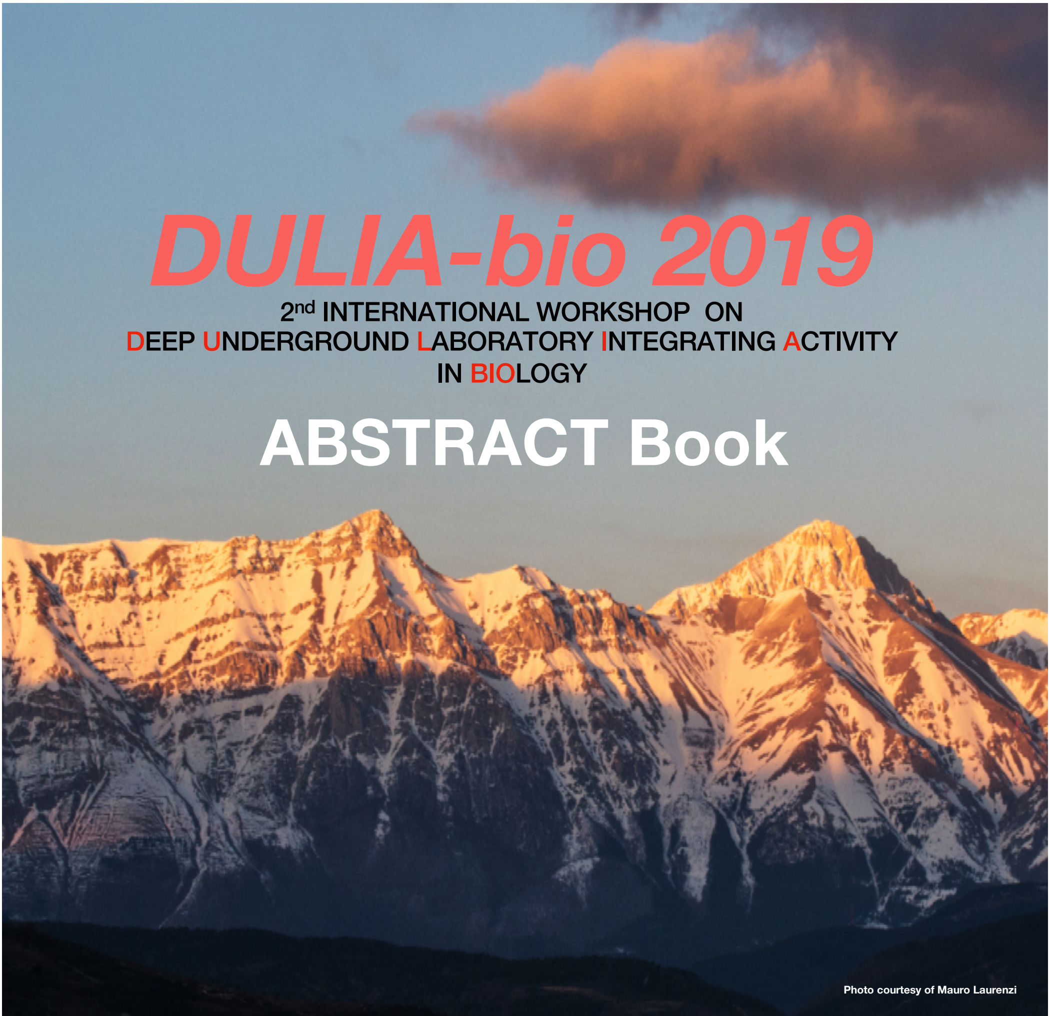
DULIA-bio 2019

2nd INTERNATIONAL WORKSHOP ON DEEP UNDERGROUND LABORATORY INTEGRATING ACTIVITY IN BIOLOGY

ABSTRACT Book



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Laboratori Nazionali del Gran Sasso

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Laboratorio Subterráneo de Canfranc

INDEX

Low Background Techniques

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In fundamental physics their application is focussed on rare phenomena, e.g. double beta decay, rare nuclear decays and Dark Matter search. In the past years there has been a growing number of underground measurements also in other fields such as environmental monitoring, surveillance of nuclear activities, benchmarking of other physical techniques, Life Science and material selection for equipment, which require materials with extremely low levels of radioactivity.
The exceptional sensitivity and high resolution of high purity germanium detectors in gamma-ray spectrometry and their use in underground laboratories (Laubenstein et al., 2004) has increasing application because of the important science and technology that they allow to be studied (Laubenstein, 2017).
Here the state of the art in ultra-low background gamma-ray spectrometry with high purity germanium detectors is described. Moreover, it is explained how to achieve the best level of background reduction taking different measures as for example installing the detector in an underground laboratory, using active and passive shielding methods, and improving intrinsic background of the detector through material selection.

