetrahymena pyriformis)	ANL[c]	0.54 mGy/yr	1.83 mGy/yr	3 fold	6 days	· Reduction in growth rate with shielding · Further reduction in growth rate when incubated with ^{39}K instead of ^{40}K	Luckey 1986 [57]
Yeast (Saccharomyces cerevisiae)	LNGS[d]	0.22 mGy/yr	1.46 mGy/yr	7 fold	At least 120 generations	· Increase in recombinant and aberrant frequency in low-background adapted cells when treated with methyl methanesulfonate	Satta 1995 [58]
	Lead , Steel, Cadmium and Copper	0.44 mGy/yr	1.1 mGy/yr	3 fold	100 generations	· Reduction in survival in shielded cells with ^{60}Co challenge (100 Gy)	Gajendiran 2002 [59]
Desiccated Yeast (Saccharomyces cerevisiae)	SNOLAB	10.1 nGy/hr	68.0 nGy/hr	6.7 fold	52 weeks	· Reduced baseline survival and post-rehydration growth and metabolic activity	Lapointe 2023

Single cell (lower) eukaryotes

11 publications in total, dating back to 1976 and as recent as 2023. Common findings relating to sub-NBR exposure are decreased growth rates, increased mutation rates, and decreased radiation resistance. Desiccated yeast elicited significantly decreased survival.



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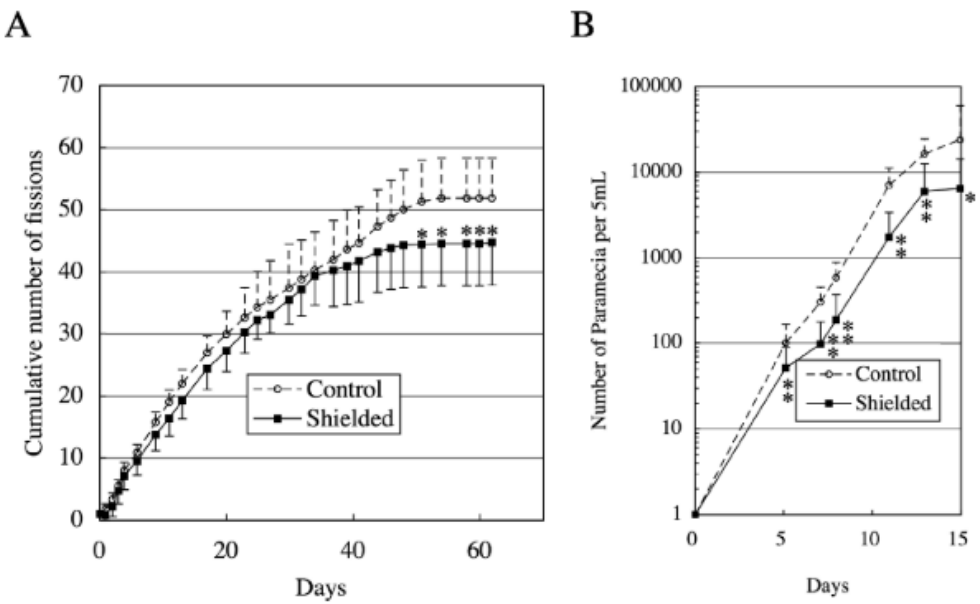

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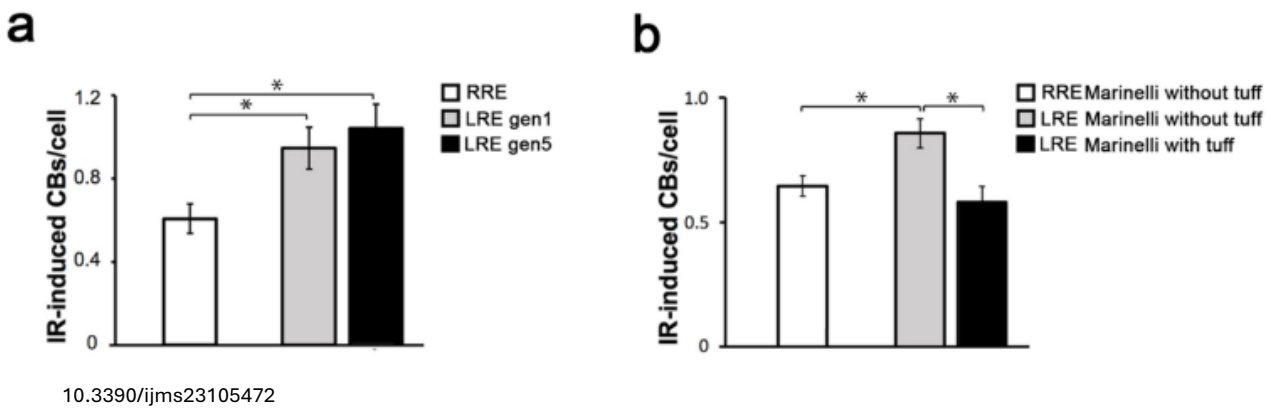
Organism	Shielding	Dosimetry				Results	Reference
		Shielded	Control	Reduction	Duration		
Multicellular eukaryotes							
Fruit flies (Drosophila melanogaster)	LNGS	0.20 mGy/yr	0.83 mGy/yr	4 fold	9 months	· Increase in lifespan with shielding · No change in climbing behavior · Reduction in fertility (30%) with shielding · Selective pressure on ATM mutant flies	Morciano 2018 [77]
	DULB-4900	0.14 mGy/yr	1.44 mGy/yr	10 fold	14 days	· 0.44% of total transcripts significantly altered from shielding · Common low background-induced transcriptional alterations with radiation doses: 4 with low dose radiation exposure, 10 with high dose radiation exposure, and one with both · Transcriptional alterations from low background exposure common with various stressors: 9 fungal treatment, 4 spaceflight, and 6 chronic circadian misalignment	Zarubin 2021 [68]
	LNGS	0.18 mGy/yr	0.58mGy/yr	3 fold	1 and 5 generations	· Two-fold increase in chromosome breaks present 4 hours after 10 Gy gamma dose from shielding · No change in chromosome break incidence between generation 1 and generation 5	Porrazzo 2022 [69]
Nematodes (Caenorhabitis elegans)	WIPP	0.14 mGy/yr	0.61 mGy/yr	4 fold	8 months	· Increased size of young larvae (48 hours) and egg laying rate · No change in egg hatch rates or adult body size · Significant upregulation in genes associated with sperm proteins, collagen and cuticle related genes, non-coding RNA, and hypothetical proteins · Significant downregulation of genes associated with collagen, cuticle, and hypothetical proteins	Van Voorhies 2020 [67]
Lake Whitefish (Coregonus clupeaformis)	SNOLAB	11.55 nGy/hr	68.04 nGy/hr	5.89 fold	160 days	· Significant increase in embryo body mass.	Pirkkanen 2020

Multicellular eukaryotes

Five total publications as early as 2018, as recent as 2022. Primary findings relating to sub-NBR exposure are reduction in fertility, large-scale dysregulation of protein expression, increased sensitivity post sub-NBR exposure to acute high dose ionizing radiation, increased size of offspring.



