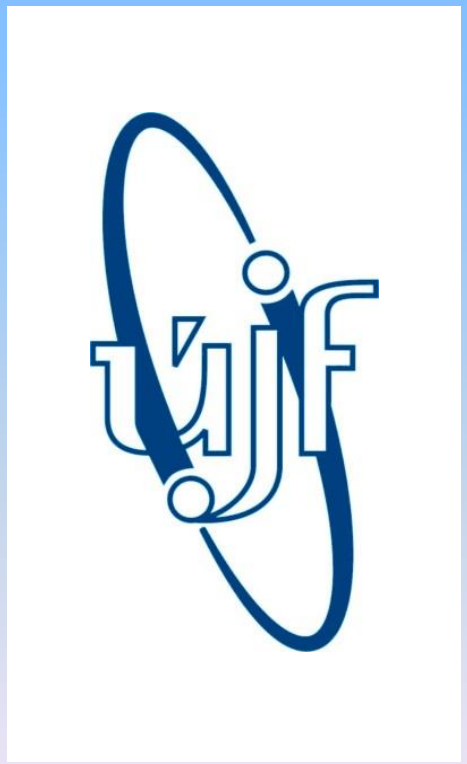
Biologické účinky nízkých dávek ionizujícího záření



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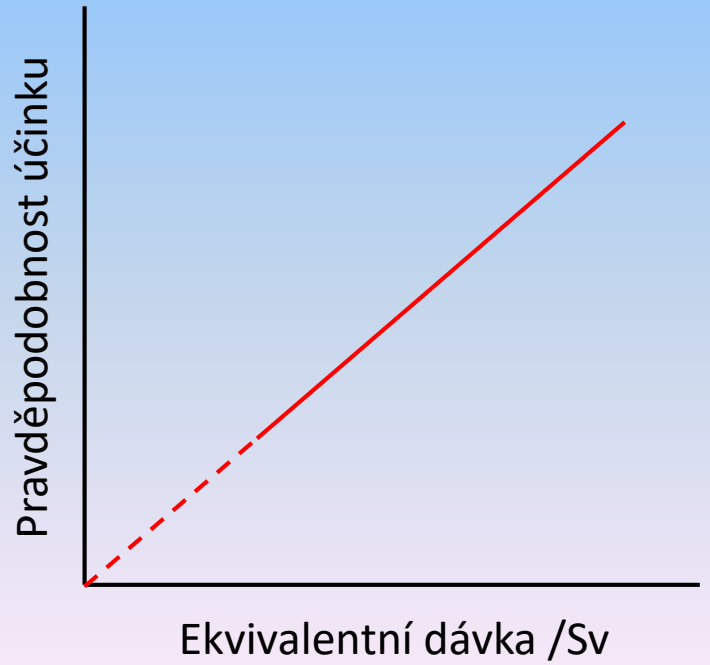
Stochastické a deterministické účinky

- Deterministické účinky se projevují jako důsledek letálního poškození buněk ozářených vysokou dávkou záření (od 100 mGy). Objevují se při překročení prahové dávky, která je podstatně vyšší než dávky z přírodního pozadí nebo v případě pracovních expozic při normálním provozu. Závažnost účinku závisí na dávce.

Stochastické účinky jsou následkem mutací genomu vzniklých ozářením nízkými dávkami záření. Prahová dávka není známa. Bylo zjištěno, že různé typy rakoviny se objeví při různém rozsahu dávek, závažnost účinku není závislá na dávce, ale četnost výskytu v exponované skupině obyvatelstva je závislá na dávce a ve většině případů se lineárně zvyšuje z dávkou. Jsou to především rakovinná onemocnění, mrtvice, onemocnění srdce, gastrointestinální a respirační nemoci.



Účinky deterministické dávky

Akutní nemoc z ozáření (celotělové ozáření)

- dřeňová, krevní forma ..... 2 Gy latence 2-3 týdny
- střevní forma ..... 6-10 Gy latence 4-6 dní
- nervová forma ..... nad 30-50 Gy bezprostředně

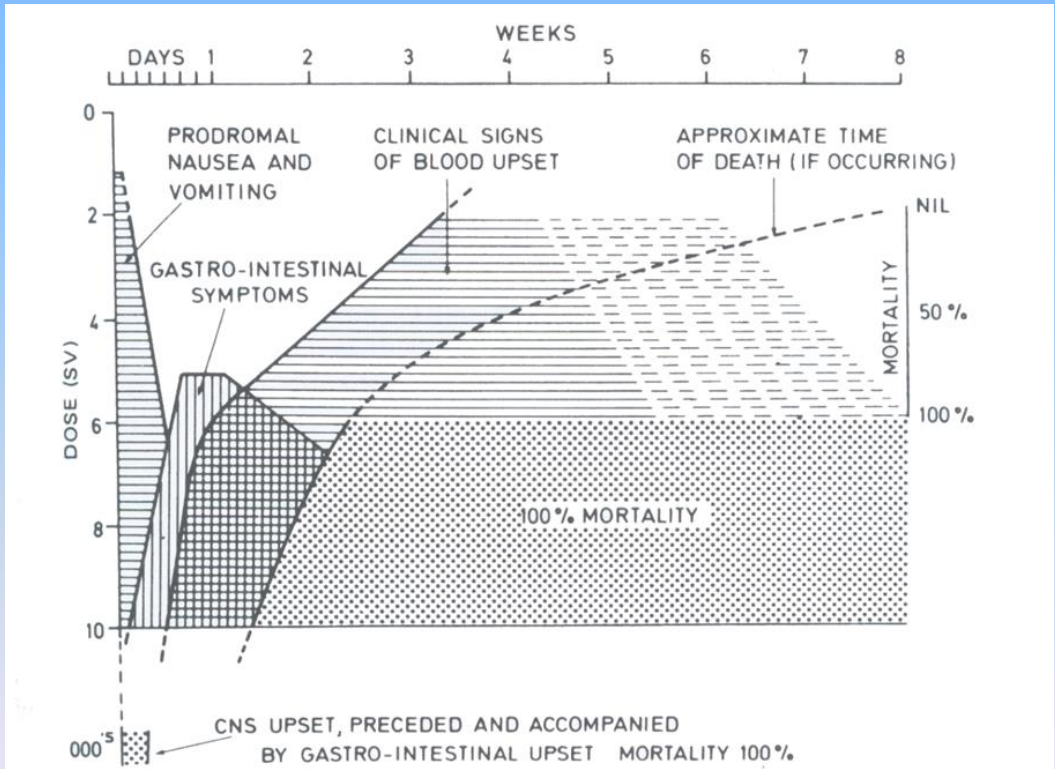


Figure 14.6. Time sequence of the main events of the total body syndrome in humans. (Reproduced from Blakely, 1968, by permission of the author.)

Lokalizované ozáření – kůže

- ICRP č.85

Typ účinku	Odhad prahové dávky (Gy)	Doba nástupu účinku
Časný přechodný erytém	2	2-24 hod
Hlavní (pozdní) erytémová reakce	6	asi 1,5 týdne
Přechodná epilace	3	asi 3 týdny
Trvalá epilace	7	asi 3 týdny
Suché olupování	14	asi 4 týdny
Odlučování pokožky s mokváním	18	asi 4 týdny
Druhotné zvředovatění	24	> 6 týdnů
Pozdní erytém	15	8-10 týdnů
Ischemická kožní nekróza	18	> 10 týdnů
Atrofie dermis (první fáze)	10	> 52 týdnů
Teleangiektasie	10	> 52 týdnů
Nekrosa dermis (pozdní vřed)	> 12	> 52 týdnů

Stochastické účinky

Deterministické účinky mají klinické projevy více méně charakteristické,

Stochastické účinky tj. zhoubné nádory a změny dědičné se klinickým obrazem neliší od obdobných spontánně se vyskytujících projevů.
Tj. klinicky (a ani biochemickými markery) nelze rozlišit případ radiačně indukovaného nádoru od nádoru spontánně vzniklého.

Ionizující záření zvyšuje pravděpodobnost jejich výskytu.

Odhad rizika nenádorových onemocnění

- Choroby srdce, mozková mrtvice, onemocnění zažívacího a dýchacího ústrojí
- Nejistota tvaru závislosti dávka-účinek v oblasti nízkých dávek
- Buněčné a tkáňové mechanismy pro vznik těchto onemocnění nejsou známy
- Dostupná data nedovolují jejich začlenění do hodnocení újmy po ozáření nízkými dávkami přibližně pod 100 mSv

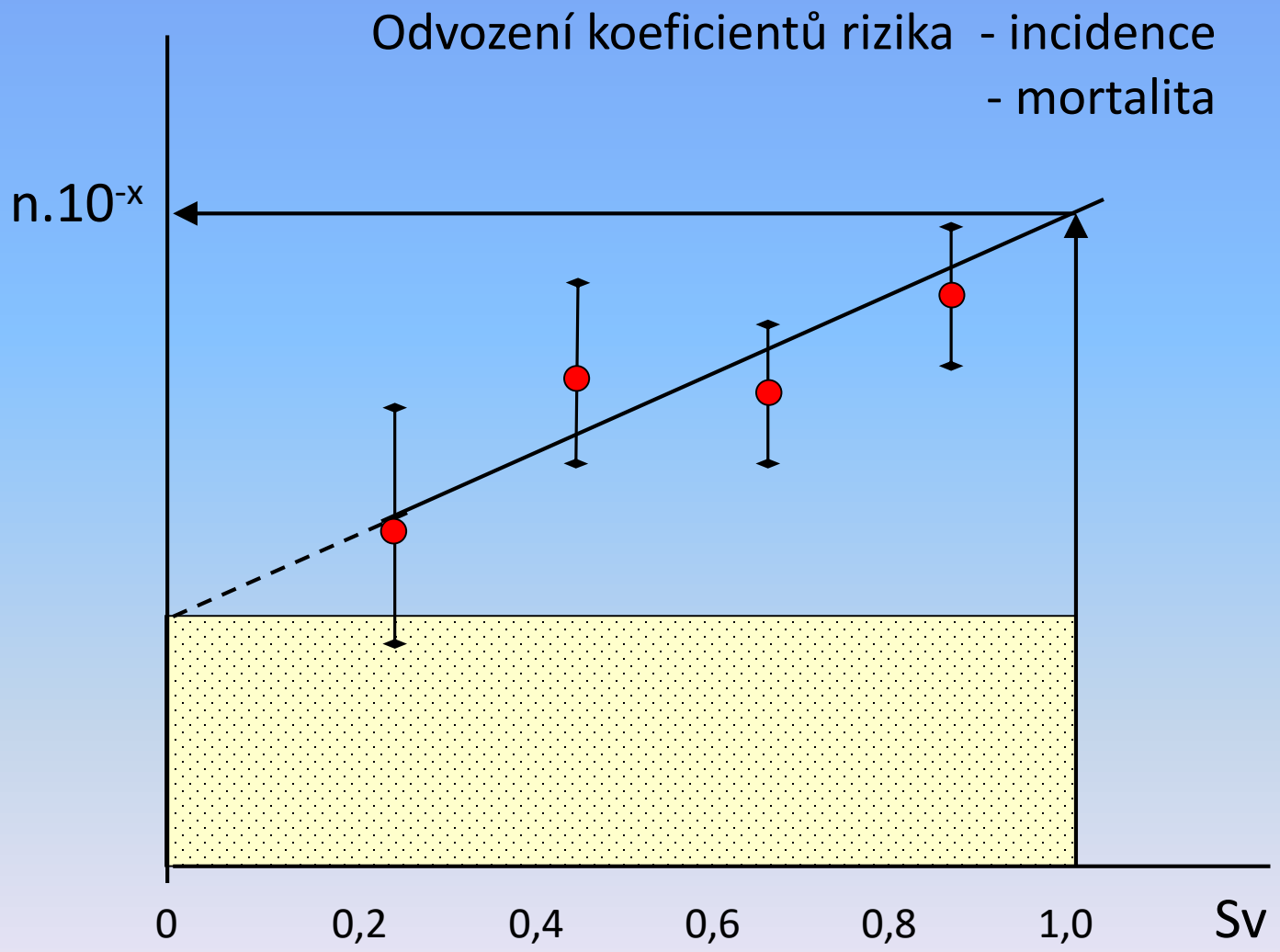
Metody průzkumu radiogenních nádorů

Skupinová - epidemiologická šetření

Vzhledem k neodlišitelnosti jednotlivých případů
od případů spontánních je třeba statistických šetření
na velkých kolektivech osob.
Skupina ozářených osob při znalosti dávky vs. kontroly
Příklady - přežívající oběti v Hirošimě a Nagasaki
- lékařská ozáření (např. Bechtěrevova choroba)
- profesionální expozice (zejména uranoví horníci)

