Low Doses of Ionizing Radiation: The Controversy of the LNT-Hypothesis

G. William Morgan Lecture, 2005
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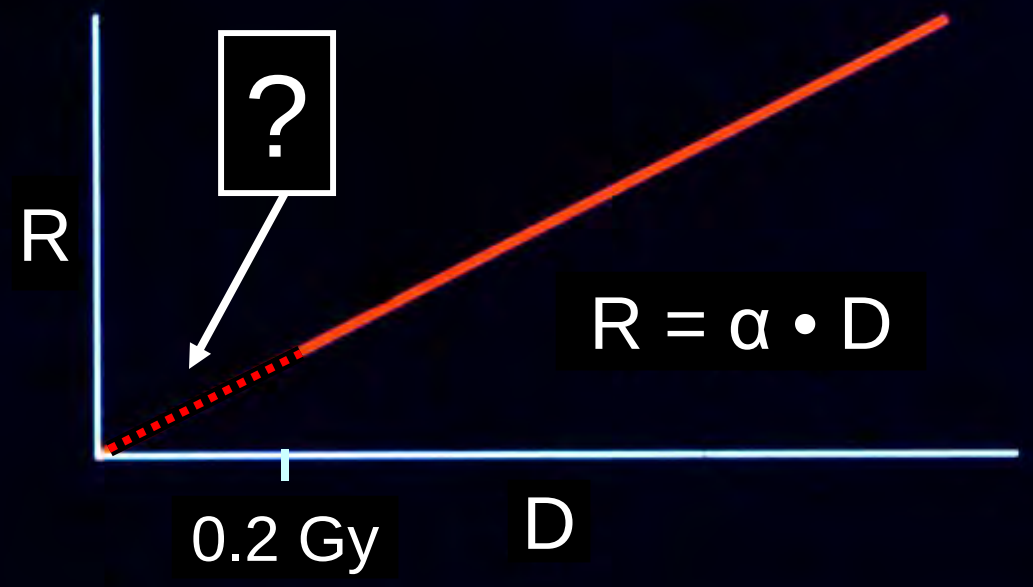
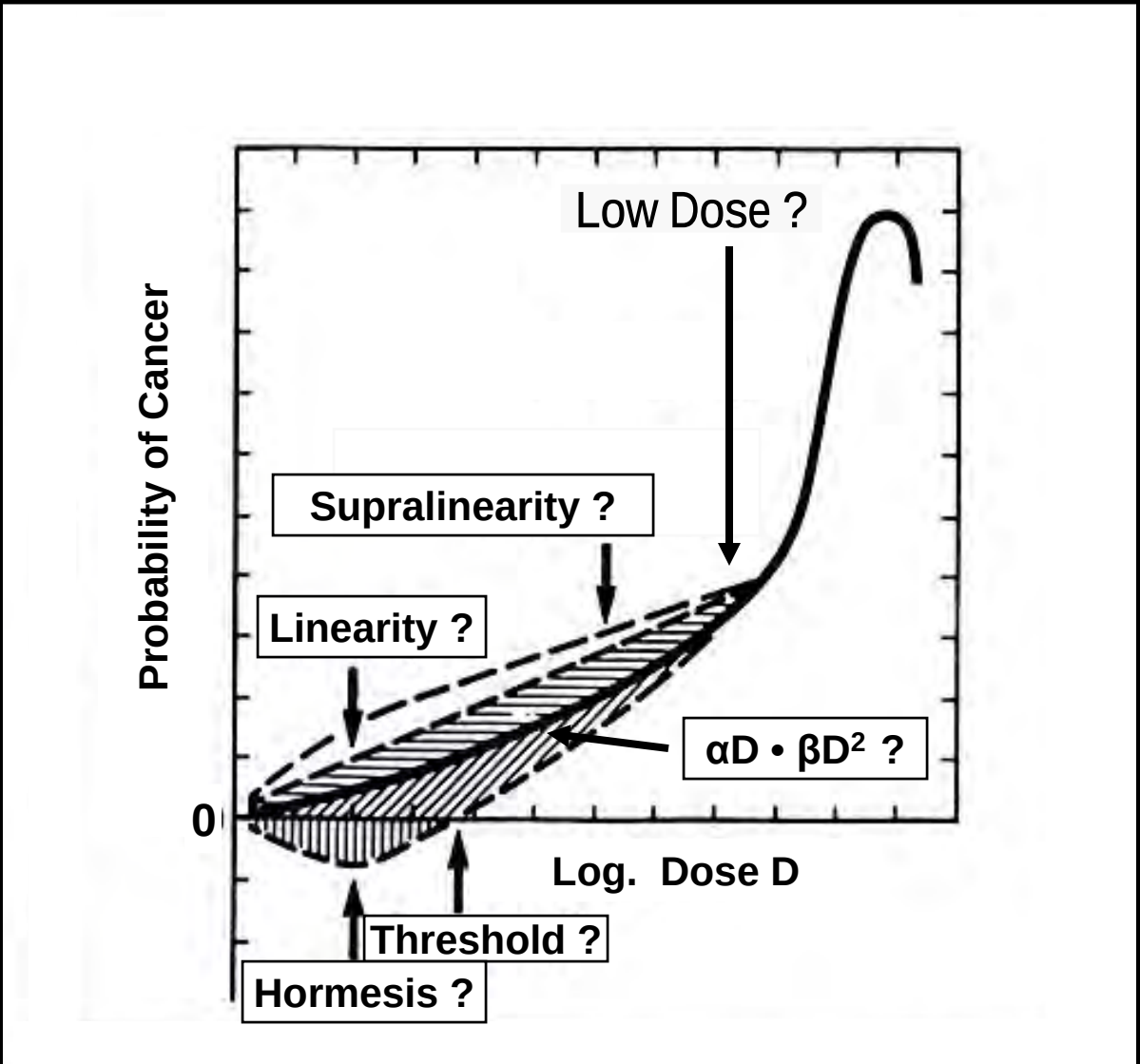
ICRP Supported by Report BEIR VII
prefers recommendations to be based on the linear, no-threshold (LNT) dose-response



R = cancer probability in exposed tissue

D = absorbed dose

Options of Low-Dose Induced Cancer Risk



Agenda

Dose	1. Dose and energy deposition events
	2. Dose to primary target
Effects	3. Damaging effects
	4. Adaptive Protection
	5. Projected net effect on cancer
Analysis	6. A Model