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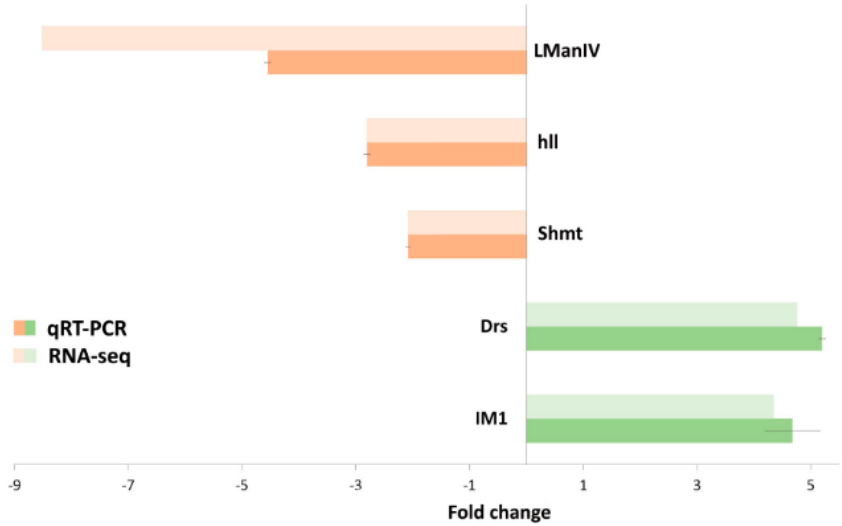

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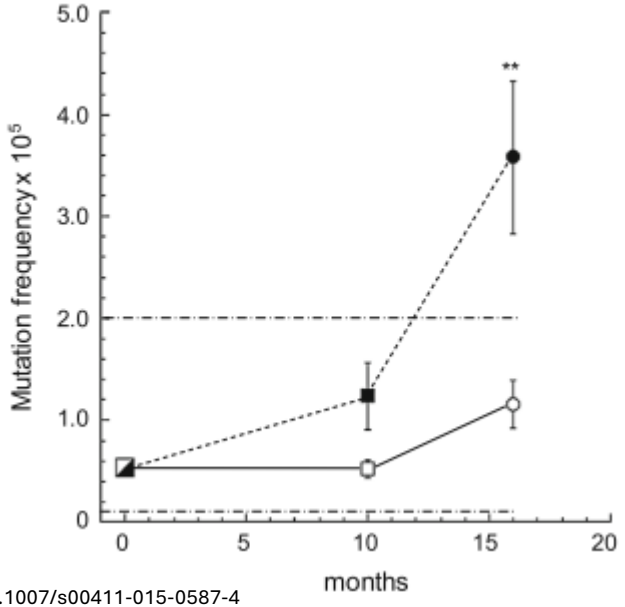
Organism	Shielding	Dosimetry				Results	Reference
		Shielded	Control	Reduction	Duration		
Mammalian cell culture							
Chinese hamster V79 lung fibroblast cells	LNGS	0.047 mGy/yr	3.17 mGy/yr	67 fold	3 and 9 months	· Increase in cell density at plateau phase with shielding at 9 months but not at 3 months · No change in cell growth rate · Increase in sensitivity to CHX induced apoptosis with shielding at 3 months but not at 9 months · Decrease in basal levels of c-myc and p53 with shielding at 9 months but not at 3 months · Decrease in SOD and increase in GSH-Px, GSSG-Rx, catalase with shielding at 9 months but not at 3 months · Increase in mutation frequency (basal and gamma ray induced) with shielding at 9 months but not at 3 months	Satta 2002 [60]
	LNGS	0.039 mGy/yr	0.612 mGy/yr	16 fold	3 and 10 months	· Increase in mutation frequency following x-irradiation with shielding at 10 months but not at 3 months · Increase in sensitivity to CHX induced apoptosis with shielding at 3 and 10 months · No change in c-myc and p53 expression · Reduced antioxidant scavenging ability with shielding at 10 months but not at 3 months · No change in micronuclei formation with shielding at 10 months with x-ray challenge (1 Gy)	Antonelli 2008 [78]
	LNGS	0.20 mGy/yr	0.83 mGy/yr	4 fold	10 and 16 months	· Reduction in GPX levels with shielding at 10 and 16 months, no change in SOD or catalase · No change in GPX, SOD, catalase or SBP1 mRNA with shielding at 10 or 16 months · Increase in mutation frequency with shielding at 10 and 16 months · GPX levels remain low and mutation frequency remains high after return to normal background radiation environment	Fratini 2015 [61]
Mouse L5178Y lymphoma cells	Lead	0.16 mGy/yr	0.48 mGy/yr	3 fold		· Reduction in growth rate with shielding	Takizawa 1992 [79]
	Iron and paraffin	0.04 mGy/yr	1.03 mGy/yr	26 fold	7 days	· Reduction in growth rate with shielding · Growth rate returns to baseline with introduction of ^{137}Cs source (0.87 mGy/yr)	Kawanishi 2012 [62]
Mouse M10 cells (XRCC4 deficient)	Iron and paraffin	0.04 mGy/yr	1.03 mGy/yr	26 fold	7 days	· No change in growth rate with shielding	Kawanishi 2012 [62]