Experimentální cesta - vliv ozáření na mutace onkogenů

Zobecnění výsledků populačních studií



Celoživotní úmrtnost populace (všech věkových skupin) na jednotlivé k smrti vedoucí zhoubné nádory po ozáření malými dávkami)			
	Pravděpodobnost fatálního nádoru (10^{-4} Sv^{-1})		
	1977	**1990**	**2007**
Močový měchýř	-	30	12
Kostní dřeň	20	50	28
Kostní povrch	5	5	3,2
Mléčná žláza	25	20	33
Tlusté střevo	-	85	31,3
Játra	-	15	28,9
Plíce	20	85	101,5
Jícen	-	30	14
Ovarium	-	10	6
Kůže	-	2	2
Žaludek	-	110	65,5
Štítná žláza	5	8	2,2
Zbývající tkáně	50	50	70,5
Gonády - dědičnost			16
Celkem	**125**	**500**	**414**

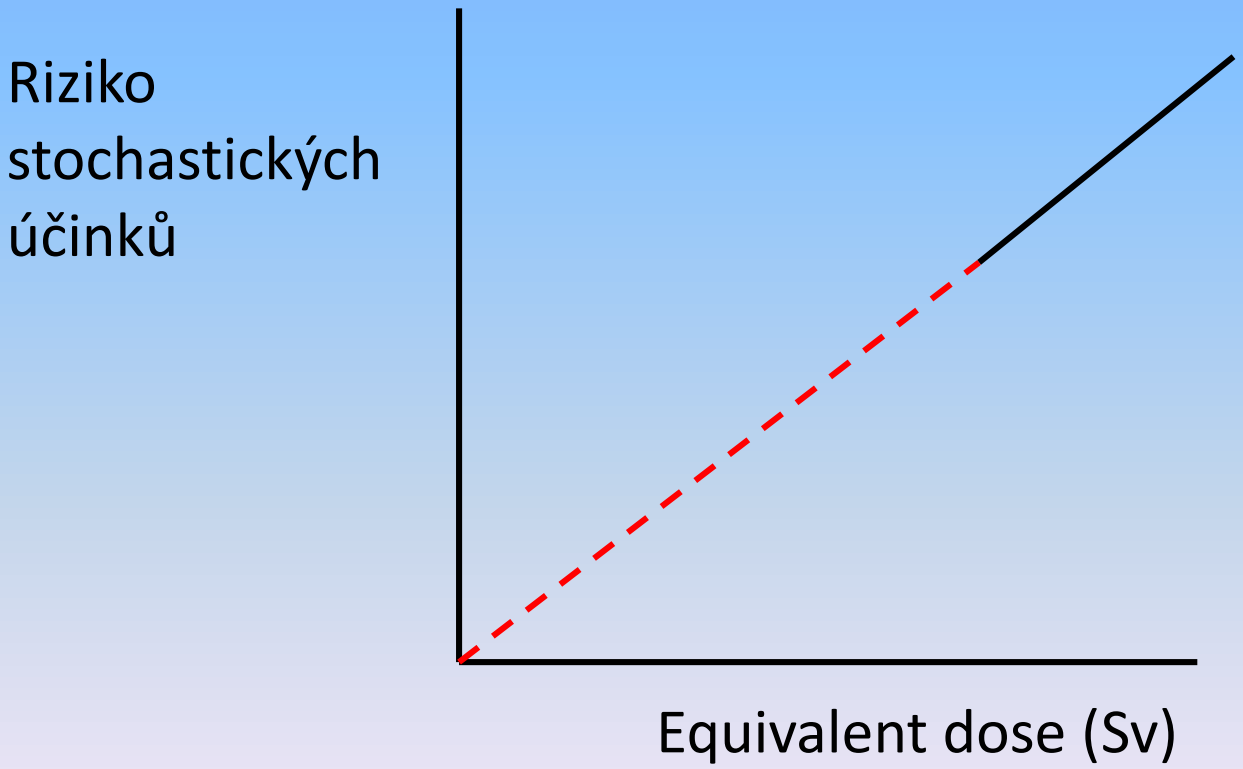
Přehled nominálních koeficientů rizika (10^{-4} Sv^{-1}): založeno nikoli na fatálních nádorech, ale na incidenci

ICRP 103 (2007)

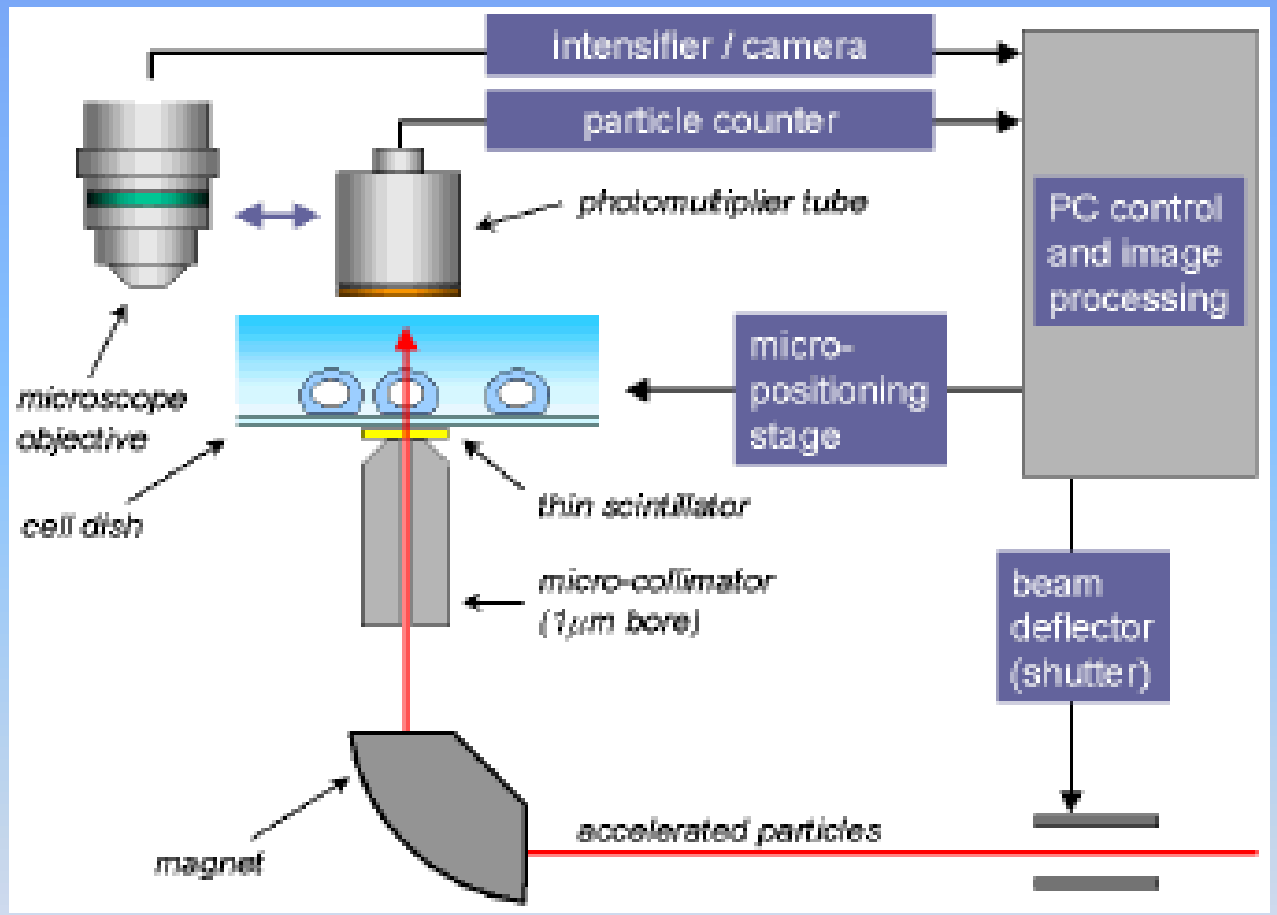
Tkáň	Obecná populace		Pracovníci (18-64 let)	
	Nominální riziko	S korekcí na letalitu	Nominální riziko	S korekcí na letalitu
Jícen	15	15	16	16
Žaludek	79	77	60	58
Tlusté střevo	65	49	50	38
Játra	30	30	21	21
Plíce	114	113	127	126
Kosti – povrch	7	5	5	3
Kůže	1000	4	670	3
Mléčná žláza	112	62	49	27
Vaječník	11	9	7	6
Močový měchýř	43	24	42	23
Štítná žláza	33	10	9	3
Kostní dřeň	42	38	23	20
Ostatní solid. nádory	144	110	88	67
Gonády - dědičnost	20	19	12	12
Celkem	**1715**	**565**	**1179**	**423**

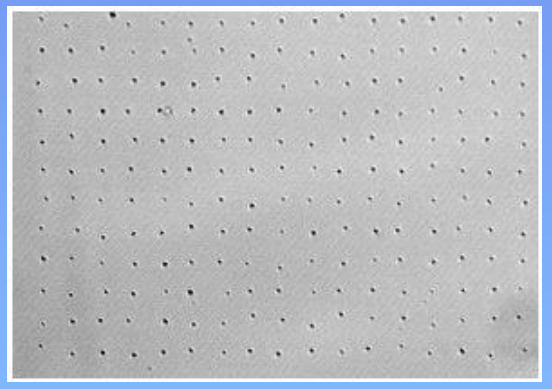
Závislost dávky a účinku

- Kvantitativní data o závislosti rizik stochastických účinků na dávce podporují lineární závislost v oblasti nízkých dávek bez existence prahové dávky

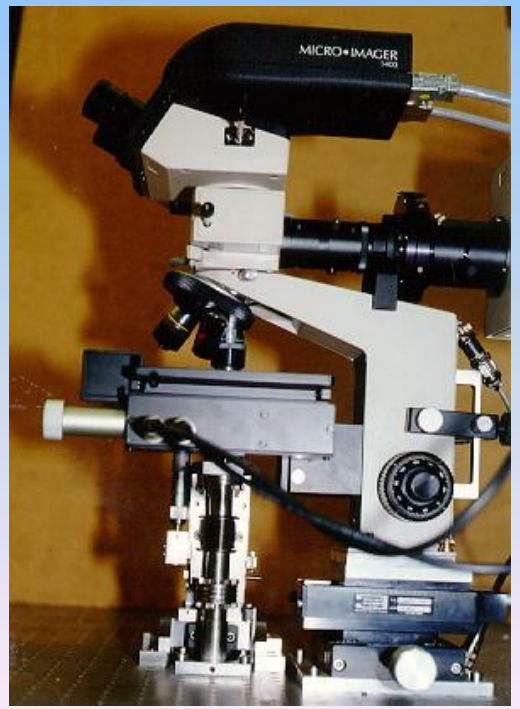


Microbeam irradiations



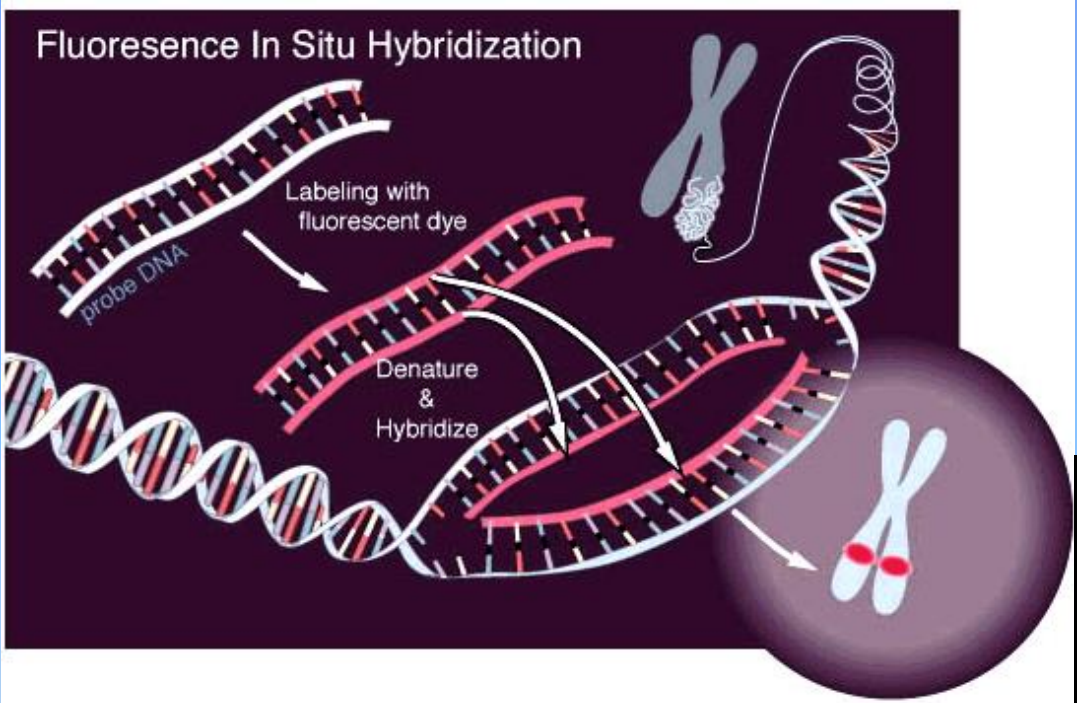


Track-etch plastic showing single hits by 3MeV protons, spaced at 20 micron intervals.