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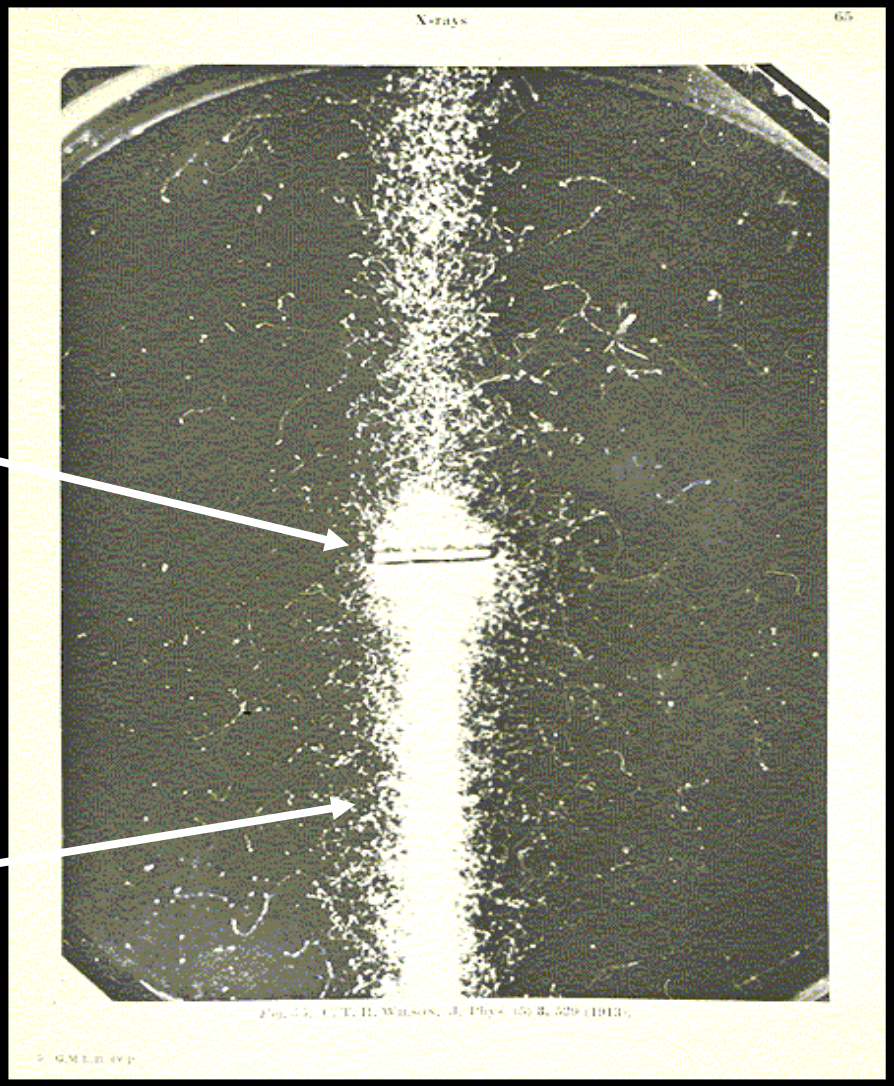
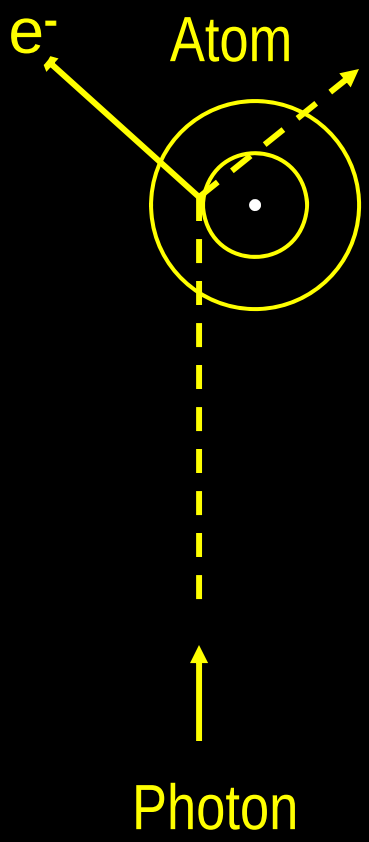
Ionizing Radiation→Energy Deposition Events