M. Laubenstein et al., Underground measurements of radioactivity, Appl. Radiat. Isot. 61, No. 2-3, 167 (2004).
M Laubenstein, Screening of materials with high purity germanium detectors at the Laboratori Nazionali del Gran Sasso, Int. J. Mod. Phys. A 32, N. 30, 1743002 (2017)

Life in Low Radiation Environment: biological response and role of background radiation

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Life has evolved on Earth for about 4 billion years in the presence of the natural background of ionizing radiation. Even if it is extremely small, nevertheless it may be significant enough for living organisms to sense it and respond to it, keeping memory of this exposure.
Our knowledge about the response of living organisms to low dose of ionizing radiation is mainly related to radiation protection needs, where the focus is on detrimental effects. In this context low doses are defined pragmatically as those typically encountered in the workplace, in the environment and in diagnostic medicine, with natural sources giving the largest fractions of the dose received by the general public.
The relevant protection principles are currently based on the assumption that any exposure to ionizing radiation can proportionally increase the risk of cancer, no matter how small the dose is. This is based on the evidence that ionizing radiation can induce can induce a series of cellular modifications from DNA damage to genetic mutations, most likely to be detrimental, within a framework called “conventional paradigm of radiobiology”. Quantitative evaluation of health risks is currently obtained by a combination of epidemiological and radiobiological data and models. Given the limited statistical power and the large uncertainties in epidemiological data below 100 mGy, radiobiology is used to give the rationale for extrapolation to low and protracted doses of the epidemiological data obtained at moderate and high doses.
However, the conventional paradigm of radiobiology has been challenged from in vitro studies that demonstrated the occurrence of non-linear responses likely related to non-DNA damage. For their explanation involvement of heritable changes in the expression of genes (epimutations) has been invoked. Therefore, the question arises as to whether the biological effects of ionizing radiation on cells, frequently assumed to be a “radiation damage” by definition, can induce various cell responses, including triggering of defence mechanisms, that are relevant at low and protracted doses, such as those given by the natural background.
Controlled long-term experiments with model organisms, conducted in underground laboratories where conditions with no or largely reduced background radiation are realized, compared with normal conditions at natural radiation background, have opened a new avenue to answer this important question. Eventually, they can provide basic information for understanding: a) the evolutionary responses of living organisms to this background, and b) the robustness of the present radiation protection system and, in case, in building a more robust one.

Low dose radiobiology at Laboratori Nazionali del Gran Sasso: results, challenges and future plans.

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Low dose radiobiology is a research field in constant evolution. One of the very exciting areas is represented by studies performed on living organisms placed in sites where background radiation is strongly reduced. Deep Underground Laboratories (DULs) represent unique places where it is possible to perform studies on the influence of background radiation on living organisms carrying out parallel experiments under- and above- ground.
An overall view on the research activities in DULs around the world shows an increased interest in the last years for underground biology with dedicated programs, meetings and proposals.
LNGS is one of the first underground laboratory to host biology experiments. So far, LNGS is the place where the majority of in vitro radiobiological data have been collected.
Here we present our recent in vivo data set obtained using Drosophila melanogaster as model organism. In the framework of the FLYINGLOW project, funded by Centro Fermi, we obtained the first evidence of a differential response below and above ground in a complex multicellular organism in terms of lifespan, fertility and motility using flies having different genetic background. Conducting parallel experiments in two different experimental sites keeping constant all the environmental parameters except radiation is a big challenge. Here we explain our efforts to manage this issue and we emphasize Drosophila as a suitable model organism for low dose radiobiology. Moreover, we present our future plans including a complementary work using the LIBIS (Low dose rate gamma irradiation facility for cell cultures) facility for analysing the effects of a chronic low dose rate slightly above the environmental background at the ISS. Our systematic analysis of chronic exposure under- and above- ground provide the opportunity to address important questions such as the role of the different component of the radiation spectrum in modulating the biological response and the shape of the dose/dose rate relationship for different biological effects in our model system. This investigation also has a potential impact on the rationale underlying the human health risks assessment for exposures at low and protracted doses, presently assumed to be linearly related to the dose.

