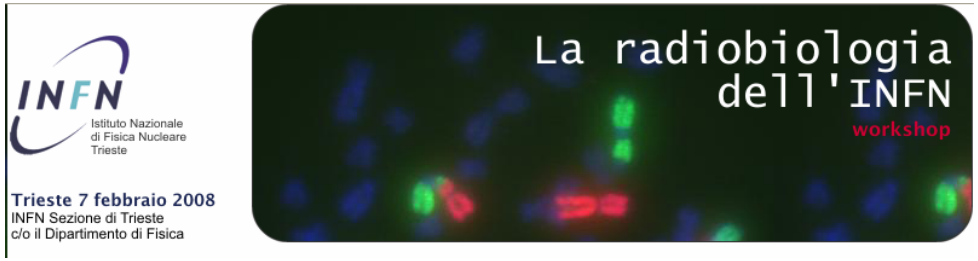

SHEILA

(*Single Hit Effects Induced by Low-dose irrAdiation*)

Collaborazione: BOLOGNA, LNL, ROMA3

Trieste, 7 Febbraio 2008

SCOPI: …studio degli effetti biologici indotti a seguito a esposizione alle basse dosi, a regime stocastico (“*broad beam*”) e di “counted particles” (“*microbeam a singolo evento*”)

1. **Contributo alla valutazione di rischio**
2. **Studio di meccanismi di trasmissione di segnale di danno indotto dalla radiazione ionizzante**
3. **Contributo allo sviluppo e validazioni di modelli biofisici**

Attività prevista:

- ***Sistematica di effetti indotti in sistemi cellulari di mammifero, in funzione della qualità della radiazione e della dose, sino al limite di una particella per cellula***

Sezioni INFN Partecipanti:

SHEILA

BOLOGNA: *(dal 2008)*

G.Baldazzi
G. Castellani (resp locale)
Masetti
Procopio
Remondini
Zironi

LNL:

L. Celotti
R. Cherubini (resp locale; rapp. naz)
V. De Nadal
S. Gerardi
D. Guryev *(EC MRTN "Cellion" post-doc fellow)*
F. Ianzini
M. A. Mackey
M. Skoczylas *(EC MRTN "Cellion" PhD-student fellow)*
D. Zafiropoulos

ROMA-3:

A. Antoccia
F. Berardinelli
A. Sgura
C. Tanzarella (resp locale)

Durata: 3 anni

FP6 -- Marie Curie Research Training Network

CELLION : Studies on cellular response to targeted single ions using nanotechnology
(UE Contract n. 511382- MRTN-CT-2003-503923)

Participant Institutions (and project leader)

1. Henryk Niewodniczañski Institute of Nuclear Physics PAN - Kraków, POLAND
Zbigniew Stachura

2. Gray Cancer Institute - Mount Vernon Hospital,Northwood, UNITED KINGDOM
Melvyn Folkard

3. Gesellschaft für Schwerionenforschung mbH**, Material Science** - Darmstadt, GERMANY
Bernd Fischer

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Roberto Cherubini

5. Jagiellonian University, Medical College - Kraków, POLAND
Jerzy Stachura

6. Centre d'Etudes Nucleaires de Bordeaux-Gradignan (CNRS - IN2P3) - GRADIGNAN, FRANCE
Philippe Moretto

7. Universität Leipzig**, Fakultät für Physik und Geowissenschaften, Nukleare Festkörperphysik** - Leipzig, GERMANY
Tilman Butz

8. Lund Institute of Technology**, Physics Department, Division of Nuclear Physics** - Lund, SWEDEN
Jan Pallon

9. The University of Surrey**, Advanced Technology Institute Dept. Physics, School of Electronics and Physical Science -** GUILDFORD, UNITED KINGDOM
Geoffrey W. Grime

10. Uppsala Universitet**, Department of Engineering Sciences -** Uppsala, SWEDEN
Klas Hjort

Ref: CELLION homepage: http://cellion.ifj.edu.pl/

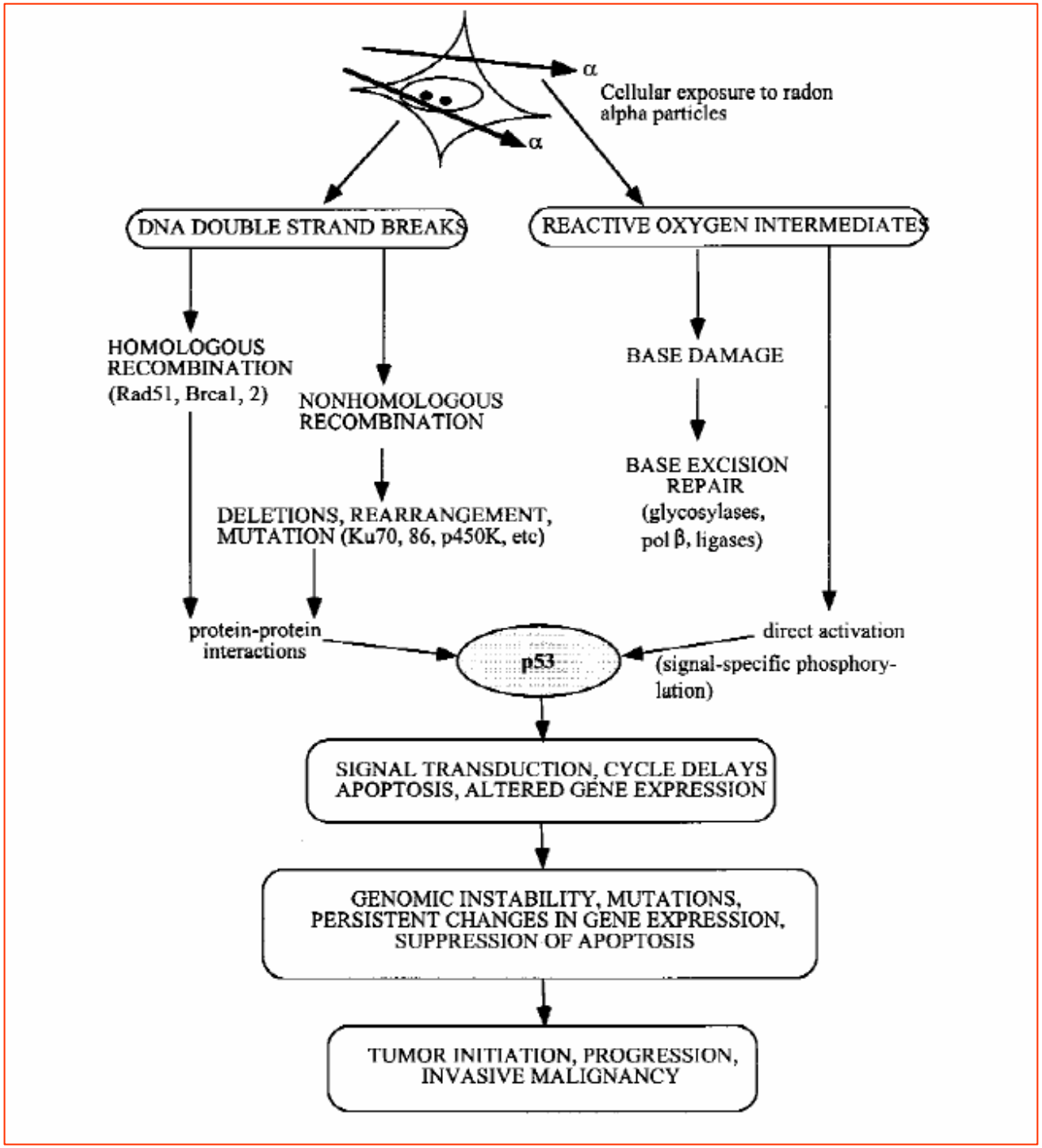


FIGURE 6.1 Flow chart showing development of malignant cells from initial α-particle damage to cells. DNA strand breaks are repaired by homologous or nonhomologous (illegitimate) double strand break rejoining, and damaged bases by base excision repair. Activation of p53 protein, initiates pathways leading to cell cycle delays and apoptosis, and surviving cells may contain gene deletions, rearrangements, amplifications, and persistant genomic instability. Mutations in oncogenes, loss of function in tumor suppressors, and loss of heterozygosity produces a heterogeneous population of cells which escapes from normal cell and tissue homeostasis to become malignant.

BEIR-VI, 1999

Low dose risk assessment

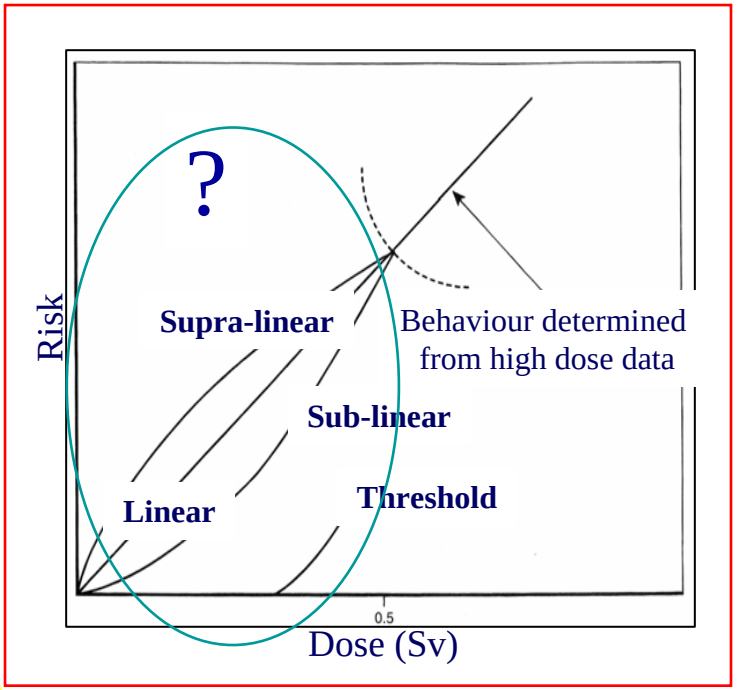
- Data collected at high doses by means of:

  - epidemiological studies (Hiroshima e Nagasaki; nuclear fallout; uranium miners..)

  - in vitro studies: conventional irradiation with broad beams

- Extrapolation to low doses:

  Linear – No threshold model



..Not linear effects of low doses?!!..
Direct investigation of low dose effects?

..but also…hormesis??!!..

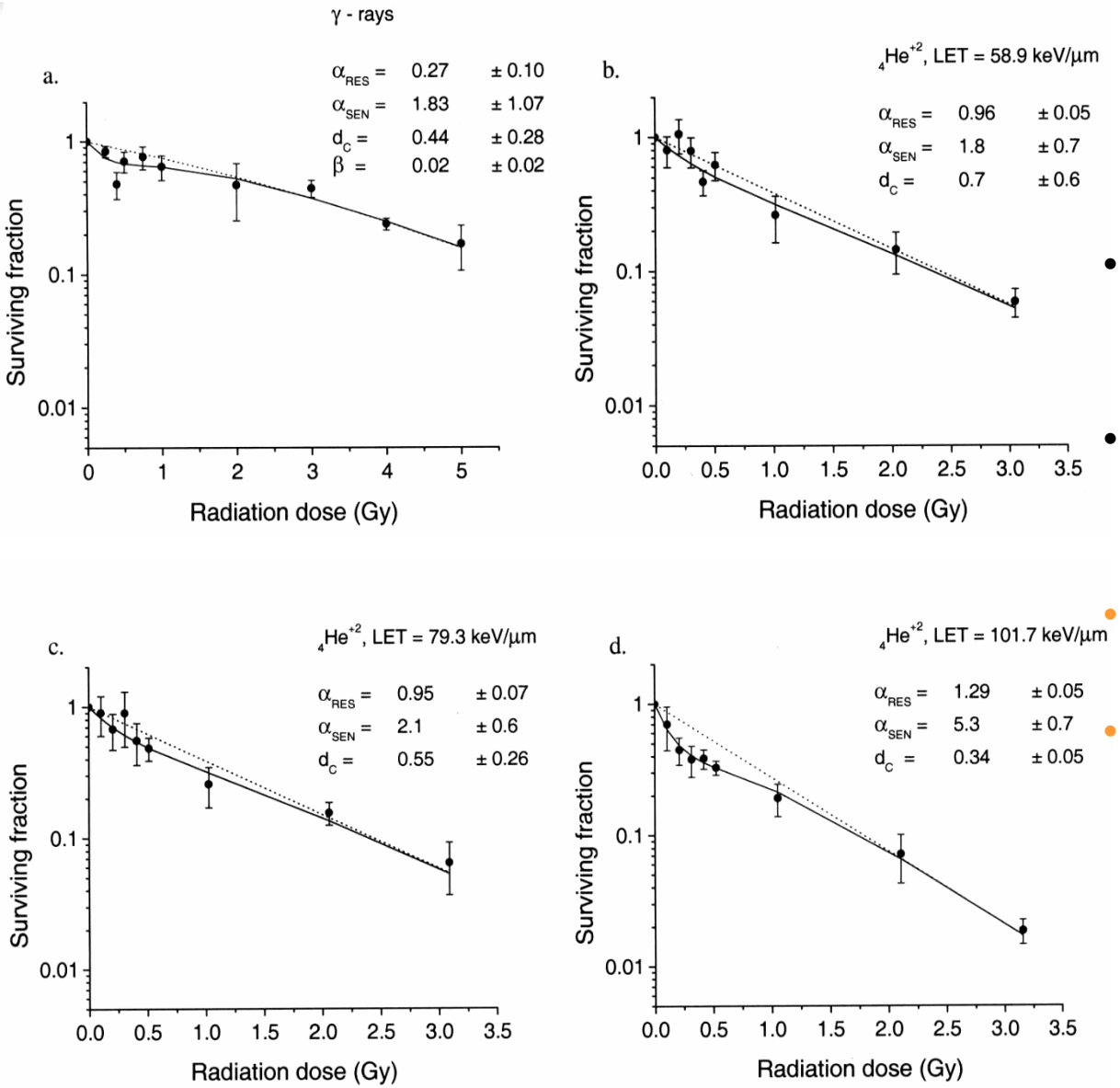
> ...our cells have developed mechanisms to detoxify harmful chemicals and exposure to radiation—in fact, low doses may even trigger responses that are beneficial

Edward J.Calabrese, EMBO reports VOL 5 | SPECIAL ISSUE | 2004

…Effetti delle basse dosi
(studi in vitro)

SHEILA

(*E.Tsoulou et al., Int. J. Rad. Biol. (2001),vol. 77, 1133-1139*)



LNL broad ion beam cell irradiations:

- **^{60}Co γ–rays and $^4He^{2+}$ ions of different energies (59, 79 and 102 keV/μm LET)**
- **Chinese hamster V79 cells**

- **Low dose hyper-radiosensitivity**
- **Induced radioresistance**

…Effetti delle basse dosi
(studi in vitro)

SHEILA

(*G. Borhnsen et al., Radiat. Prot. Dos.(2001),vol. 99, 255-256*)