Wilson cloud chamber, compton electrons

attenuating metal plate

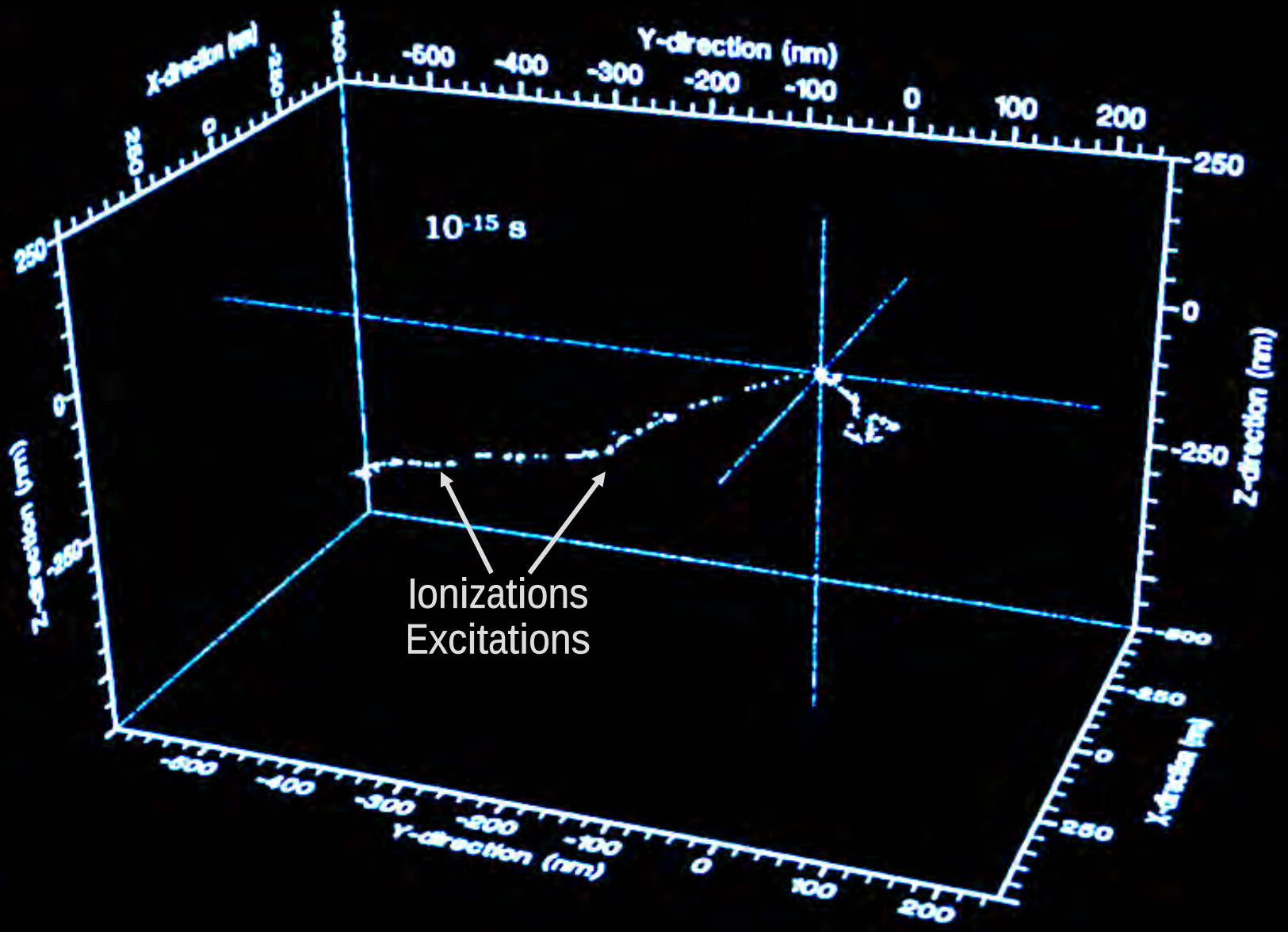
x-ray beam from below





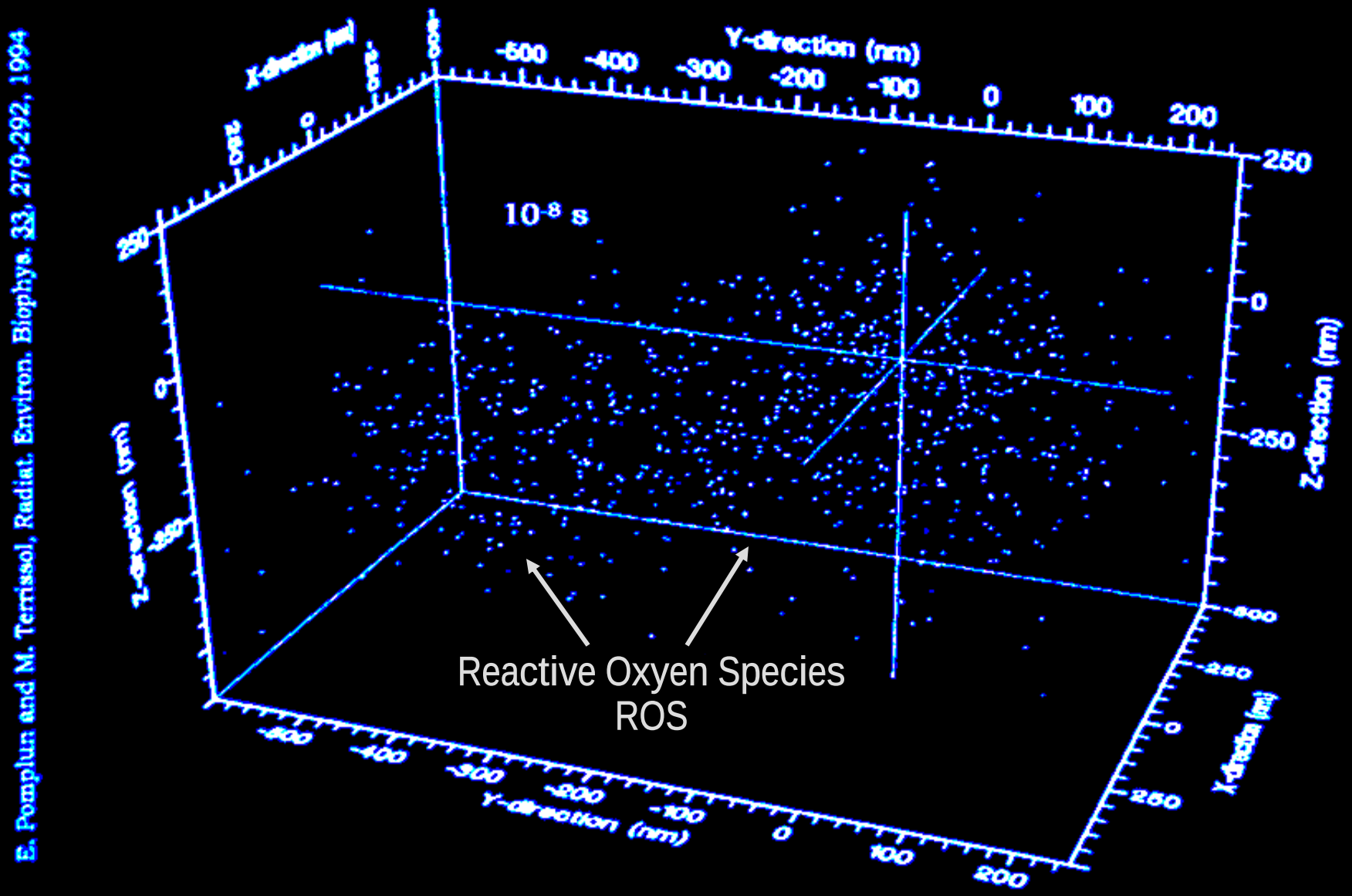
Wilson CTR, J. Phys., 1913

Electron Track in Water at 10^{-15} sec



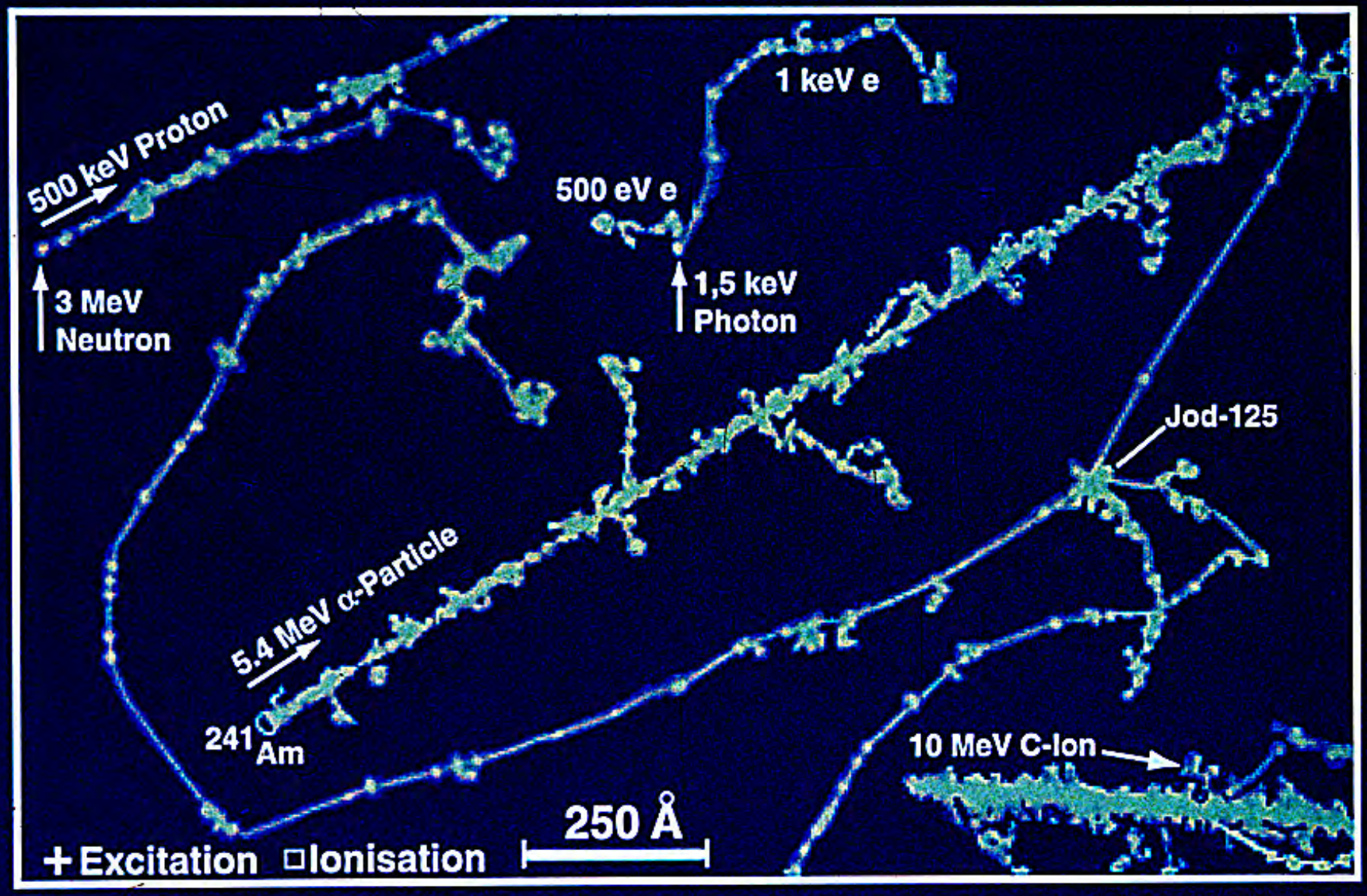
E. Pomplun and M. Terissol, Radiat. Environ. Biophys. 33, 279-292, 1994