Research on proliferation inhibition in Chinese hamster V79 cells and highly differentiated laryngeal squamous cell carcinoma FD-LSC-1 cells in deep underground (rock cover 1470m) environment and its mechanism based on transcriptomics and proteomics

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Resources in the shallow depths of the earth have gradually become exhausted. Therefore, history has progressed to a new era of exploring and exploiting the deep-underground space. The environmental factors in the deep-underground might have biological effects on humans and other organisms. However, little is known about the effect of these factors on the health of humans or other organisms that work or live in the deep-underground space, even the nature of the deep Earth itself. To understand the biological effects of working or living in the deep-underground on humans or other organisms, a deep-underground cell culture laboratory (DUGL) was set up in a goaf of a gold mine that is under 1470 m of rock and 820 m below sea level in a deep gold mine in China. Control experiments are conducted in an above-ground laboratory (AGL) in an office building near the entrance of the mine. The main environmental parameters of AGL and DUGL were measured respectively.The Chinese hamster V79 cells(V79) and laryngeal squamous cell carcinoma FD-LSC-1 (FD-LSC-1) cells were cultured in AGL and DUGL. Their growth curve were measured by CCK-8. Also, transcriptomic and TMT proteomic techniques were used to investigate and integrate the differences of molecular pathways and biological processes of the cultures grown in AGL and DUGL. The air pressure［AGL/DUGL: 951.9（949.65-953.9，947.8-960.3）/1118.2（1117.3-1119.6，1080-1120.7）hPa, p <0.001］, relative humidity［AGL/DUGL: 57.2（46.9-63.6,29.8-87.7）/99（99-99,97-99）%, p <0.001］,Rn［AGL/DUGL: 1.25(1-1.47,0.8-1.7)/4.0(3.9-4.1,3.7-5.5) pCi/L, p <0.001］and CO2 concentration ［AGL/DUGL: 540.11±110.39 / 951.9±137.56 ppm, p <0.001］ in DUGL were higher than AGL, and total γradiation dose-rate［AGL/DUGL:0.15(0.13-0.18,0.135-0.16)/ 0.04(0.035-0.045,0.03-0.05)μSv/h, p =0.005］lower than AGL. There was no change in oxygen concentration［AGL/DUGL:20.6(20.6-20.8,20.5-20.8)/ 20.8(20.7- 20.9,20.4-20.1)%, p =0.079］between the AGL and DUGL. And theγ radiation dose-rate, relative humidity and concertation of Rn in DUGL of CJEM were similar with Gran Sasso National Laboratory (LNGS).
The CCK-8 growth curves showed that the growth of two cell lines incubated in DUGL were inhibited. In the transcriptomic analysis, a total of 1758(mRNA 1504) differentially expressed genes (DEGs) of V79 cells were identified between AGL and DUGL groups, among which 1338 DEGs (mRNA 1118) were up-regulated and 420 (mRNA 386) were down-regulated. As to FD-LSC-1 cells, the DEGs were

903(mRNA 666) ,280（mRNA 206）increased and 623（mRNA 460）decreased. The correlation of DEGs between qRT-PCR and RNA-Sqe was well and showed that the result of RNA-Sqe was accurate（V79 cells R2=0.8716, FD-LSC-1 cells R2=0.9726）. In V79 cells, the GO analysis of DEGs suggested that most of them were mainly assigned to terms associated with metabolic process. And DEGs in FD-LSC-1 cells were mainly annotated to biological progress. The KEGG pathway enrichment analysis of DEGs of V79 showed that significantly enrichment pathways of were ECM-receptor interaction（DEGs；16 up-regulated and 0 down-regulated, q=0.007）, Arachidonic acid metabolism（DEGs；10 up-regulated and 2 down-regulated, q=0.011）,Linoleic acid metabolism（DEGs；5 up-regulated and 1 down-regulated, q=0.032） and Ovarian steroidogenesis（DEGs；4 up-regulated and 4 down-regulated, q=0.046）. In FD-LSC-1 cell lines, the DEGs significantly enriched in the pathways of Hippo signaling pathway（DEGs；3 up-regulated and 13 down-regulated, q=0.014） and Pathways in cancer（DEGs；7 up-regulated and 17 down-regulated, q=0.036）.

