Non-Targeted Effects of Ionizing Radiation

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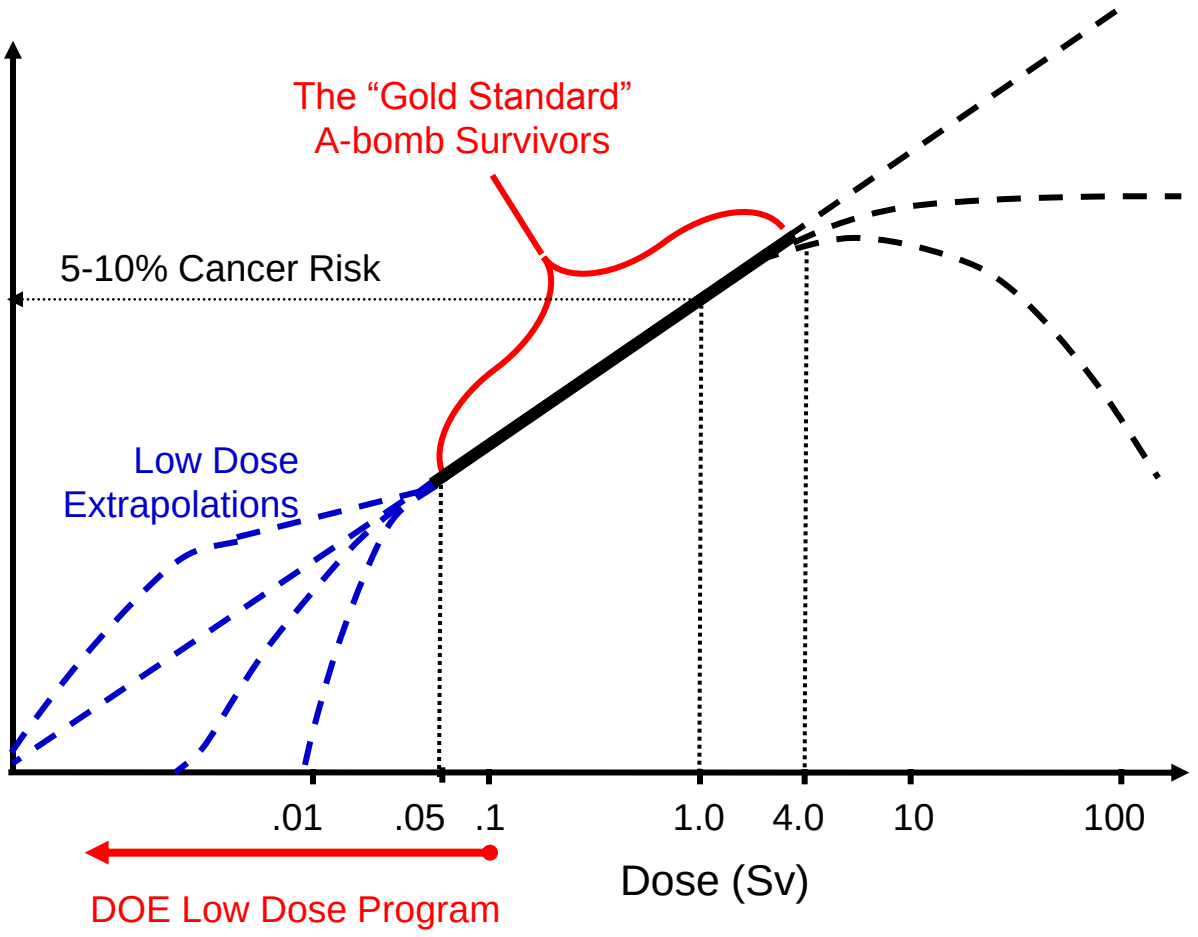
The dilemma for radiation protection: what is the scientific basis for radiation standards to protect the public from exposures to low levels of ionizing radiation (<0.1 Sv) where there are considerable uncertainties in the epidemiological data.

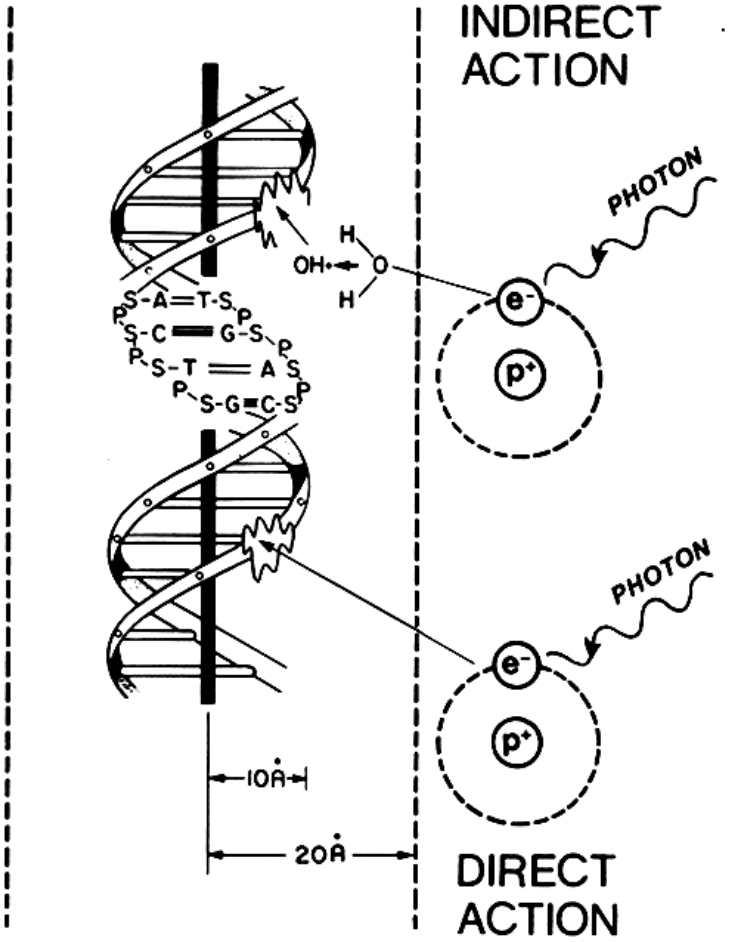


Implicit in evaluating radiation effects is that the nucleus is the target, and that the deposition of energy induces the effect.

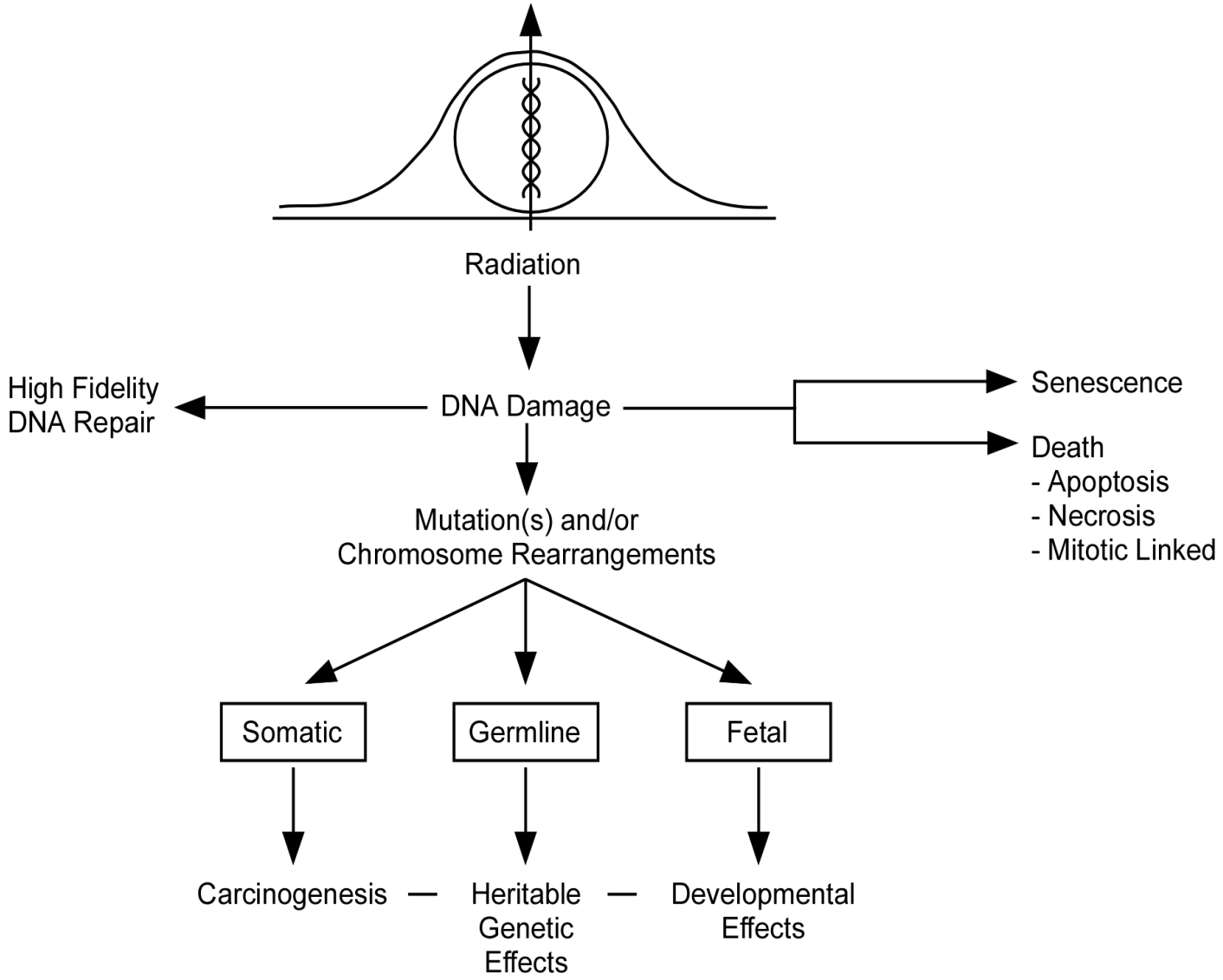
DNA damage is the result of direct and indirect effects of radiation