- precision sub-micron up to several microns
- up to 10000 cells per hour
- X and g rays, electrons, ions up to argon nuclei

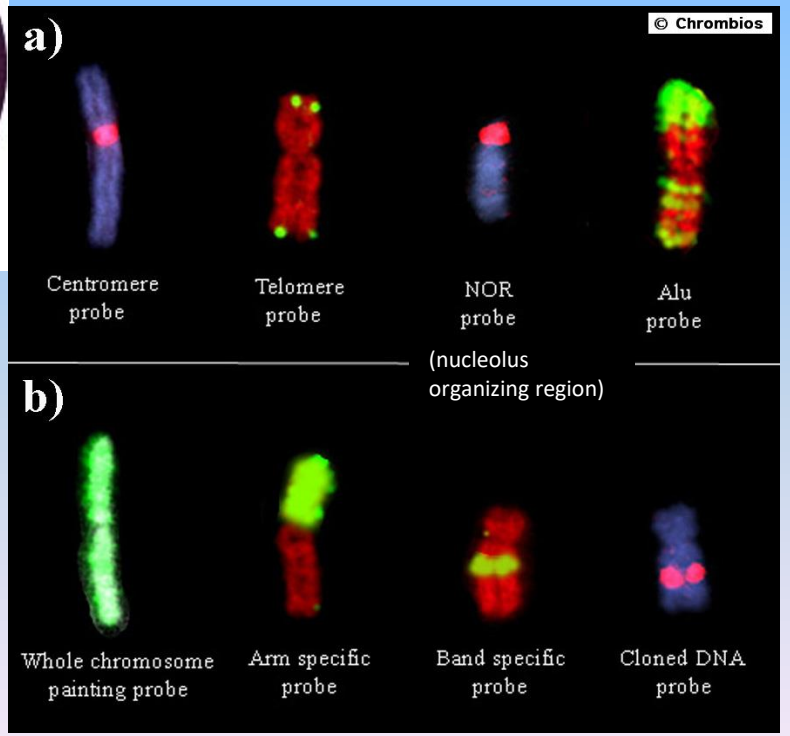
Fluorescence *in situ* Hybridization (FISH)



The probes: repetitive and "unique" sequences.

Targets:

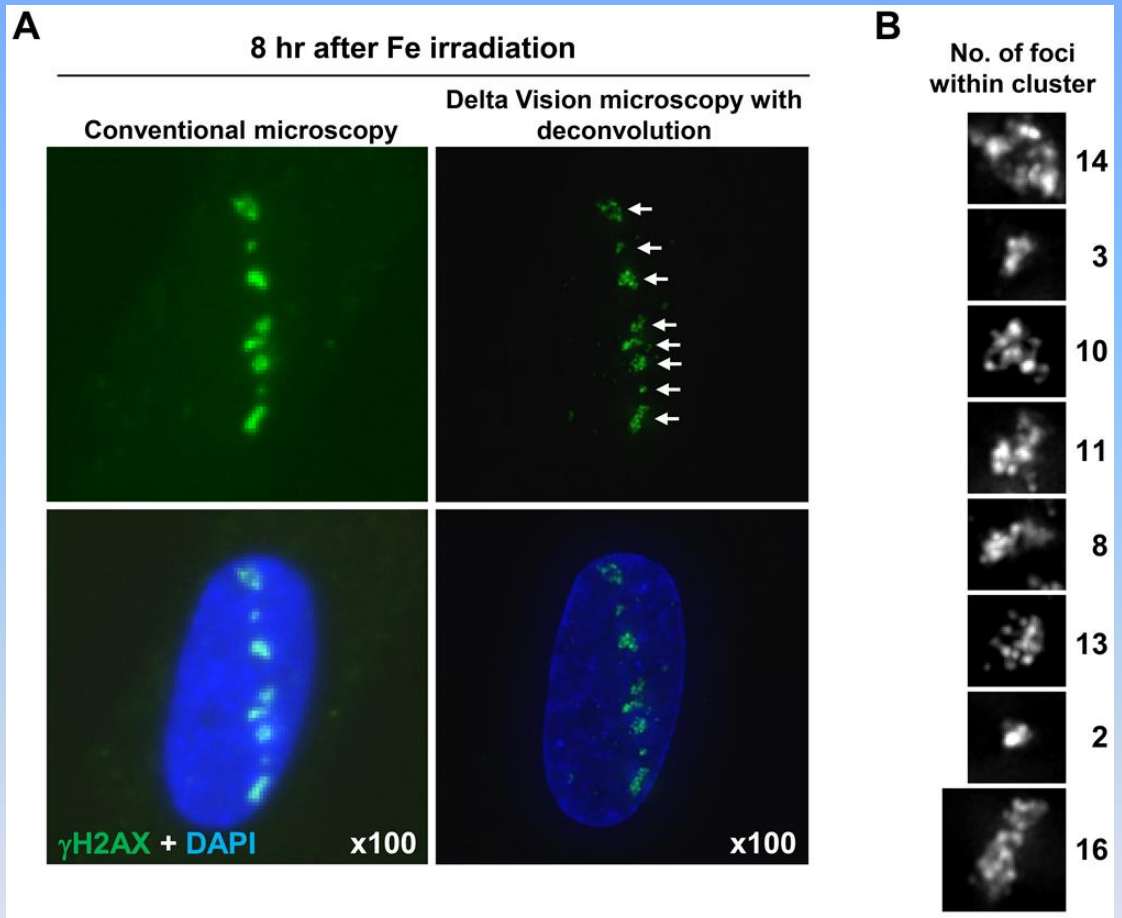
- Metaphase chromosomes
- Interphase nuclei
- Extended chromatin fibers
- Entire Cells/RNA
- Tissue sections



- FISH applies fluorescently labeled DNA probes to detect gene or chromosome abnormalities that are generally beyond the resolution of routine cytogenetics. The DNA is first *denatured*. The fluorescently labeled probe is added and hybridizes with the sample DNA at the target site as it *reanneals* back into a double helix. The probe signal can be seen through a fluorescent microscope.

Measurement of γH2AX foci

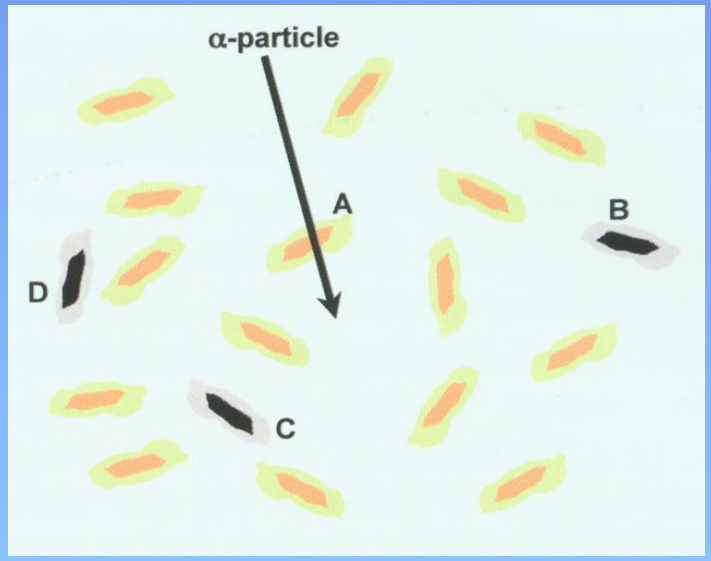
- H2AX represents 2 – 10% of the H2A subfamily of histone proteins in chromatin
- Phosphorylated rapidly in response to DSB at serine 139
- ~1% of the H2AX phosphorylated per Gy and number of gH2AX foci
  ≈ number of DSB
- Acts as major recruiter of repair enzymes



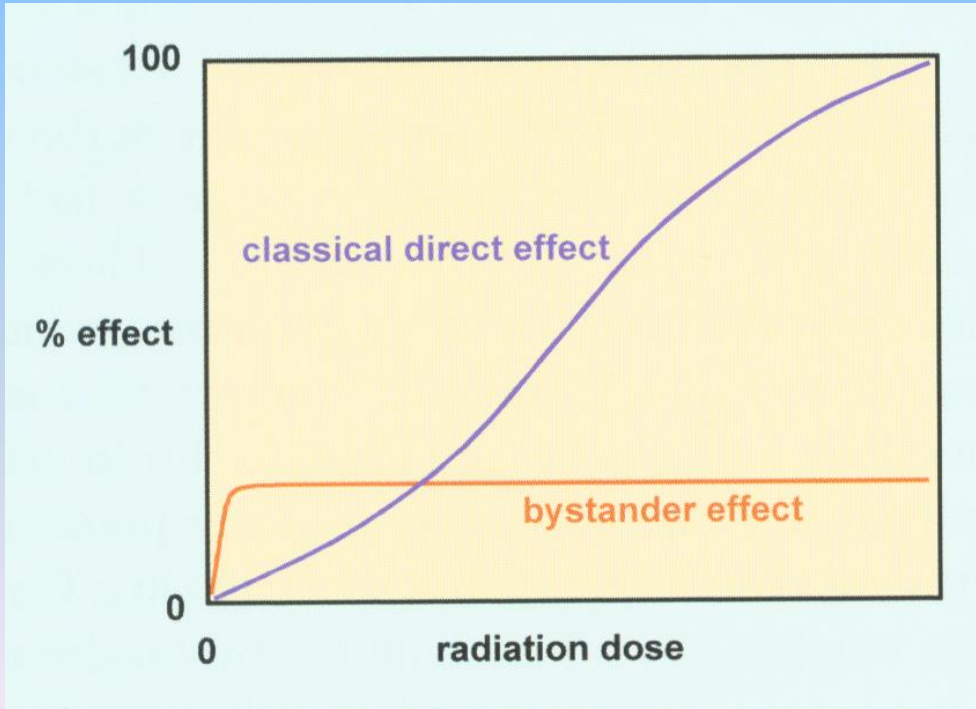
High resolution microscope analysis revealed clustered γH2AX foci formation within the tracks following Fe irradiation 48BR (WT) primary G0/G1 cells.