PRM analysis the DEPs were consistent with the findings from TMT proteomic analysis. Herein, further analysis was conducted.115 DEPs (52 increased and 63 decreased) were detected in the V79 cell lines. However, 146 DEPs detected in FD-LSC-1 cells, only 26 up-regulated, other 120 down-regulated. GO enrichment analysis showed that DEPs of V79 cells enriched in the term of ATP synthesis coupled proton transport, and FD-LSC-1 cells in the terms of all three main categories, and mainly in cell component. In the KEGG pathway enrichment analysis, DEPs of V79 cells were significantly enriched in the pathway of oxidative phosphorylation（DEPs;3 increased and 1 decreased, $p<0.001$）, and FD-LSC-1 cells in complement and coagulation cascades（DEPs;0 increased and 10 decreased, $p<0.001$） and mineral absorption（DEPs;0 increased and 3 decreased, $p<0.001$）.

By the combination analysis of DEGs and DEPs, 14 genes displayed differential expression at both mRNA and protein levels in V79 cells, and 9 genes in FD-LSC-1 cells. Among of these genes and proteins, most of them with the function of promoting cell proliferation decreased in DUGL group. In contrast, the genes and proteins with inhibition of proliferation increased in DUGL group. All those changes in genes and proteins level might explain the proliferation inhibition of cultures in DUGL.

Conclusion

The environmental parameters showed that relative humidity, concertation of Rn and total γradiation dose-rate was similar with Gran Sasso National Laboratory. And DUGL was suitable for cells culture. According the growth curve of the cultures in AGL and DUGL, the environment of deep underground could inhibit the proliferation of cells. Among the environmental parameters measured, low background radiation might play the main role in inhibiting the proliferation of cultures in DUGL. However, the different cells might have different mechanisms in coping with low background radiation. V79 cells present the disorder of energy synthesis and oxidative metabolism in DUGL. FD-LSC-1cells growth inhibition mainly caused by the genes and protein with function of promoting cell proliferation decreased, and the genes and proteins with inhibition of proliferation increased in DUGL group.

A case for cells sensing the absence of background levels of ionizing radiation: Overview of the transcriptional response of four species at the Waste Isolation Pilot Plant (WIPP).

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The study of depriving cells from background ionizing radiation for the past decades has provided a valuable new insight on its role in cellular homeostasis control. The Low Background Radiation Experiment (LBRE) at the Waste Isolation Pilot Plant (WIPP) in Carlsbad, NM., has employed four taxonomically unrelated biological models to identify potential physiological and transcriptional endpoints to help elucidate the sensing mechanisms that cells have for something as ubiquitous as radiation. In two bacteria, Deinococcus radiodurans and Shewanella oneidensis, an approximately 80X reduction in dose rate caused the deceleration of growth in the first but not the second specie. The study of their differential gene expression, using RNASeq, showed varying degrees of genomic regulation, suggesting different sensitivity to detect and respond to ultra-low levels of radiation. In general, S. oneidensis response suggests a lower translational rate and a higher rate of transmembrane transport, while D. radiodurans extended lag phase could be explained, in part, by its partial loss of proteome integrity and its diminished affinity for ammonium assimilation. In Cricetulus griseus (V79 cells), a lower cell viability was accompanied by the upregulation of various gene families related to translation and protein targeting and transport, as well as cellular response to stress and by the downregulation of ribosome assembly and RNA processing-coding genes. For Caenorhabditis elegans, the withdrawal of normal radiation levels increased egg-laying rate and resulted in the regulation of collagen and sperm genes. All four species respond to the presence and absence of normal radiation fields, indicating the significance of background radiation as a selective environmental cue.