Damage / Gy of X-rays:

- 40 DSBs
- 150 DNA crosslinks
- 1,000 SSB
- 2,500 base damages



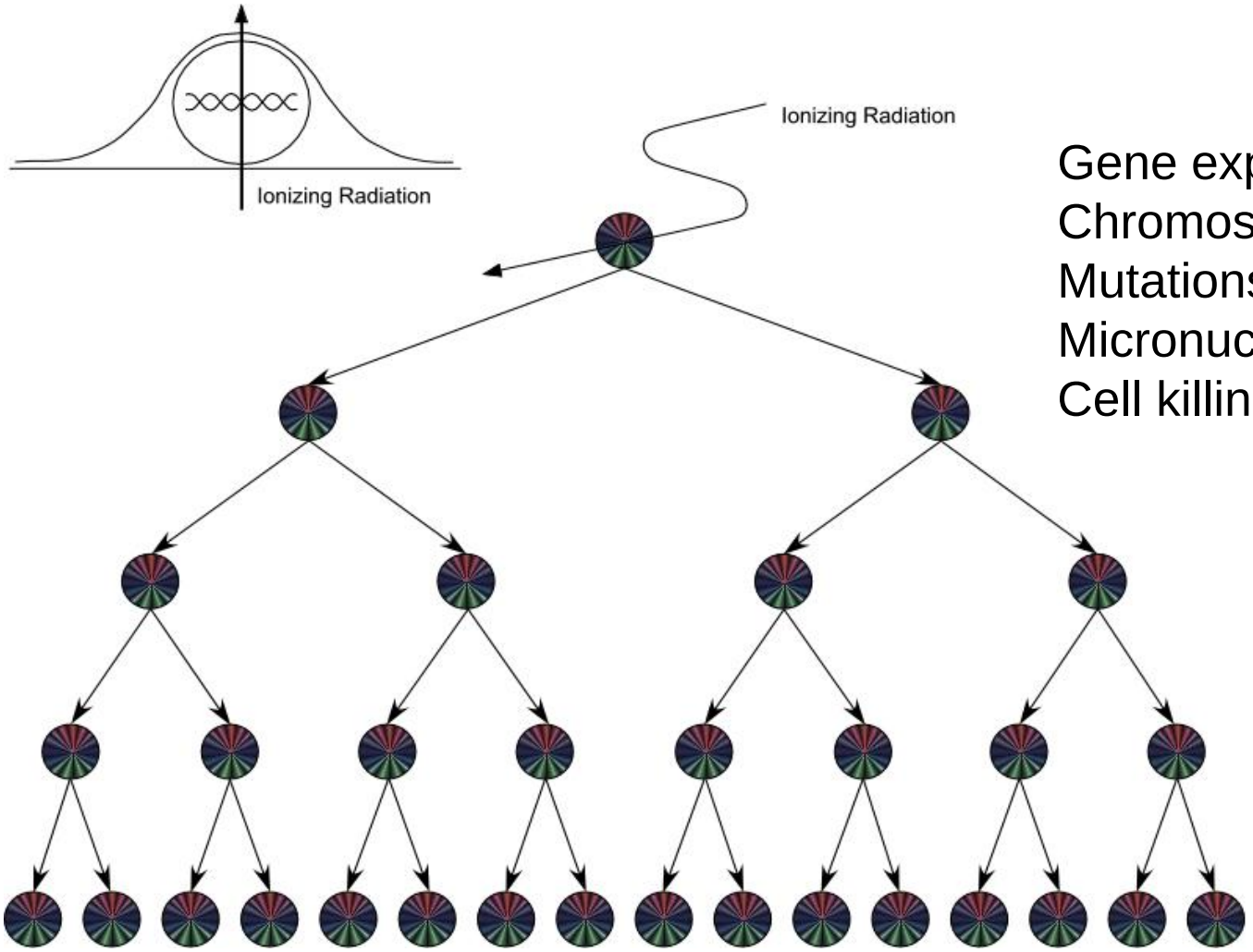
From: Hall, “Radiobiology for the Radiologist”



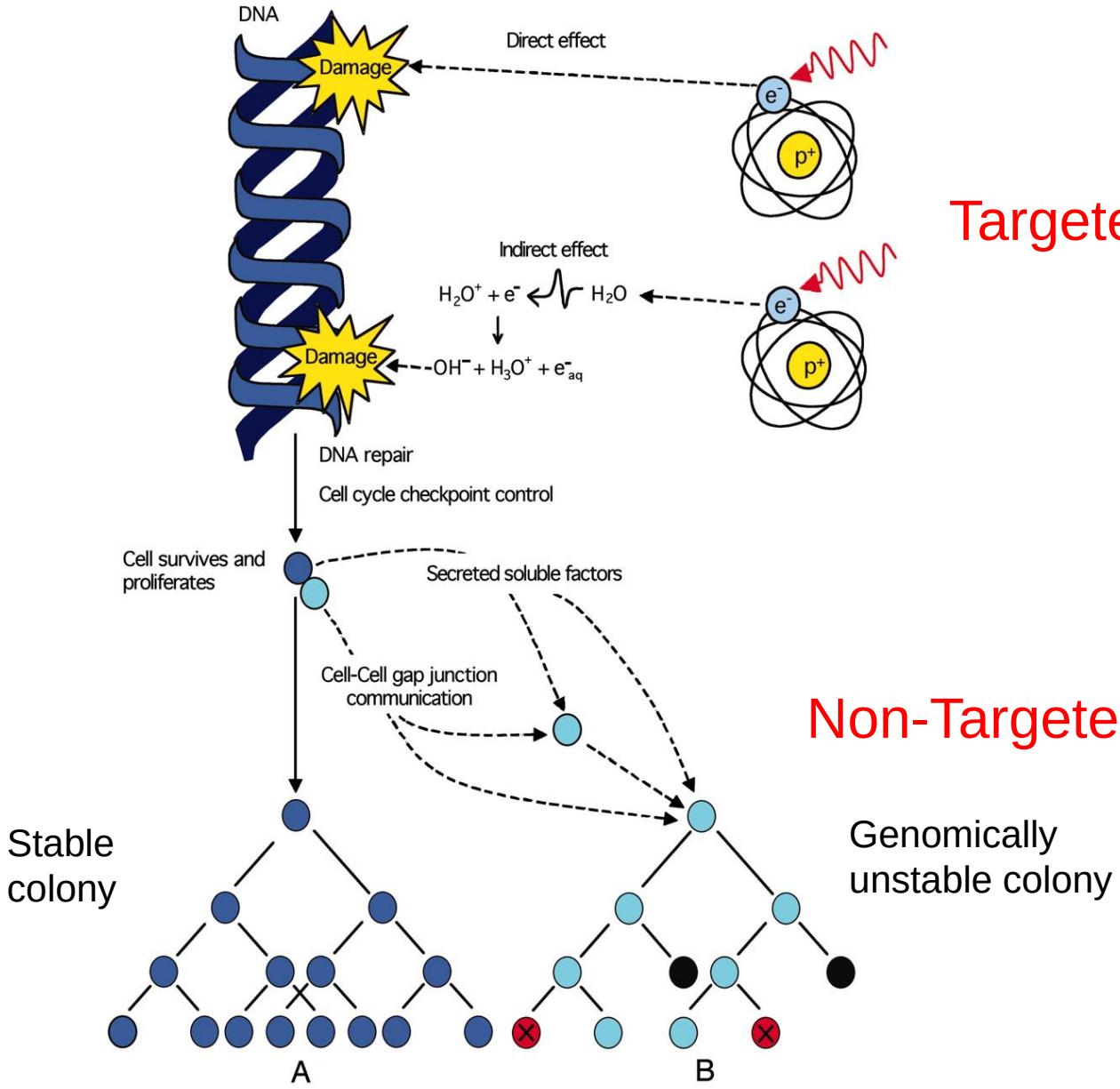
Any exposure has the potential for risk

Genetics
Environment
Diet

Conventional paradigm for radiation effects: Effects occur in “hit” (targeted) cells