Electron Track in Water at 10^{-8} sec



E. Pomplun and M. Terissol, Radiat. Environ. Biophys. 33, 279-292, 1994

Individual Particle Tracks in Water



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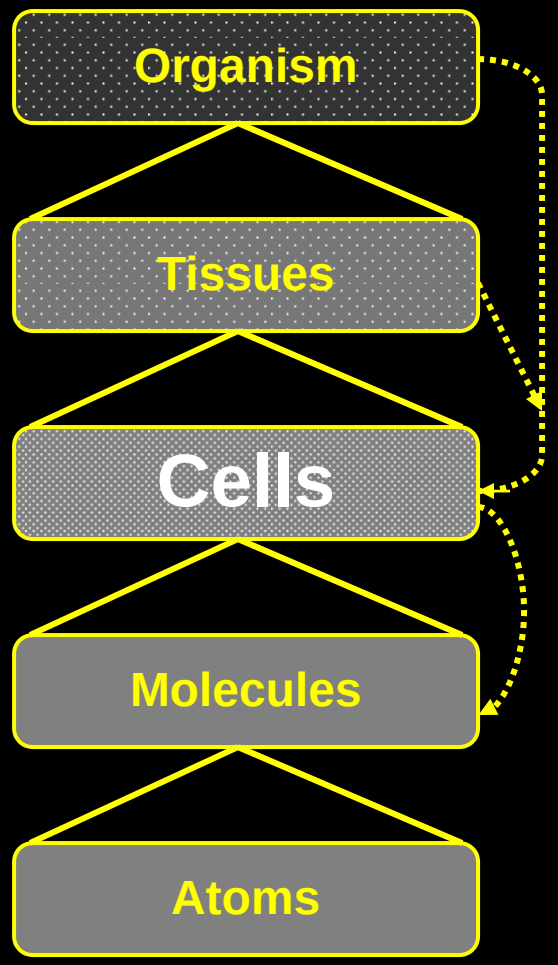
Complex Adaptive Systems at Various Levels