Biotechnological applications of extremophiles

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Since the discovery that life can develop in extreme conditions researchers have tried to find different applications, from catalysis at high temperatures following the Arrhenius equation to the production of compatible solutes in halophiles. In this communication we will review some of the biotechnological applications of extremophiles, paying special attention to lithoautotrophic microorganisms developing in the continental deep subsurface or acidophiles associated to metal mining activities.

Exploring microbial biosignature in Mn-patinas of deep biosphere: a cross-disciplinary approach to investigate geomicrobiological interactions in Grotta Grande del Cervo (Abruzzo, Italy)

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The terrestrial subsurface offers privileged sites both to search for microbial life and to observe characteristic lithologies which are still mostly unknown. In particular, caves represent natural laboratories where to investigate unique minerogenetic processes and biotic interactions, connected to these phenomena. Manganese mineralizations in cave environment provide a window to understand the complex Mn cycle and the development of microbial communities in special conditions, such as low constant temperature, absence of light and, particularly, low-energy environments. In the current study, we used a multidisciplinary approach to characterize mineralogy and microbiology of Mn-samples collected in the Grotta Grande del Cervo, at the aim of understanding the biogeochemical processes in environments considered extreme.
Crossing SEM-EDS, XRPD, FT-IR, Raman spectroscopy and µ-XRF data, a proper mineralogical characterization of the samples was achieved. They consist of a very fine mixtures of todorokite [(Ca,Na,K)(Mn4+,Mn3+)6O12·nH2O], a Mn oxide that can be related to biological processes, quartz, phyllosilicate-like minerals and calcite.
SEM investigations revealed microbial imprints, showing cell-like structures and suggesting that the cell-like shapes occur within internal laminae.
To assess the possibility that biotic factors may be involved in the production of these mineralizations and to investigate the nature of the microbial community in these materials a culture-independent approach was used.
A molecular approach is the first step to investigate the role of microorganisms in forming manganese oxides, associated with water bearing rocks.
DNA from the black deposits was extracted using a soil DNA isolation kit following the manufacturer's instructions, and sequencing analyses of specimens are undergoing.
Our data support the hypothesis that microorganisms may contribute to the formation of manganese mineralizations in this environment, providing new encouraging insights in the understanding of the Mn cycle and over the processes of energy acquisition in unfavourable conditions, with relevant implications for astrobiology.

Deep Life, Volatiles and the Search for Extraterrestrial Life

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The past decade of subsurface microbiology has revealed that subsurface environments, both marine and continental, harbor one the largest ecosystem of our planet, with diversity and biomass that rivals surface ecosystems. What's more, subsurface life has been shown to significantly contribute to the planet biogeochemistry, and recent studies show that it might have an outsized impact on the flux and composition of volatiles recycled between the surface and the Earth interior. Taken together, the last 10 years of studies suggest a prominent role of subsurface life, with broad implications for our search of life beyond our planet. Current targets for the search of extraterrestrial life are planetary bodies with largely inhospitable surfaces, such as Mars, Europa, Titan, and Enceladus. In these locations, subsurface environments might provide niches of habitability, making the study of the deep life a priority target for future astrobiological missions.

Astrobiology and Planetary Science at the Boulby Mine

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In 2013, we established a deep subsurface astrobiology laboratory as part of the existing Boulby Underground Laboratory. The facility is located in 250 Myr old Permian evaporites. Using this facility, we have undertaken studies on the limits to life in the deep subsurface and the preservation of biomarkers in ancient salts. For five years, we have run the MINAR (Mine Analogue Research) programme, which tests technology that links mining and planetary exploration. The programme has involved teams from ESA, NASA, Abdul Kalam Technical University, India and other institutions. Technology testing has involved new underground rovers, mapping and life detection technologies. MINAR has also been used to advance an education programme that has involved over 100,000 pupils across India.