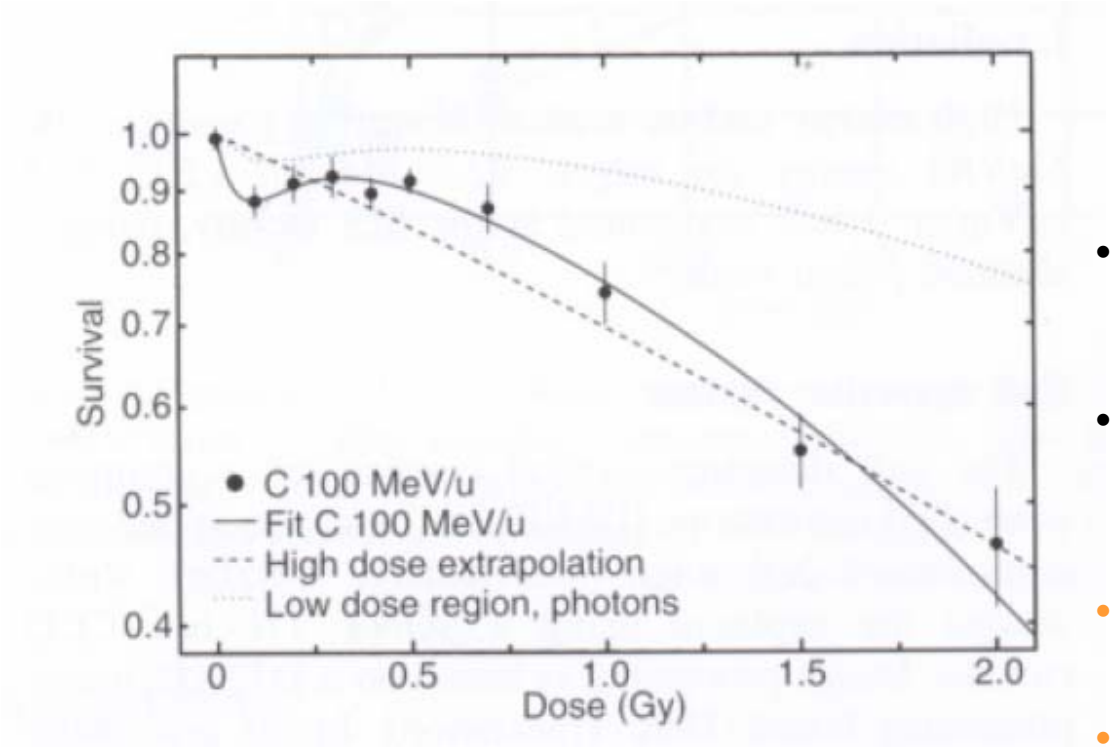
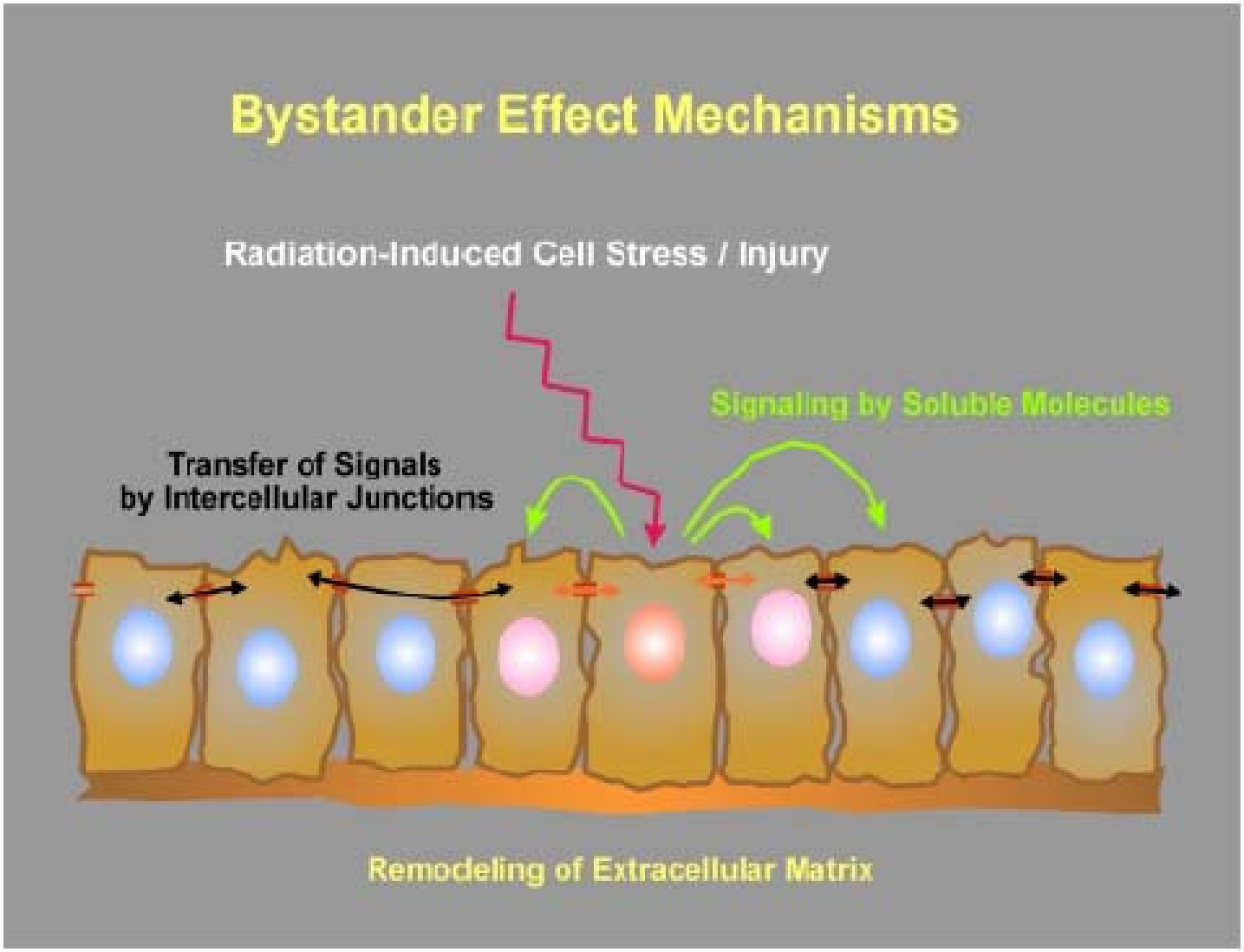


Figure 1. Survival of V79 cells after irradiation with low doses of 100 MeV/u carbon ions. Dashed line: expectation value for survival according to a LQ-fit to data obtained by the dilution assay over a larger dose range[6]. Full line: fit according to the Induced-Repair formulation[7]. Dotted line: corresponding fit to the survival curve obtained after irradiaton with 6 MV photons.

GSI SIS facilty
scanned pencil beam
cell irradiations:

- **6MV photons and $^{12}C^{6+}$ ions of 92.5 MeV/amu on target (27.5 keV/μm LET)**
- **Chinese hamster V79 cells**

- **Low dose hyper-radiosensitivity**
- **Induced radioresistance**

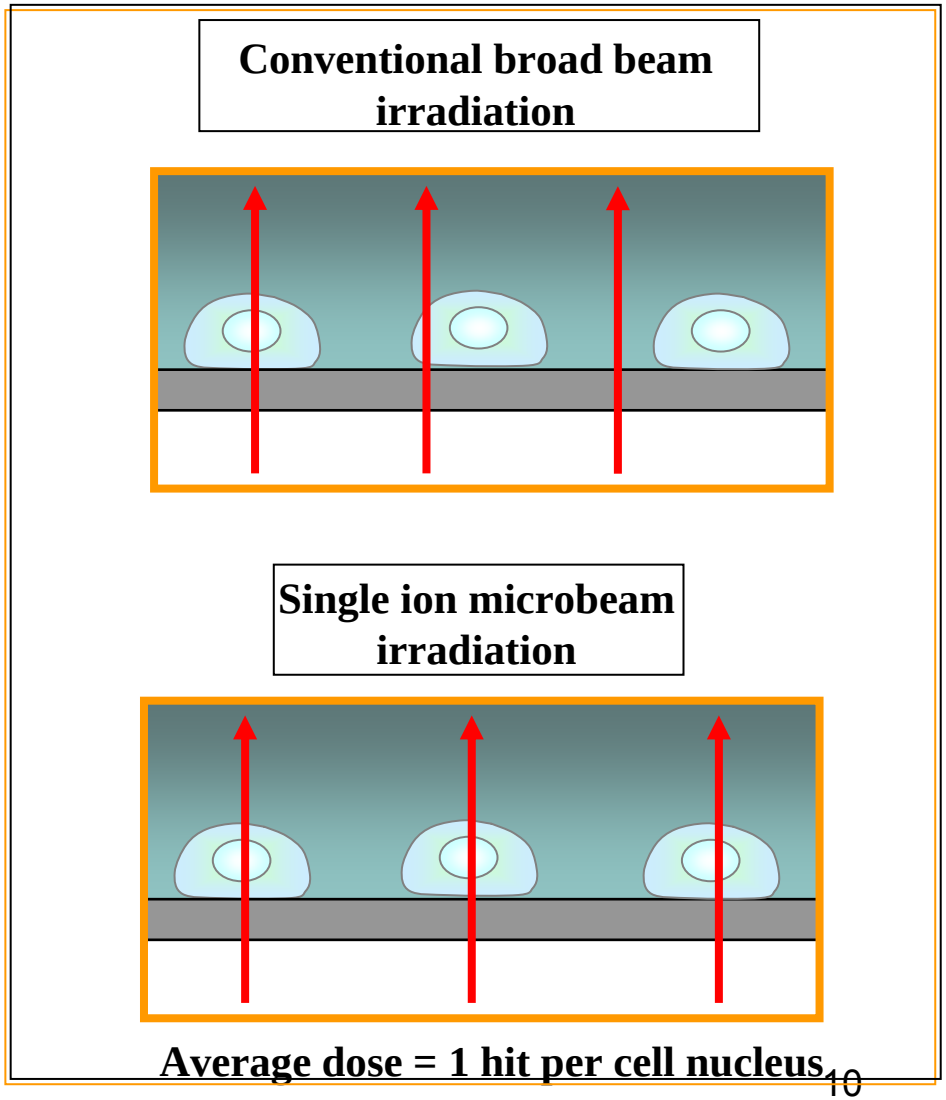


(G. Nelson, 2000)

Recenti evidenze sperimentali mostrano che cellule <u>non</u> direttamente irradiate esprimono un danno a seguito di irraggiamento di cellule della stessa popolazione cellulare… *anche se non direttamente adiacenti*!!

… approccio sperimentale proposto per lo studio degli effetti delle basse dosi

Sistematica di effetti indotti in sistemi cellulari di mammifero, in funzione della qualità della radiazione e della dose, sino al limite di una particella per cellula, in regime stocastico (“*broad beam*”) e di “counted particles” (“*microbeam a singolo evento*”)



Available INFN-LNL facilities for radiobiological studies:

- Light ion broad beam irradiation facility at the 7MV Van de Graaff CN electrostatic accelerator
  (protons, deuterons, helium-3 and helium-4 ions; E: 0.8-12 MeV)

- Heavy ion broad beam facility at the Tandem-ALPI accelerator complex
  (A>4; E: 5-26 MeV/amu)

- Light ion microbeam facility at the 7MV Van de Graaff CN electrostatic accelerator for "single-ion single-cell" irradiation
  (protons, deuterons, helium-3 and helium-4 ions; E: 0.8-12 MeV)

- Fully equipped cell biology Laboratory

→*Under development*: Heavy ion microbeam facility at the Tandem-ALPI accelerator complex for "single-ion single-cell" irradiation
(A: 6-20; E: few-20 MeV/nucleon)

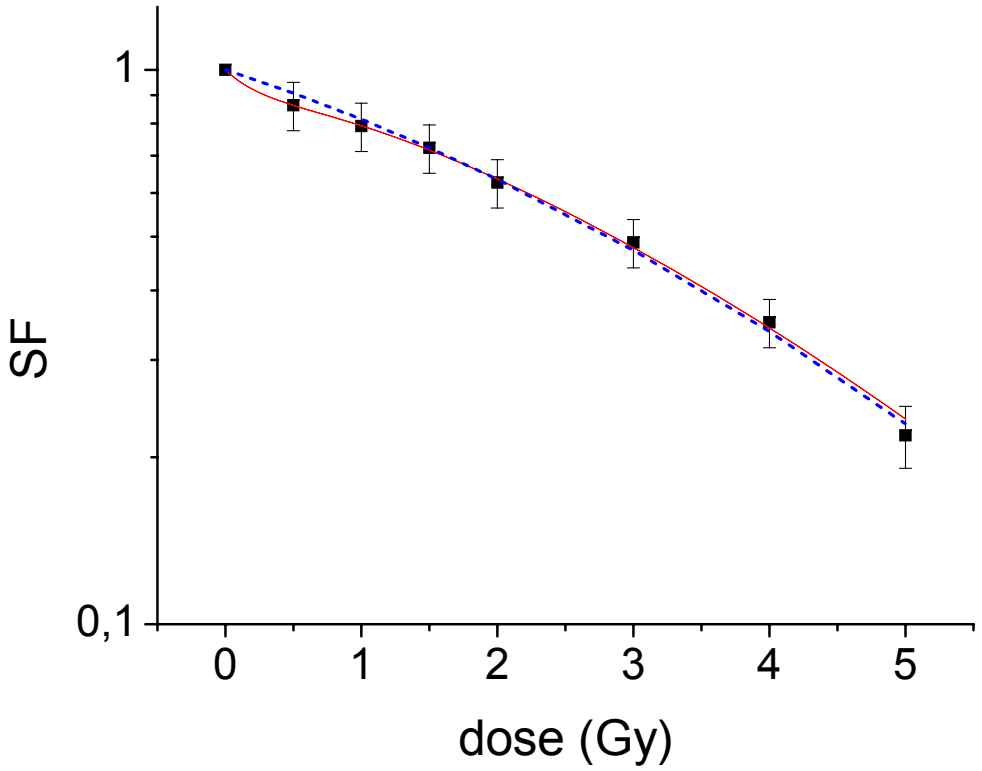
Cellule di mammifero usate nella sperimentazione:

Roditore : V79, MEFs

Umane: fibroblasti primari

Misure del danno al DNA	Misure di risposta cellulare alle radiazioni
✓ Sopravvivenza cellulare ✓ Foci dell'istone H2AX ✓ Micronuclei ✓ Aberrazioni cromosomiche	✓ Telomeri ✓ Patch clamp ✓ Espressione genica ✓ P53 e CDKN1A