Gene expression
Chromosomal damage
Mutations
Micronuclei
Cell killing



Targeted

Non-Targeted

Stable colony

Genomically unstable colony

Morgan & Sowa, PNAS 102, 14127-8 (2005)

In addition to these “targeted effects” the new biology reveals “non-targeted effects” of ionizing radiation

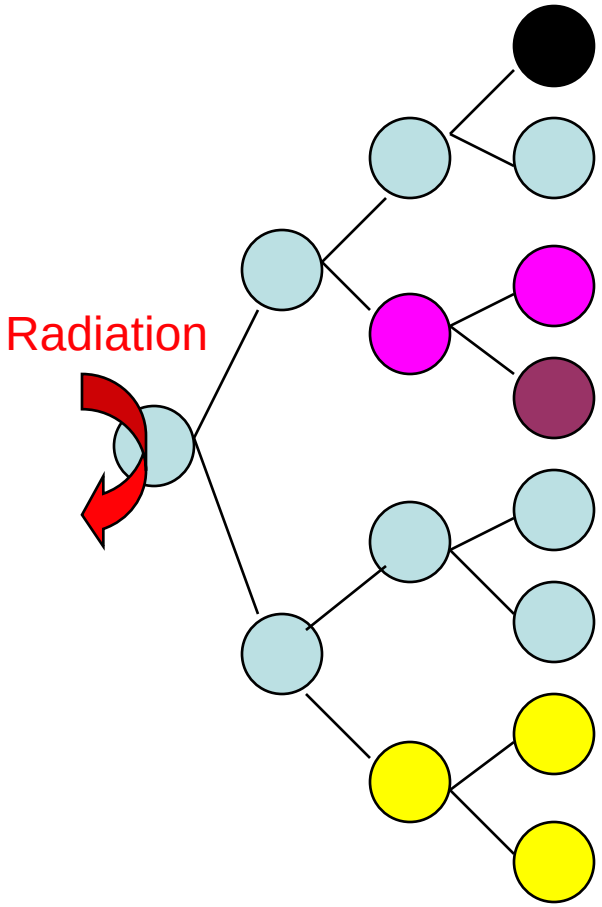
Induced genomic instability: observed in the progeny of an irradiated cell that may / may not have been subject to energy deposition events.

Bystander effects: occur in cells that were not traversed by radiation and are induced by signals from irradiated cells.

Implications for radiation protection?