Organism	Shielding	Dosimetry				Results	Reference
		Shielded	Control	Reduction	Duration		
pKZ1 A11 mouse hybridoma cells	LNGS + Iron	-	-	- + 4 fold*	4 weeks	· No change in growth rate in the low dose environment · PARP1 cleavage was reduced in overgrown cells within the low dose environment · The cellular response to overgrowth shifted towards autophagy in lieu of apoptosis	Fischietti 2021 [63]
Human TK6 lymphoblast cells	LNGS	0.033 mGy/yr	2.91 mGy/yr	87 fold	6 months	· No change in growth rate with shielding · Increase in micronuclei formation with shielding and x-ray challenge (2 Gy) · Reduction in catalase and Se-GPx with shielding · No increase in Se-GPx levels with shielding and x-ray challenge (1 Gy) · Reduction in ROS scavenging efficiency with shielding	Carbone 2009 [80]
	LNGS	0.033 mGy/yr	2.91 mGy/yr	87 fold	6 months	· Reduction in spontaneous micronuclei formation with shielding · Increase in micronuclei formation with shielding and x-ray challenge (2 Gy) · Reduction in ROS scavenging efficiency with shielding	Carbone 2010 [81]
Primary human lung fibroblast cells	Lead	0.3 mGy/yr	1.75 mGy/yr	6 fold	10 passages	· Increase in Hsp 90B and Hsp 70 expression with shielding · Further increase in Hsp expression with x-ray challenge (100 mGy)	Smith 2011 [72]
Bronchial epithelial cells	Lead	0.3 mGy/yr	1.75 mGy/yr	6 fold	10 passages	· Increase in Hsp 90B and Hsp 70 expression with shielding · Further increase in Hsp expression with x-ray challenge (100 mGy)	Smith 2011 [72]
CGL1 Human Hybrid Cell Line	SNOLAB + Lead + Rn mitigation	2.48 nGy/hr	68.04 nGy.hr	27.4 fold	16 passages	· Increased iALP expression, indicative of higher neoplastic transformation	Pirkkanen 2024

Mammalian cell culture

12 publications to date as far back as 1992, as recent as 2024. Primary results from sub-NBR exposure are increased mutation frequency and sensitivity to apoptotic stimuli, reduced antioxidant and ROS scavenging capacity, altered stress protein expression, and shifts in cellular stress responses. In most cases, these effects occurred with increasing intensity and/or frequency with prolonged exposure (increased over time).



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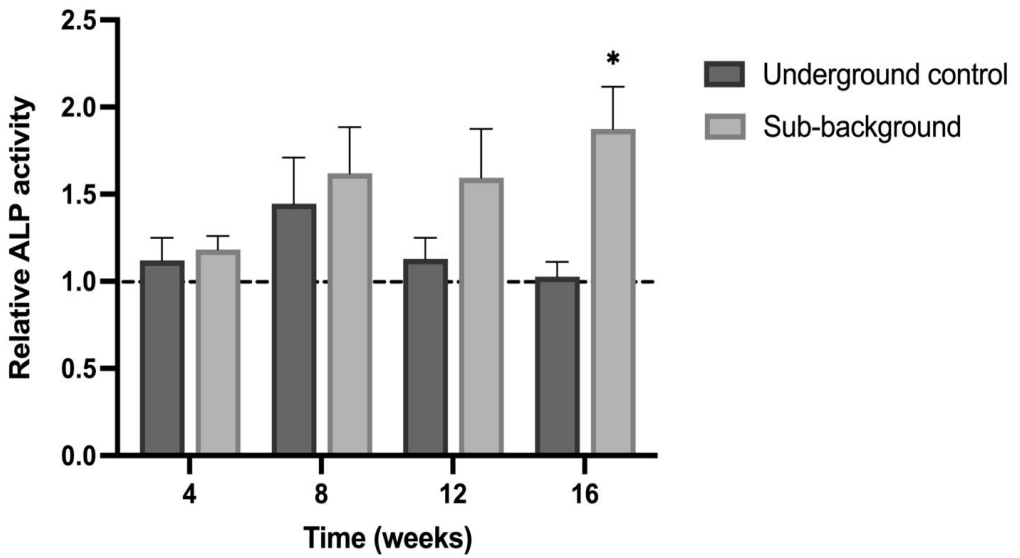

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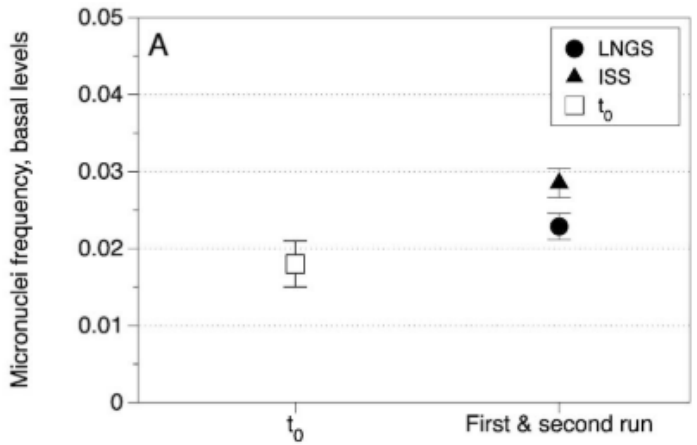