From Matter to Life



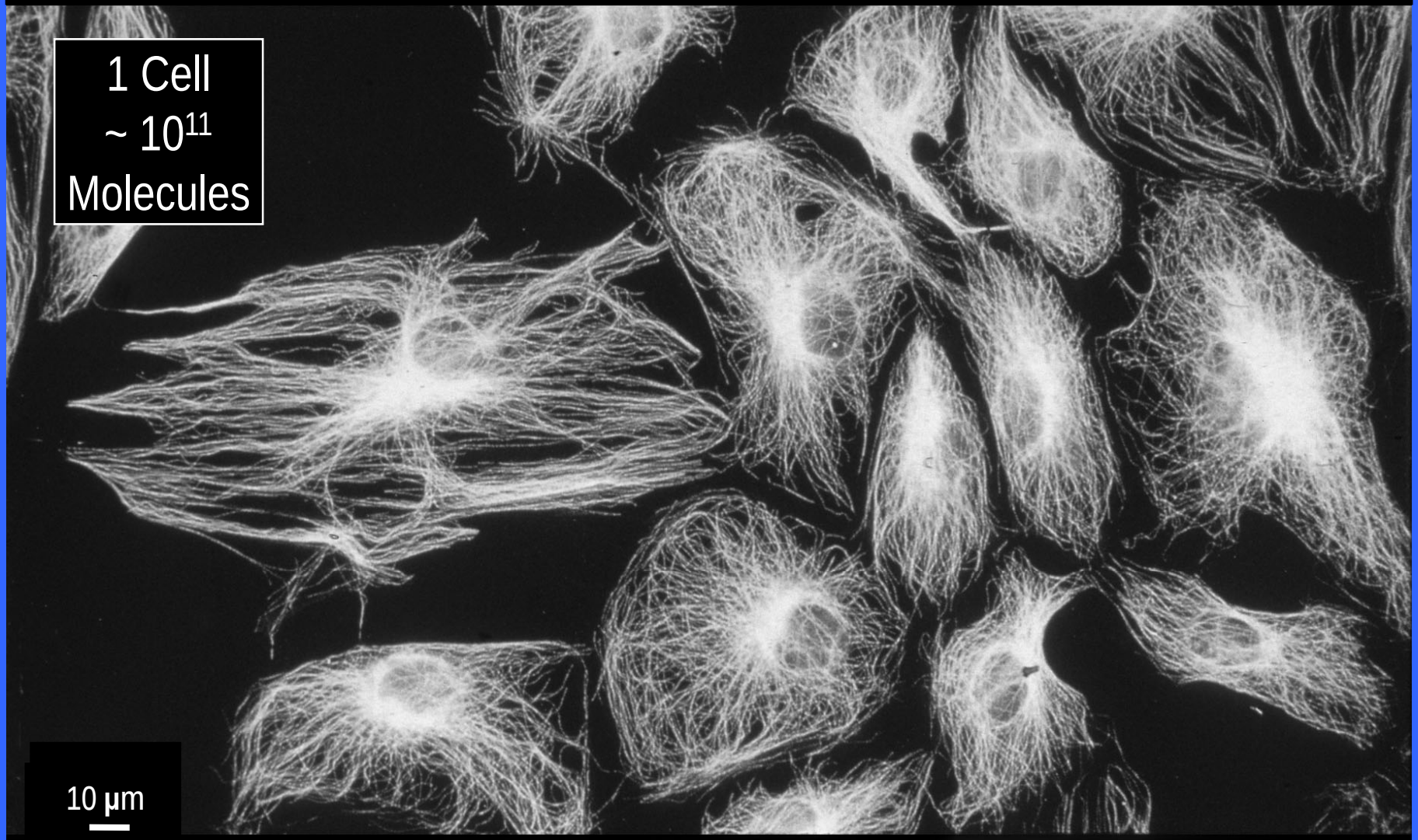
Living Organism

Cells are Fundamental Units of Life

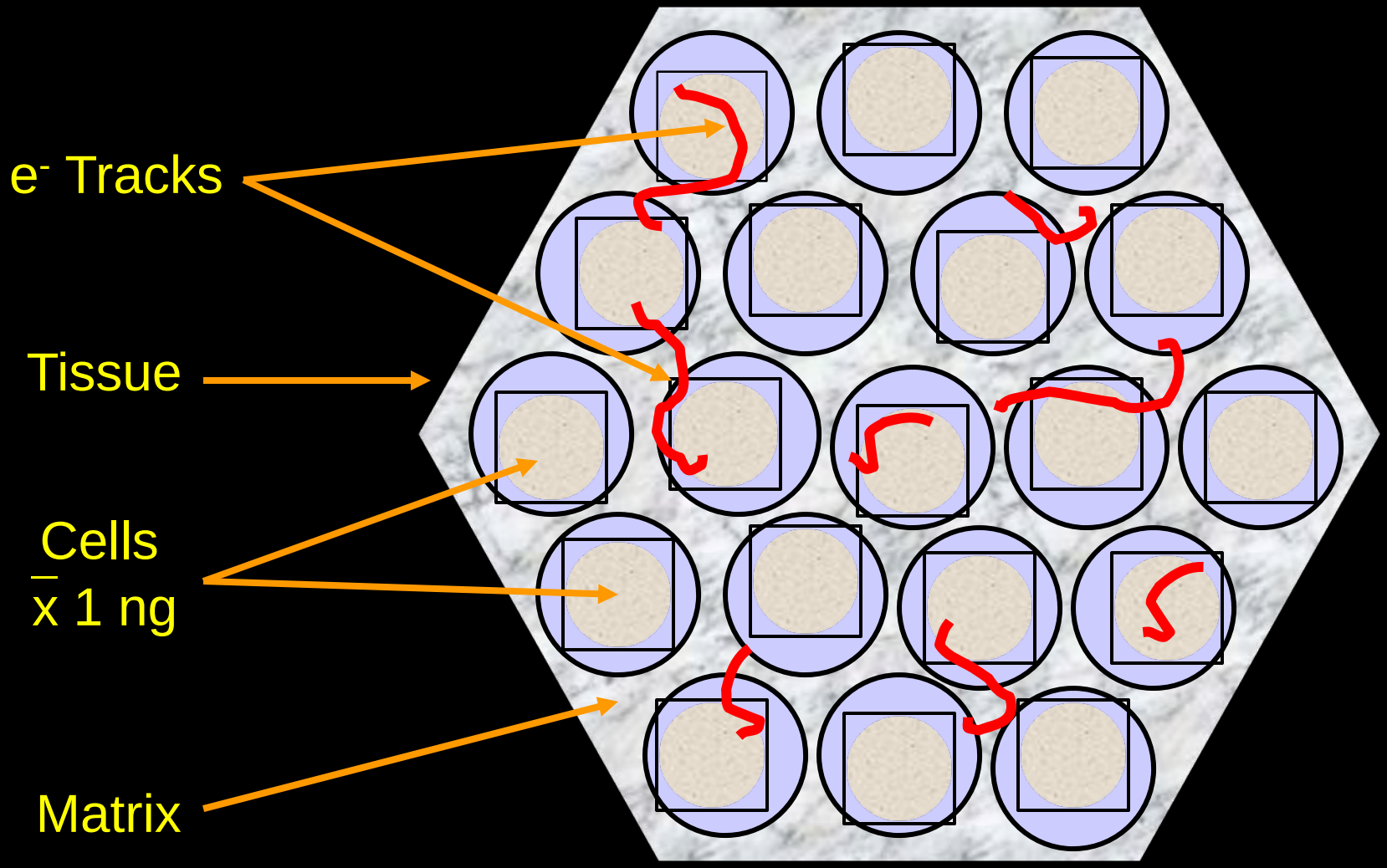
Building Blocks of Life

Cells React as Entities

average cell mass in mammals is 1 ng



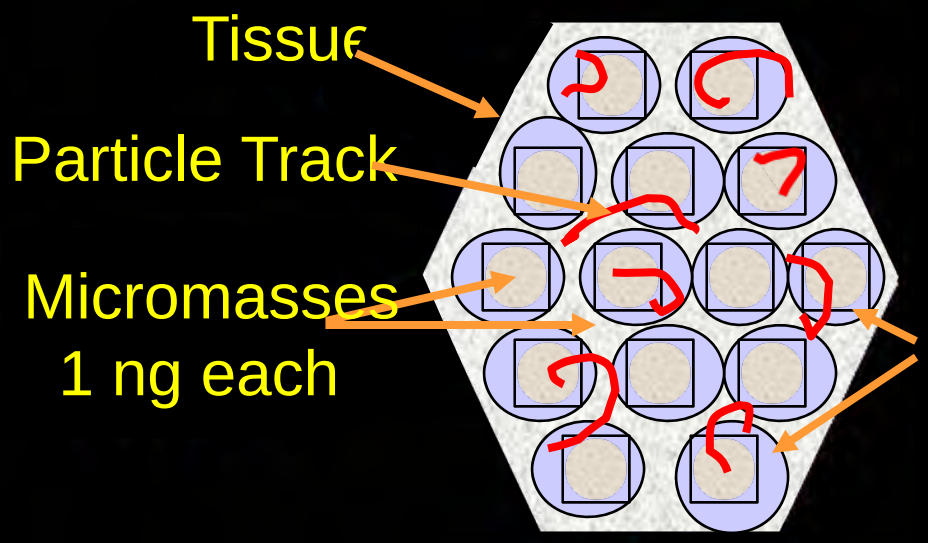
X-Ray Induced Electron Tracks in Tissue



Dose is proportional to number of tracks / exposed mass

From Absorbed Dose (D) to Total Energy (E)

absorbed in exposed micromasses



M = Mass of exposed tissue

N_E = No of exposed micromas

N_H = No of track hits in microm

z_1 = Energy absorbed per hit in micromass (Specific En

$$E / M = \bar{D} = [\ \bar{z}_1 \bullet N_H\ ] / N_E$$

Dose expresses multiple hits of $\bar{z}_1$ per N_E

Bond et al., 1988

Some $\bar{z}_{F1}$ Values (mGy) Commonly Used

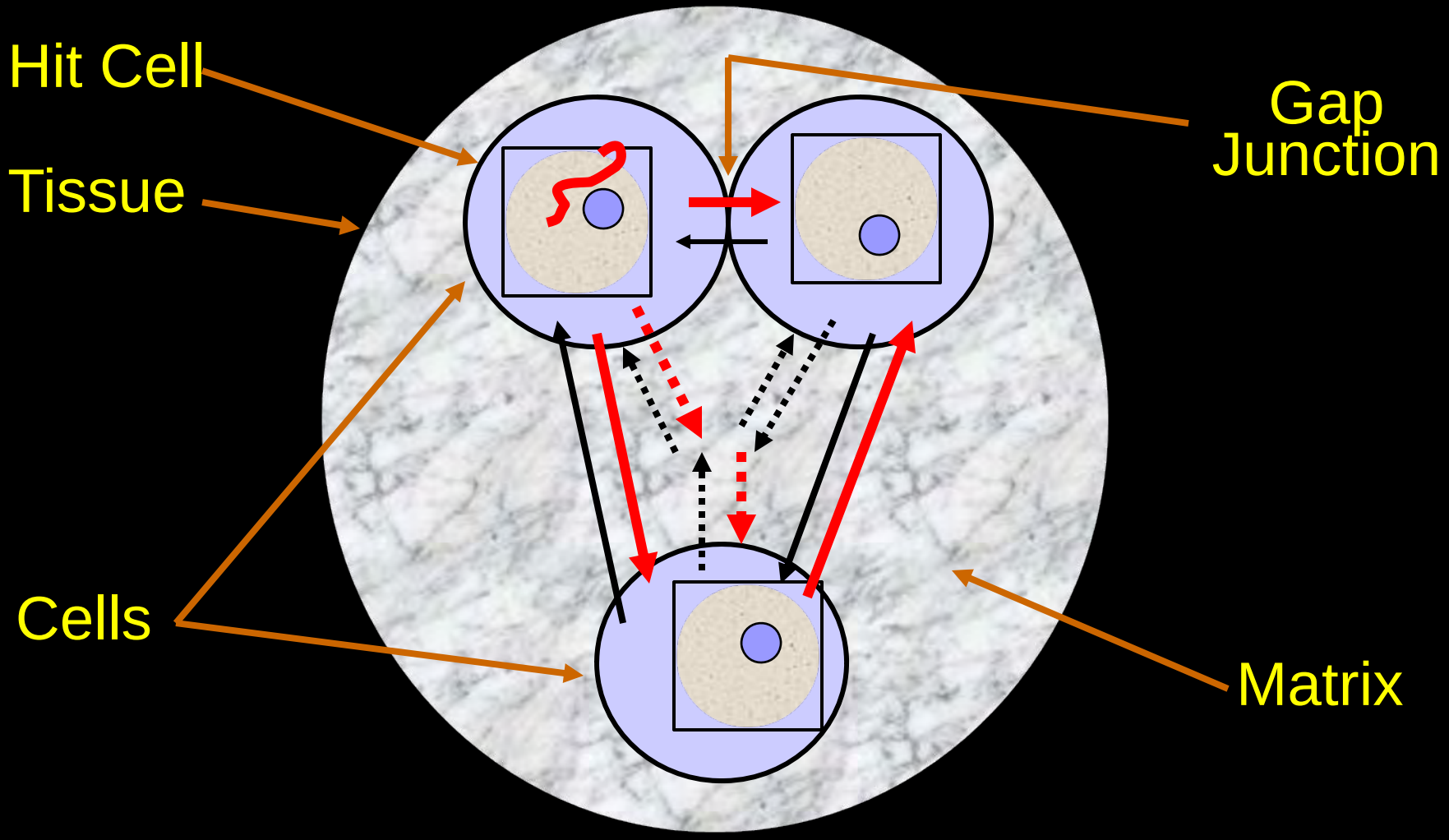
mean energy absorbed per particle track

in micromass of 1 ng (Microdose)