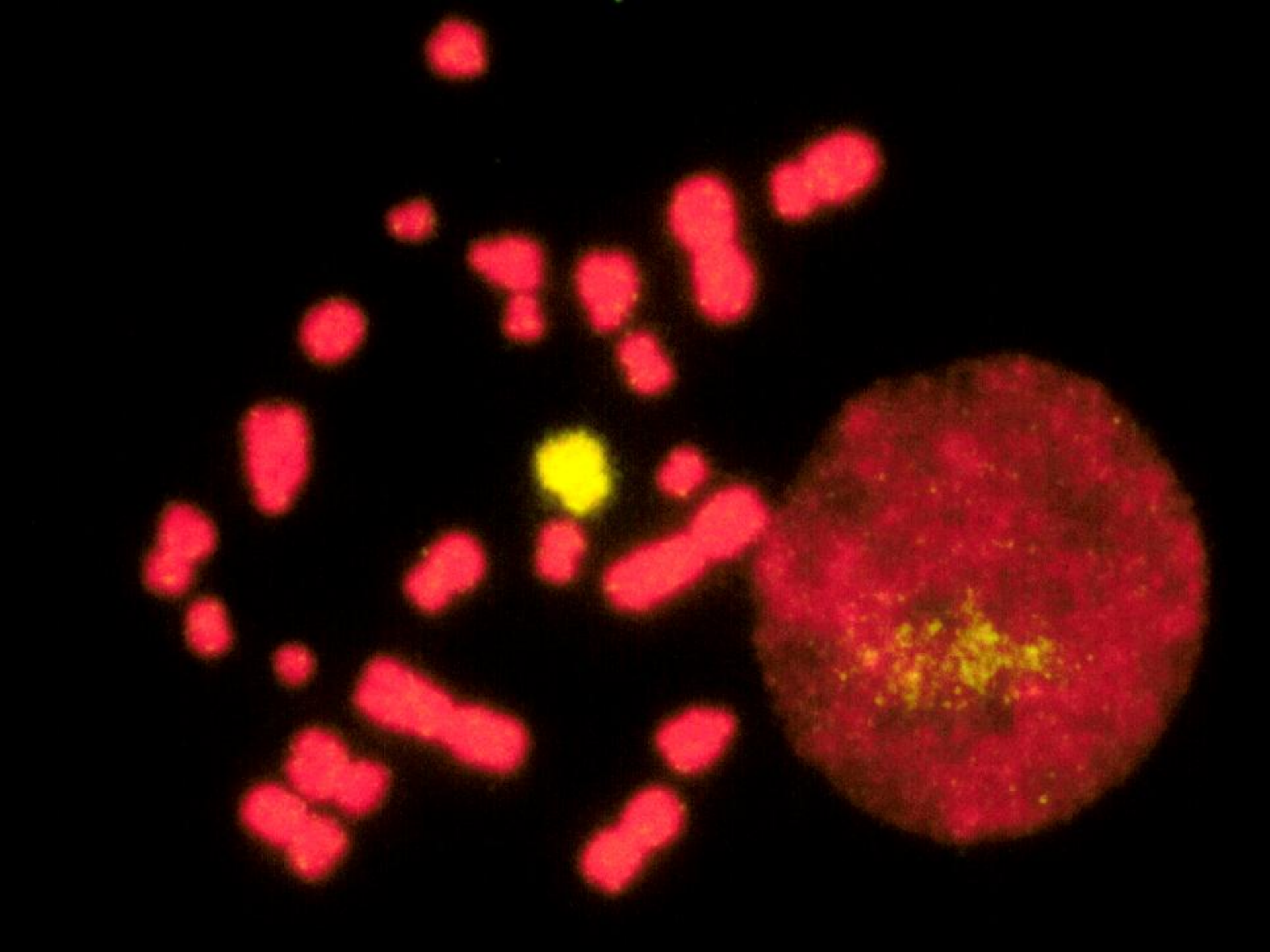
RADIATION-INDUCED GENOMIC INSTABILITY

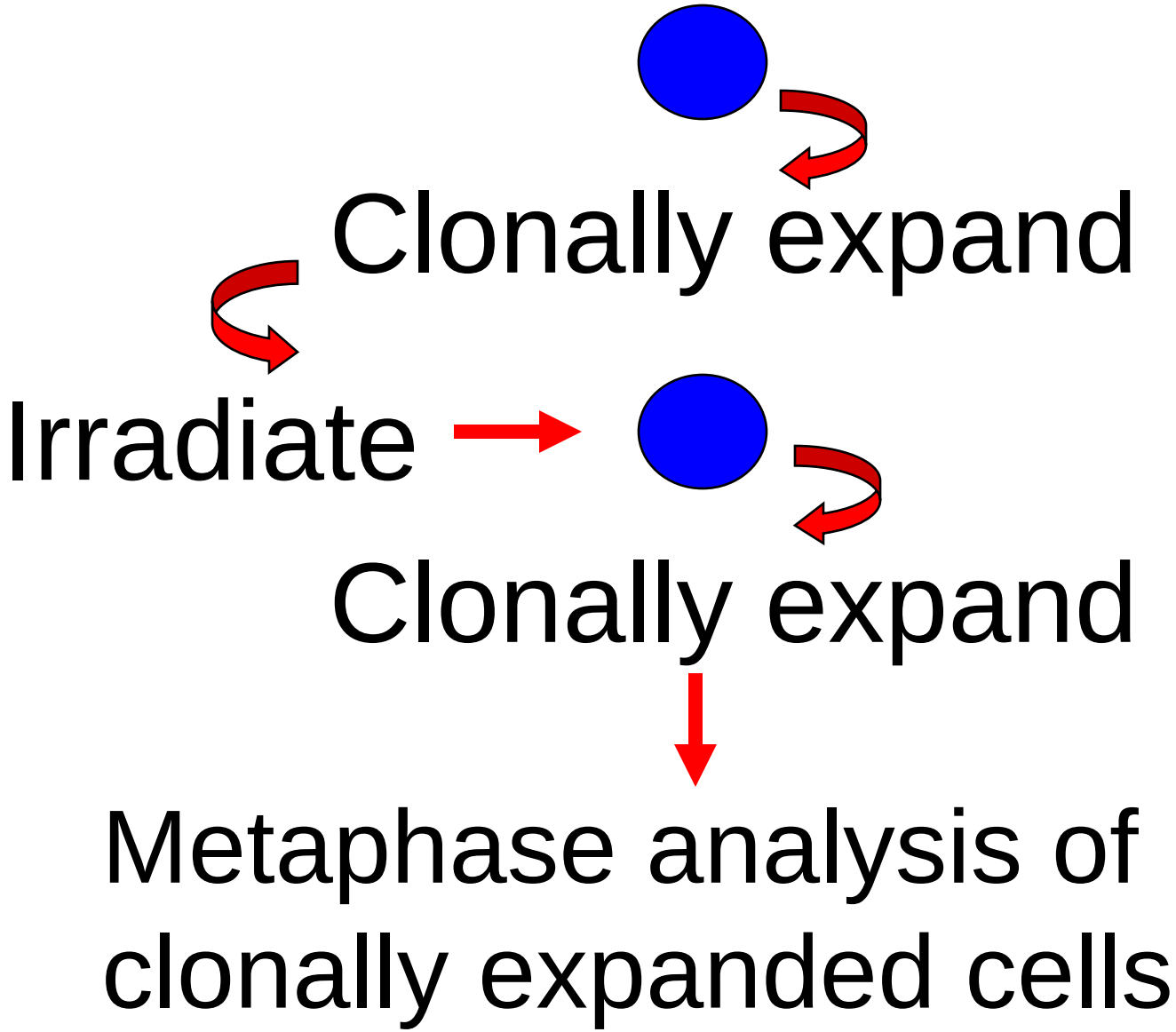
Increased rate of genomic alterations in the progeny of irradiated cells

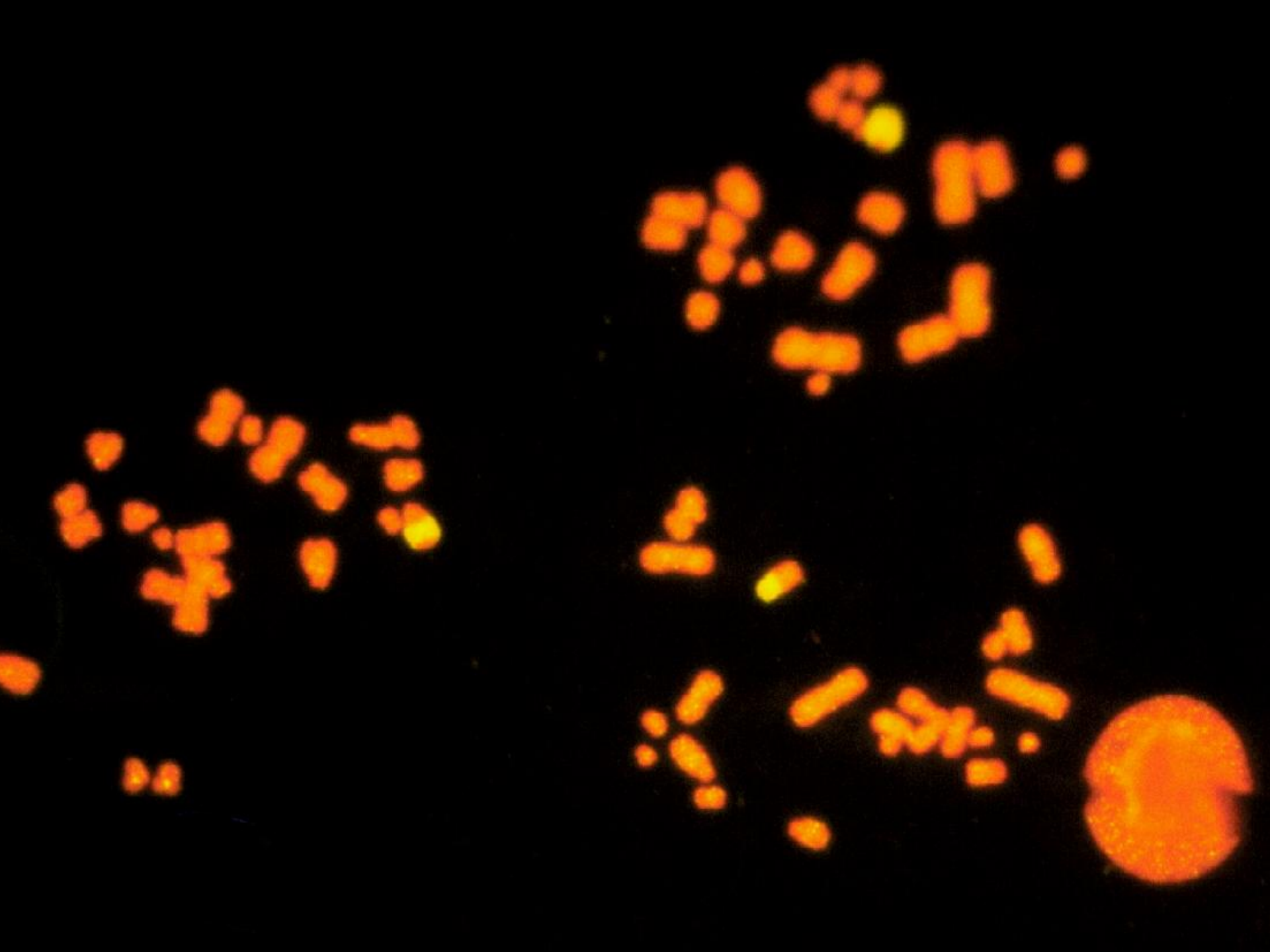


Manifests as:

chromosomal rearrangements
micronuclei
aneuploidy
delayed mutation
(spectrum different)
gene amplification
cell killing




Clonally expand
Irradiate
Clonally expand
Metaphase analysis of
clonally expanded cells



Radiation-induced instability can occur in bystander cells:

Instability observed in cells not traversed by an alpha particle

Kadhim et al. Nature 355, 738-40 (1992)

Shielded grid experiment

Lorrimore et al. PNAS 95, 5730-3 (1998)

secreted factor?

cell to cell gap junction communication*?

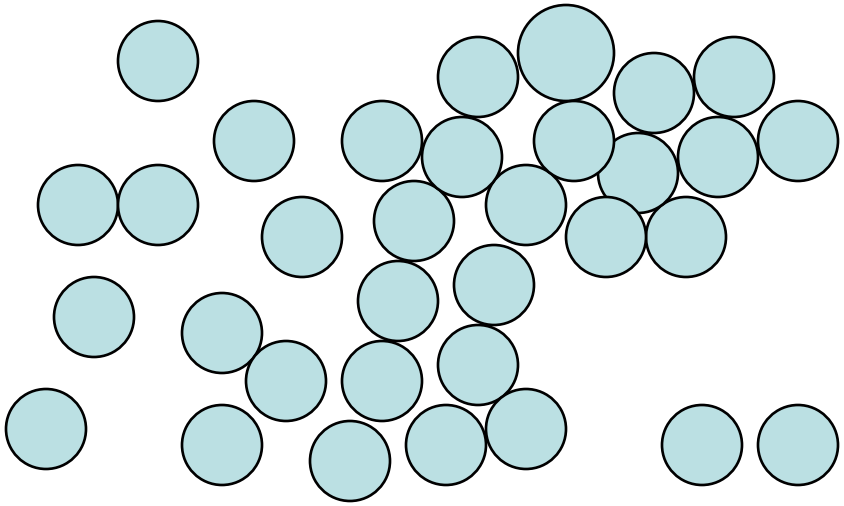
dead / dying cells*?