Cellule V79 – Raggi γ Co-60



Model: M&Jg
Chi^2 = 0.07209
R^2 = 0.99905

asen	0.53768	±1.42605
dc	0.4034	±0.87849

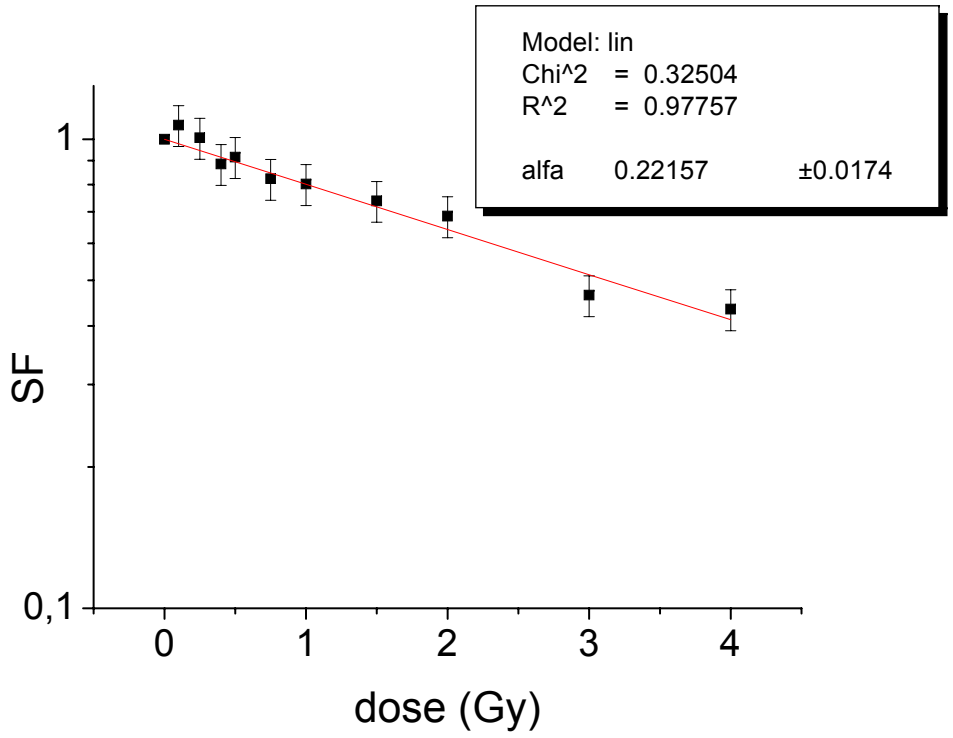
Model: lin.quadr.
Chi^2 = 0.12576
R^2 = 0.99835

alfa	0.18262	±0.04875
beta	0.02233	±0.01226

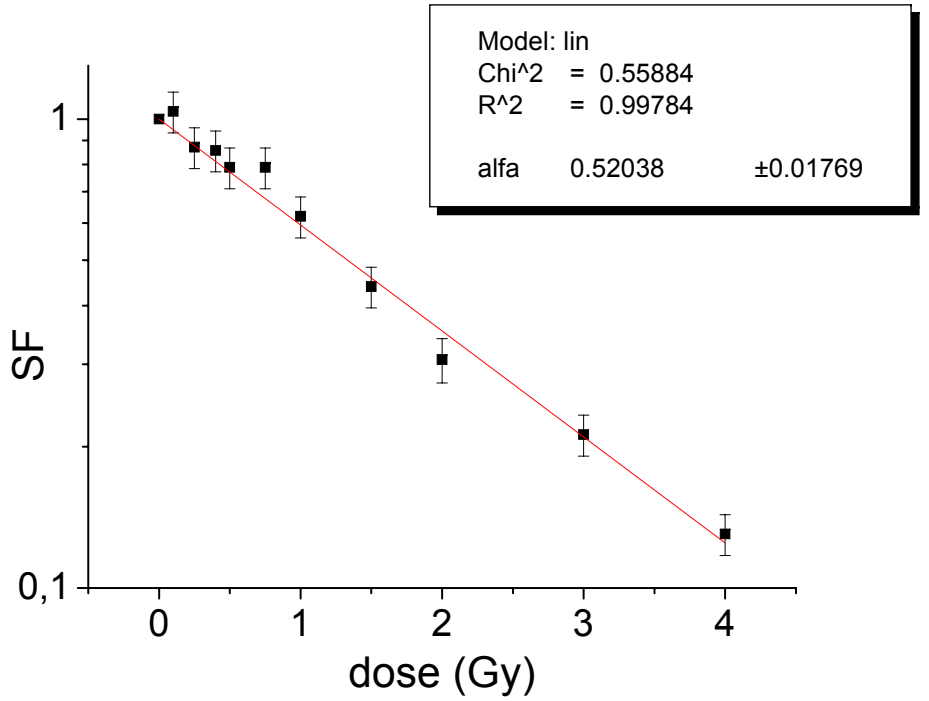
Cellule V79 – protoni

Risultati preliminari

LET= 7.7 keV/µm



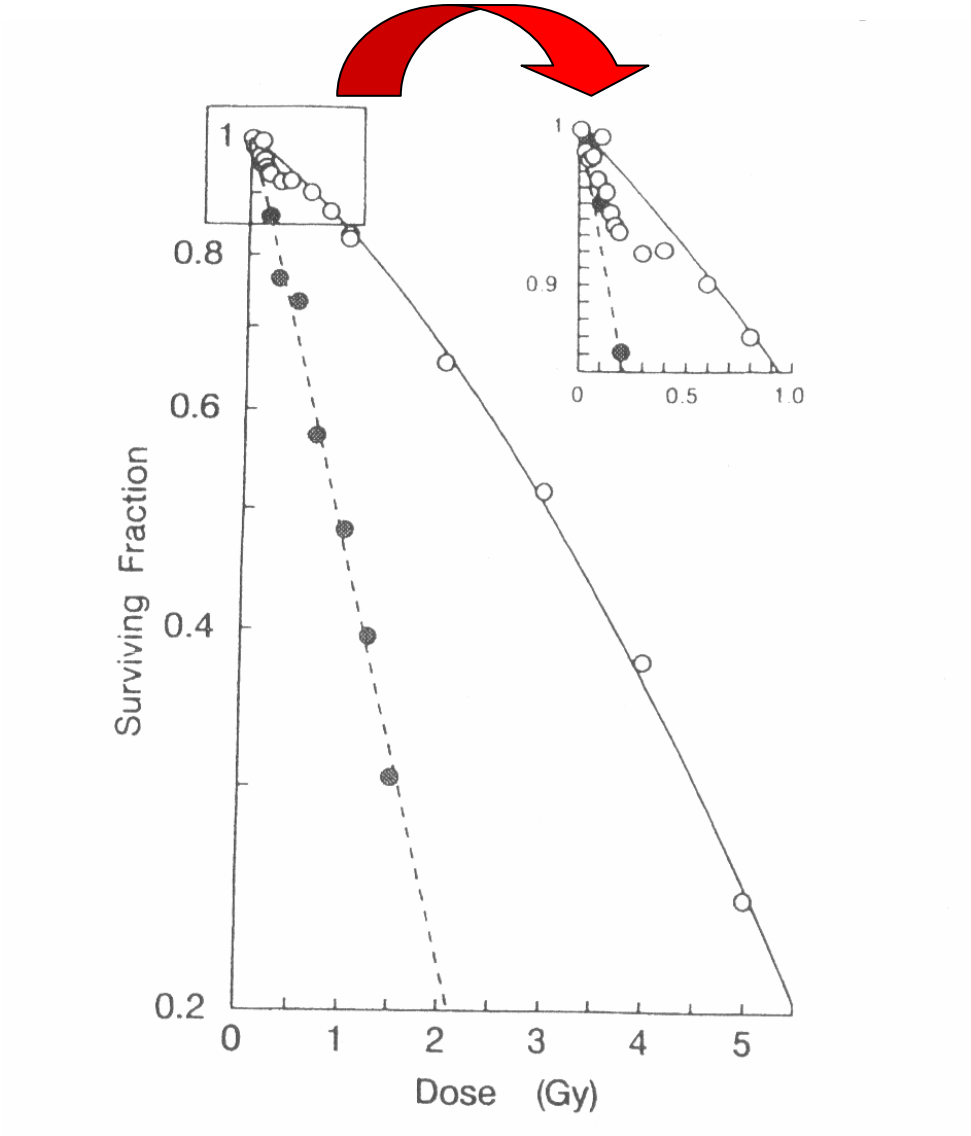
LET= 28.5 keV/µm



LNL

Cell survival curves after irradiation with neutrons and X-rays

B. Marples and M.C. Joiner: *The response of Chinese Hamster V79 cells to low radiation doses: evidence of enhanced sensitivity of the whole cell population* Radiat. Res. 133 (1993) 41-51



Cell survival curves after irradiation with neutrons (•) and 250 kV_p X-rays (◦).
The solid line shows the fit of the linear-quadratic (LQ) model to the X-rays data above 1 Gy. The inset shows the low dose region of the survival curve expanded and the increased effectiveness of X-rays below 0.5 Gy compared to the LQ prediction.

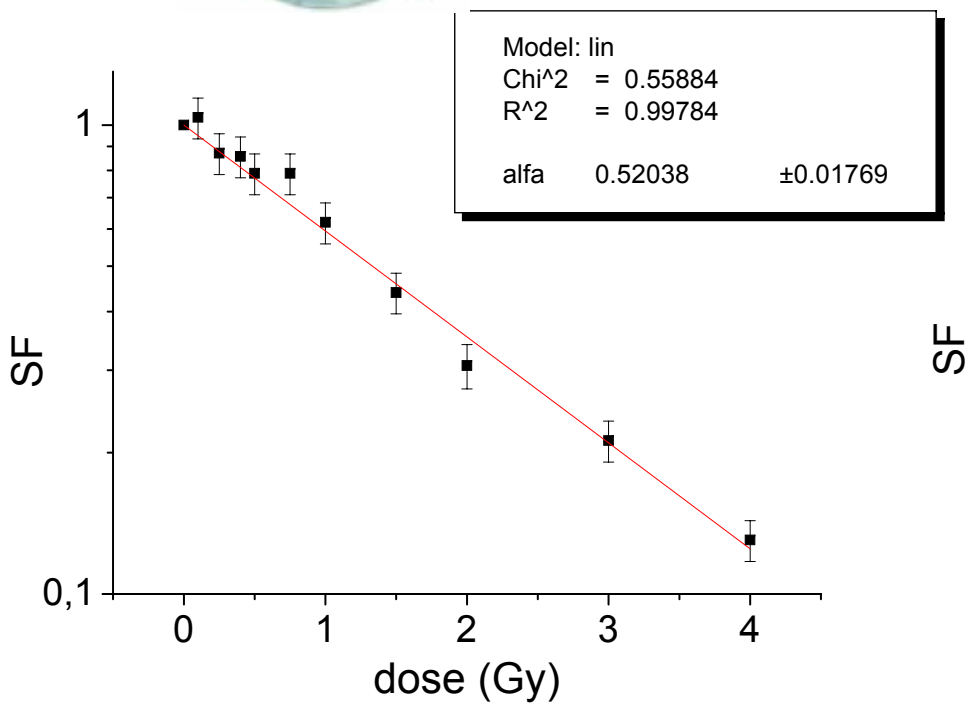
Cellule V79 – protoni, LET= 28.5 keV/μm
Half-shielded Irradiation

SHEILA

Risultati preliminari

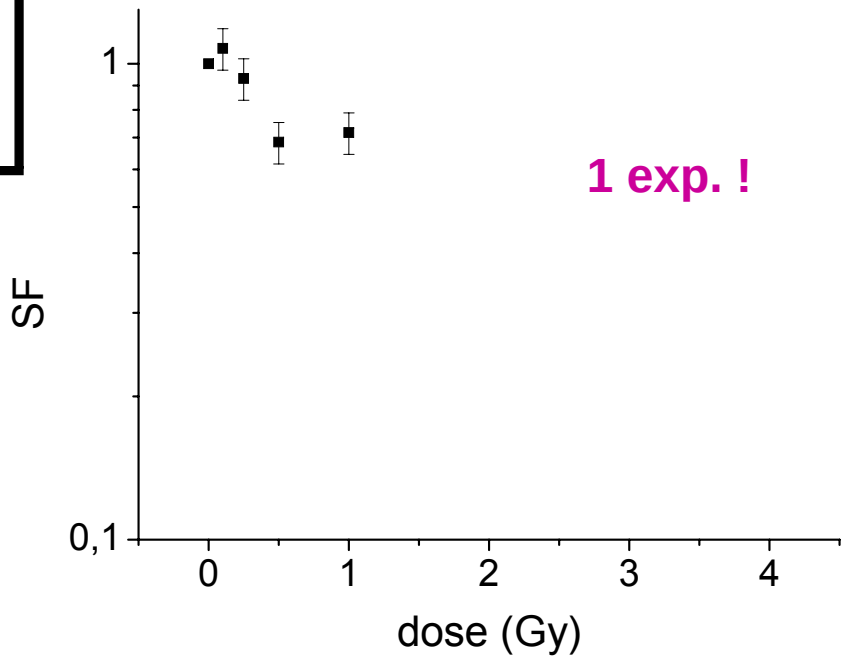
Irraggiamento del 100% della popolazione cellulare

Beam-spot
Su gafcromico



Irraggiamento del 59% della popolazione cellulare

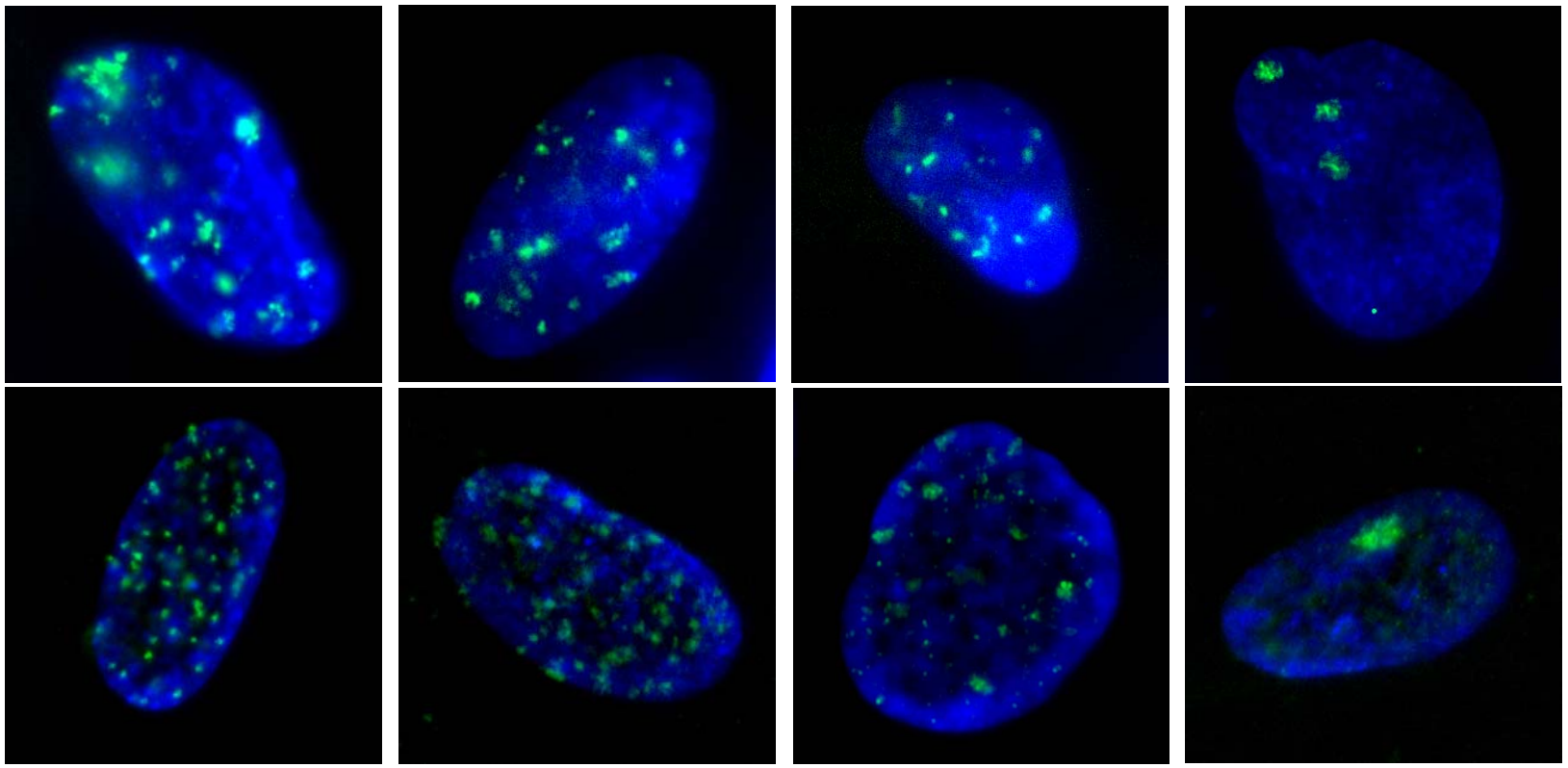
Beam-spot
Su gafcromico



1 exp. !