Nakajima et al. (2013) PLoS ONE 8(8): e70107

By-stander effects
Non-targeted effects



- Up to the early 1990's: important biological effects are produced only very close neighborhood of the tracks and within cell nucleus;
- Cellular responses induced via by-stander effect include chromosomal aberrations, mutations, cell death, apoptosis, malignant transformation and genomic instability.
- 1-30% of nonirradiated cells
- found in different cell systems and signaling mechanism appear to be involved (role of cytokines, ROS = reactive oxygen species)



Hall, Brenner, Columbia University, NY

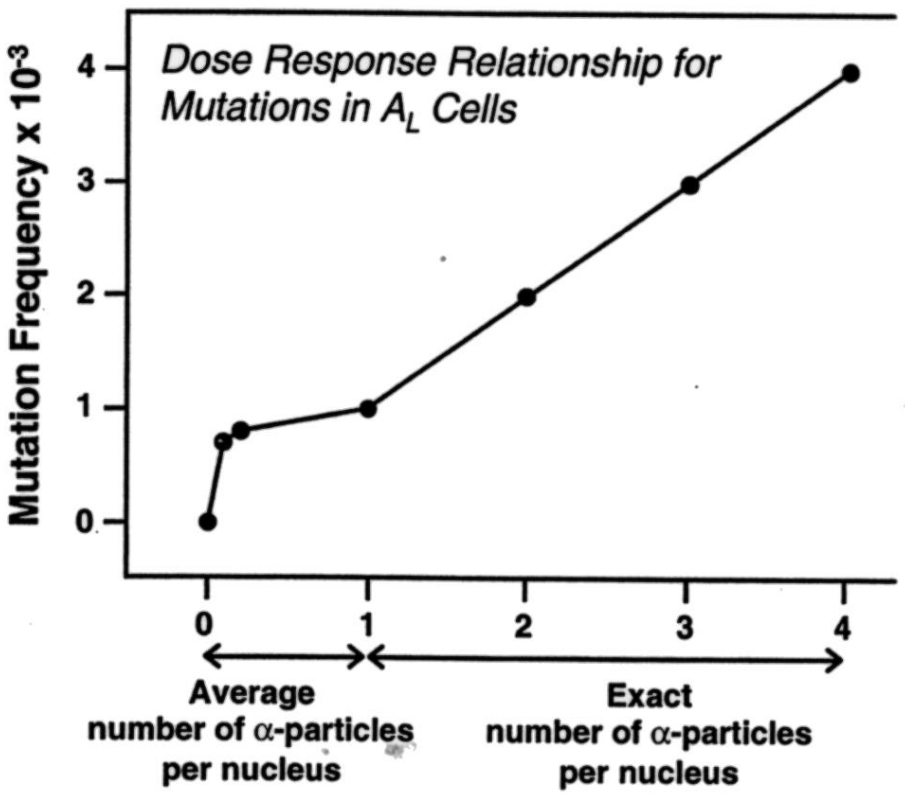
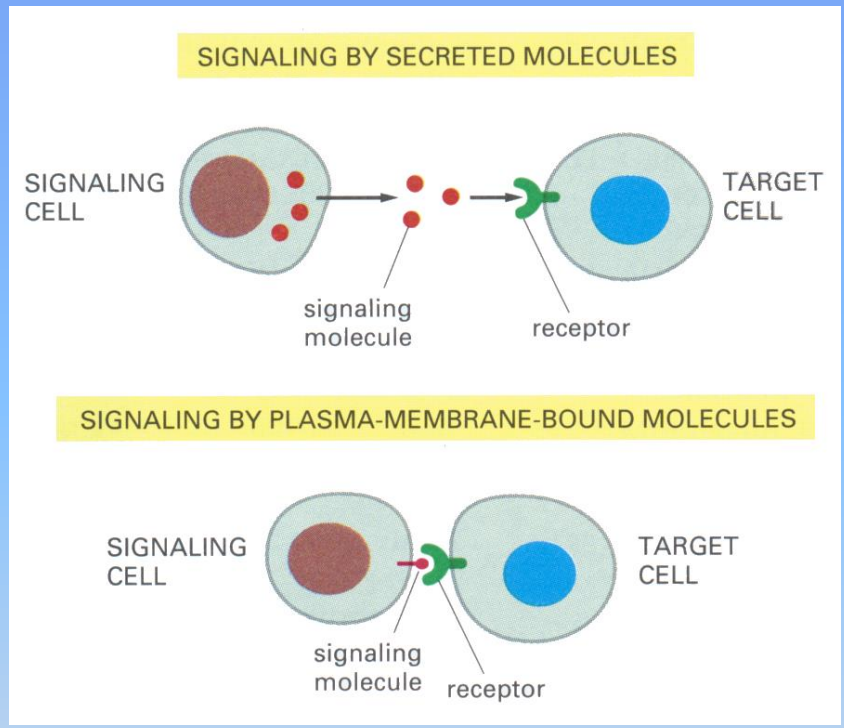


Fig. 6. Mutation frequency as a function of the number of alp particles per nucleus (data fractions of the cell population w exposed to a single alpha particle). Due to the bystander eff which is evident when only a proportion of the population exposed, the risk at low doses is higher than predicted by a lin extrapolation from high doses (based on the data of Zhou et 2000, 2001).



Adaptive response and bystander effect

Adaptive response
= pre-exposing cell to a low priming dose appear to protect these cells from the deleterious effects of second, larger dose

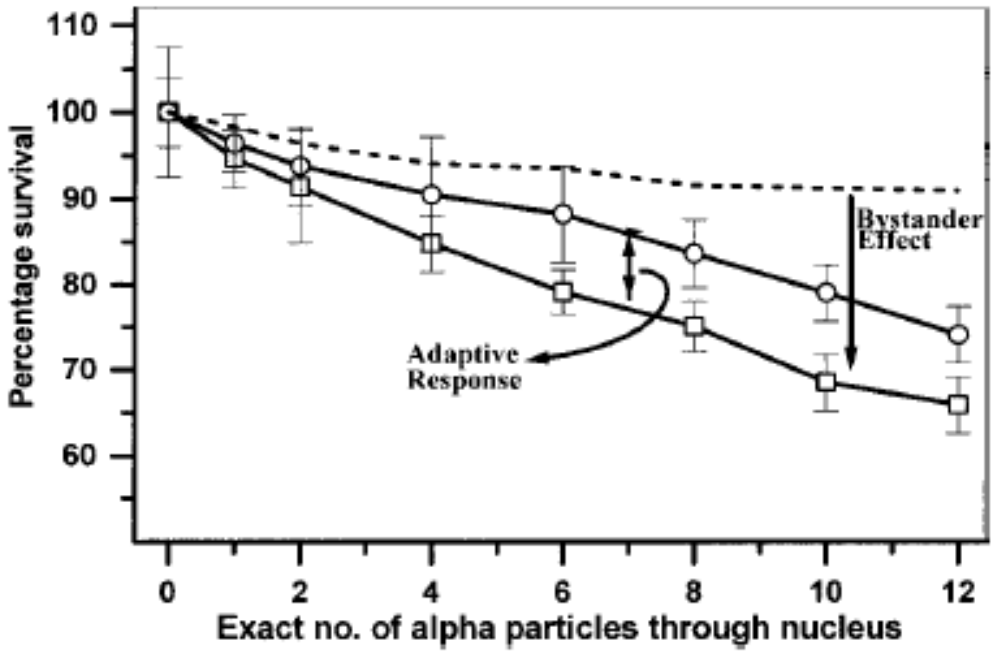
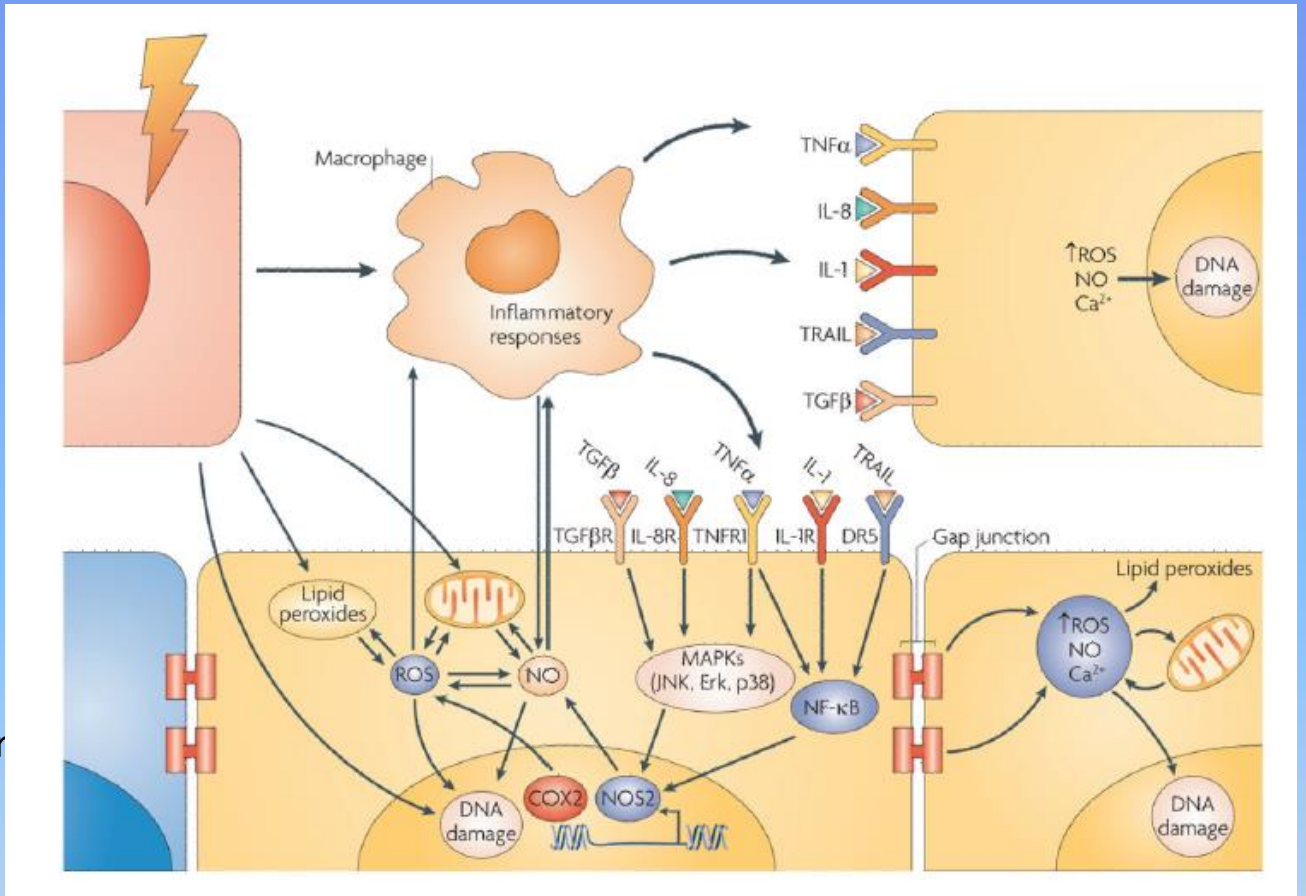


FIG. 1. The adaptive response and the bystander effect for cell survival in C3H 10T½ cells. The dotted line shows the percentage of cells that would be expected to survive when 10% of the cells are exposed to various numbers of α particles calculated from the survival curve for all cells irradiated. The squares show survival for various numbers of α particles, from 1 to 12, traversing 10% of the cell population. The extent to which this falls below the dotted line is an indication of the magnitude of the bystander effect. The circles show survival for cells exposed to 2 cGy of γ rays, 6 h before exposure to various numbers of α particles traversing 10% of the population. The extent to which the circles are above the squares reflects the adaptive response.

Bystander signals

COX2 - cyclooxygenase 2
DR5 - death receptor 5
IL interleukin
JNK - Jun N-terminal kinase
NO - nitric oxide
NOS2 - NO synthase 2
ROS - reactive oxygen species
TGFβ transforming growth factor
TGFβR - TGFβ receptor
TNFα - tumor necrosis factor α
TRAIL - TNF related apoptosis inducing ligand