*Not in our cell system

Radiation induced bystander effects:

Effects observed in cells that were not irradiated but were “bystanders” at the time of irradiation

Single cell microbeam irradiation



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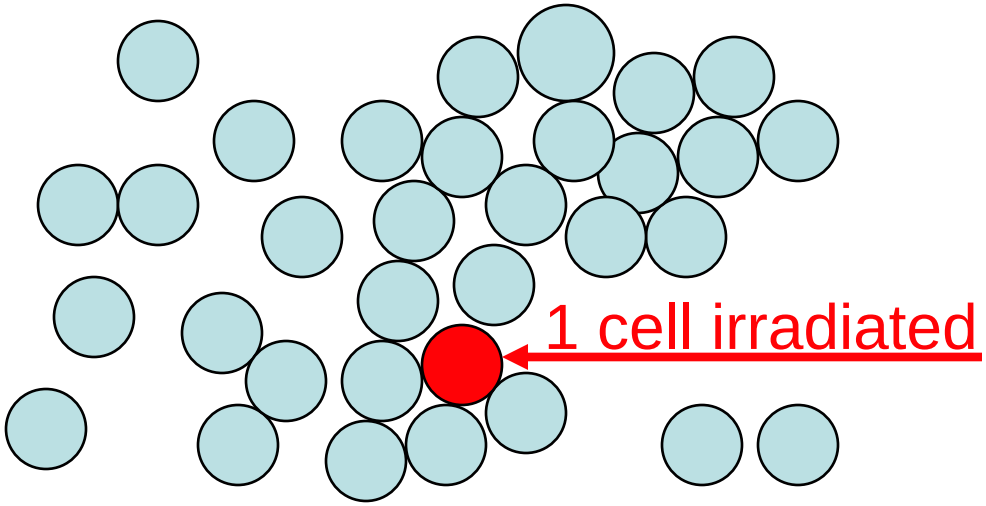

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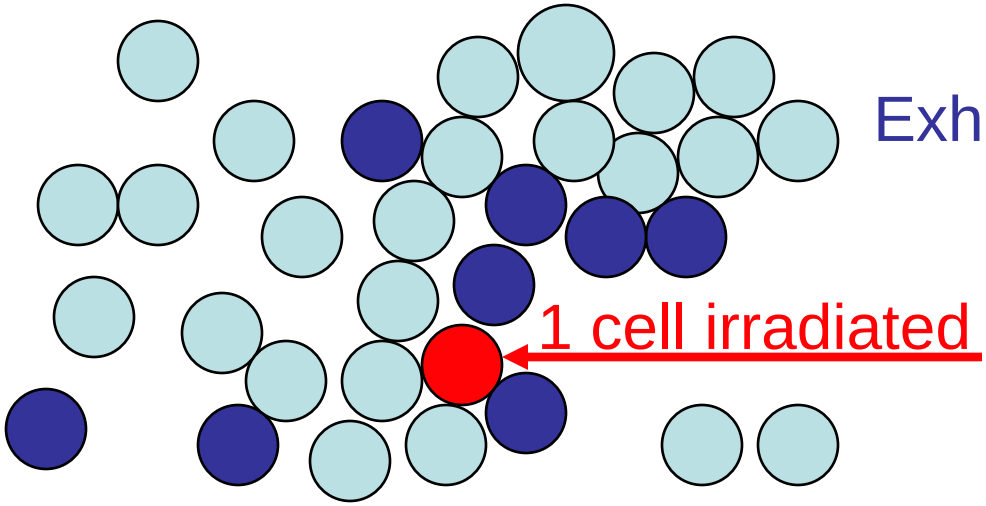
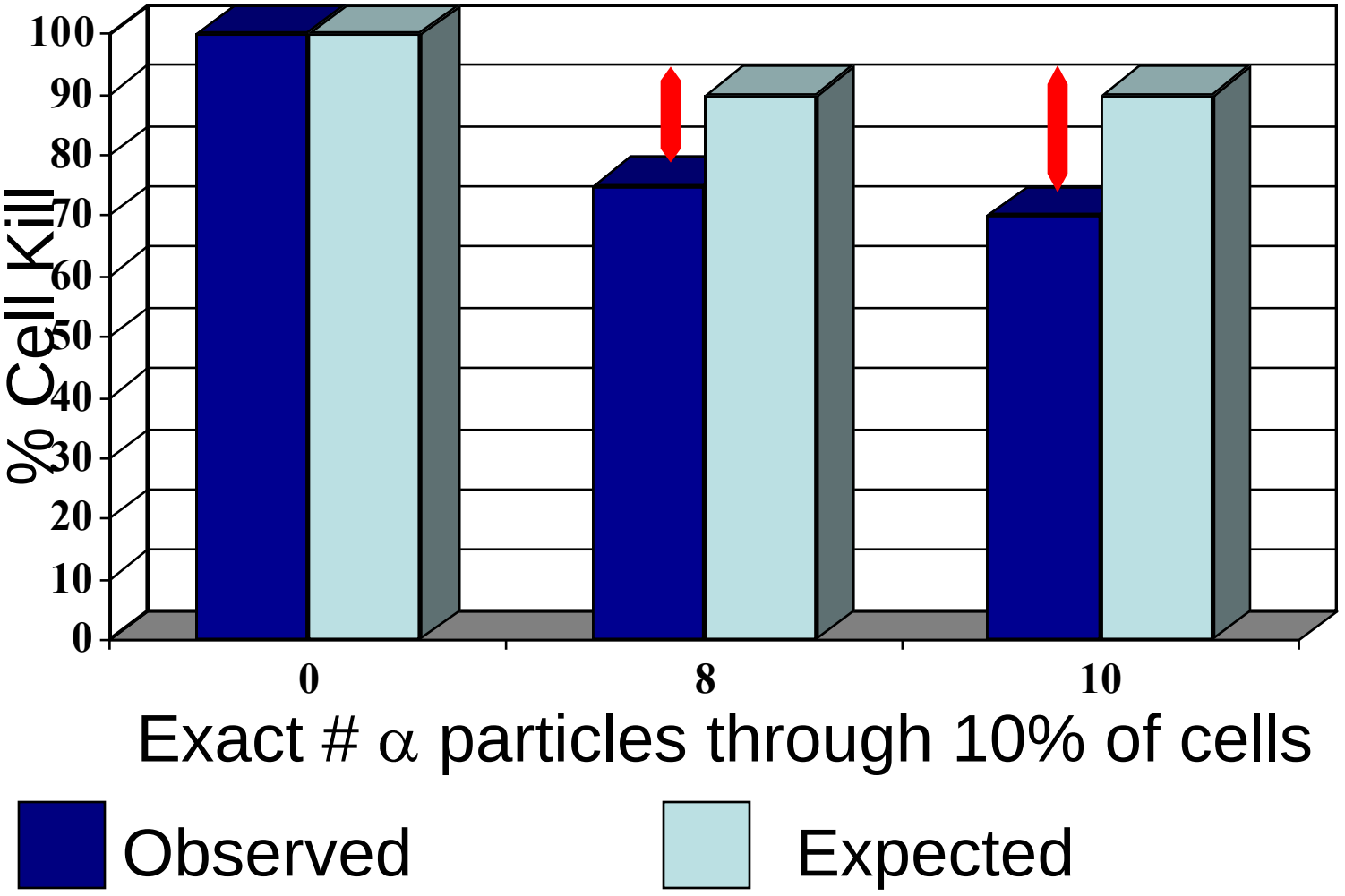


Exhibit bystander effect

- gene expression
- mutation
- transformation
- micronuclei
- cell killing

Bystander effect for cell survival



Cell kill due to bystander effect

Sawant et al. Radiation Res. 156, 177-180 (2001)