Boulby Geo and Bio Science status and future plans (BOULBY Review)¶

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For more than three decades astro-particle physicists have been operating experiments to search for Dark Matter 1100m below ground in a purpose-built 'low-background' facility at Boulby mine in the North East of England. This facility - the Boulby Underground Laboratory - is one of just a few places in the world suited to hosting these and other science projects requiring a 'quiet environment', free of interference from natural background radiation. The race to find Dark Matter continues and Boulby, in the meantime the range of science projects looking for the special properties of deep underground facilities is growing and new projects operating at Boulby range from astro & particle physics to studies of geology/geophysics, climate, the environment, life extreme environments on Earth and beyond. This talk will give an overview of the Boulby Underground Laboratory, the science currently supported and plans for science at Boulby in the future. Particular attention will be given to current and future planned studies at Boulby of geology, geophysics, biology, astrobiology and planetary exploration.

Cosmogenic Background and Mitigation of Radiactive Backgrounds

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Experiments which require very low levels of background radiation are located deep underground as the deep underground facilities provide significant rock overburden and commensurate reduction in the cosmic ray flux and cosmic ray-spallation induced products, such as neutrons. However, even when an experiment is deep underground there are still backgrounds present, these can include high-energy cosmic ray muons which pass through the rock overburden that then interact with the experiment or rock nearby the experiment, and the detector environment itself, which can include the radioactivity naturally emitted from the surrounding rock and the materials used to build the experiment itself. Since some of these backgrounds may be present, it is highly desired to measure these backgrounds and to determine if further work is required to reduce the backgrounds further to meet the desired scientific goals of the experiment. This presentation will describe the different techniques currently used to measure these backgrounds and show that these methods can predict the background observed by the experiments. In addition, a proposed program to cross calibrate detectors at several laboratories will be described, and a searchable database used to store radioactivity measurements of experimental materials will be introduced.

Low Radon Cleanroom for Underground Laboratories

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The need to achieve ultra-low radon concentration in air was realizes only recently due to the necessity to suppress radioactivity caused by the presence of radon in the air (e.g. the study of behaviour of cells or DNA in the radiation free environment, or the study of the influence of „single event effect“ caused by alpha particles from radon decay product contamination in nanoelectronics). The demand for radon concentrations in air in such special cases should be at the level of mBq/m3 and radon decay products below ▯Bq/m3. In addition, there may be a request for such spaces to comply with the requirements for minimum aerosol concentrations according to the ISO standard (so-called cleanrooms). State of art in radon corrective measures in buildings represents radon concentration reduction typically by factor of 10. However, since the radon concentrations in buildings are at tens and hundreds of Bq/m3 and in the outdoor atmosphere of tens of Bq/m3, to achieve indoor air radon concentration on the level of mBq/m3 requires special system able to reduce radon concentration by factor more than 1000 times in comparison to the standard environment. Such system for creating and maintaining radon-free and aerosol-free cleanrooms were built at SURO and at the underground laboratory LSM (Modane). Both systems of cleanrooms are based on a set of 4 separated spaces, arranged in a row from entering room, dressing room, air-shower room to ultra- clean room, where all experiments are performed. Rooms are built from specially sealed sandwich metal panels with thermal insulation. Into the last room with highest class of cleanliness (ISO 5) is delivered the radon-free air from antiradon facility delivering 20 m3/h or 150 m3/h, respectively. The rooms are also intensively filtered by HEPA filtration system with filtration rate of 8000 m3/h. For control of low radon concentration is used continuous radon monitor with sensitivity of mBq/m3 developed by IEAP CTU. The cleanrooms are also equipped by detector to check inside atmosphere and by IP camera as safety measures. The effect of human body radon exhalation and its contribution to indoor radon was analysed. At present, cleanroom in underground laboratory LSM is planned to be substantially extended also for purpose of new experiment DAMIC-M (dark matter search) using CCD detectors. The cleanroom will be used for preparation of CCD detectors and testing to avoid any surface contamination of the detectors.

Modane Underground laboratory as a shield to protect stem cells from cosmic rays and radioactivity during cryogenized storage