10.1393/ncb/i2010-10889-y

Summary of Current Sub-NBR Findings

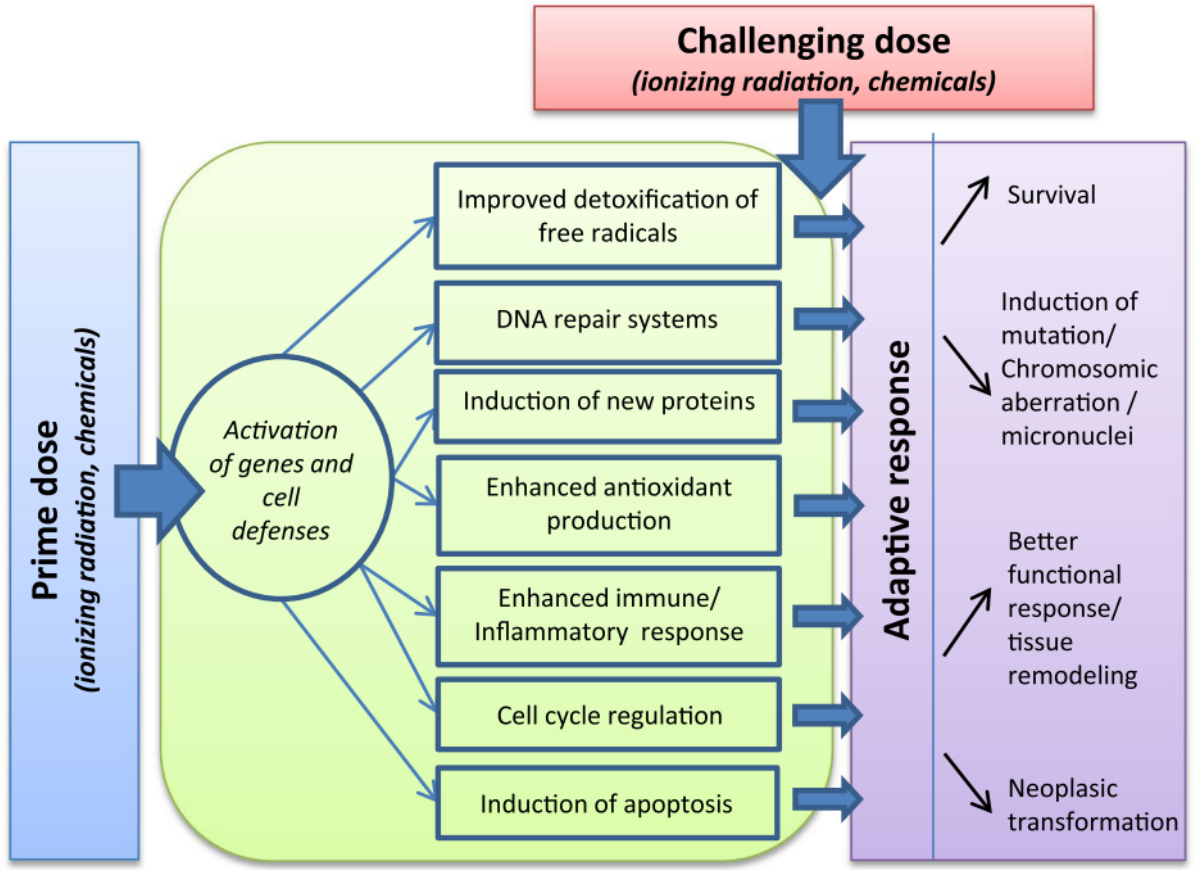
General summary:

- Reduced growth and/or reproductive fitness,
- Increased genomic stress at baseline,
- Impaired antioxidant defenses,
- Increased sensitivity to subsequent stressors such as acute doses of ionizing radiation

These effects are often exacerbated with prolonged exposure and are apparent from molecular signaling and organismal-level outcomes.

Interpretation Of The Data

Fundamentals of Radiobiology: Priming Stressors and Adaptive Response



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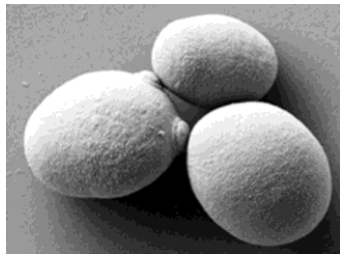
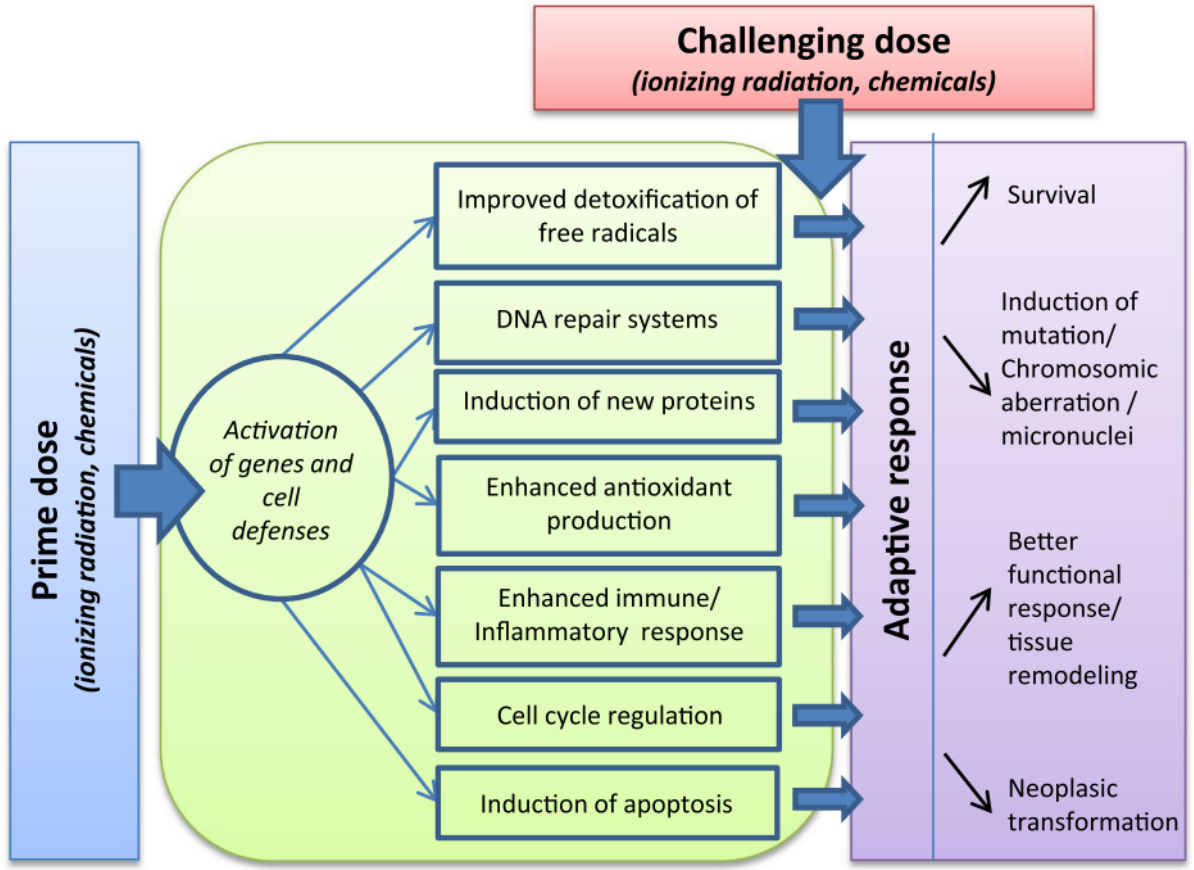
Fundamentals of Radiobiology: Priming Stressors and Adaptive Response