LNL

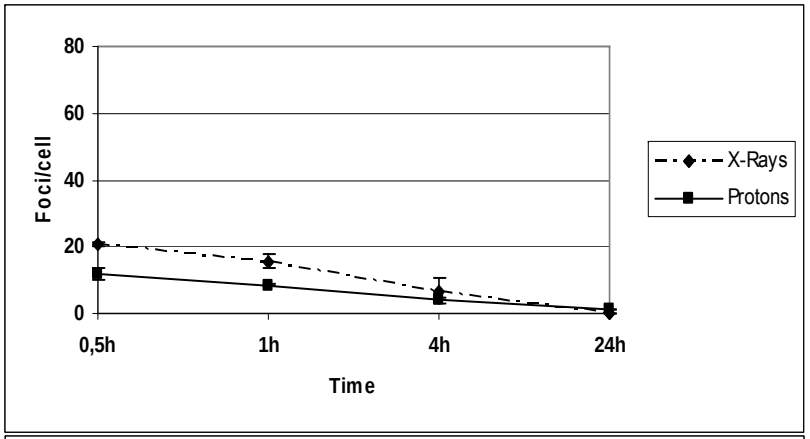
Misure di danno al DNA



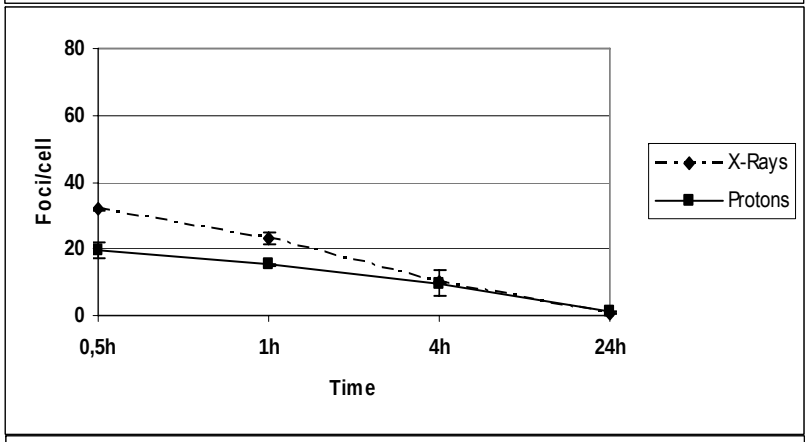
Induzione di DSBs e cinetica di ricongiungimento

Induzione di DSBs e cinetica di ricongiungimento

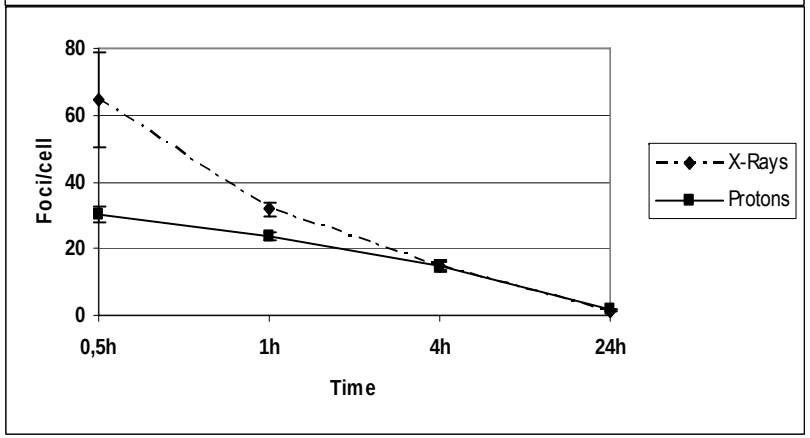
0,5 Gy



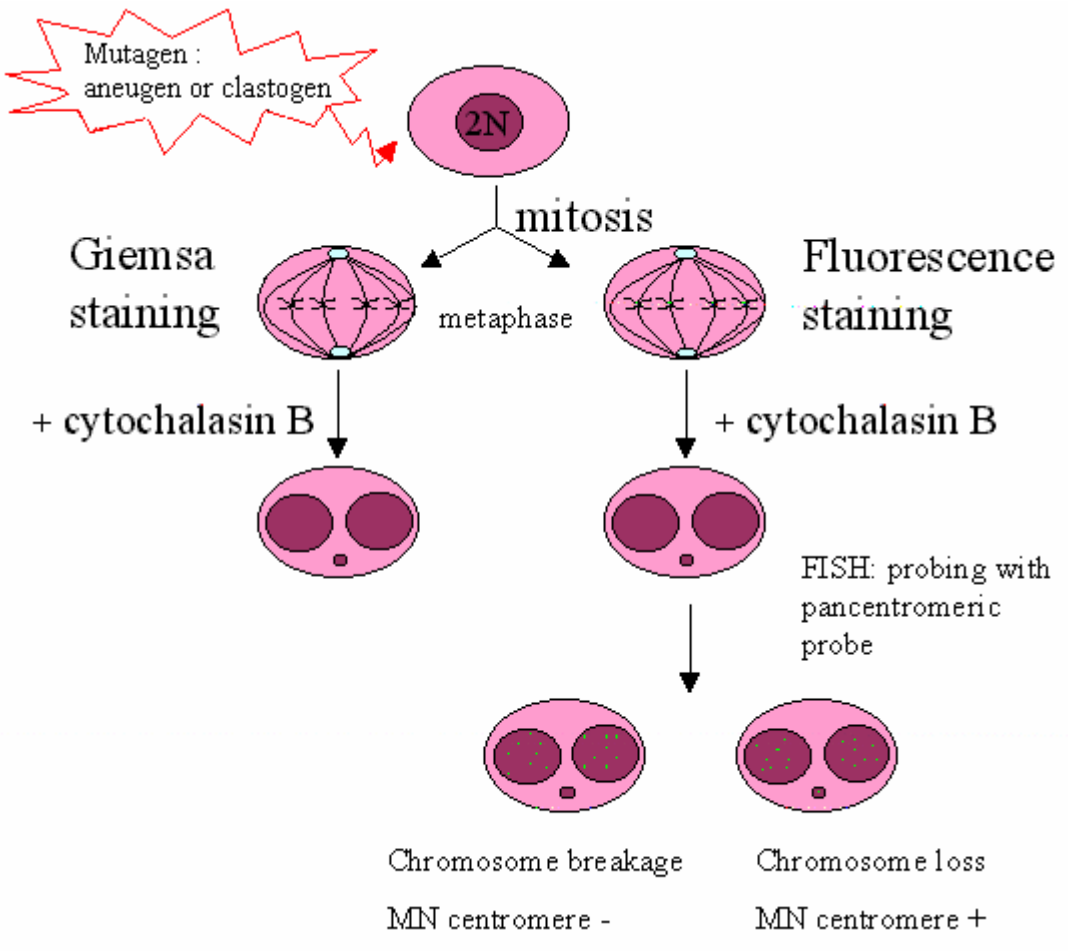
1 Gy

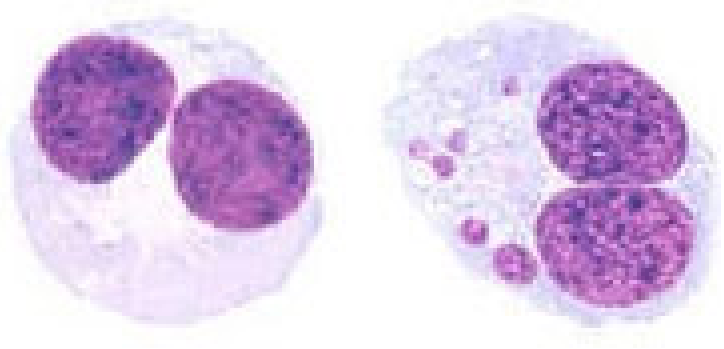


2 Gy



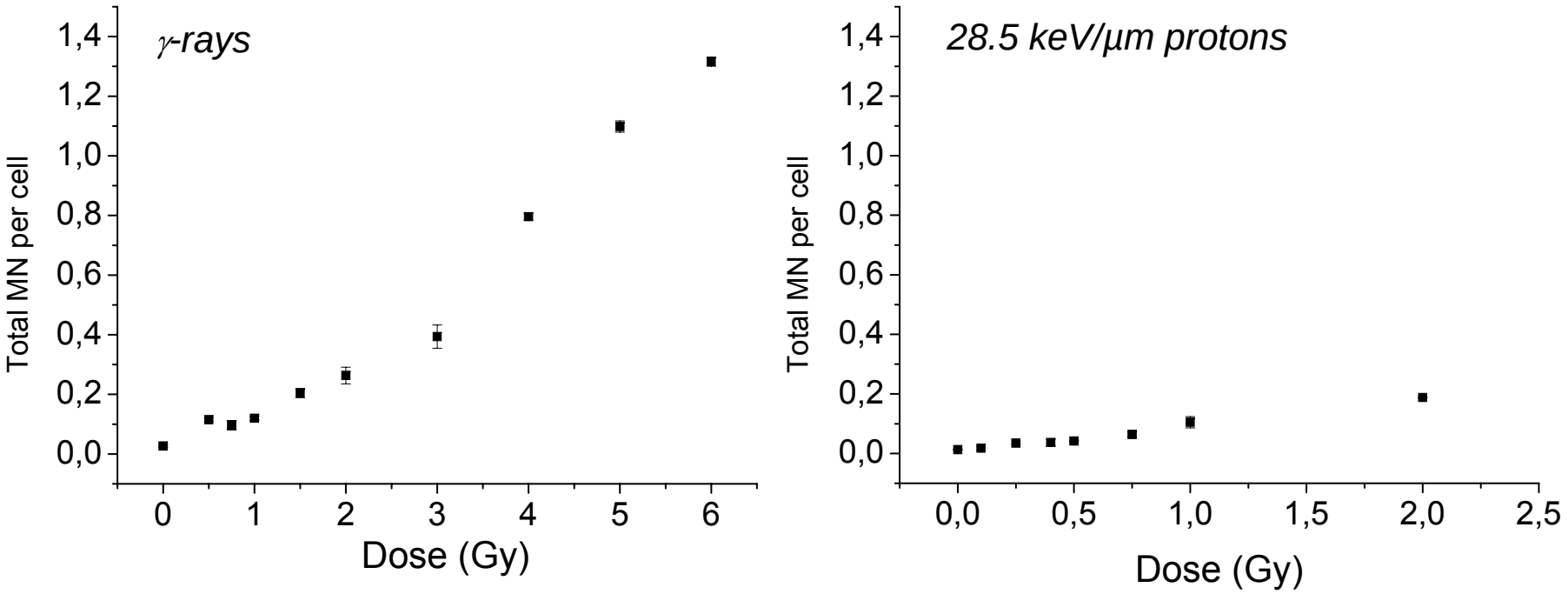
Misure di danno al DNA



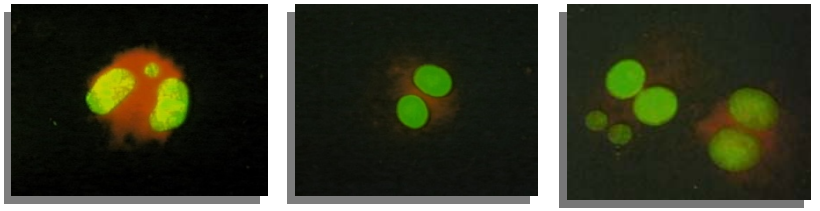


Micronuclei in cellule Binucleate

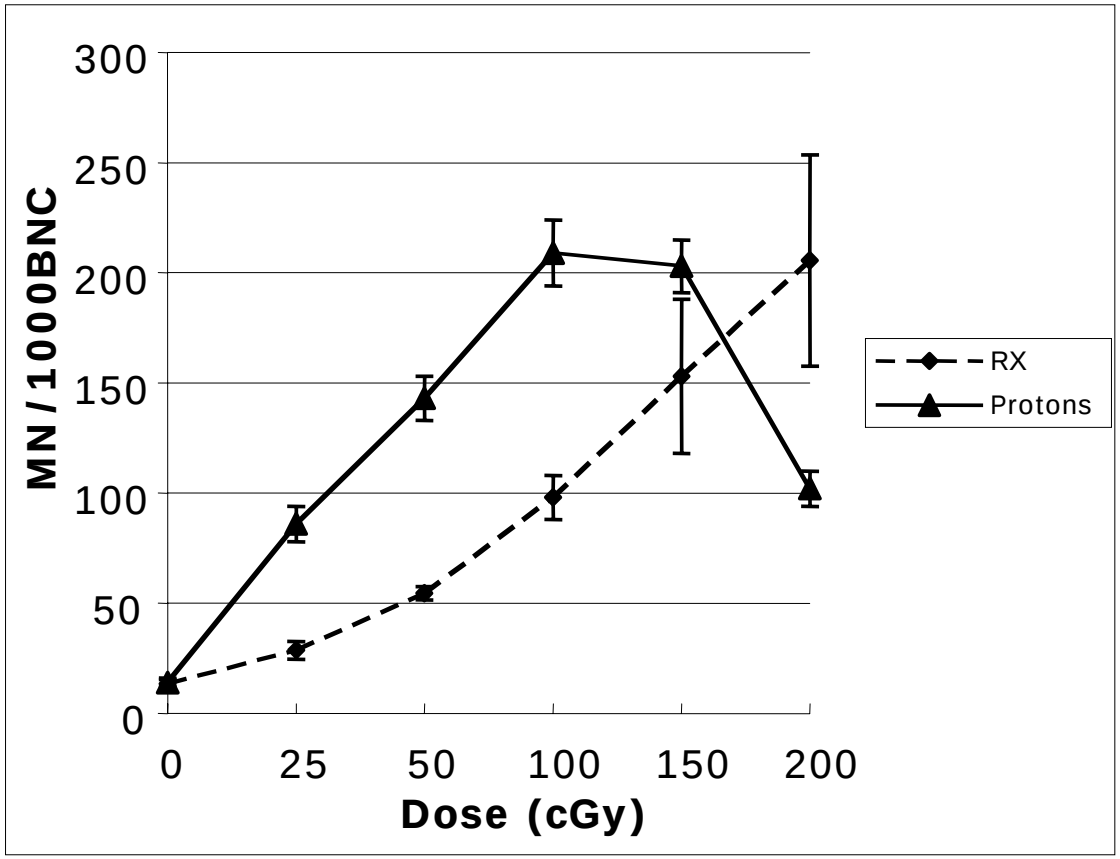
V79 cells – *Risultati preliminari*



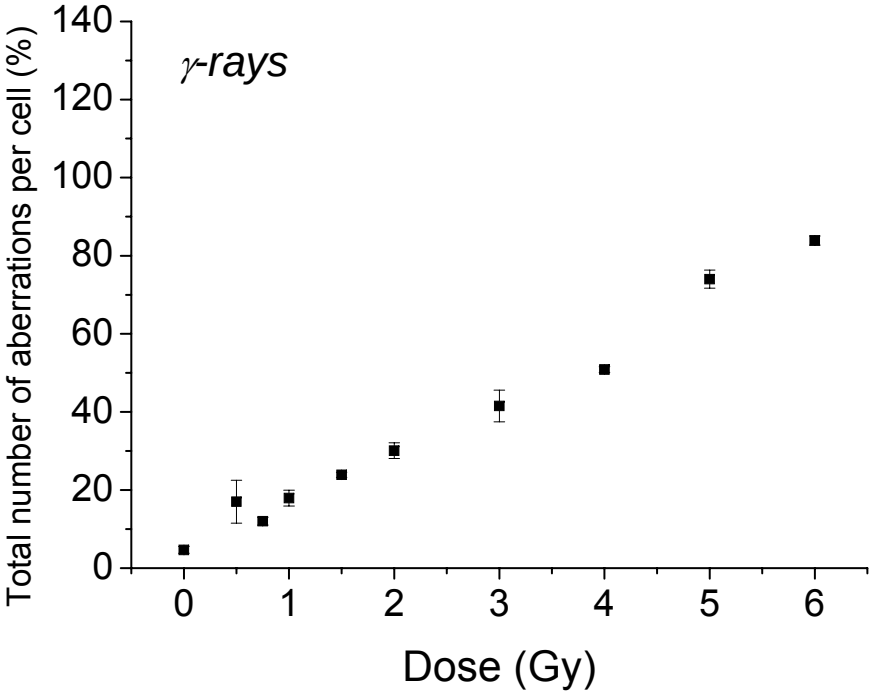
The frequency of MN per BN cell after exposure to different types of radiation