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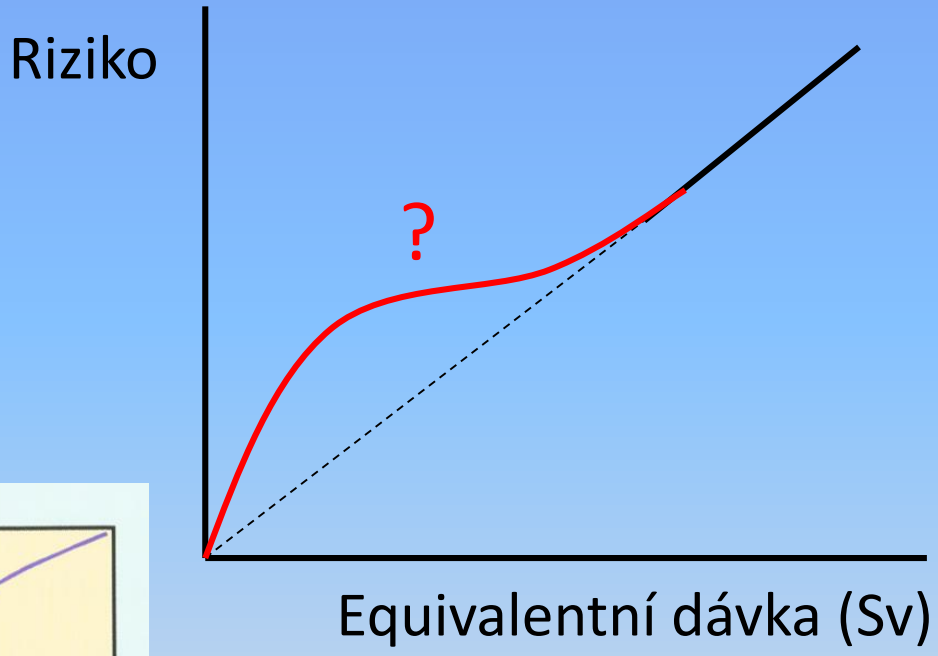
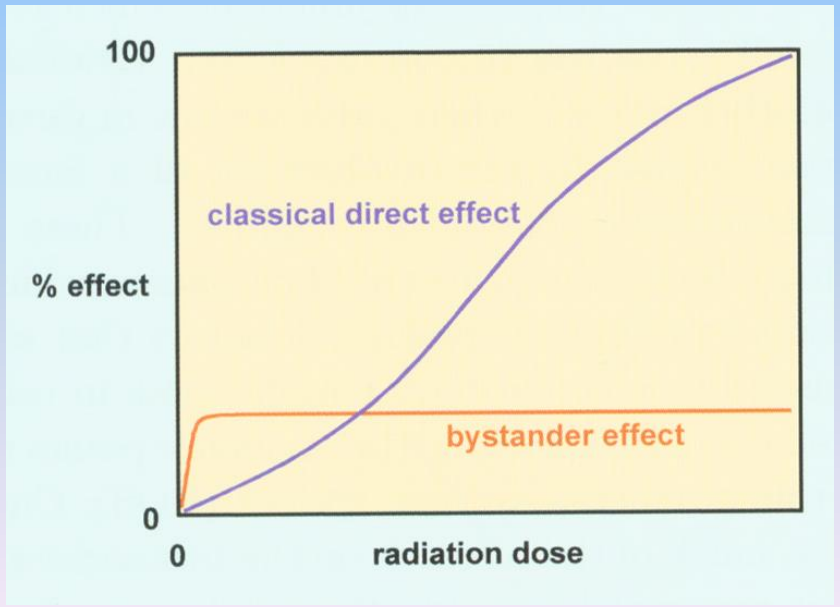
- Through gap junctions and the cytokine signals into the extracellular matrix.
- **In vivo:** macrophages may be important mediators, which release bystander signals affecting nonirradiated cells. Cytokine-mediated signaling, signal transduction through MAPKs and nuclear factor κB alongside the production of reactive oxygen and nitrogen species.

Riziko stochastických účinků?





Adaptivní odezva a neterčové efekty

Adaptivní odezva

= buňky předzářené nízkou dávkou gama záření lépe odpovídají na následné akutní ozáření vyšší dávkou (vyšší přežití buněk v porovnání se stejnými buňkami bez adaptační dávky)

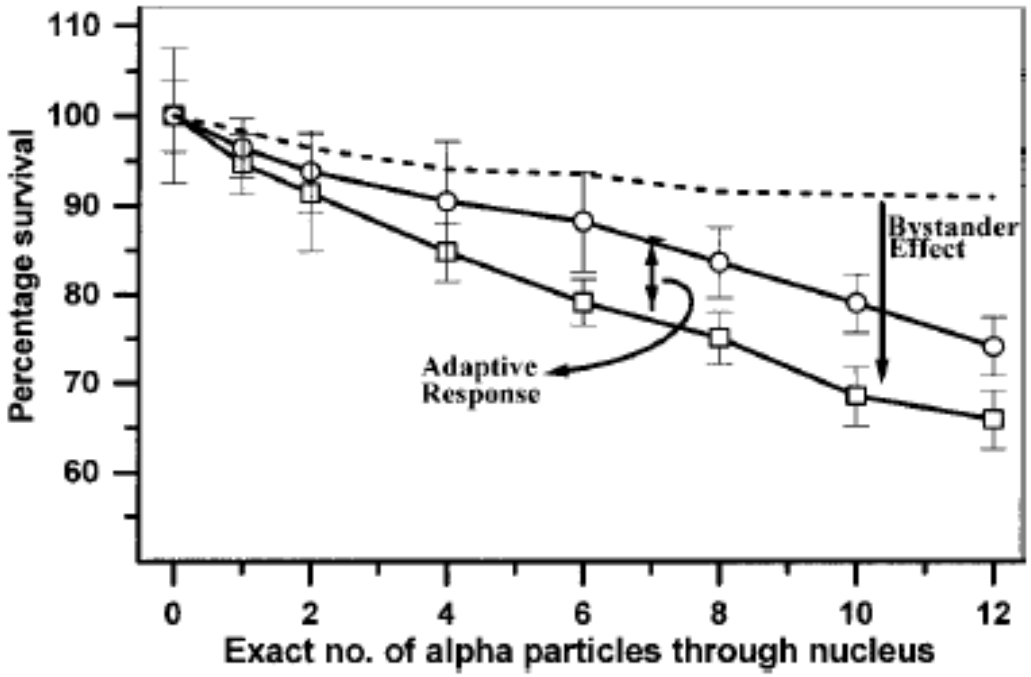
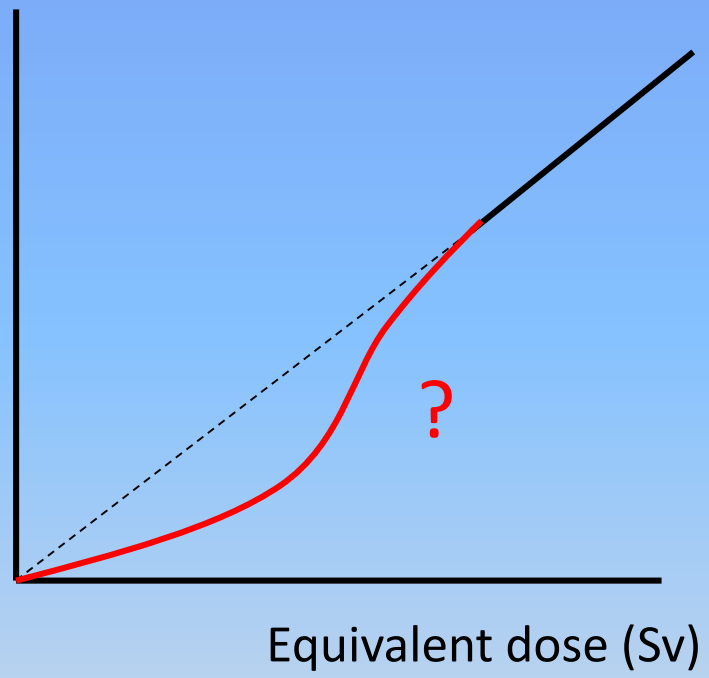


FIG. 1. The adaptive response and the bystander effect for cell survival in C3H 10T½ cells. The dotted line shows the percentage of cells that would be expected to survive when 10% of the cells are exposed to various numbers of α particles calculated from the survival curve for all cells irradiated. The squares show survival for various numbers of α particles, from 1 to 12, traversing 10% of the cell population. The extent to which this falls below the dotted line is an indication of the magnitude of the bystander effect. The circles show survival for cells exposed to 2 cGy of γ rays, 6 h before exposure to various numbers of α particles traversing 10% of the population. The extent to which the circles are above the squares reflects the adaptive response.

Sawant et al., Radiat. Res. 156, 177, 2001

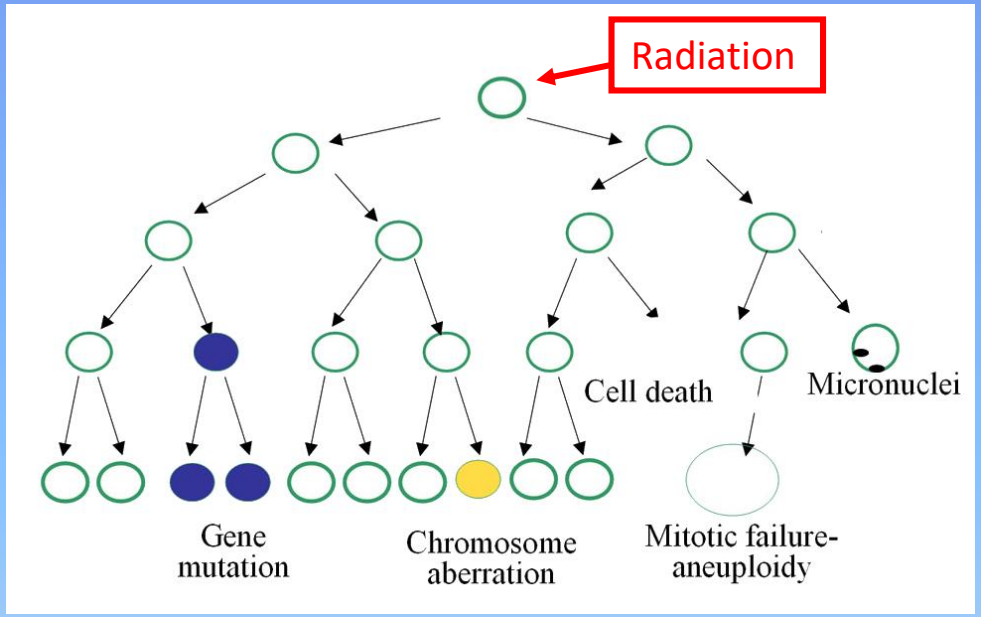
Adaptive response

- Both *in vivo* and *in vitro*
- Humans and animals
- In normal and tumour cells
- For different endpoints: chromosomal aberrations, micronuclei formation, gene mutations, cell killing
- Doses in the range 50 - 200 mGy, decline above 200 mGy, disappear above 500 mGy
- Hypothesis: enhance DNA repair ability and cellular antioxydant activity
- Adaptive response and apoptosis possibly constitute a complementary defense mechanism



Genomic instability

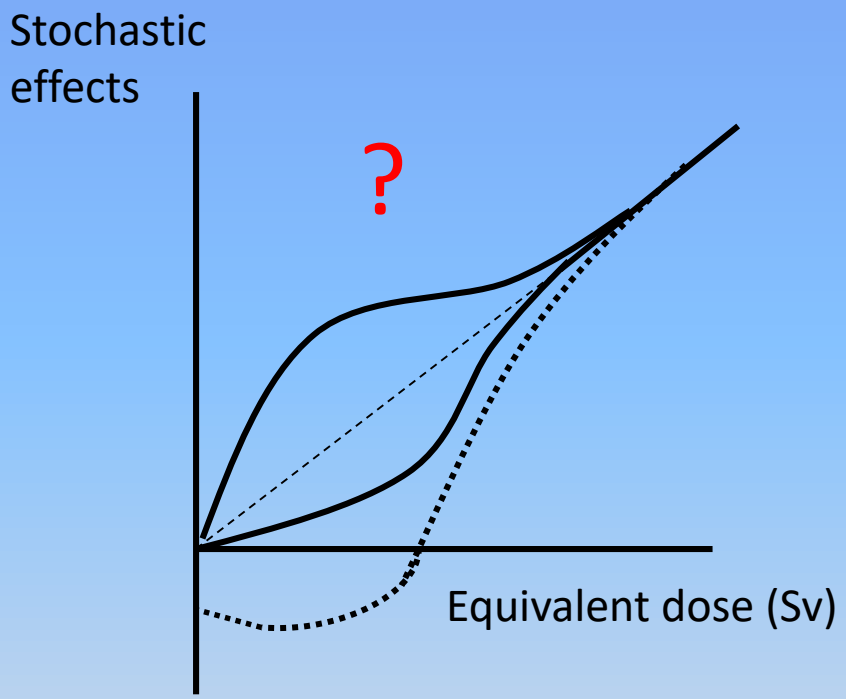
- Observed in number of different cellular systems whereby radiation exposure induces a type of instability in individual cells that is transmitted to their progeny leading to a persistent enhancement in the rate at which genetic changes arise in the descendants of irradiated cells after many generations of replication



- diverse endpoints: large-scale chromosomal rearrangements, deletions and aberrations, gene amplification (extra copies of specific DNA segments), aneuploidy (wrong number of chromosomes), micronucleus formation and gene mutation.
- The capacity of radiation to induce genomic instability depends to a large extent on radiation quality or linear energy transfer (LET) and dose.
- There appears to be a low dose threshold effect with low LET, beyond which no additional genomic instability is induced. Low doses of both high and low LET radiation are capable of inducing this phenomenon.