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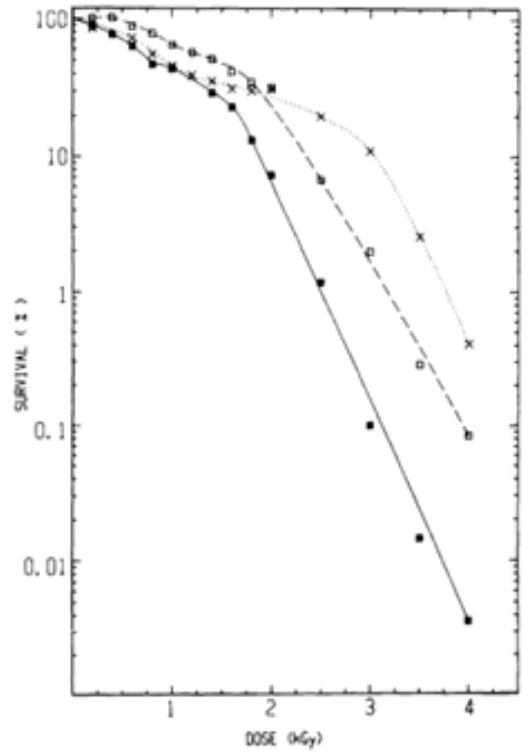

FIG. 3. Ionizing radiation survival curves of wild-type strain MJ67 after exposure to a heat shock at 38°C. Radiation survival prior to the shock, ■; radiation survival after 20 min at the shock temperature, □; radiation survival after 60 min at the shock temperature, ×.

https://doi.org/10.2307/3575853

Fundamentals of Radiobiology: Priming Stressors and Adaptive Response

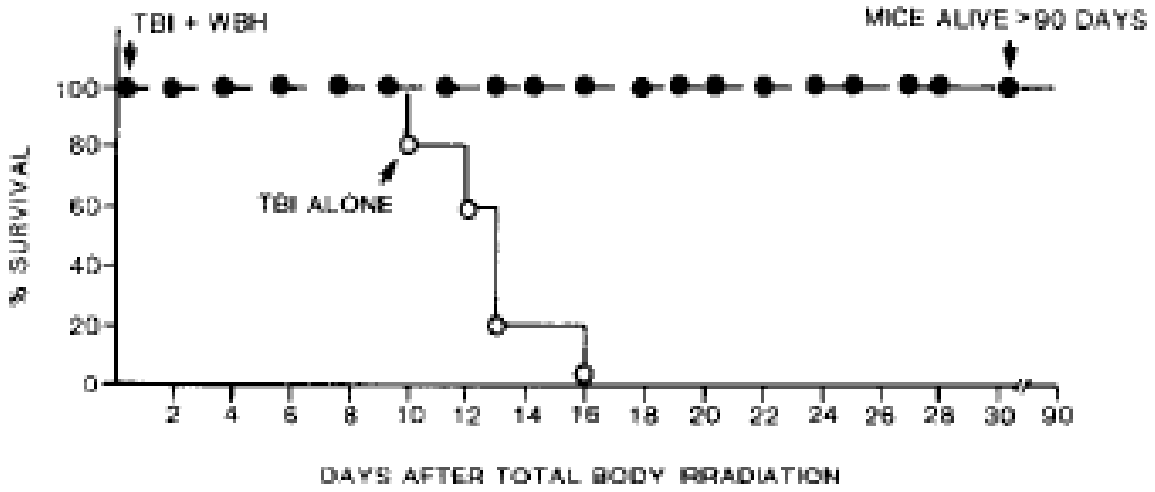

Fig. 1. Radioprotective effect of WBH on mice given lethal doses of TBI: (○) Mice receiving 900 cGy TBI, (●) Mice given WBH 20 hr before 900 cGy TBI. The data represent three separate experiments involving a total of 45 mice in each group. Statistical Analysis, TBI alone vs WBH + TBI $p < 0.001$.

ELSEVIER

International Journal of Radiation Oncology*Biology*Physics

Volume 20, Issue 3, March 1991, Pages 525-530

Original contribution

Whole body hyperthermia: A potent radioprotector *in vivo* ☆

Rong-Nian Shen M.D. * § , Ned B Hornback M.D. ‡, Homayoon Shidnia M.D. ‡, Bo Wu M.D. ‡, Li Lu M.D. * §, Hal E Broxmeyer Ph.D. * † §

WBH - 40°C for 60 minutes 20 hours prior to 9 Gy radiation

Challenges In Translating Results

Translation Issues: Dosimetric Reporting

Volume 118 (2013) http://dx.doi.org/10.6028/jres.118.021
Journal of Research of the National Institute of Standards and Technology

The Importance of Dosimetry Standardization in Radiobiology

Marc Desrosiers[1], Larry DeWerd[2], James Deye[3], Patricia Lindsay[4], Mark K. Murphy[5], Michael Mitch[1], Francesca Macchiarini[6], Strahinja Stojadinovic[7], and Helen Stone[3]

1. Biologists and physicists should collaborate on study design and execution.
2. Study design should indicate the accuracy and precision required to meet the expected experimental result.
3. A qualified radiation physicist should help to establish the methods needed to achieve the required accuracy and precision.
4. The physicist should help to establish an ongoing dosimetry constancy program with traceability to National or International standards.
5. Authors should include in their publications sufficient detail concerning the setup and dosimetry used for the study, including references to written standards and/or protocols used. This will require journal editors and reviewers to ensure compliance.
6. The radiobiology community should publish a list of the minimum dosimetry information to be included within publications (see examples in the Appendix).
7. The radiobiology community should determine where gaps exist in written standards and protocols and publish standards to fill those needs. The workshop participants recommended formation of 3 working groups tasked to develop protocols for routine radiobiology experiments: one each for cells, small laboratory animals, and large laboratory animals.
8. The radiobiology community should decide whether a formal dosimetry intercomparison program needs to be implemented for the radiobiology researchers and, if so, how will it be established and sustained.
9. One suggested mechanism for implementation of many of these recommendations would be to establish continuing education venues in both the radiobiology and physics communities to foster communication and arrive at agreed upon standards.