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On a daily basis the DNA of living cell is modified by external or internal phenomenons such as random physiological fault, chemical agression or natural ionising radiation. But the DNA is constantly repaired to avoid dramatical degradation of the cells functions. Stem cells are stored for therapeutics or research purposes at liquid nitrogen temperature. This storage prevents physiological and chemical degradation and also stops the reparation mechanism but ionising radiation continue to impact the cells. Therefore, the cryogenized stem cells accumulate these damages over the time of storage. These damages have been measured by a collaboration LSM/Institut Pasteur counting double strand breaks(DSB)over time storage. 4 cryogenic setups allow to measure the influence of cosmic rays and of natural radioactivity. A regular cryogenic storage in the institut pasteur is used as a reference condition. Then 3 other setups were built to vary the ionising radiation dosis : a fully shielded underground storage, a regular dewar located at 1000m high increasing the cosmic ray flux and a Dewar with additionnal radioactivity to mimic 20 years of storage in 7 months. Moreover, Reactive Oxygen Species(ROS) are generated during the cryostasis and will have an action upon thawing. We also measure this concentration increase due to radioactivity exposure. Finally, a comparison of engraftment potential was made between cells stored underground and cells exposed to 20 years of radioactivity.

New Technologies for Acoustic, electromagnetic and radioactivity Silence Characterization (LSC Activities)¶

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The Laboratorio Subterráneo de Canfranc (LSC) is one of the Spanish research and technology installations, the second biggest deep underground laboratory in Europe. Current activities are focused on a) the Gollum Project, where we identified and characterized the ultraoligotrophic endolithic microbial communities living in a range of different host rocks throughout the length of the Somport international tunnel and b) construction of a laboratory for biology experiments underground to host low radioactivity experiments while monitoring radioactivity, electromagnetic and acoustic fields. I will discuss the microbial composition seen in the samples studied in the Gollum project and its correlation with the metal composition in the substrate . I will also present our current plans for the new bio-facility, current activities of the Spanish teams, first users of the new facility, and the first proposed projects on studying the effects of low radiation on biological systems.

The RENOIR Experiment at the LNGS

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Little information is known about the influence of environmental radiation on living matter. A useful approach to deepen this topic is the analysis of possible differences between parallel biological systems, one maintained in the radiation environment at the Earth's surface and the other underground, in a low-radiation environment. So far, biology experiments are ongoing or planned in a few underground laboratories around the World. Among them the Laboratori Nazionali del Gran Sasso (LNGS) of the National Institute of Nuclear Physics (INFN), are those where the largest in vitro and the first in vivo evidence of a differential response below and above ground have been obtained. In particular, using the fruit fly Drosophila melanogaster we found that the reduction of the environmental radiation perturbs basic biological processes such as lifespan and fertility.
The overall results so far obtained in underground studies carried out in different Deep Underground Laboratories (DULs) have consistently shown that natural radiation is necessary to trigger biological responses that make biological systems more capable to cope against stress responses. To get more insights on the underlying biophysical mechanisms it is mandatory a continuous improvement in the characterization of radiation spectrum. Actually, it is not known whether all the components of the natural radiation field weigh in the same or in different way to trigger the biological responses.
The main aims of RENOIR ("Radiation ENvirOnment triggers bIological Responses in flies: physical and biological mechanisms"), a 3-year experiment funded by INFN-Commissione Scientifica Nazionale V, are: (i) to improve the knowledge of the radiation field inside the underground and the external laboratories, with dosimetric and spectroscopic measurements and simulations, and (ii) to obtain information about the involvement of the different components of the radiation field in the biological responses of fruit flies. We will present the strategy and the devices we are developing to modify the underground as well as the aboveground environments with shielding or with the presence of natural sources to reduce or increase the gamma component of the radiation spectrum.

The Phenotypic and Genotypic Response of the Caenorhabditis elegans nematode to Background and Below-Background Radiation Underground at the Waste Isolation Pilot Plant.