Summary

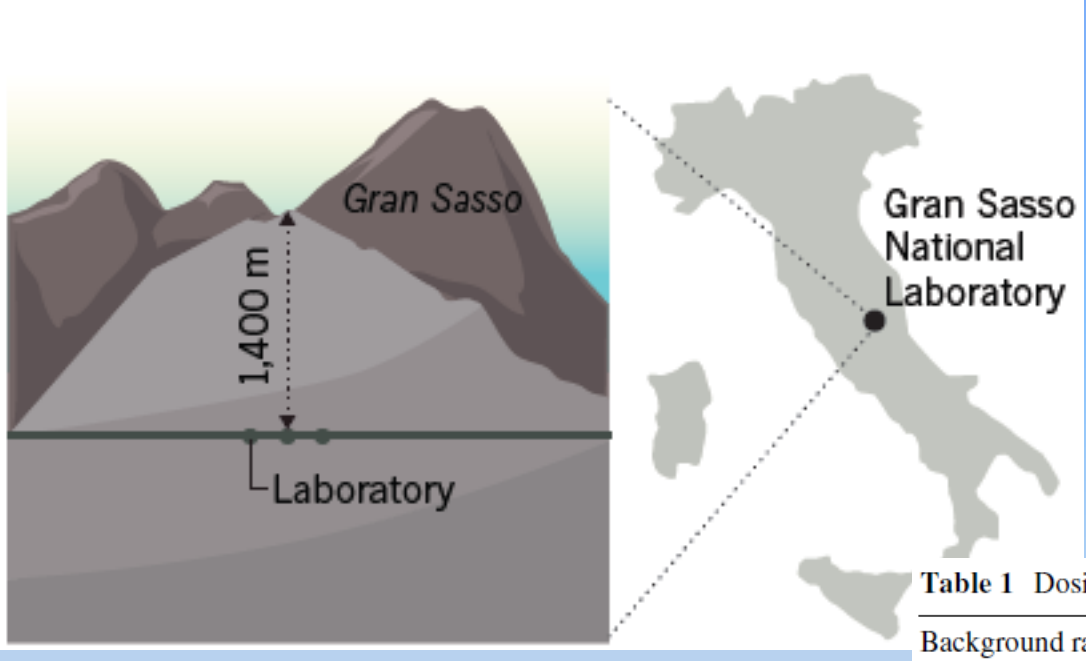
- Chromosome aberrations and somatic cell mutations suggest linear dose-effect relationship at low dose region.
- There exist the by-stander effect, adaptive response and genomic instability which may influence the nature of dose response relationship at low doses and low dose-rates.
- Dose response at low doses of ionizing radiation is at present uncertain and a simple extrapolation from high doses may not be appropriate.



- The better understanding of the mechanisms for these phenomena, the extent to which they are active *in vivo* and how they are interrelated is necessary before they can be confirmed as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

Cell response to extremely low levels of IR

Gran Sasso National Laboratory INFN, Italy



Adopted from Nature 485, 435, 2012

Table 1 Dosimetry estimates

Background radiation component	Reduced background (underground) (nGy/h)	Normal background (external) (nGy/h)
^{222}Rn and daughters[a]	0.17	1.7
All γ-rays[b]	3.6	300
Cosmic rays	Negligible	30[c]
Total dose-rate	3.8	331.7

[a] Based on the application of the model by Jostes et al. (1991)

[b] TLD measurements

[c] Based on UNSCEAR 2000 Report, Sources, Annex E

Carbone et al. Radiat. Environ Biophys. 48, 189, 2009

First radiobiology experiments in LNGS (1992-3)

yeast cells *(Saccharomyces cereuisiae* D7)

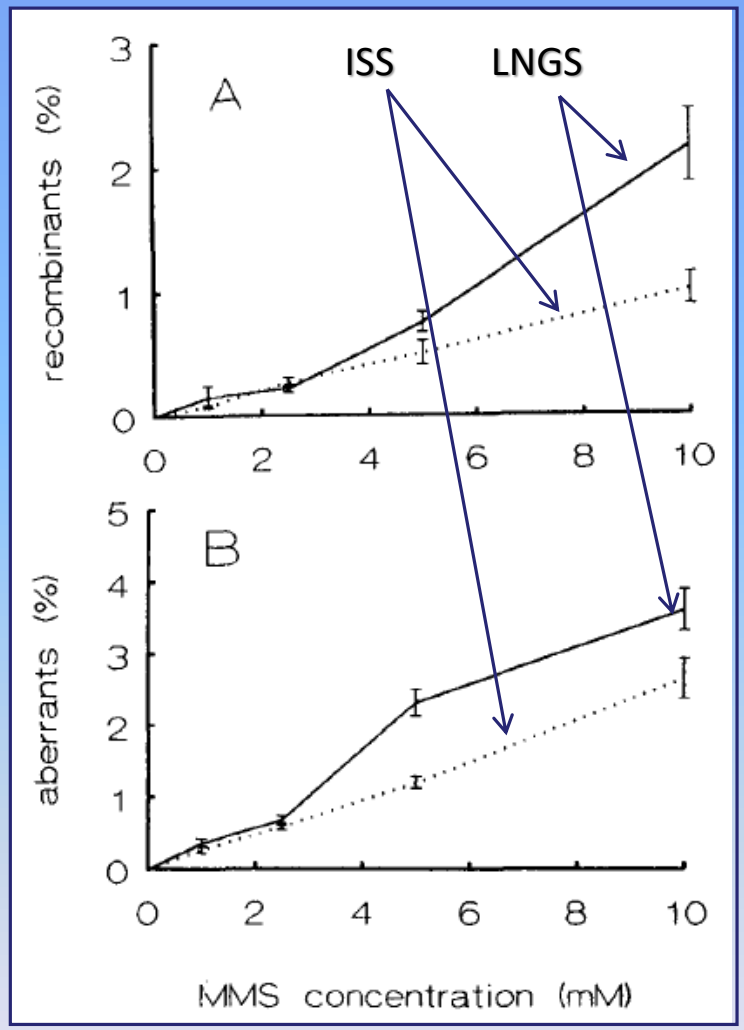
120 generations

- 4 mSv in ISS, 0.6 mSv in LNGS

- cells treated by different concentrations of metyl methasulfonate (MMS, an alkylating agent and a carcinogen)

Induction of Ade-2 reciprocal recombinants or aberrant has been followed (single event of crossing-over at the Ade-2 locus provokes formation of a twin spot red-pink colonies, other recombinational rearrangements lead to red, pink or sectored colonies).

Mitotic intergenic recombination is a signature of DNA damage → cells cultivated at extremely low radiation background lose their ability to deal with DNA damage



Satta et al. Mutation Research 347, 129-133, 1995

Chinese hamster V79 cells

3 months 9 months

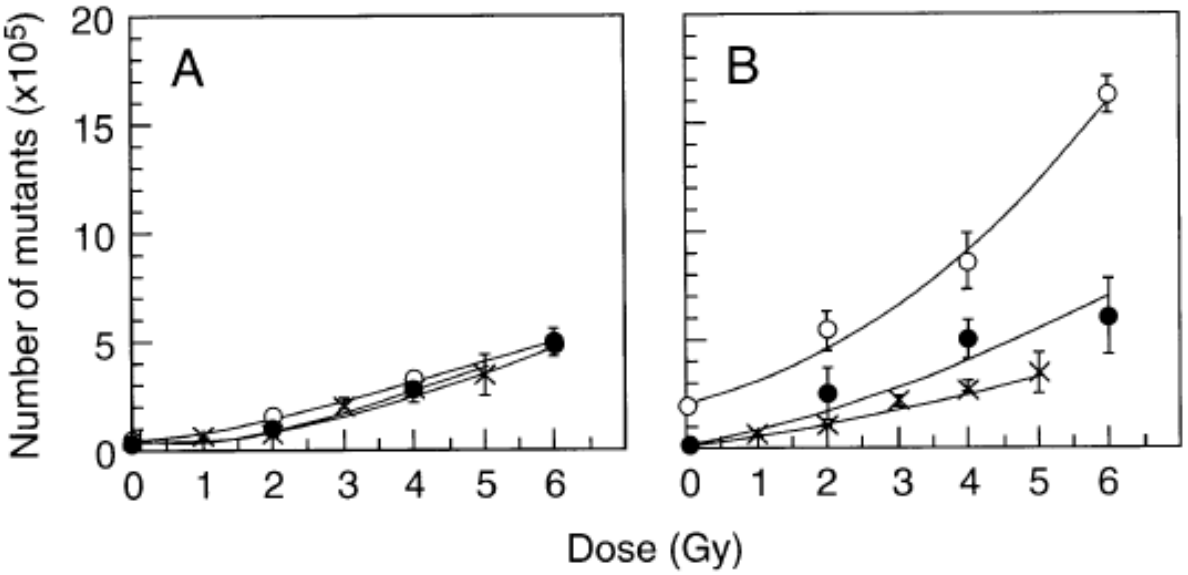


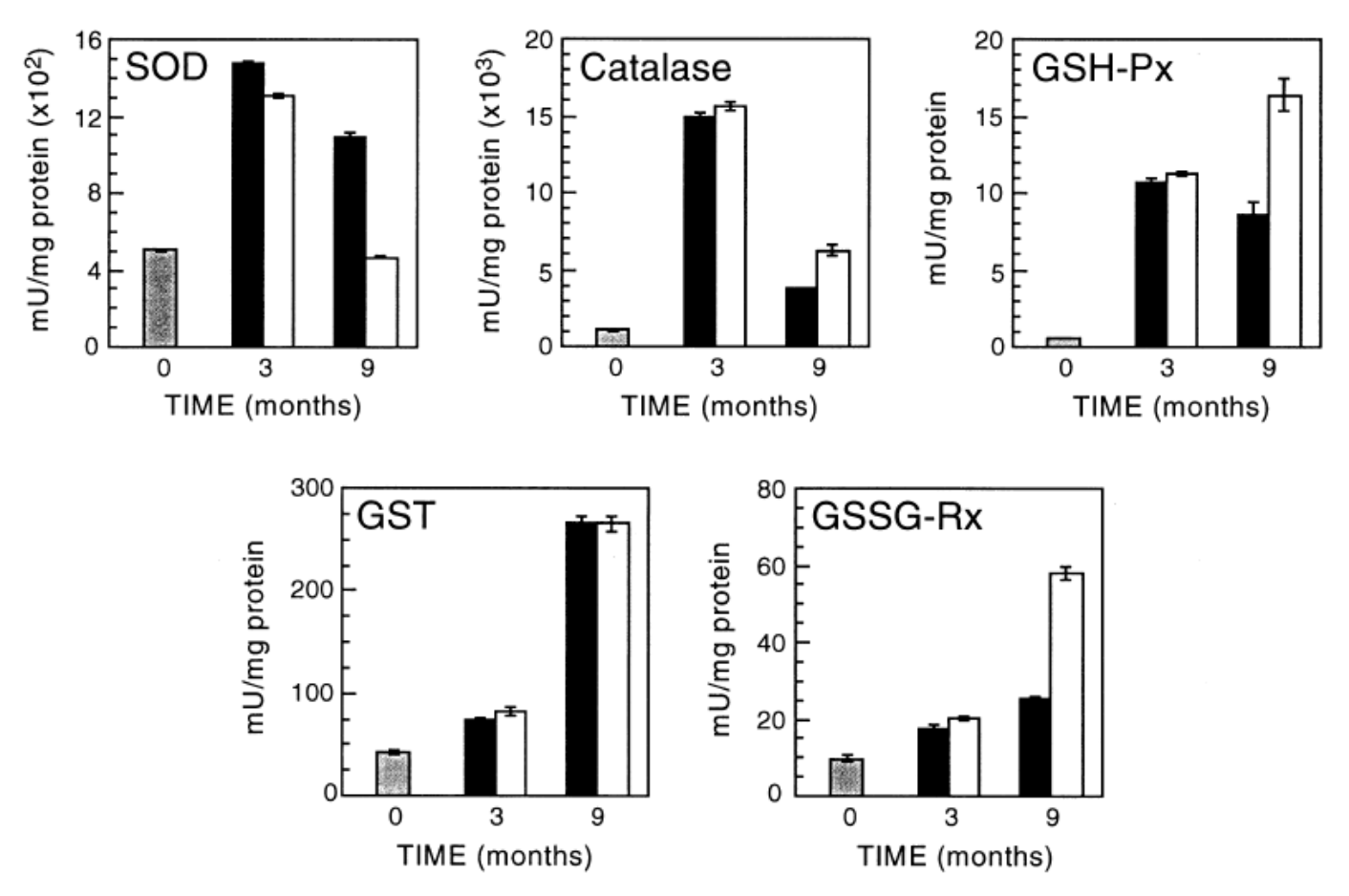
Fig. 6 Number of mutants as a function of different γ-ray dose. Cells grown for 3 (*panel A*) and 9 (*panel B*) months at the ISS (*closed circle*) and at the LNGS (*open circle*). Results obtained at zero time of culture (X) have been included in both panels. Each data point is the mean ±SE of 3 different determinations, performed on day 6, 8 and 10 after irradiation