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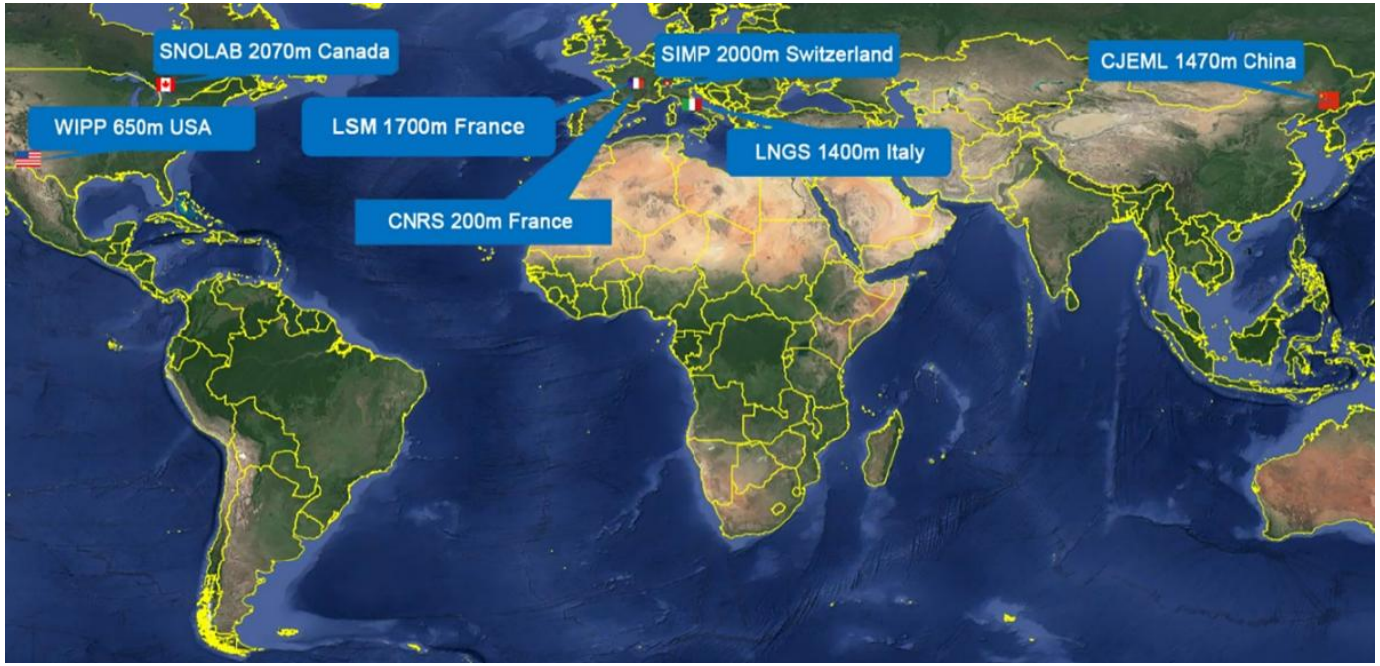
Has not been done well, historically.

Next Step:
Multi-DUL Biology

Global Deep Underground Biology Collaborative Project

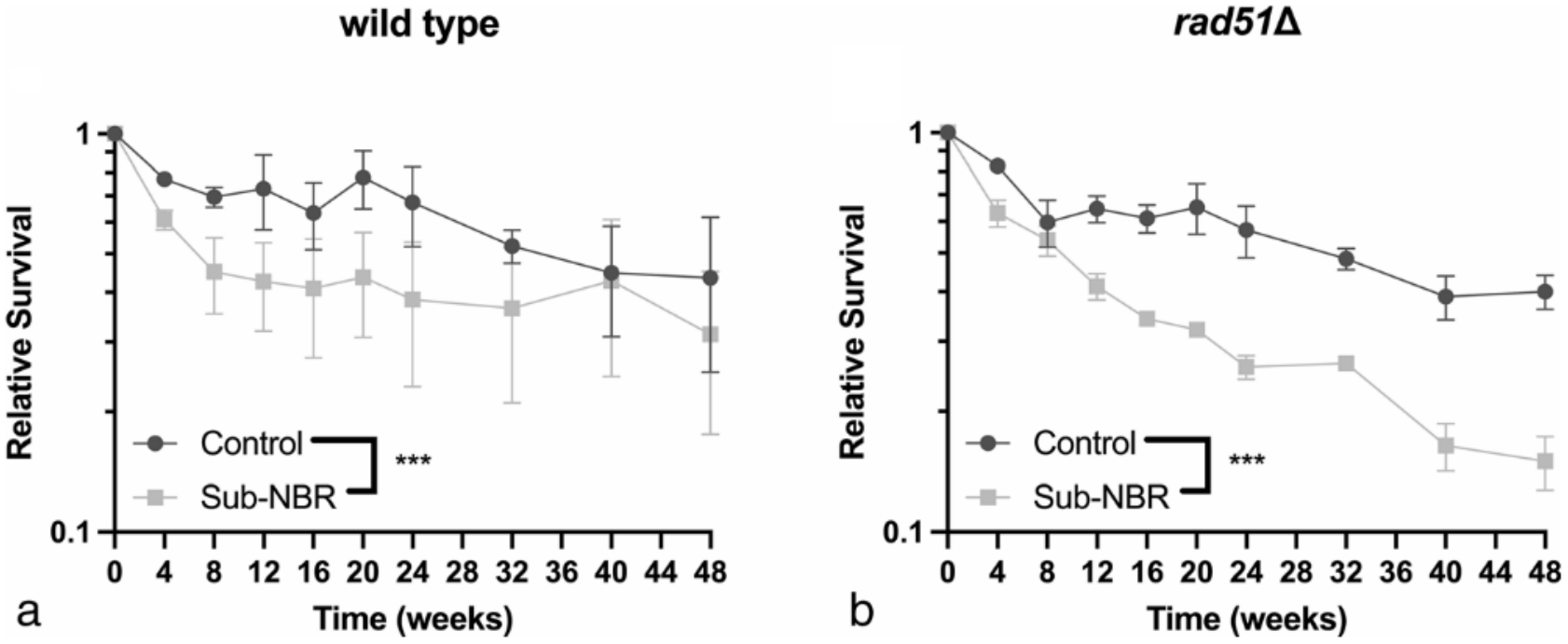
- 6-10 Deep Underground Laboratories (DULs)

- The pilot project:
  - identical experiment
  - run in parallel



10.1016/j.envint.2018.07.031

Global Deep Underground Biology Collaborative Project



10.1097/HP.0000000000001804

Fig. 1. Survival of yeast, stored in the control (dark circles) and sub-background (light squares) environments for the wild type (A) and *rad51*Δ (B) strains. Survival was determined via plating for colony-forming units immediately after rehydration. Relative survival was calculated with respect to pre-desiccation plating controls. Data represent the mean of three independent replicates, and error bars represent the standard error of the mean, significance was determined via ANCOVA. Note: some error bars are smaller than the symbols. (** $p \leq 0.01$, *** $p \leq 0.001$).