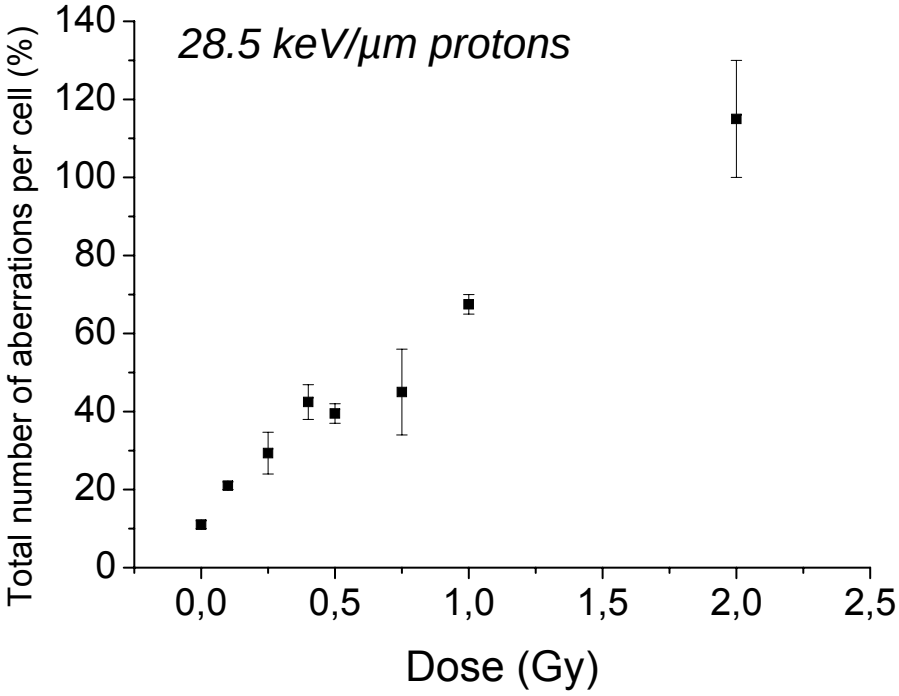


MN in fibroblasti primari umani HFFF2

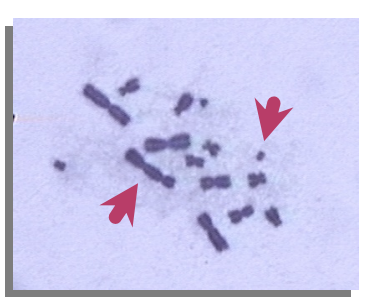


V79 cells – *Risultati preliminari*



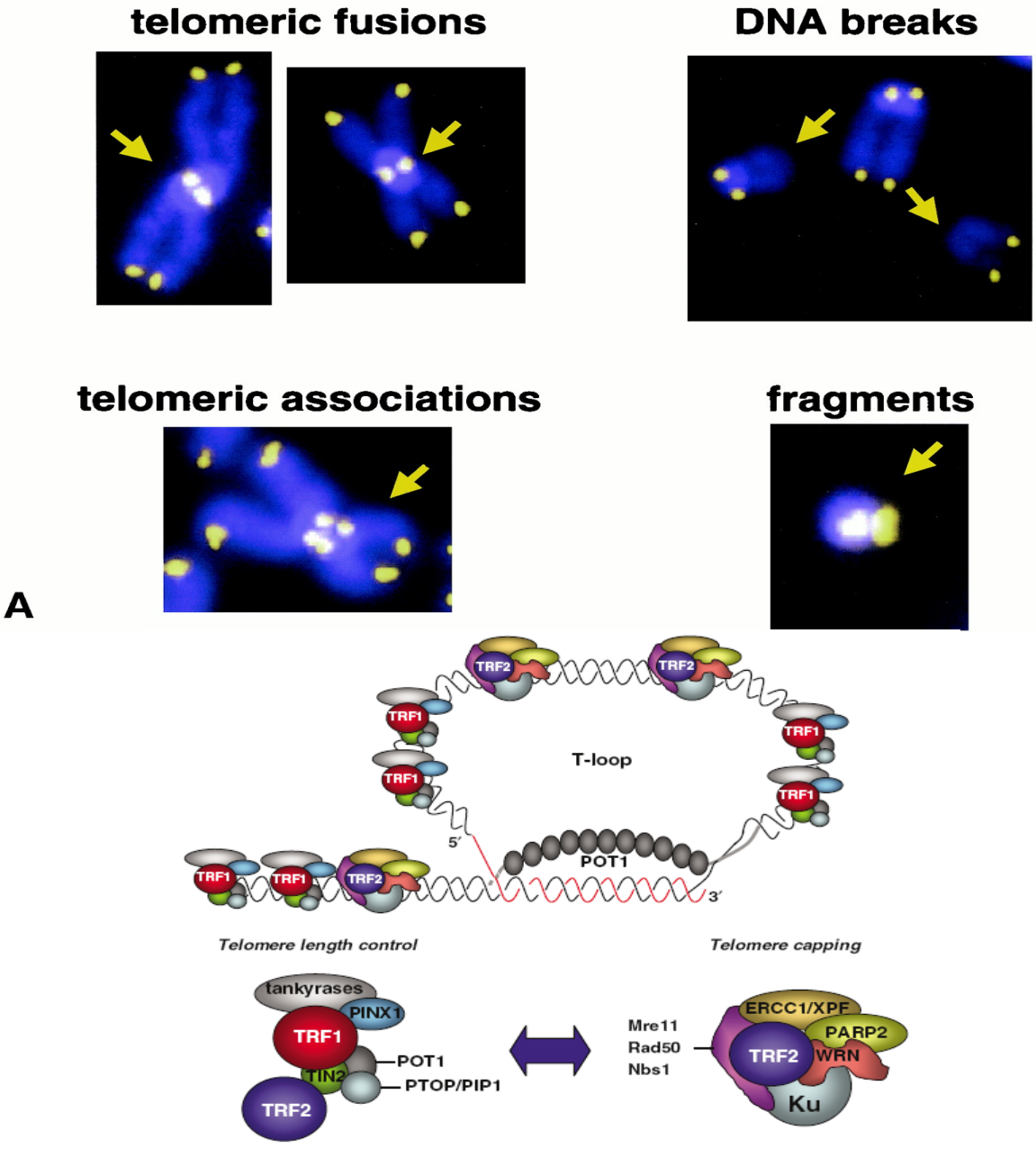


The total number of aberrations per cell after exposure to different types of radiation



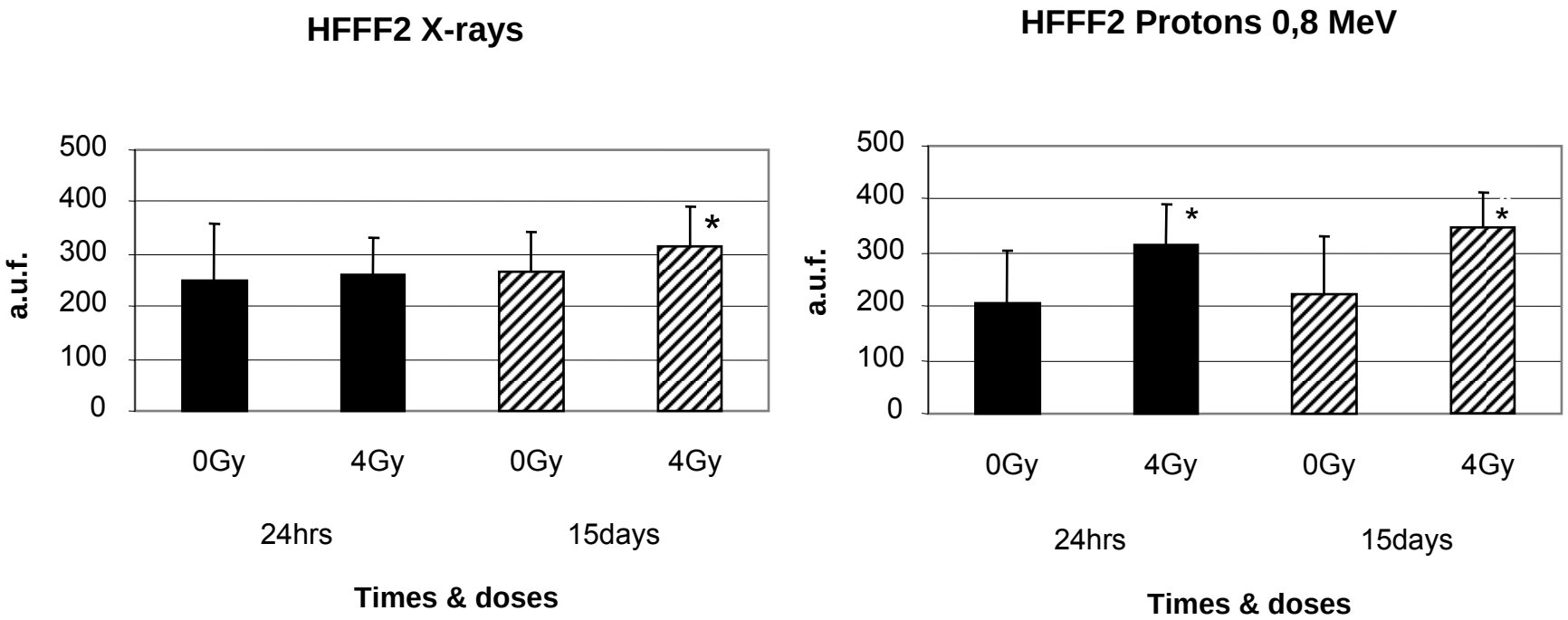
LNL

Anomalie del telomero sono responsabili della instabilità cromosomica



ROMA 3

Analisi Q-FISH (Quantitative FISH) in Nuclei

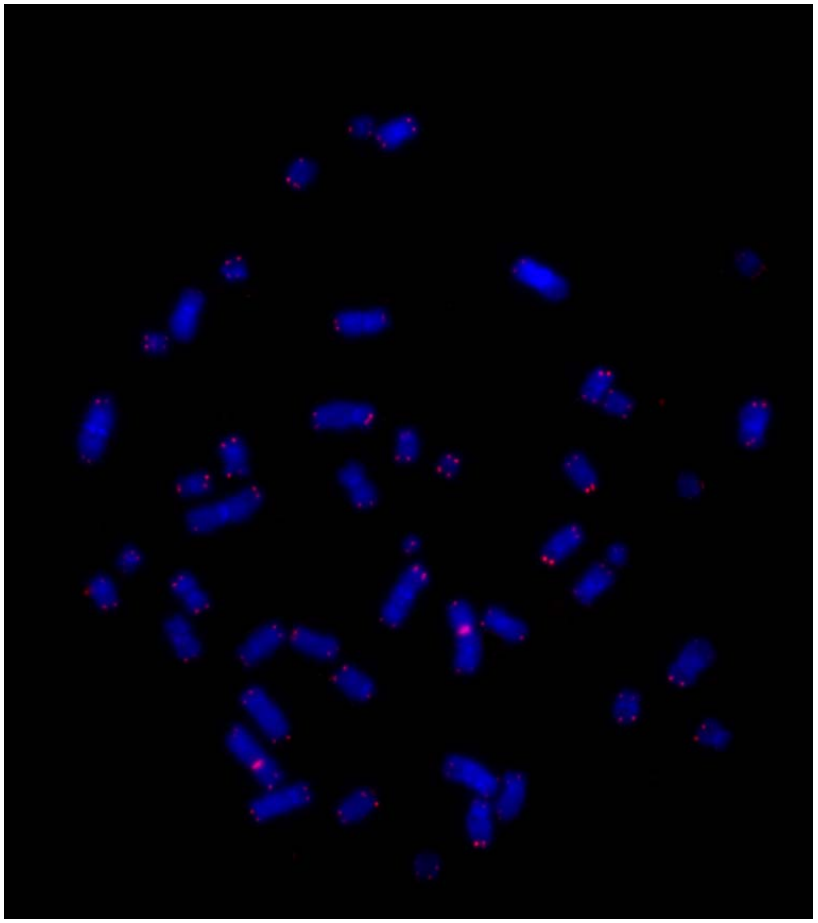


Sgura et al, Radiat. Prot. Dos. 122(2007)