Adopted from Satta et al. Radiat. Environ. Biophys. 41, 217-224, 2002

Mutation frequency in Chinese hamster V79 cells at *hprt* locus

Chinese hamster V79 cells

ISS LNGS



Adopted from Satta et al. Radiat. Environ. Biophys. 41, 217-224, 2002

- Specific activities of antioxidant enzymes: SOD - superoxide dismutase, catalase, GSH - Px glutathione peroxidase, GST - glutathione-S-transferase, GSSG-Rx - glutathione reductase

LNGS vs ISS – human lymphoblastoid TK6 cells

Table 1 Dosimetry estimates

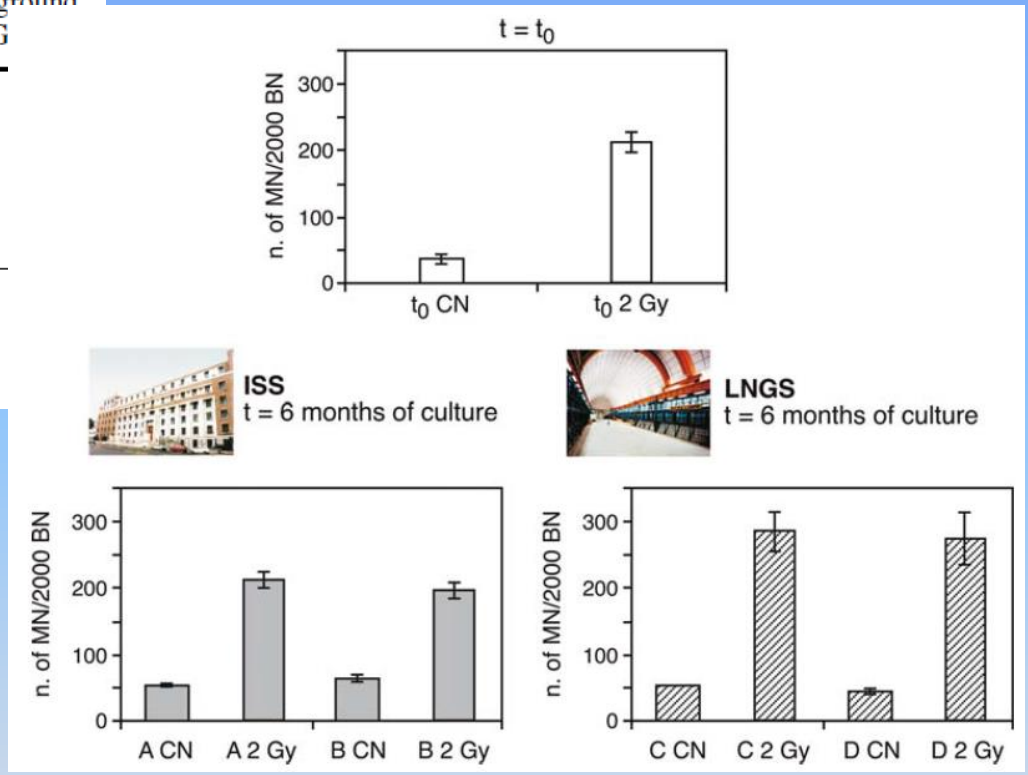
Background radiation component	Reduced background (underground) (nGy/h)	Normal background (external) (nG
^{222}Rn and daughters[a]	0.17	1.7
All γ-rays[b]	3.6	300
Cosmic rays	Negligible	30[c]
Total dose-rate	3.8	331.7

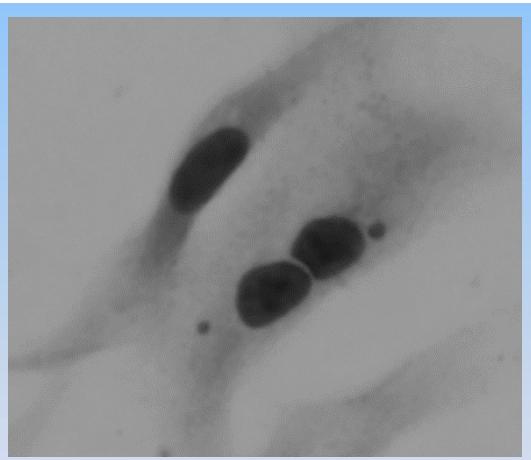
[a] Based on the application of the model by Jostes et al. (1991)

[b] TLD measurements

[c] Based on UNSCEAR 2000 Report, Sources, Annex E

Carbone et al. Radiat. Environ Biophys. 48, 189, 2009





- increase in micronuclei formation after 2 Gy X-rays

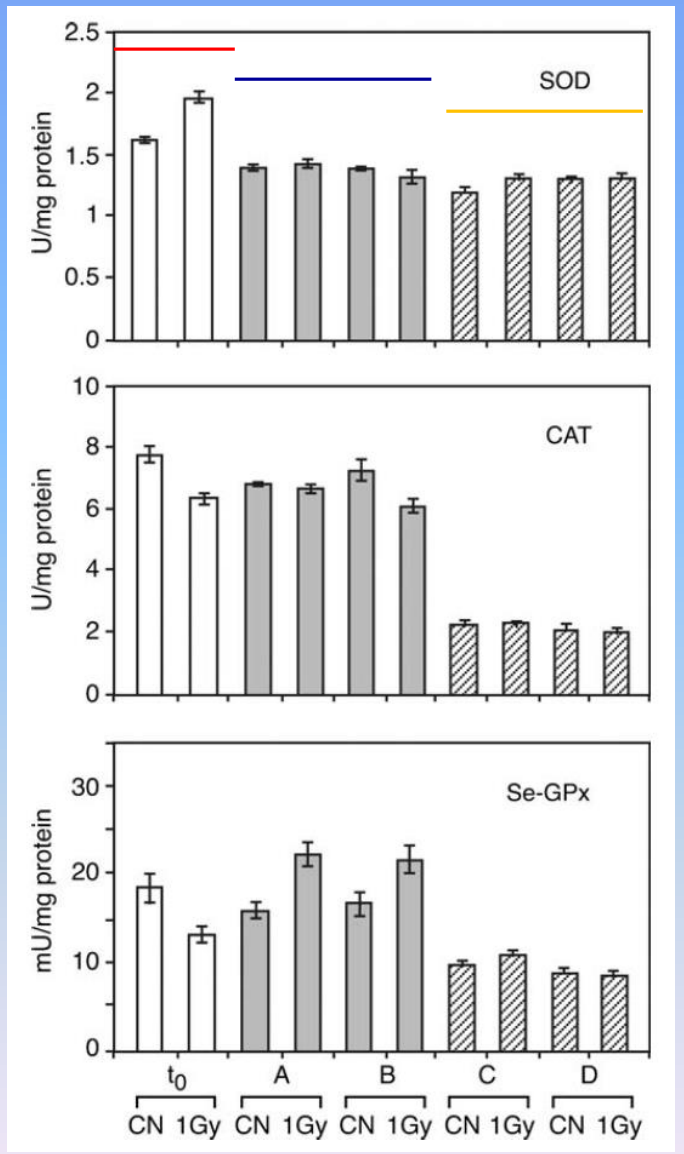
→ cells cultivated at extremely low radiation background lose their ability to deal with DNA damage

Human lymphoblastoid TK6 cells

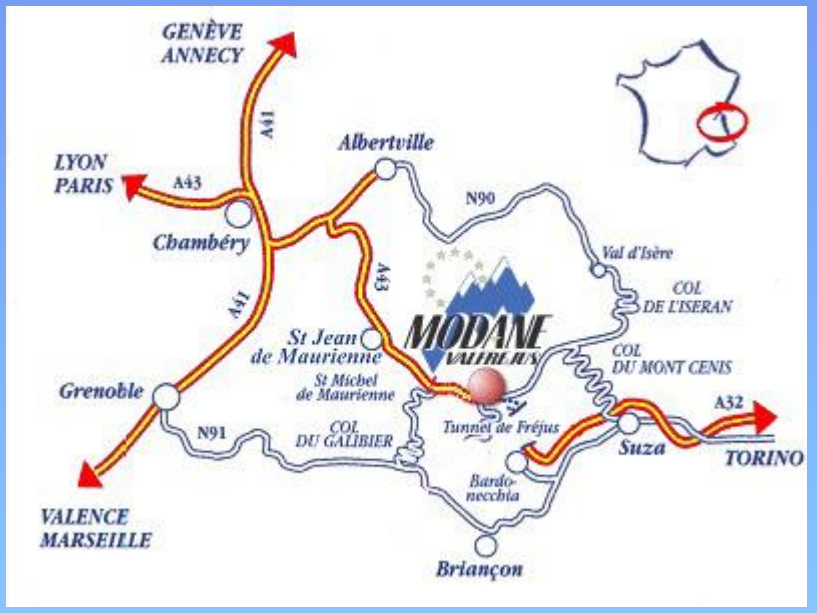
Carbone et al. Radiat. Environ. Biophys. 48, 189-196, 2009

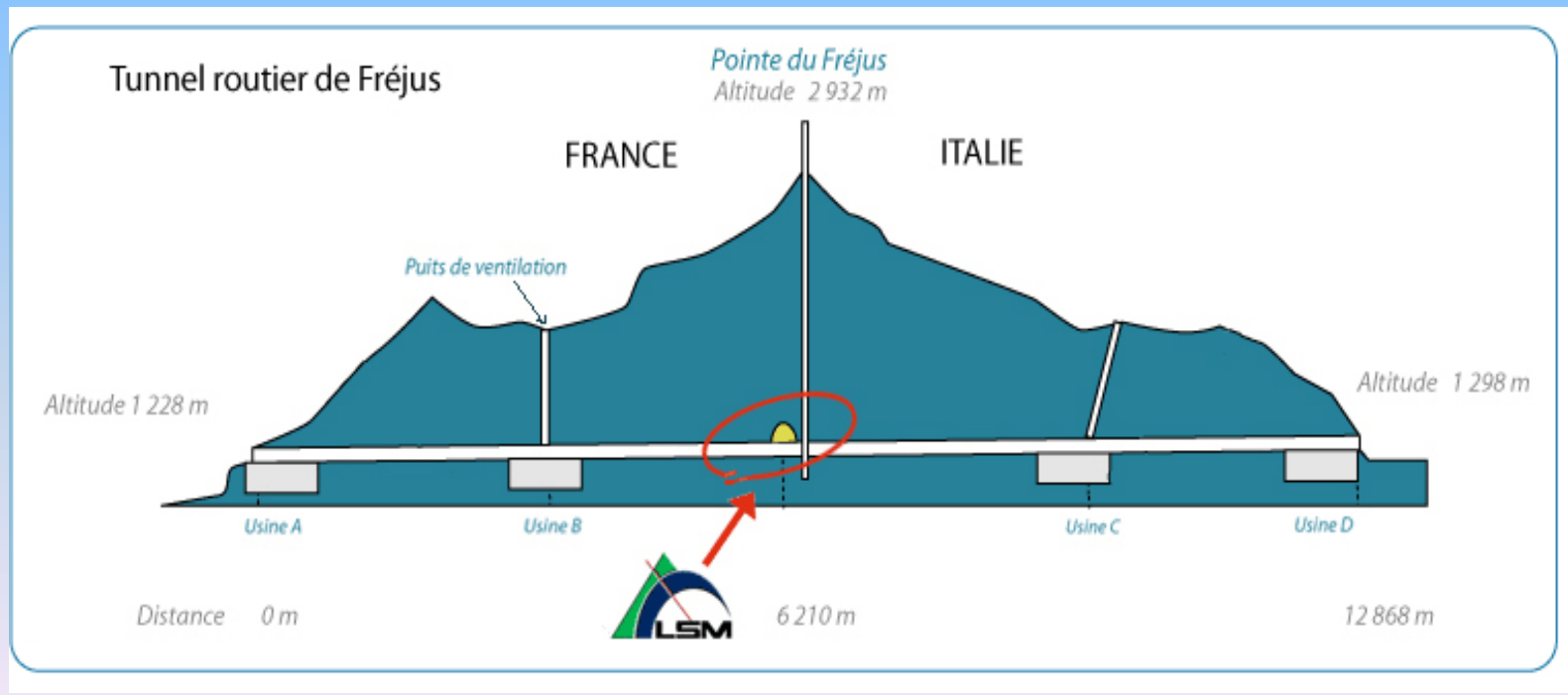
- decrease of antioxidant enzymatic activity after 6 months growth at the ISS and LNGS

- SOD - superoxide dismutase
- CAT - catalase
- SE-GPx - selenium-dependent glutathione peroxidase



Underground Laboratory Modane (Laboratoire Souterrain de Modane, LSM) CNRS/IN2P3, France (1700 m underground)





Adopted from http://www-lsm.in2p3.fr

Future perspectives

- Further decrease of natural radiation background level in the Underground Laboratory in Modane, France
- investigate the mechanisms involved in low dose radiation response of human/rodent cell cultures kept in conditions of extremely low levels of background ionizing radiation or in “reference” background radiation laboratories:
  - changes of the cell cycle regulation, cell proliferation and stability of the genome, gene expression of stress response enzymes

The main outcome of the research shall be determination of mechanisms regulated by low radiation doses which are necessary for physiological functioning and ability to cope with DNA damage.

http://www.odz.ujf.cas.cz/

Department of Radiation Dosimetry
Nuclear Physics Institute
Academy of Sciences of Czech Republic

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- People
- Research
  - Radiation biophysics
  - Mixed radiation fields
  - Radioecology
- Education
- Resources
  - Microscope HSP-1000
  - Equipment for detector etching
- Contacts

The research activities of the department lie at the border of basic and applied research in the domain of dosimetry and microdosimetry of ionizing radiation and their applications in radiation protection, radiotherapy and radioecology. Research studies are often interdisciplinary and include physics, chemistry and biology, for more details please see **Research**. Several available unique equipments are summarized in **Resources**.