^{60}Co γ-rays	~ 0.3
^{137}Cs γ-rays	~ 0.4
250 kVp x-rays	~ 0.9
100 kVp x-rays	~ 1.0
10 MeV protons	~ 6.0
4 MeV α-particles	~ 350.0

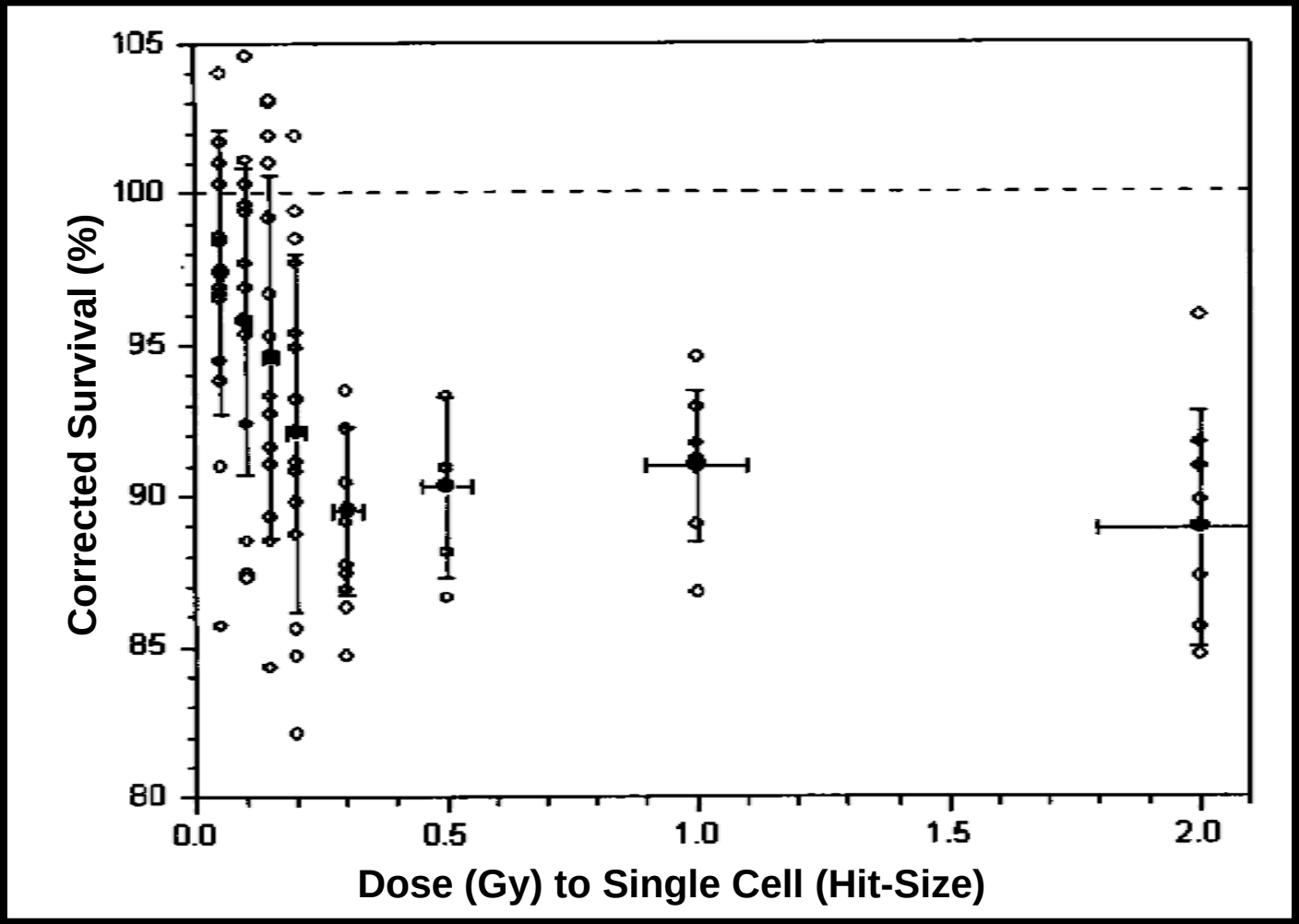
Modified from: ICRU Report 36, 1983, 1993

Signaling between Tissue Cells and Matrix



→ = Bystander Effects

Bystander Killing Effect from Single Cell
C_K x-irrad. of 1 cell in dish with ~160 cells



Schettino G. et al., Radiat. Res. 163: 332 - 336, 2005

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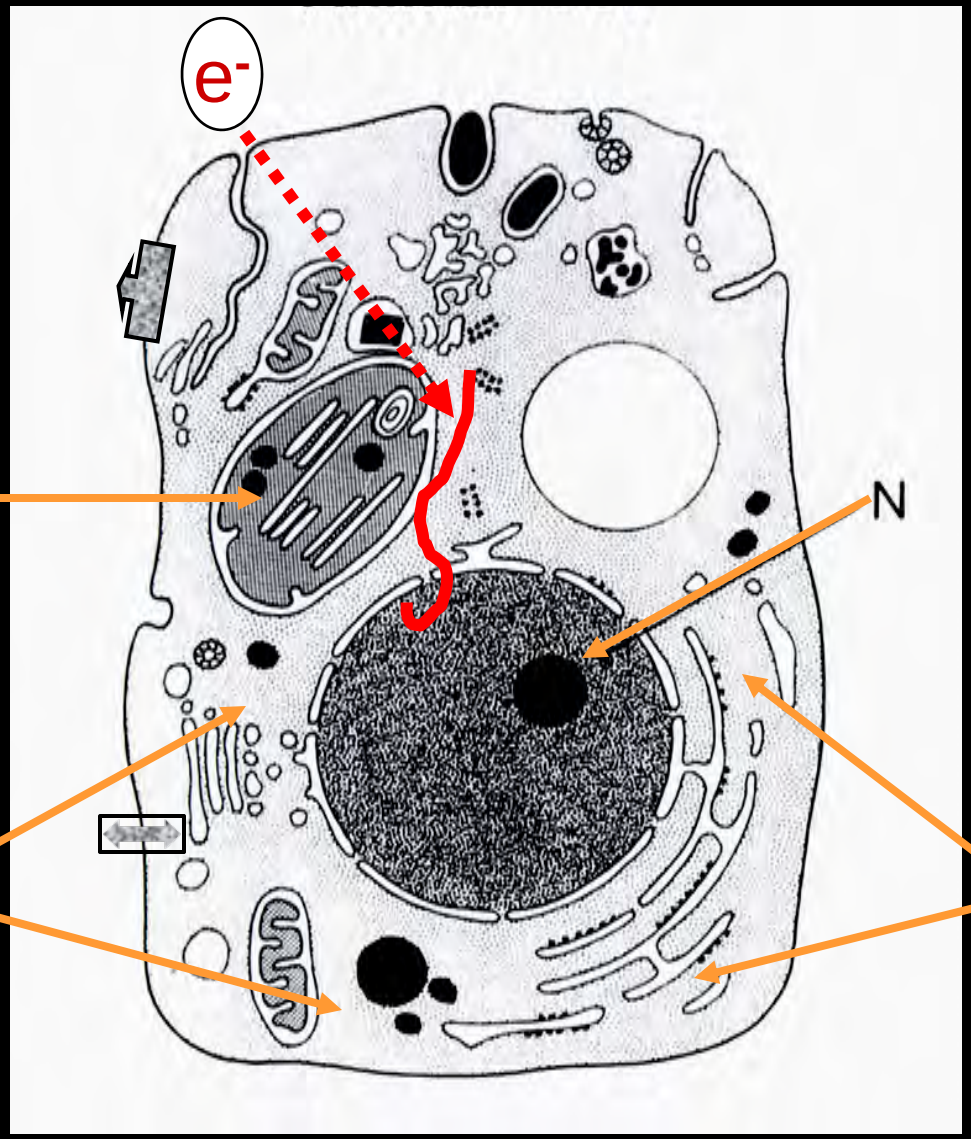
Animal Cell Hit by Electron