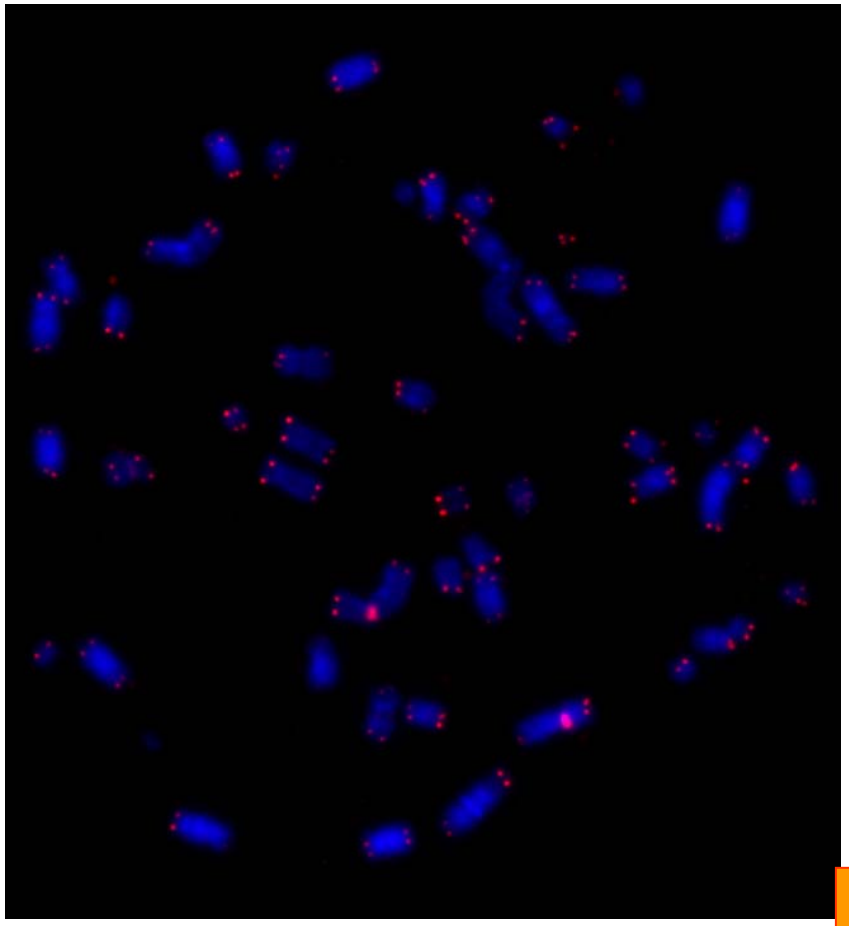
Analisi: 50 nuclei per punto sperimentale in 3 esperimenti indipendenti

Analisi Q-FISH in Metafase

4Gy X-rays



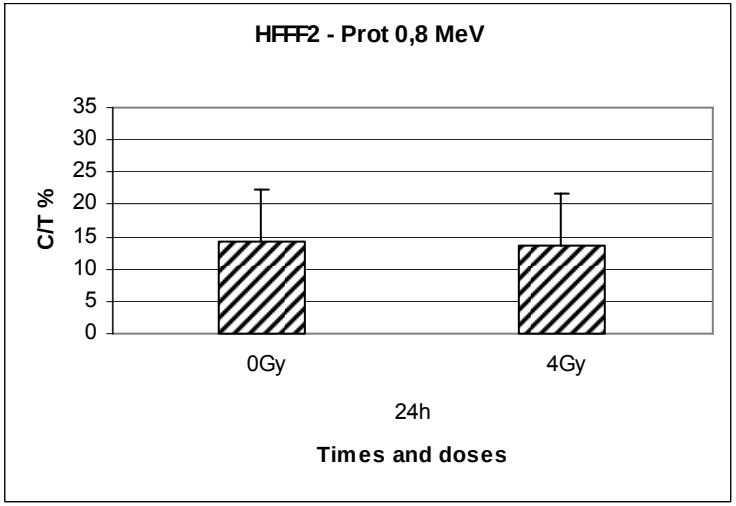
4Gy Protons

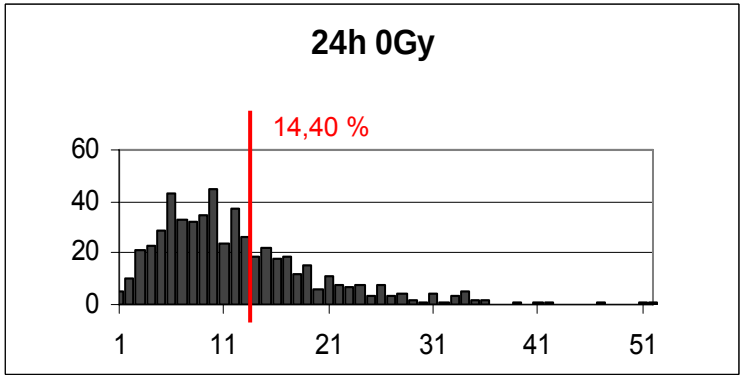


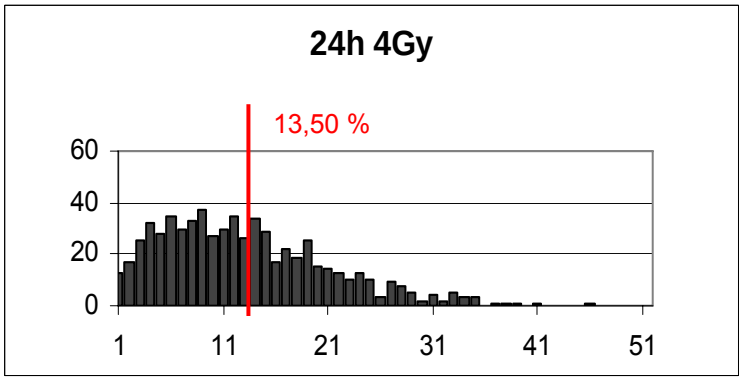
Analisi: 930 segnali telomerici per punto sperimentale