The founder and the first director of our institute was well known Czech physicist Frantisek Behounek. It has been established in 1953 as an independent institute; since 1994 it has became a part of Nuclear Physics Institute AS CR as the Department of Radiation Dosimetry. In the same year Frantisek Spurny has been appointed as the new director and had held the role until his death in 2010. The department headed by Marie Davidkova is located within the premises of Bulovka Hospital; currently the building undergoes an extensive reconstruction. Some of us are lectures on Department of Dosimetry and Application of Ionziing Radiation at the Faculty of Nuclear Sciences and Physical Engineering, CTU in Prague. We are also engaged in supervising of bachelor's, master's, and doctoral reserach projects (more you find in **Education**).

News

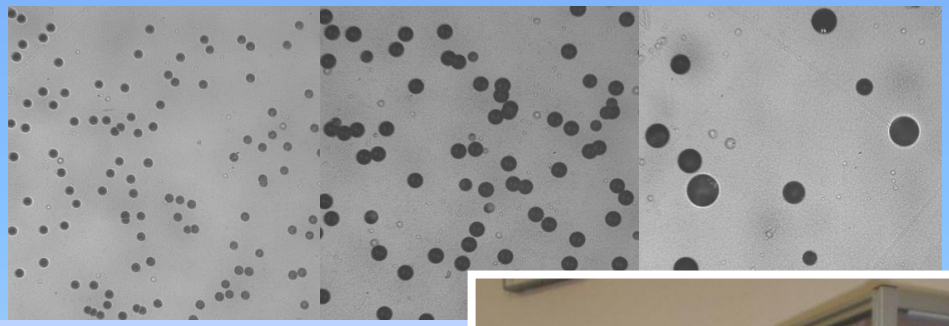
4.6. 2014
We are pleased to invite you to the lecture "Charged Particle Transport Simulations for Radiotherapy and Space Dosimetry" by Prof. Lembit Sihver from Chalmers University of Technology. The lecture will be held on Wednesday at 3 p.m.

3.6. 2014
Martin Sefl succeeded to defend his diploma theses, congratulation!

18.6. 2014
Jan Kubancak succeeded as well to defend his PhD thesis. Congratulation!

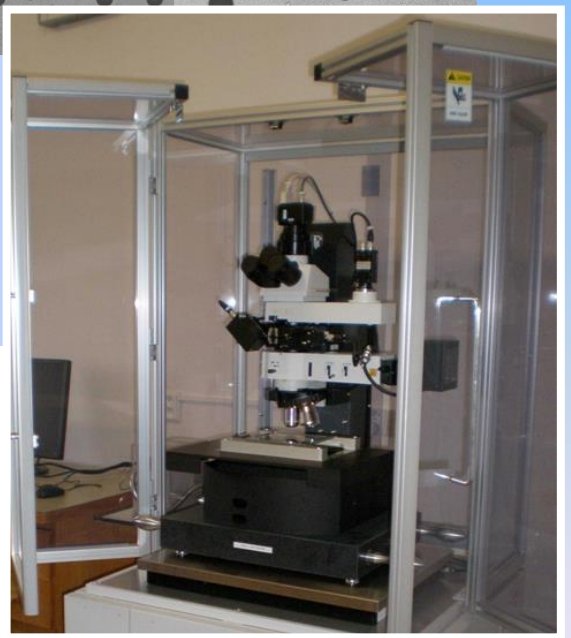
Development of new dosimetric methods for

- LET spectrometry
  (track-etched and semiconductor detectors)
- measurements in mixed radiation fields



Monitoring of cosmic radiation in space and at mountain observatories for research on

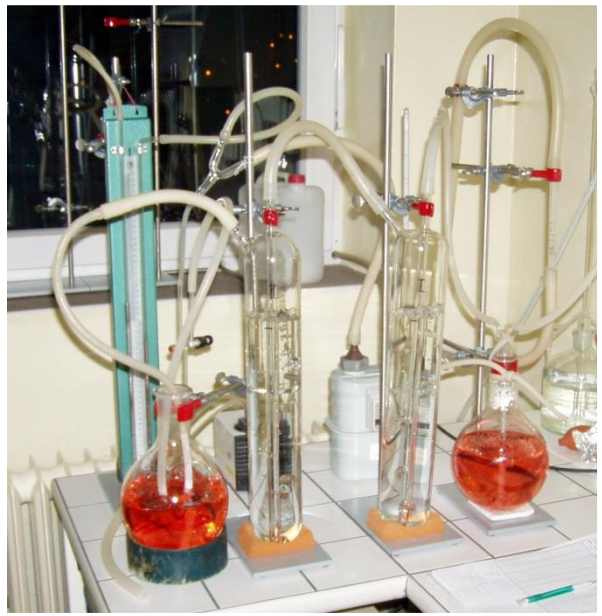
- space weather
- aircraft and spacecraft crew dosimetry



Radioecology and radiocarbon dating

- Radiocarbon dating laboratory CRL (in co-operation with Archaeological Institute CAS)
- Fossil fuel combustion and atmospheric $^{14}CO_2$ and CO_2
- Past environmental changes and ^{14}C
- ^{14}C in the vicinity of NPPs and in reference areas

Low background liquid scintillation spectrometers QUANTULUS 1220



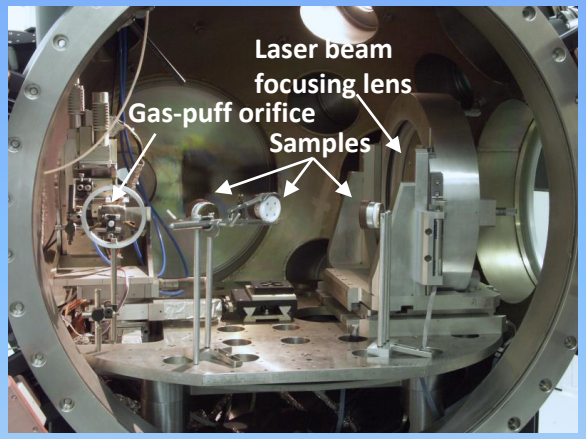
Monitoring of atmospheric $^{14}CO_2$

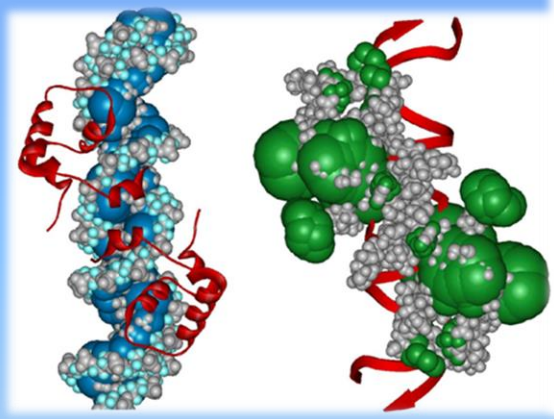
Sampling in the vicinity of NPP Temelín

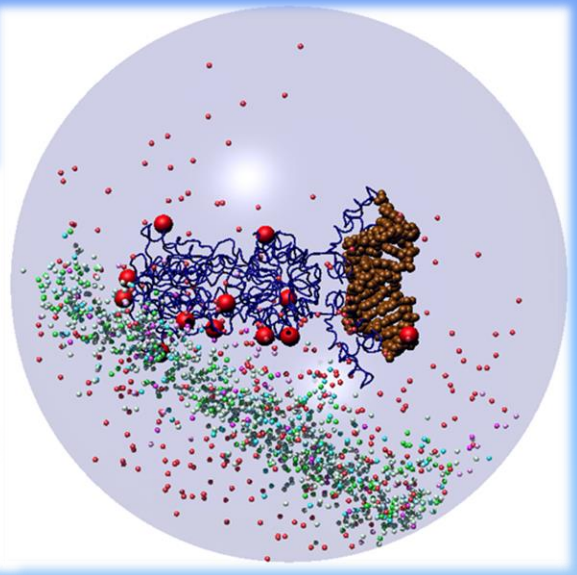
- Development of new analytical methods
- Theory of formation of liquid scintillation pulse spectra

Research on biological effects of radiation

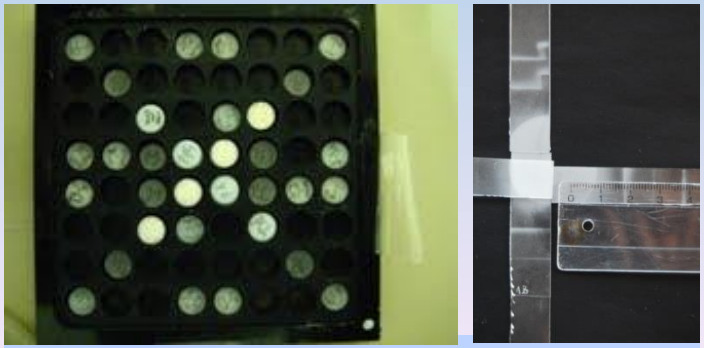
- Radiation damage to DNA and DNA-protein complexes
- Biological effects of soft X rays and XUV



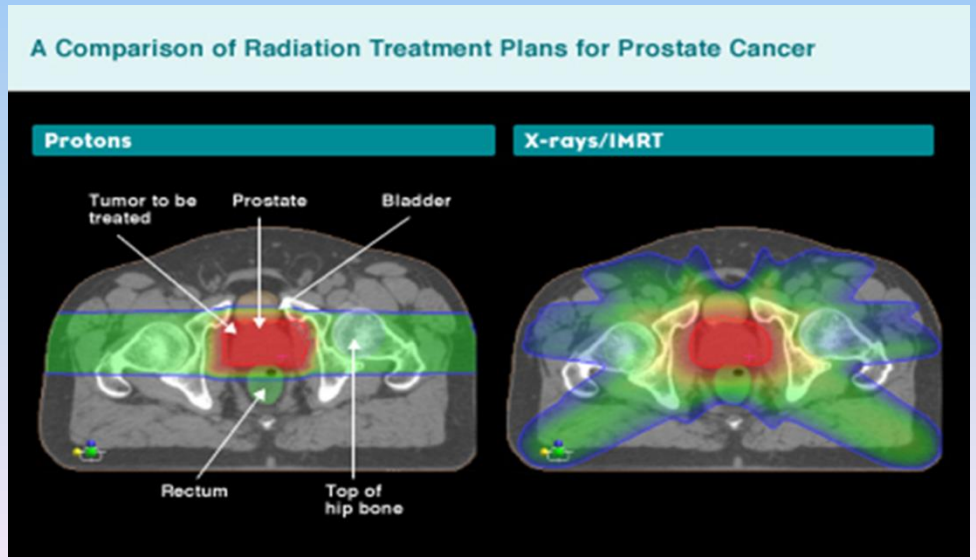




- Radiation therapy of cancer:
  - Induction of secondary cancers
  - Nuclear fragmentation



TLD and PADC in proton beam



Sháníme studenty na všechny tématiky

Děkuji za pozornost