Bystander effect after low fluences of α-particles

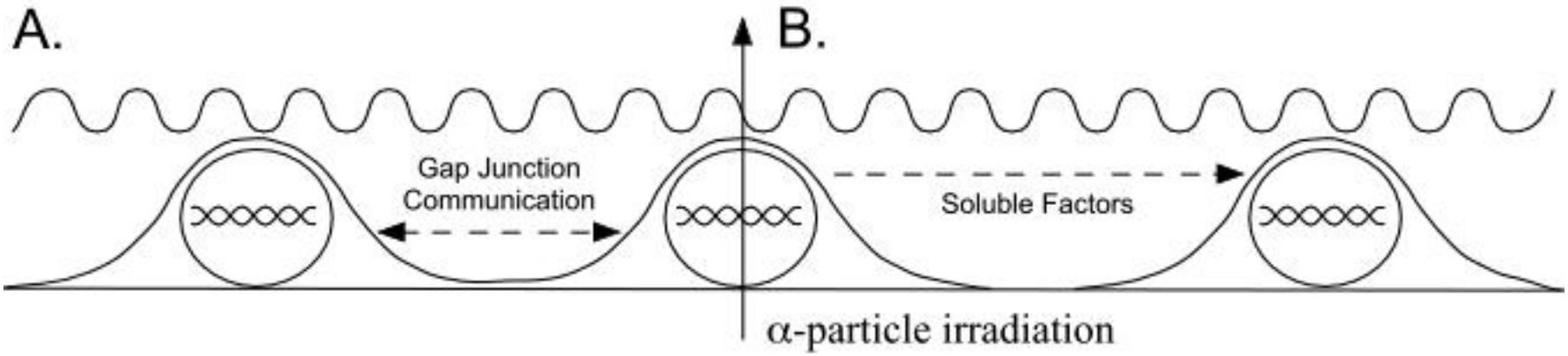
Azzam, Little and colleagues

or targeted high LET microbeam irradiation

Columbia University, Gray Cancer Institute

Media transfer experiments - low LET irradiation

Mothersill, Seymour and colleagues



A. Mediated by cell to cell gap junction communication
B. Secretion of soluble factors into culture medium

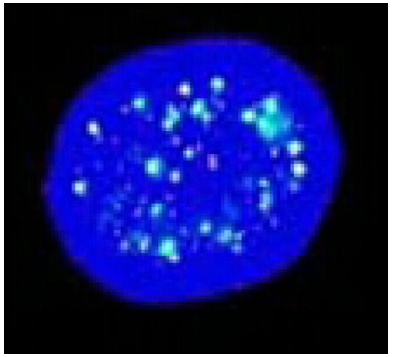
What is the nature of the signal generating bystander effects?

Reactive oxygen/nitrogen species

What is the interaction of that signal with the bystander cell?

Can lead to DNA double strand breaks

- γ-H2AX foci
- chromosomal aberrations
- micronuclei
- apoptosis



Biological significance of bystander effects … are they good or bad?

Bad - genetic damage and cell killing

Good - > proliferation, radio-protection

Any effect occurring outside irradiated zone - bad

Do bystander effects occur *in vivo*?

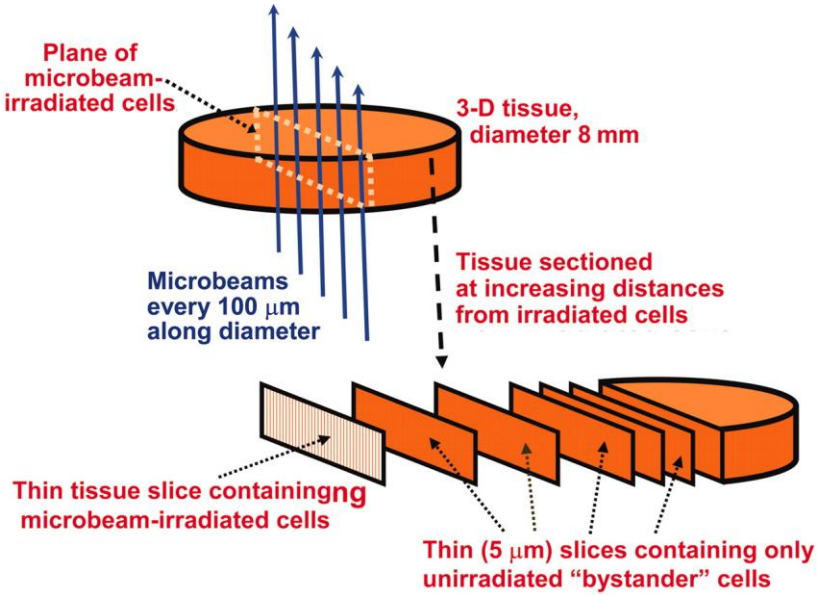
Is there a role for bystander factors in communicating the radiation response in the real world?

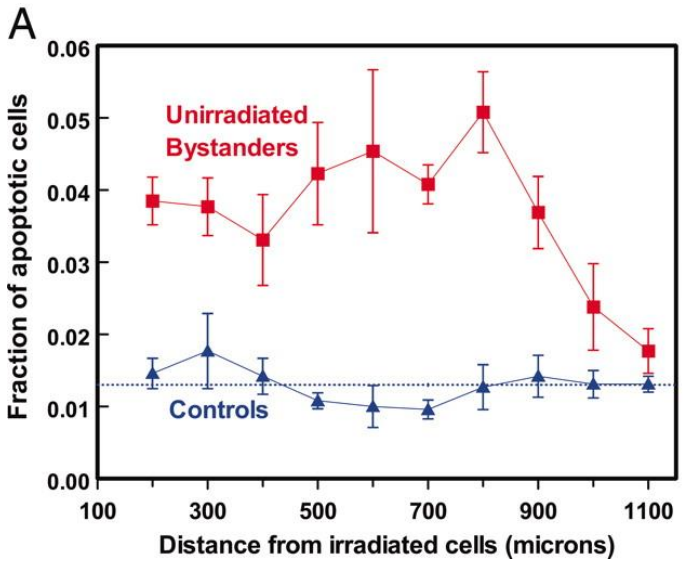
Clastogenic factors

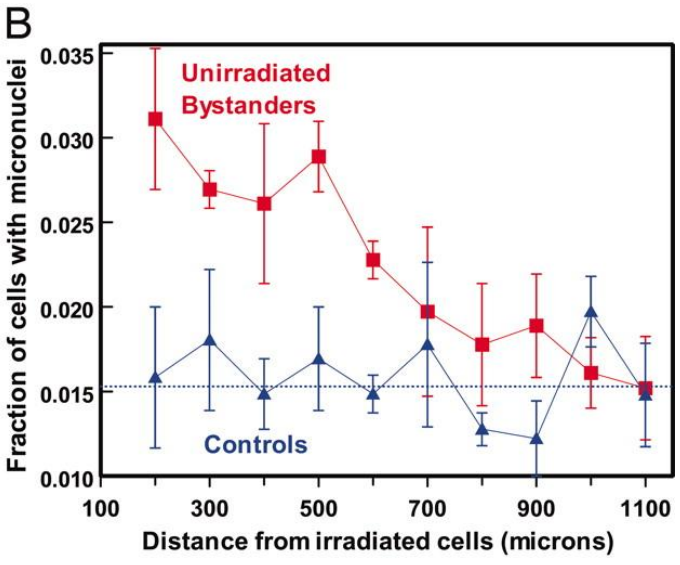
Abscopal effects

Bystander effects in an *in vivo* human skin model (3D).

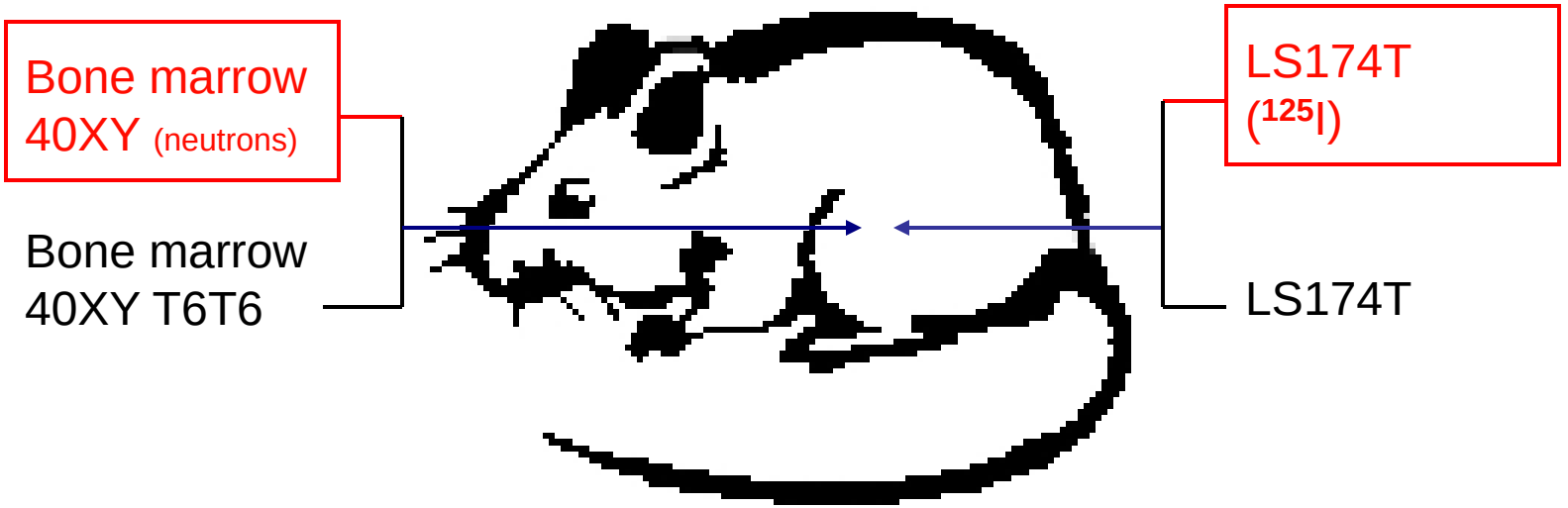
Belyakov et al. PNAS 102, 14203-7 (2005)