Mitochondrium generates energy (has own DNA)



Nucleus = site of genetic material (DNA)

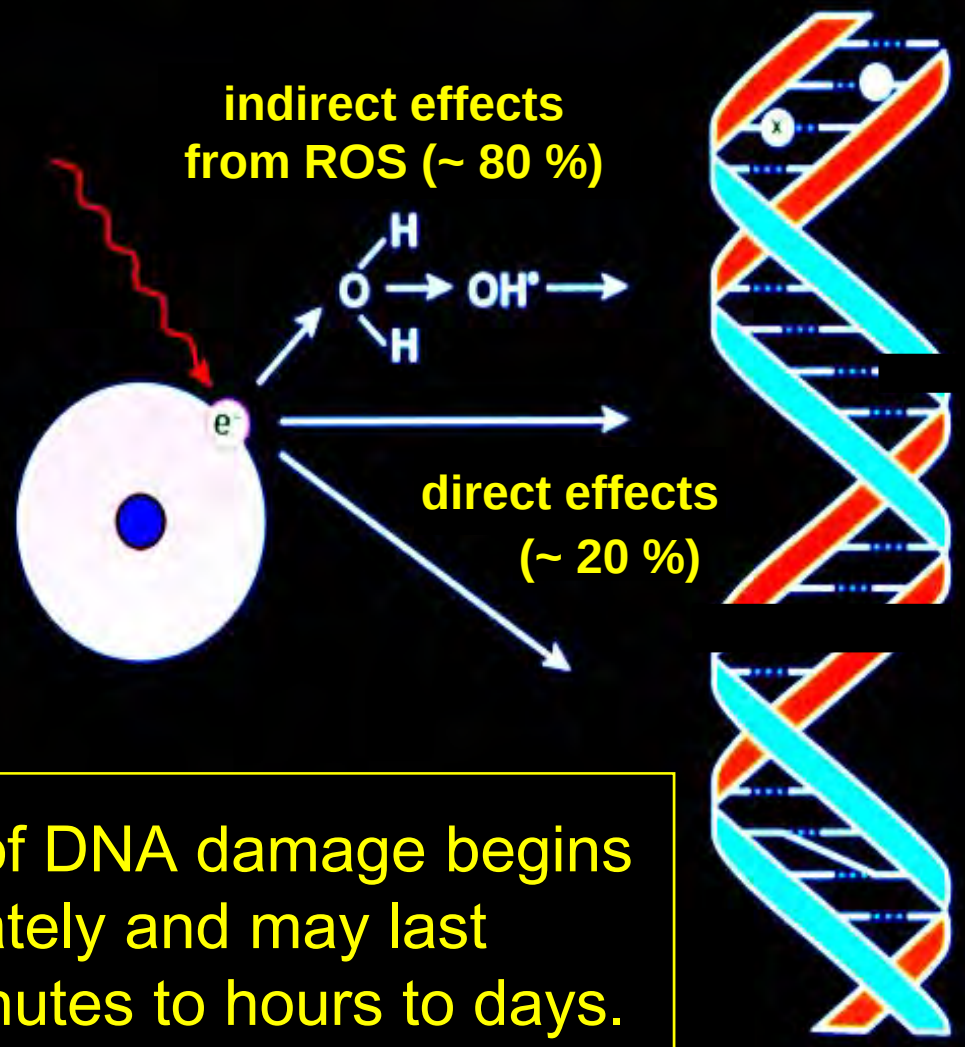
Cytoplasm

Cytoplasm

Average cell mass = 1 ng

N = Nucleus ($\bar{x}$ 8 µm ∅)

Radiation Effects on DNA



base loss
base change
(~10/0.01 Gy)

single strand breaks
SSB (~ 10/0.01 Gy)

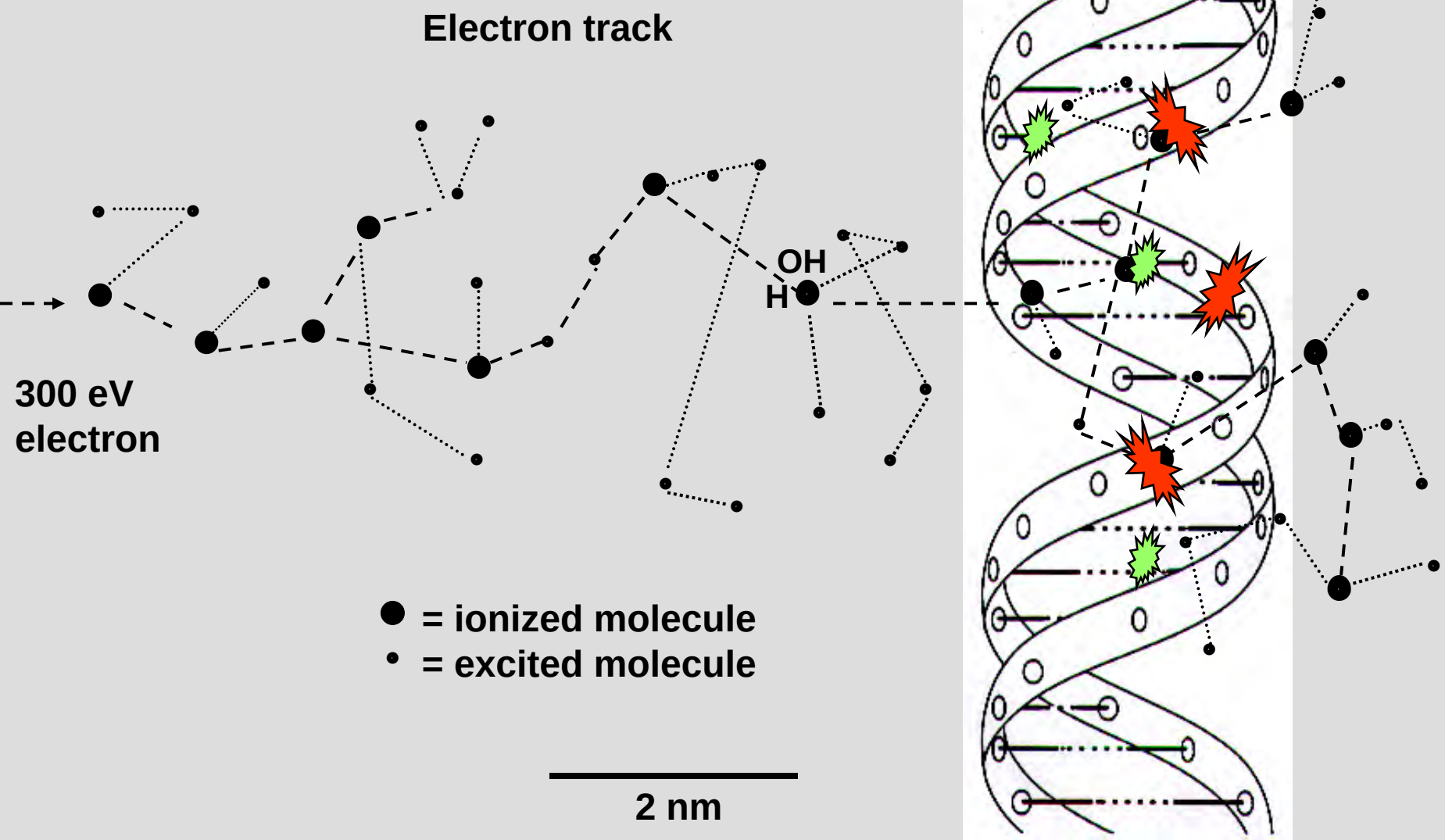
double strand breaks
DSB (~ 0.4/0.01 Gy)

cross links
(~ 1-2/0.01 Gy)

Repair of DNA damage begins immediately and may last from minutes to hours to days.