SHEILA

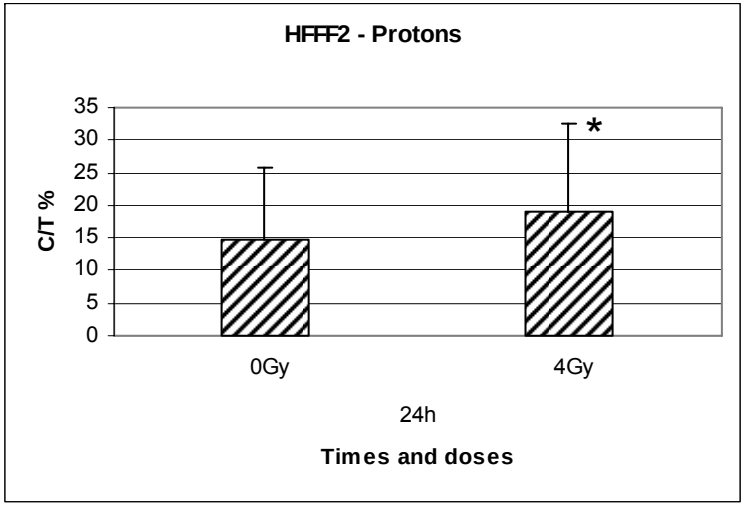
X-rays

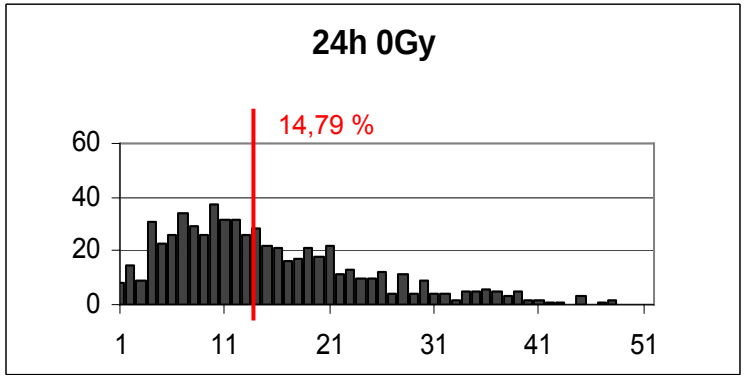


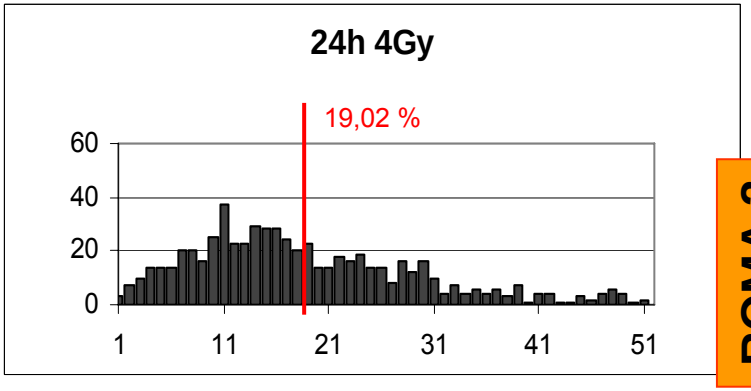




Protons





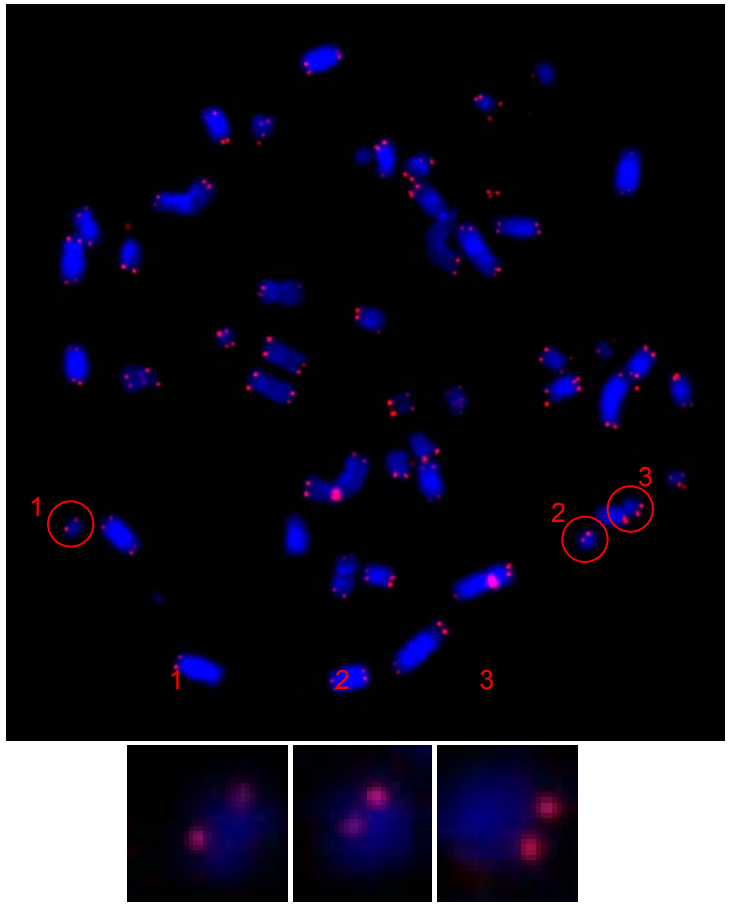


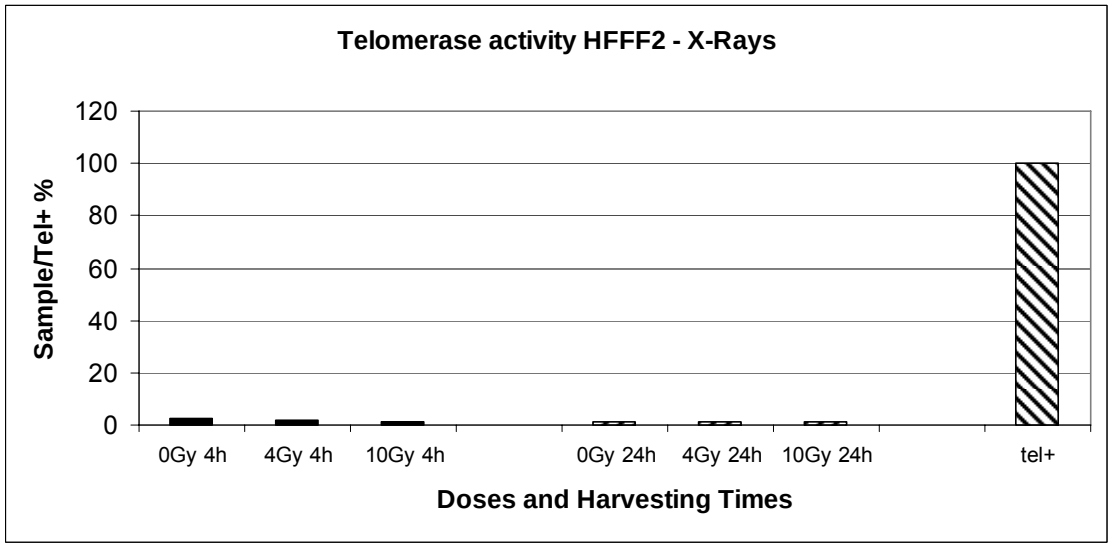
Mann-Whitney Test

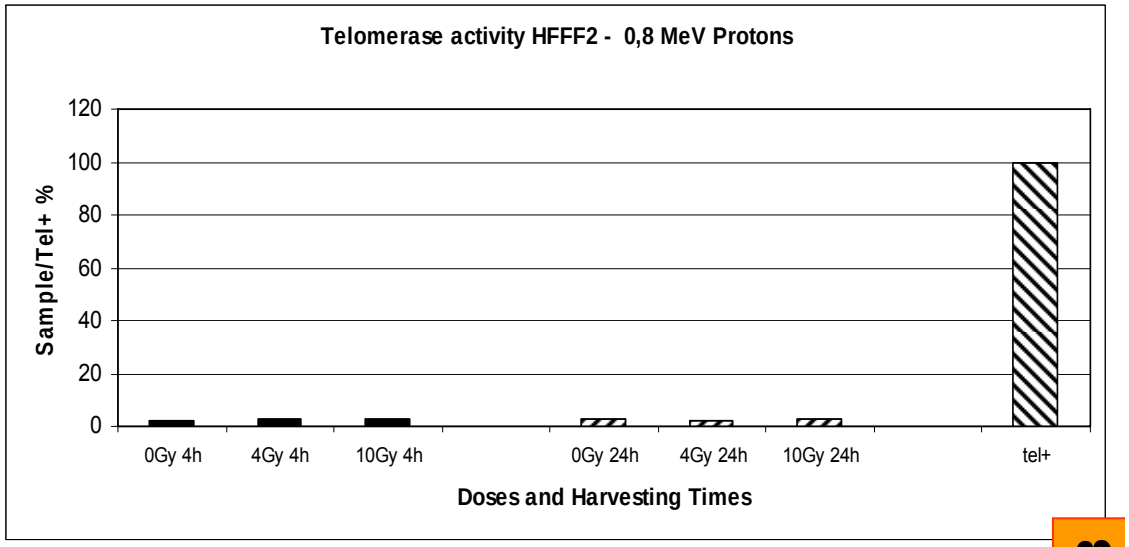
*** = P < 0,0001**

ROMA 3

Attività telomerasica

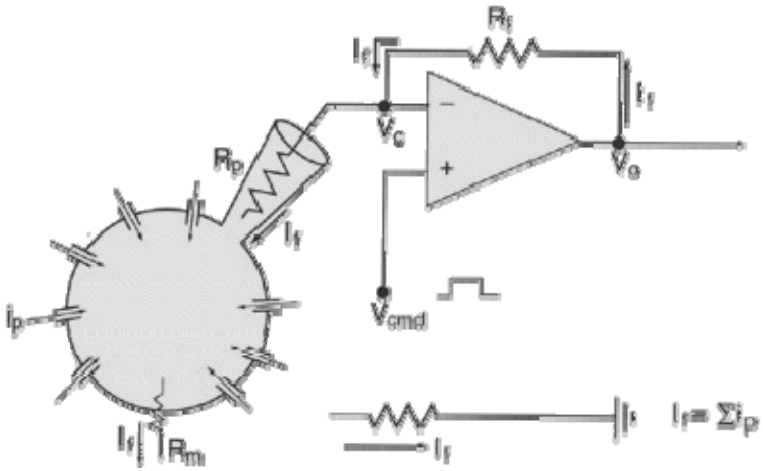


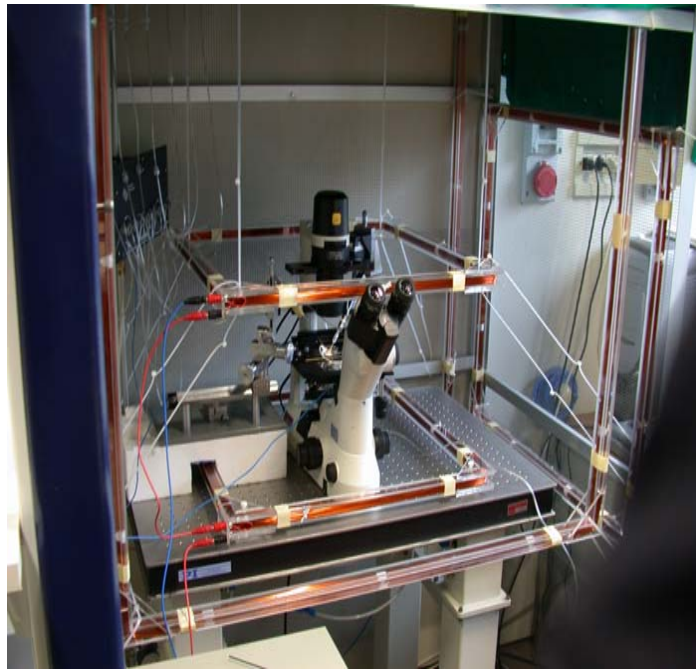


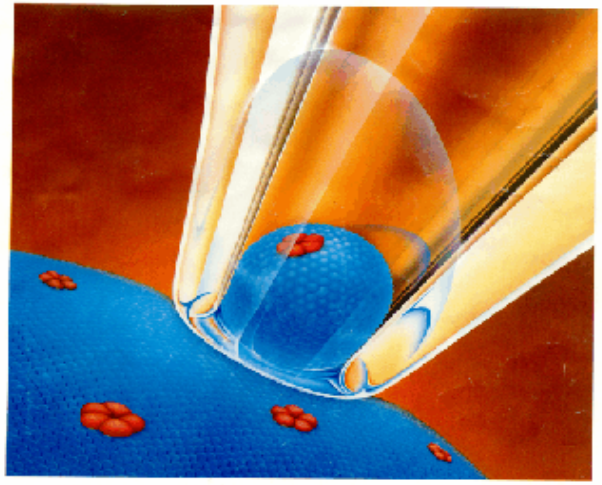


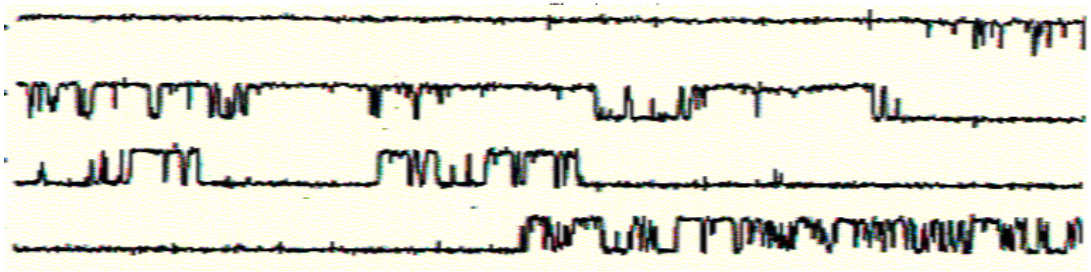
TECNICA DEL PATCH CLAMP

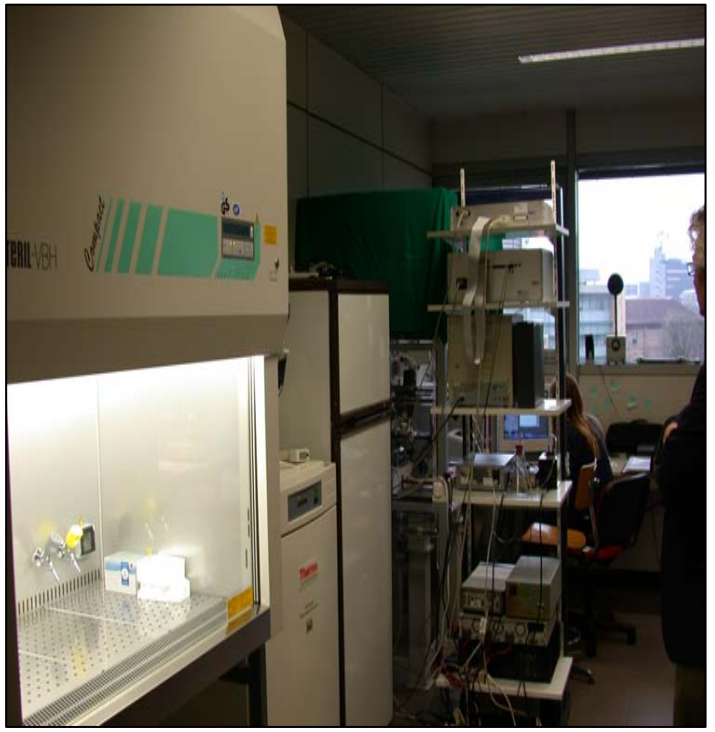
- E’ una tecnica biofisica molto raffinata per lo studio dell’attività dei canali ionici.
- Il canale ionico è una macromolecola con un poro (diametro dell’ordine dei nm) che consente la permeazione selettiva di ioni (Ca, Na, K, Cl, etc)
- I flussi ionici sono presenti in tutte le cellule, mediano funzioni fondamentali quali l’omeostasi e l’eccitabilità elettrica (comunicazione neuronale e contrazione muscolare)
- “Patch Clamp“ significa imporre ad un tratto di membrana (patch) un voltaggio definito (*voltage clamp*) e misurarne il flusso di corrente





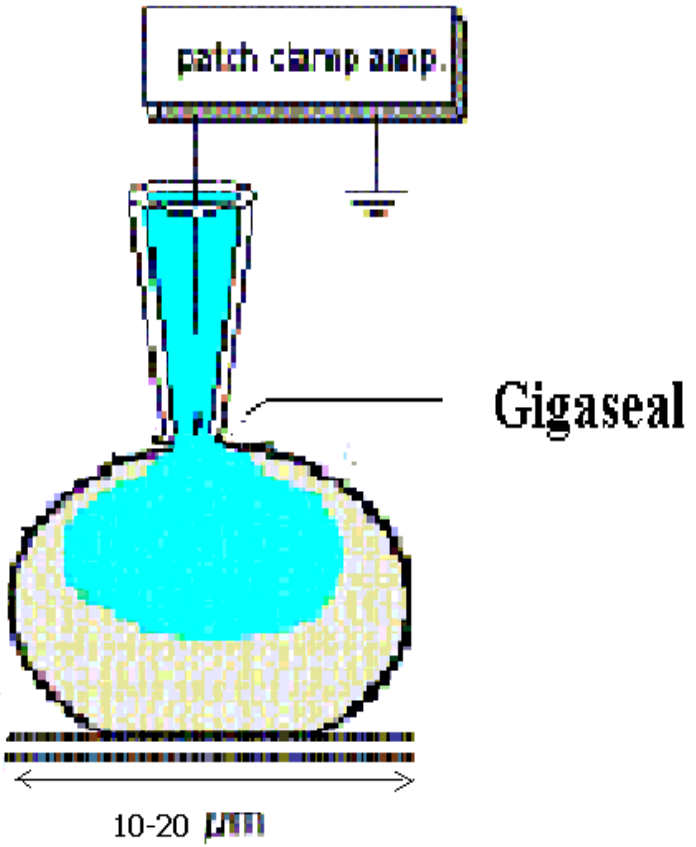


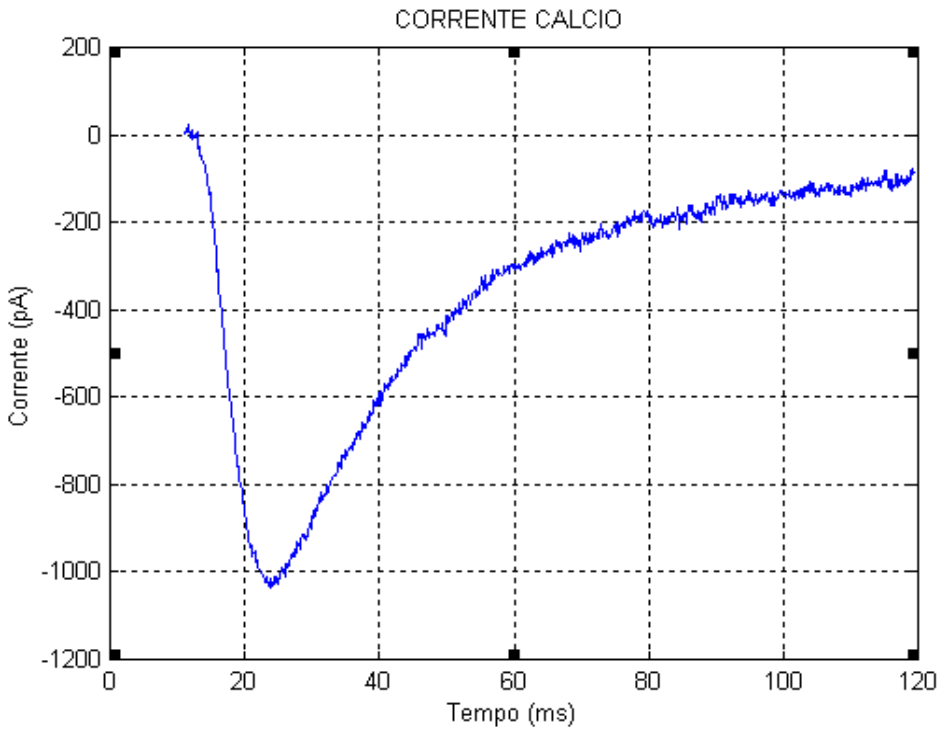




BOLOGNA

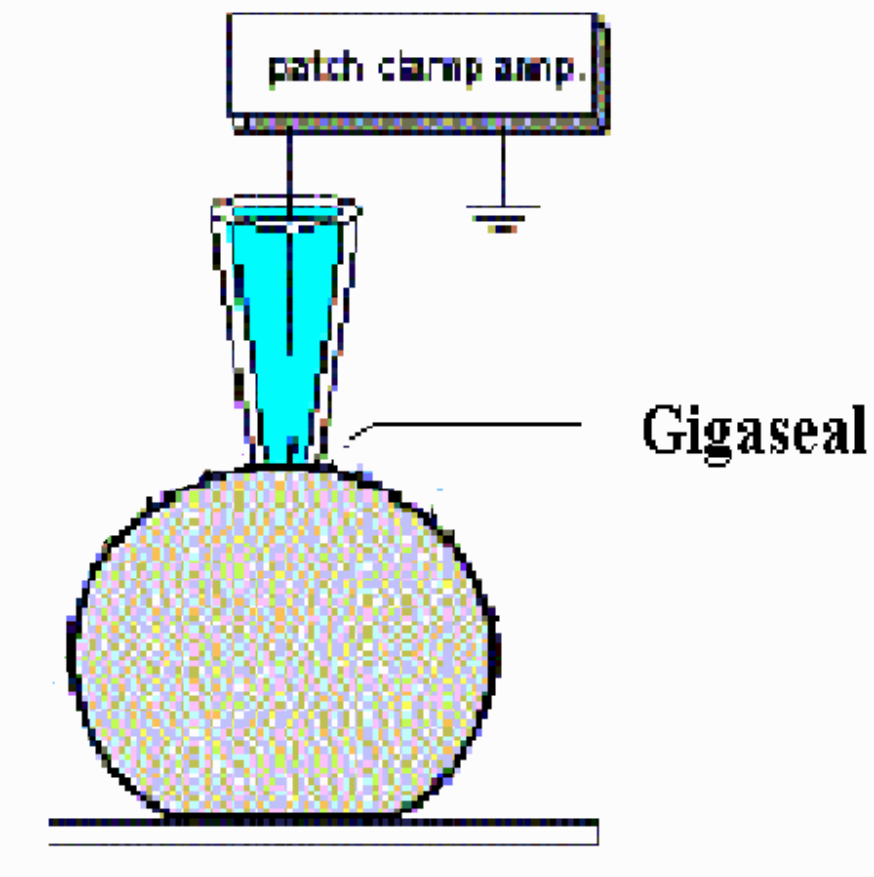
Con la tecnica del Patch Clamp si può misurare la corrente risultante da tutta la superficie cellulare - *whole-cell*

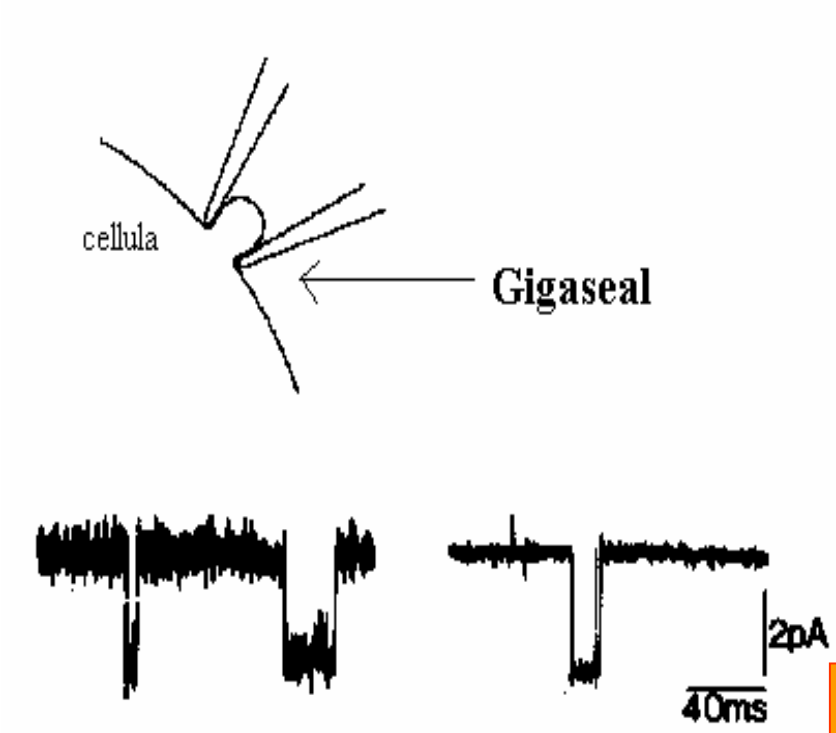




BOLOGNA

Con la stessa tecnica si può misurare la corrente passante attraverso un singolo canale ionico – *single channel*