Bystander effects *in vivo*



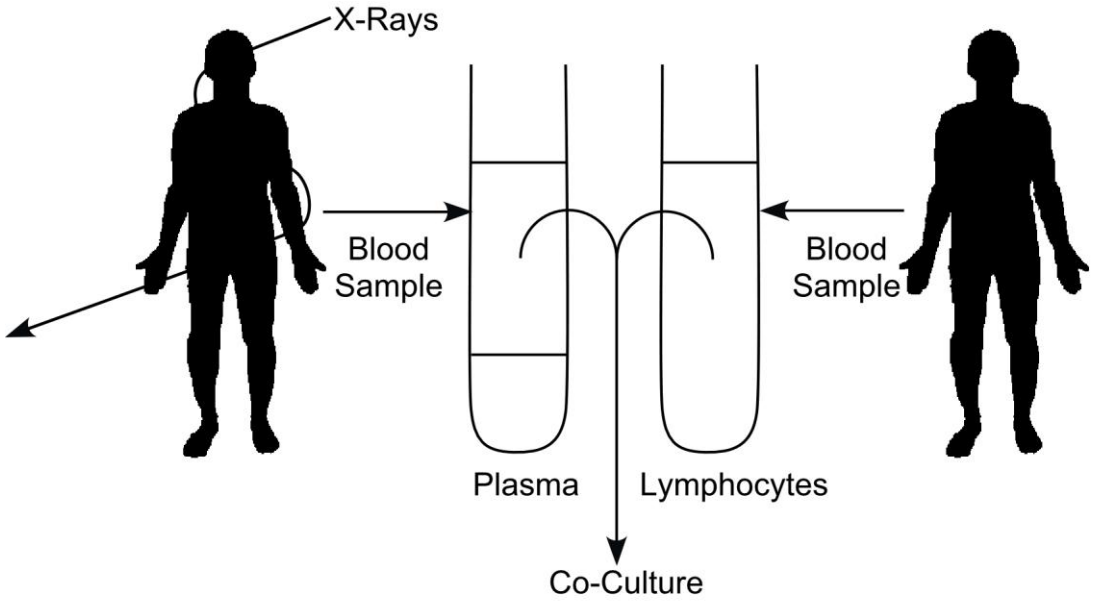
Chromosomal instability in progeny of non-irradiated hemopoietic stem cells

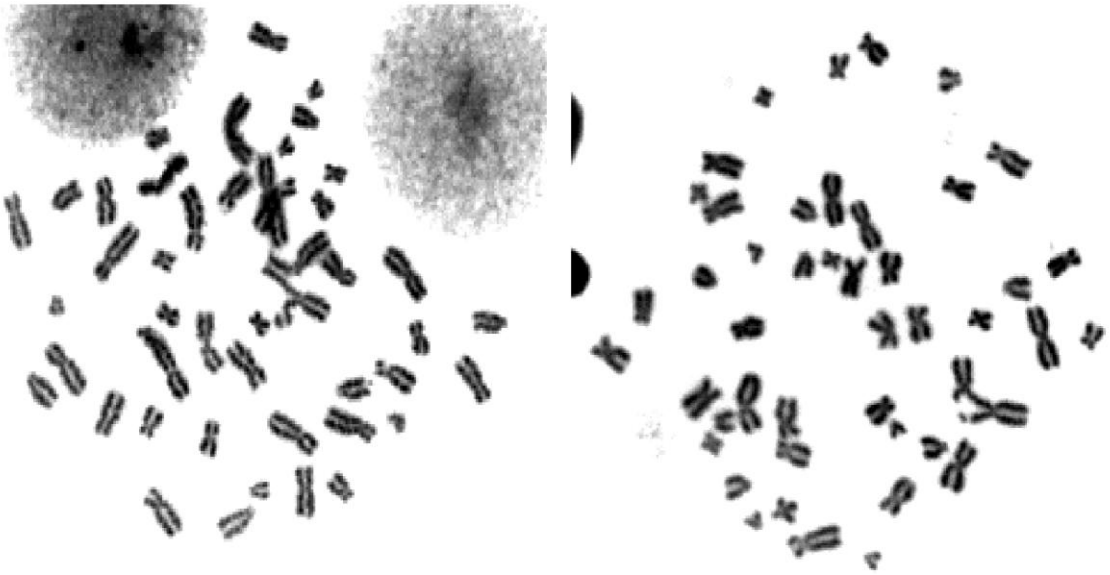
Watson et al., Cancer Res. 60, 5608 - 5611 (2000)

Inhibitory effect on tumor growth

Xue et al., PNAS 99, 13765-70 (2002)

Clastogenic Factors





Clastogenic factors in plasma from:

Accidentally irradiated individuals

Goh & Sumner, Radiation Res. 35, 171-181 (1968)

Therapeutically irradiated individuals

Hollowell & Littlefield, PSEBM. 129, 240-244 (1968)

A-bomb survivors

Pant & Kamada, Hiroshima J. Med. Sci. 26, 149-154 (1977)

Chernobyl clean up workers

Emerit et al., J. Cancer Res. Clin. Oncol. 120, 558-561 (1994)

Children exposed after Chernobyl

Emerit et al., Mutation Res. 373, 47-54 (1997)

Human blood irradiated *in vitro*

Scott, Cell Tissue Kinet. 2, 295-305 (1969)

CF-Nelson rats

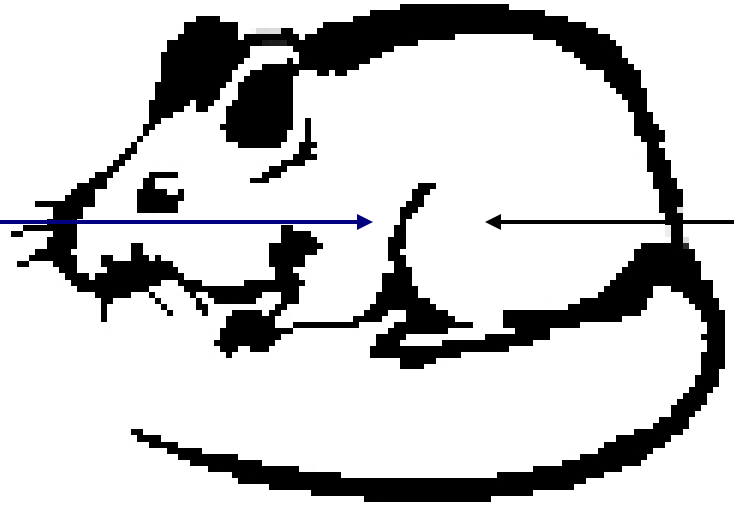
Fagnet et al., Cancer Genet. Cytogenet. 12, 73-83 (1984)

Patients with chromosome fragility syndromes

Bloom syndrome, Fanconi anemia, xeroderma pigmentosum

Abscopal “anti-tumor” effects *in vivo*

Implanted LLC cells



5 x 10Gy
12 x 2Gy
fractionated

Significant delay in LLC cell growth.

Camphausen et al. Cancer Res. 63, 1990-1993 (2003)

Further focused studies required

Mechanism of transmission?

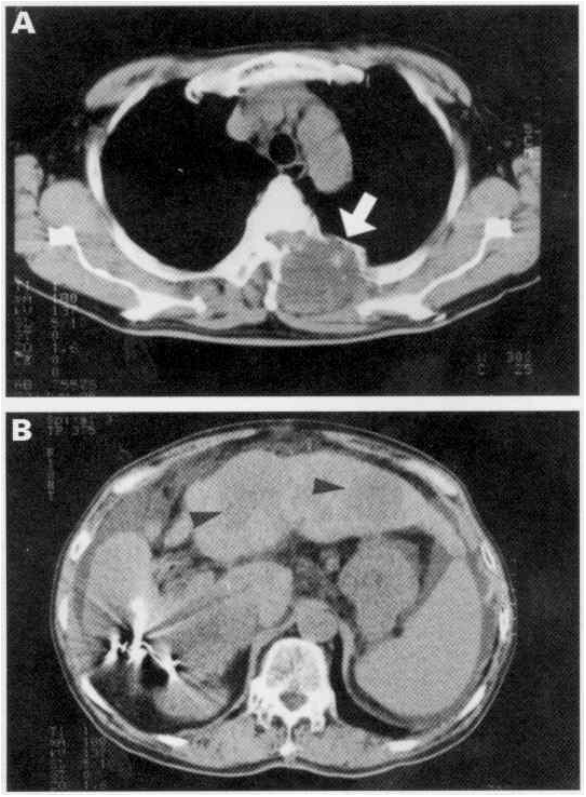
What is the factor?