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The biological effects of low-level and below-background radiation continues to be important due to conflicting results and the potential effects of these levels on humans. To complement our previous work on the bacterial stress response to below background radiation, we report on the response of the nematode Caenorhabditis elegans. C. elegans is one of the best-studied organisms in the world, with extensive knowledge available on its genetics and gene function, developmental biology, rapid life cycle, and physiology. Two experiments were carried out underground at the Waste Isolation Pilot Plant (WIPP) in New Mexico USA, one on naïve nematodes with data gathered within one week of being placed underground, and the second, from worms that had incubated eight months underground in below background radiation levels. Nematode eggs were hatched and life cycles were monitored in two incubators, one without supplemented radiation (ca.16 nGy/hr) and another in an incubator lined with KCl (ca. 120 nGy/hr) to approximate natural background. Phenotypic variables measured in these experiments were: 1.) egg hatching success 2.) body size of worms from larval development to adulthood, 3.) developmental time from egg to egg laying adult, and 4.) egg laying rate of young adult worms. Transcriptome analyses were done on adult worms 72 hours after egg hatching. In the 1'st experiment, there was a non-statistical trend of decreased egg-laying rate in the radiation-supplemented incubator and this trend became statistically significant ($p=0.04$) in the 2'nd long-term experiment. Transcriptome analyses of 72-hour old nematode RNA showed that the expression of a group of sperm protein and collagen genes responded to this radiation treatment. Results demonstrate that C. elegans exerts a phenotypic and genotypic response to this radiation treatment and, instead of a stress response to the absence of radiation as observed with bacteria, egg-laying rate was inhibited in the presence of a source of radiation designed to mimic natural background.

FLIES IN A MINE: UNDERSTANDING THE PHYSIOLOGY OF DEEP UNDERGROUND MINING

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Mining is a physically and mentally strenuous occupation. Underground miners are often exposed to environmental extremes, including temperatures of 35°C or more, air filled with mine dust, and atmospheric pressure exceeding 20% greater than surface pressure. These environmental challenges can lead to changes in biology such as accelerated tiring, increased appetite, and difficulty thinking. While we know that the mining environment leads to these biological changes, we do not know the molecular and biochemical basis of these changes. We are using fruit flies, Drosphila melanogaster, and a deep underground laboratory to explore the molecular basis of these biological changes. Using the facilities at SNOLAB, a controlled laboratory facility located 2 kilometers underground, and a novel fly exercise machine, the Flygometer 2.0, which uses the innate negative geotaxis of fruit flies to exercise flies, we simulate mining conditions of high atmospheric pressure and physical activity. We measured the longevity of both sexes in a pair of D. melanogaster lines when exposed to this mining environment proxy and found a significant decrease in their average lifespan, in some, but not all flies. To further study the overall effects of the mining environment, we are using a broad-spectrum approach analyzing both the transcriptomic (gene expression) and metabolomic (metabolite) responses. We quantified the metabolomic response using liquid chromatography - mass spectrometry to separate and identify a broad suite of metabolites and quantify changes in response to exposure to our simulated mining conditions. We have also measured the broad transcriptomic changes using next generation mRNA sequencing (RNA-seq) and differential expression analysis to quantify changes in gene expression. Changes in the metabolic pathways, determined by associations between metabolomic and transcriptomic analysis, will provide a basis for future targeted exploration of specific metabolic pathways, genes and metabolites that are altered under underground mining conditions. Ultimately, we hope to make suggestions to limit or mitigate the negative impacts of working deep underground.

The REPAIR project: Investigating the biological effects of sub-natural background radiation exposure

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Considerable attention has been given to understanding the biological impacts of low-dose ionizing radiation exposure at levels slightly above background. However, relatively few studies have examined the inverse, where natural background radiation is removed. The limited available data suggest that organisms exposed to sub-background radiation experience reduced growth and an impaired capacity to repair genetic damage. The REPAIR project (Researching the Effects of the Presence and Absence of Ionizing Radiation) is utilization the cosmic radiation shielded environment within SNOLAB to investigate the biological impacts of sub-background radiation exposure. SNOLAB is a Class 2000 cleanroom laboratory space located 6,800ft (2km) underground and is ideally suited for these research endeavours. We hypothesize that natural background radiation is essential for life and maintains genomic stability, and that prolonged exposure to sub-background radiation environments will be detrimental to biological systems. We are testing this hypothesis using both whole organism and cell culture models, which are being grown underground and in our surface control laboratories. Lake whitefish embryos are known to be sensitive to low-dose radiation exposure and represent a good model for examining whole organism growth and development. Cell lines will be measured for baseline levels of DNA damage, oxidative stress and cancer frequency. Low background adapted cells will then be examined for their ability to repair induced damage. A custom engineered fully sealed tissue culture glovebox incubator allows for control and reduction of natural background radiation components below ambient surface levels. The results of this research will provide insight into the biological effects of low-dose radiation exposure as well as elucidate some of the processes that may drive evolution and/or selection in living systems.