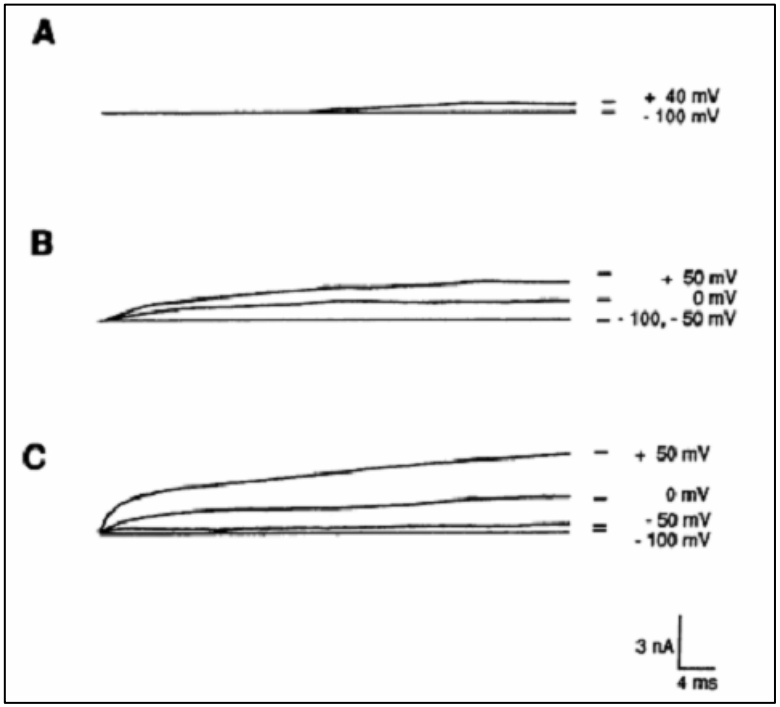


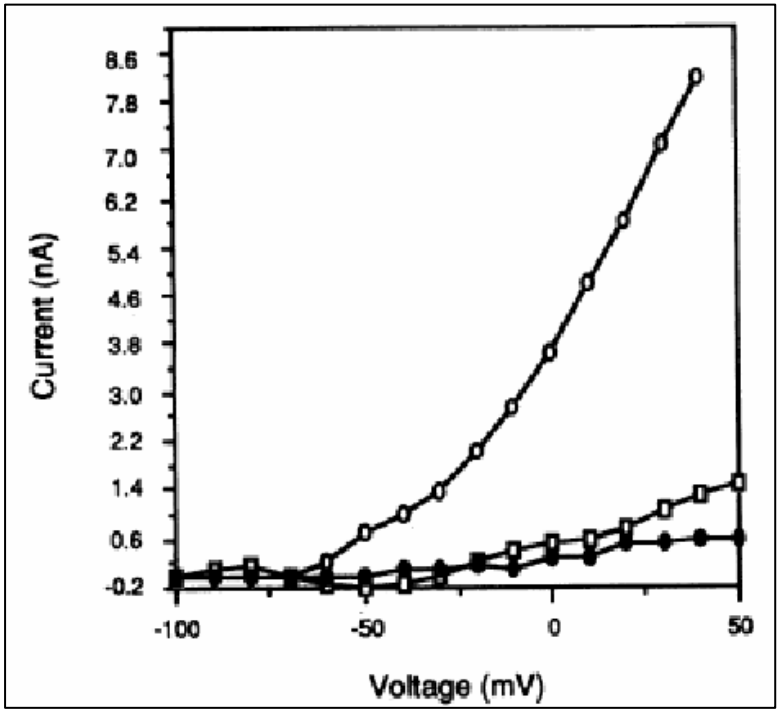
Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 908–912, February 1993
Cell Biology

Potassium-channel activation in response to low doses of γ-irradiation involves reactive oxygen intermediates in nonexcitatory cells

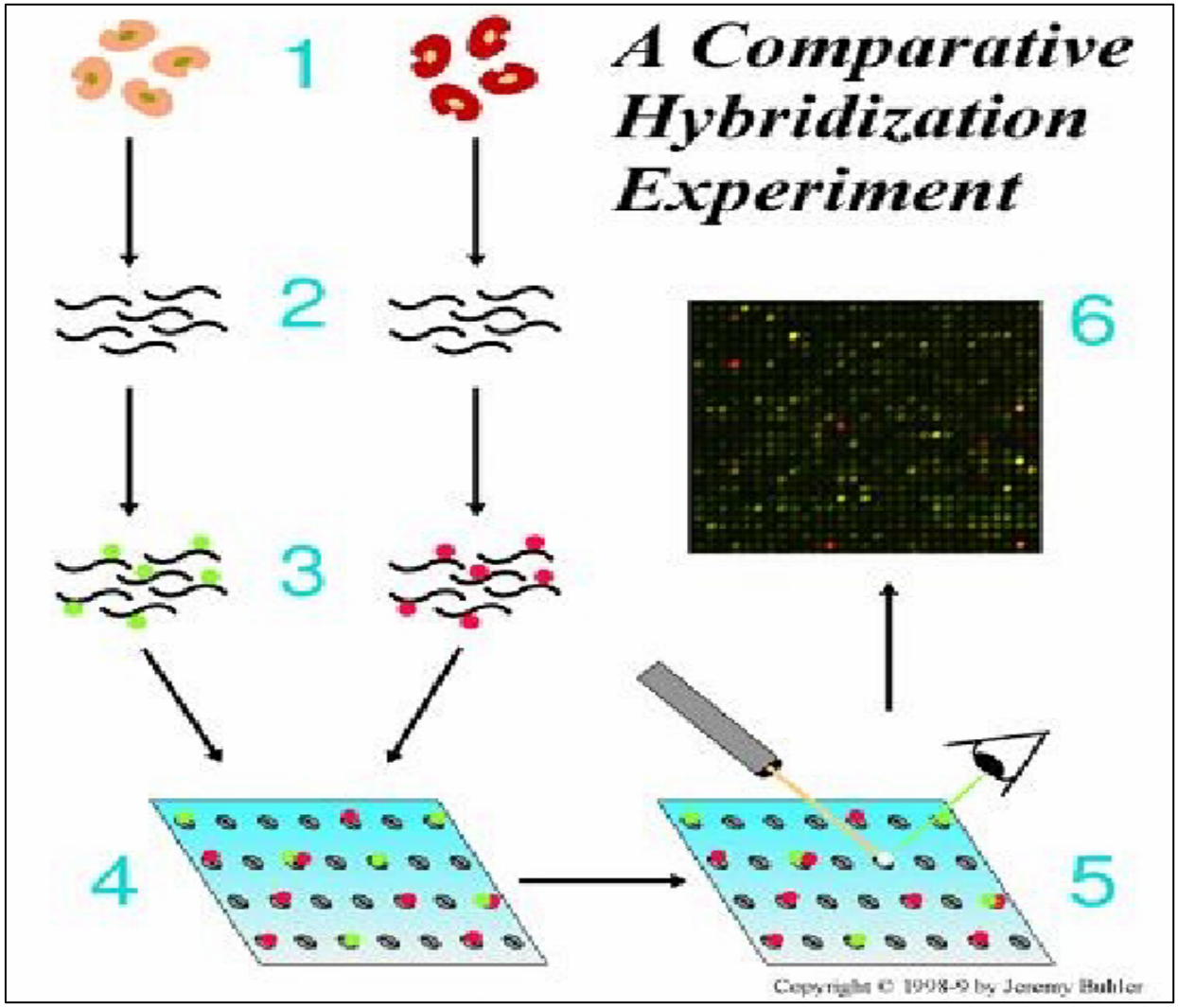
(ionic channels/reactive oxygen intermediates/second messengers/free radical scavengers)

SOPHIA S. KUO, ALIE H. SAAD, ALBERT C. KOONG, GEORGE M. HAHN, AND AMATO J. GIACCIA*





cDNA Microarrays



Significance analysis of microarrays applied to the ionizing radiation response

Virginia Goss Tusher*, Robert Tibshirani†, and Gilbert Chu*‡

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Communicated by Bradley Efron, Stanford University, Stanford, CA, February 6, 2001 (received for review December 1, 2000)

Microarrays can measure the expression of thousands of genes to identify changes in expression between different biological states. Methods are needed to determine the significance of these changes while accounting for the enormous number of genes. We describe a method, Significance Analysis of Microarrays (SAM), that assigns a score to each gene on the basis of change in gene expression relative to the standard deviation of repeated measurements. For genes with scores greater than an adjustable threshold, SAM uses permutations of the repeated measurements to estimate the percentage of genes identified by chance, the false discovery rate (FDR). When the transcriptional response of human cells to ionizing radiation was measured by microarrays, SAM identified 34 genes that changed at least 1.5-fold with an estimated FDR of 12%, compared with FDRs of 60 and 84% by using conventional methods of analysis. Of the 34 genes, 19 were involved in cell cycle regulation and 3 in apoptosis. Surprisingly, four nucleotide excision repair genes were induced, suggesting that this repair pathway for UV-damaged DNA might play a previously unrecognized role in repairing DNA damaged by ionizing radiation.

IR & microarrays

Esperimento:

- confronto tra 3 campioni di controllo (sham) e 3 trattati con irraggiamento.
- analisi di geni e pathway biologici coinvolti

Future direzioni:

- espressione genica dose-dipendente
- dinamica della risposta genica (time series design)

SHEILA

PNAS

Targeting c-Myc-activated genes with a correlation method: Detection of global changes in large gene expression network dynamics

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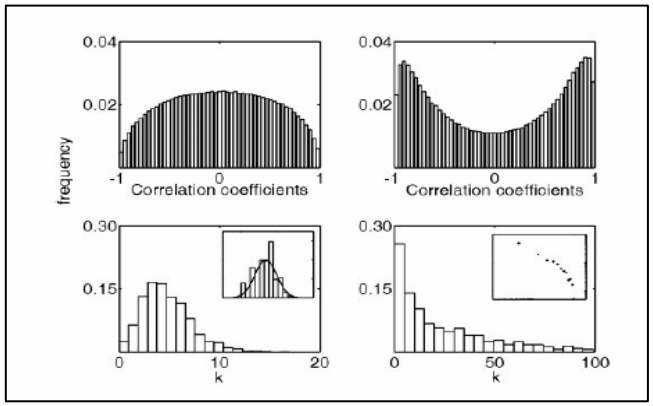
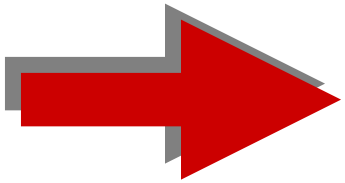
Contributed by L. N. Cooper, March 14, 2005





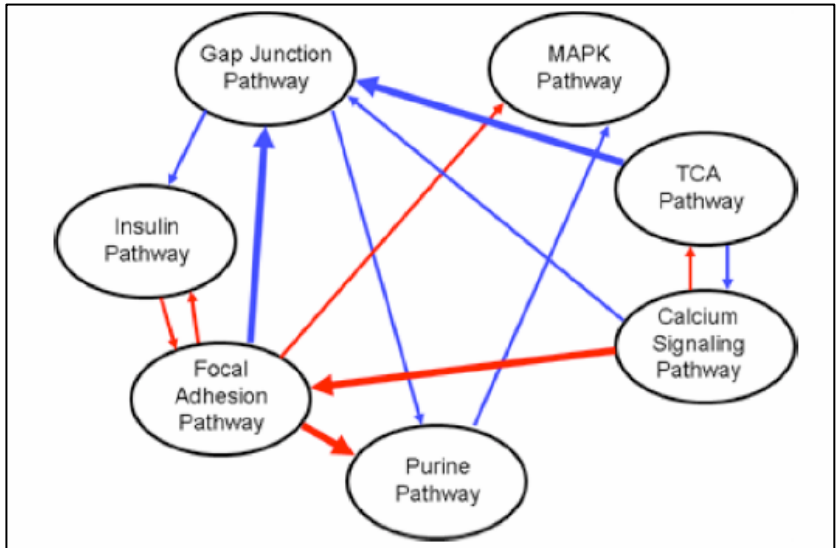
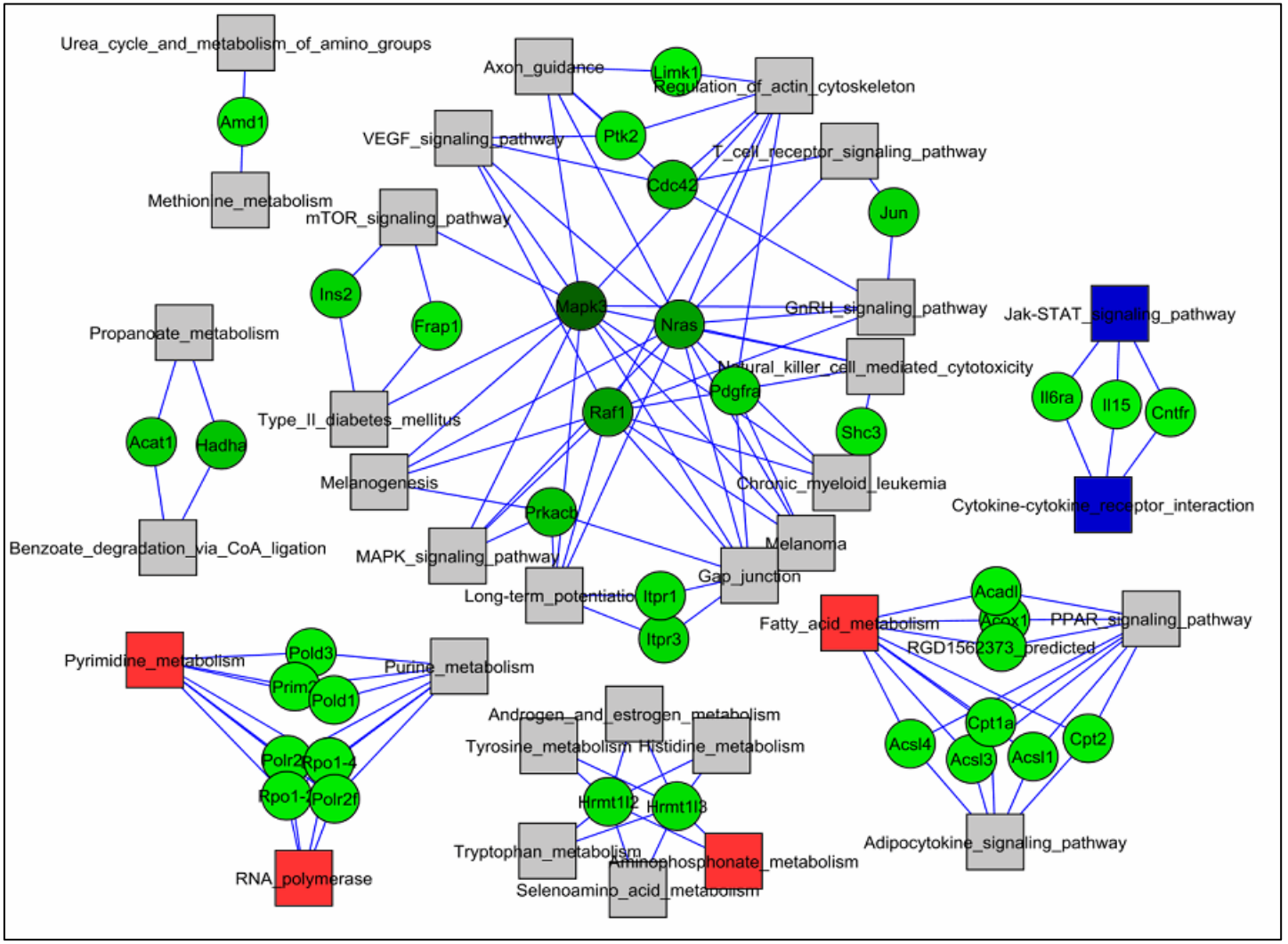


Fig. 3. Network of selected Myc-influenced pathways showing positive and negative correlations. The red and blue arrows denote positive and negative co-regulation, respectively. The thickness of the arrows is proportional to the magnitude, or absolute value, of the co-regulation. A network with these properties is called a weighted directed graph.

BOLOGNA

Geni & Pathways



..Grazie dell'attenzione..

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