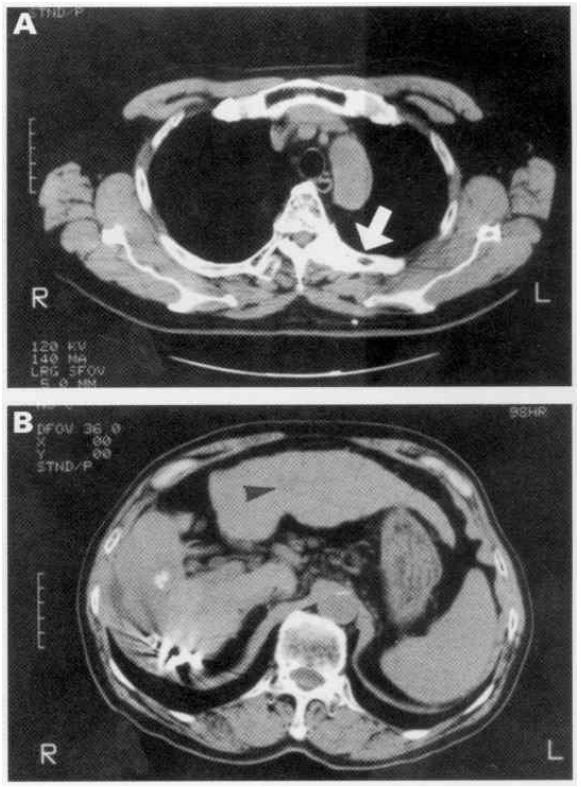
Organ specific or whole body at risk?

Abscopal Effects:

76 year old male with back pain
Thoracic and abdominal CT scans
thoracic vertebral bone metastasis and hepatocellular carcinoma
36Gy to the bone mass
regression of hepatic lesions

Ohba et al. Gut 43, 575-577, (1998)





Retrospective analysis of serum concentrations of IL-1 beta, IL-2, IL-4, IL-6, HGF, and TNF-alpha

Issues with the new biology:

Primarily (not exclusively) demonstrated in *in vitro* model systems

Relationship between genomic instability and radiation carcinogenesis? Not expected in healthy radiation exposed individuals!

Bystander effect primarily a low dose phenomena - reconciling this with the cell survival curve?

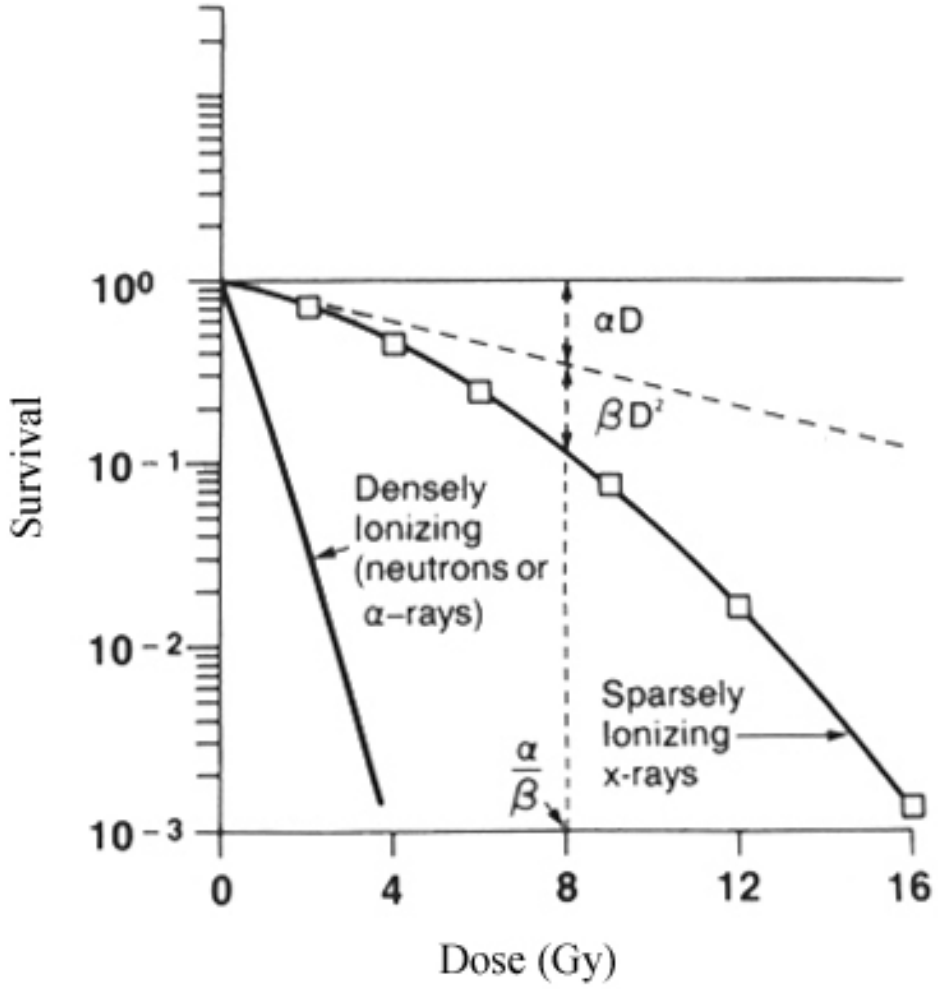
Clearly demonstrated after high LET radiation, but is 1 α particle a low dose?

Ambiguous data for low LET radiation. Reporting of negative data, reproducibility.

How modified by individual susceptibilities?

Bystander effects a low dose phenomena

Inherent scattering of electrons



How do you reconcile a very low dose cytotoxic bystander effect with the cell survival curve?

No direct irradiation effect <30mGy, but a significant bystander effect 3mGy. Liu et al. Rad. Res 166, 19-23 (2006)

Protecting mankind important

Regulations must be practical and relevant…animal studies?

Non-targeted effects tell us that:

cells communicate

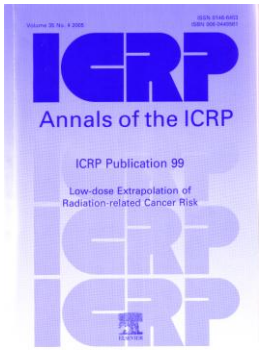
target > than irradiated volume

beneficial or detrimental?

Already “built into” organ risks?…….

If limited to a specific organ!

ICRP Publication 99, (2005) Conclusions, page 112



tion has not been answered scientifically and remains open.

(264) When considered as a whole, the emerging results with regard to a radiation-related adaptive response, genomic instability, and bystander effects suggest that the risk of low-level exposure to IR is uncertain, and a simple extrapolation from high-dose effects may not be wholly justified in all instances. However, a better understanding of the mechanisms for these phenomena, the extent to which they are active in vivo, and how they are inter-related is needed before they can be evaluated as factors to be included in the estimation of potential risk to the human population of exposure to low levels of IR. It should be recognised that information from direct epidemiological measure of cancer risk will, by definition, include any potential contribution from these mechanistic processes, and may therefore provide insights about them, subject to the constraints of low statistical power at low doses.

Challenges for the future:

Mechanistic studies essential

- DNA repair at low doses / low dose rates
- Technologies (significance of foci formation?)
- Differences between high and low LET

Tissue & animal studies - important information on signaling pathway(s)

- always be caveats - inbred, diet, strain specific

Genetic susceptibility *

- *appropriate model systems
- genetic and epigenetic component
- individual differences

Challenges for the future:

Non-targeted effects *in vivo*

what is the relationship between non-targeted effects?
what is the signaling molecule(s)?
why is signal amplified in tissue model systems?
what are the receptors for the signal?
why the lack of a dose response?
why don't all cells in a tissue respond?
biological significance of bystander effects
eliminate initiated cells? Good
generate initiated cells? Bad
random effects?
reconciling low dose bystander effects on cytotoxicity with the cell survival curve
do targeted and non-targeted cells respond differently?

Implications for radioprotection:

- Target at risk is greater than the volume actually irradiated (radio-therapy)
- Do non-targeted effects amplify the detrimental effects of radiation?
- How do we build non-targeted effects into radiation risk estimates?
- Why have they evolved and are they good or bad?

Comments or Questions

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