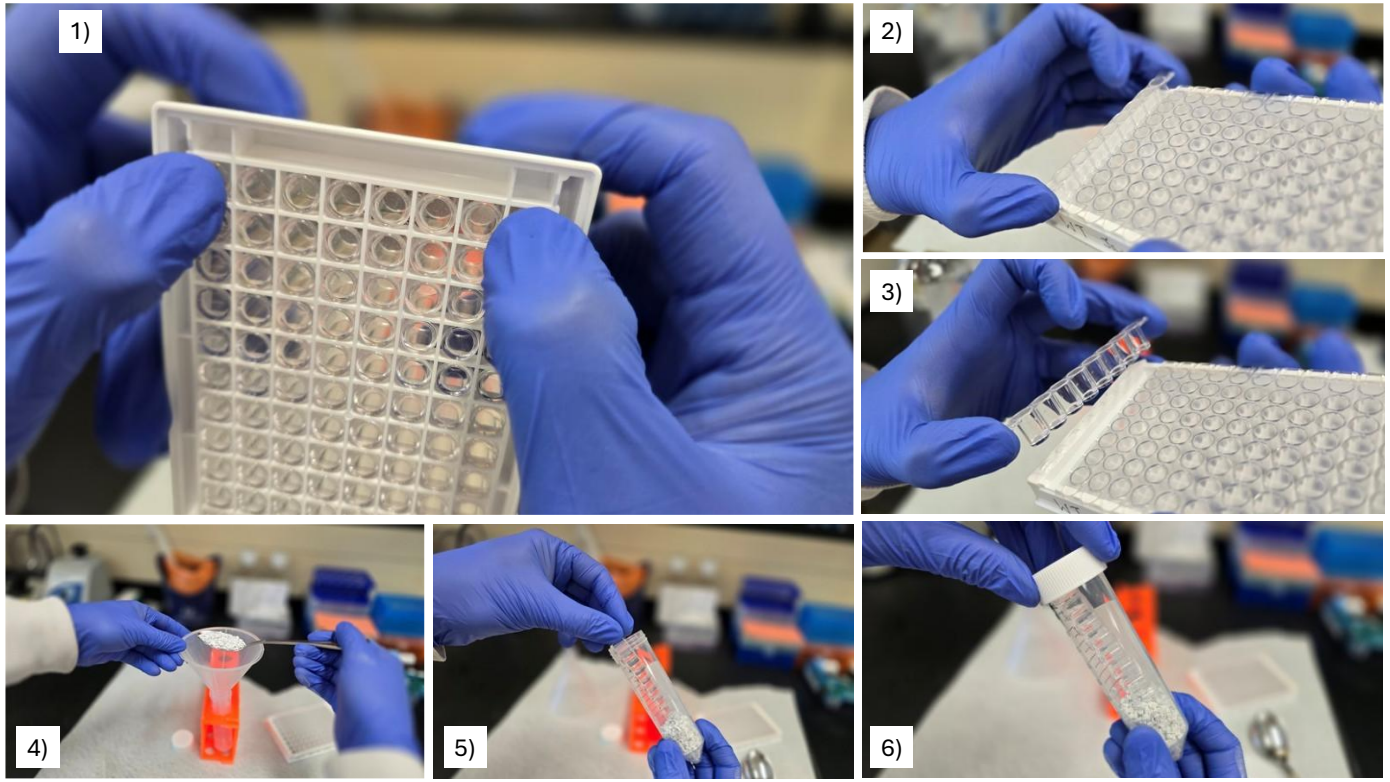
Global Deep Underground Biology Collaborative Project

Timeline and Key Steps:

Steps	Responsible Group	Lead Time/Timepoint	Dependency
Notice of readiness	Receiving Lab	≥ 2 weeks	Lab-specific
Yeast culturing	REPAIR	7 days	Notice
Desiccation preparation	REPAIR	3 days	Complete culturing
Sample shipping	REPAIR	Variable	Desiccation completion
Receipt of desiccators with samples	Receiving Lab	-	Shipping
Travel-control sample return and data logger addition	Receiving Lab	Immediately upon receipt	Shipping
Long-term storage (surface & sub-surface)	Receiving Lab	16-week intervals	Sample reception and handling
16-week sampling and desiccant refresh	Receiving Lab	< 1 day	16-week storage period
Post-return sample storage	REPAIR	Up to 7 days since sample collection	Samples return
Sample rehydration and assaying	REPAIR	1-5 days, depending on assay	7-day post-collection period complete
Data sharing	REPAIR	Ongoing	Analysis completion

Global Deep Underground Biology Collaborative Project

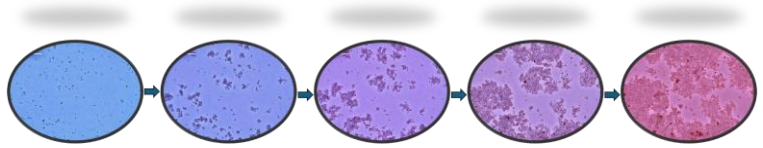
Visual protocol of sample collection

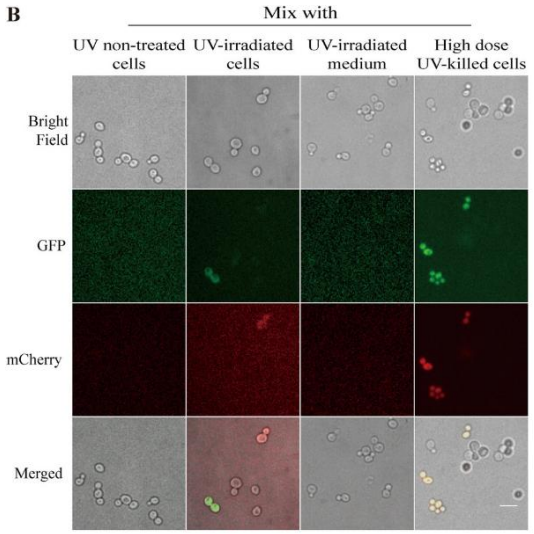


Global Deep Underground Biology Collaborative Project

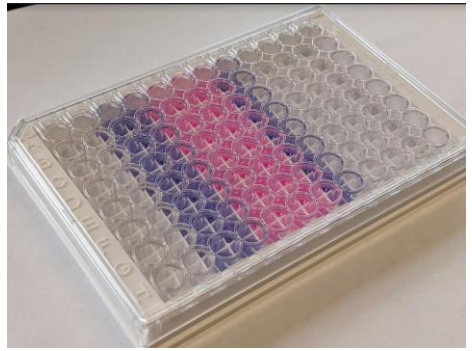
Return-sample testing:

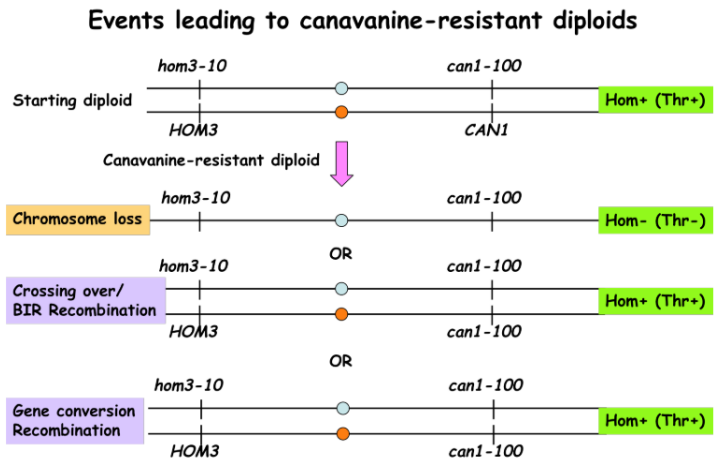
- Baseline survival (CFU)
- Post-rehydration growth rate (2 ways)
- Post-rehydration metabolic activity (aB)
- CAN1-mutation/fluctuation assay
- P_{RNR3} – genotoxicity assay





10.1128/aem.02194-21





https://med.nyu.edu/klein/fluctuation.html

Global Deep Underground Biology Collaborative Project

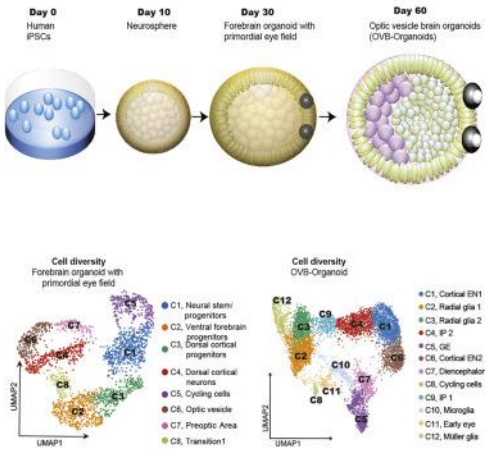
Task Summaries by Institution

REPAIR	Receiving Group
Yeast culturing	Provide notice of readiness
Yeast Desiccation	Upon receipt of samples: return 2 strips from each sample plate (labelled) to REPAIR & add relative humidity data logger to desiccators
Sample distribution	Store desiccators in experimental conditions
Return-sample testing: survival by CFU, metabolic activity by alamarBlue, growth by OD600 measurement & colony size (from survival), CAN1 mutation assay, Optional: PRNR3-EGFP genotoxicity assay	In 16-week intervals: return 2 strips from each sample plate (labelled) to REPAIR & refresh/replace desiccant
Data sharing	Maintain local storage logbooks (dates, changes to conditions, handling incidents, etc.)
Data analysis and dissemination of results	

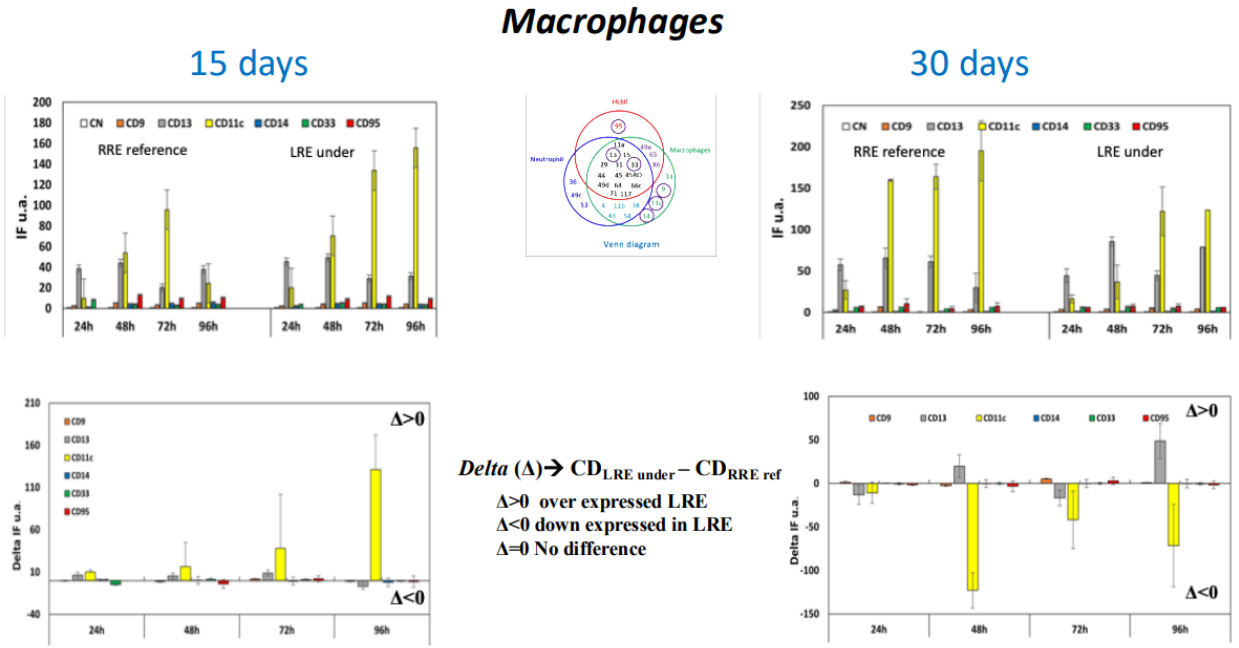
Global Deep Underground Biology Collaborative Project

Future projects could include:

- Immune cell culture (LNGS model)
- Organoids
- Etc.



https://doi.org/10.1016/j.stem.2021.07.010



https://indico.stfc.ac.uk/event/1058/contributions/6732/attachments/2331/4169/DULIAbio%202024_Morciano.pdf

FIN

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- All SNOLAB staff and scientists
- All participating DUL Biology groups

Questions?

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