~ 25 - 40 % of DSBs from x-rays have complex structure

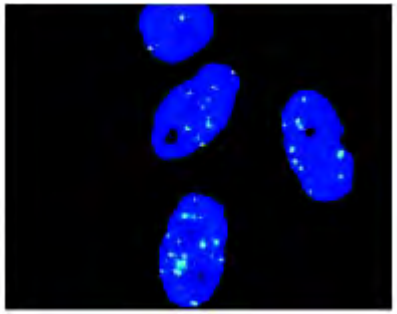
Clustered DNA Damage

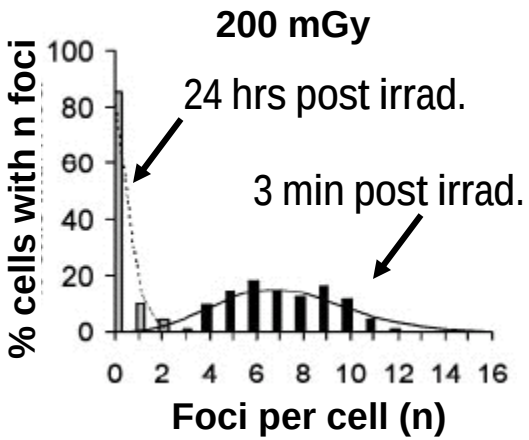


Goodhead D, pers. comm., 21. 08. 2003

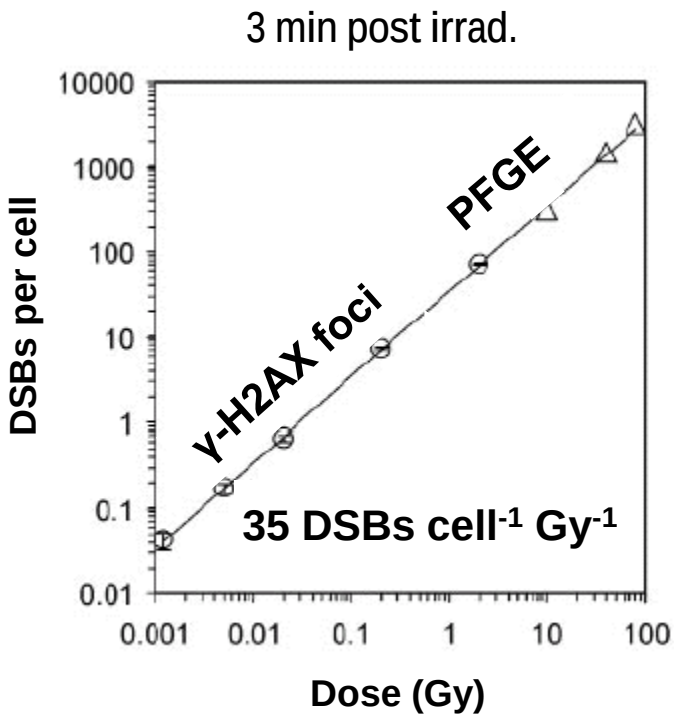
DTG 21.8.03

Radiation-Induced DNA-DSB
in γ-irradiated MRC-5 cells in culture





γ-H2AX foci distribution



spont. DSB ≅ $\bar{x}$ 0.04 – 0.06 / cell at steady state

Rothkamm K, Löbrich M, PNAS, 2003

Radiation-Induced Chromosome Aberrations
in human leukocytes

ring chromosome

dicentric, fragment chromosome

Hall E., Radiobiology for the Radiologist, Lippincott et al., 2000

Radiation-Induced Genomic Instability

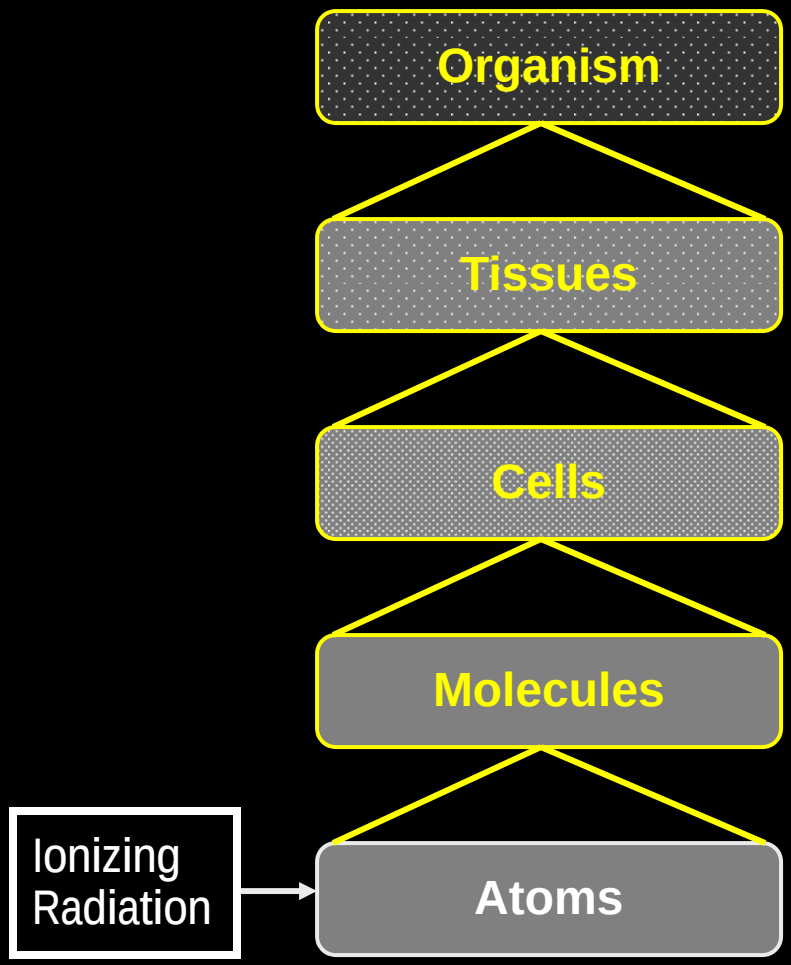
DNA damage may cause genomic instability in the progeny of the affected cells, likely in a dose dependent manner.

Genomic instability *in vivo* tends to

1. enhance malignant cell transformation,

2. tag damaged cells for removal
   by immune response or apoptosis.

Complex Adaptive Systems at Various Levels



Risk per Human Stem Cell per 1 mGy from 100 kV x-rays projected for blood forming tissue by extrapolation from high to low D

~ 10^{-14}	Malignant transformation with death of individual
	?
~ 10^{-4}	Chromosomal aberr.
~ 10^{-2}	DNA - DSB
~ 2	$\sum$ DNA alterations
~ 150	ROS

Feinendegen et al., Stem Cells 13 Suppl 1: 7-20 (1995)

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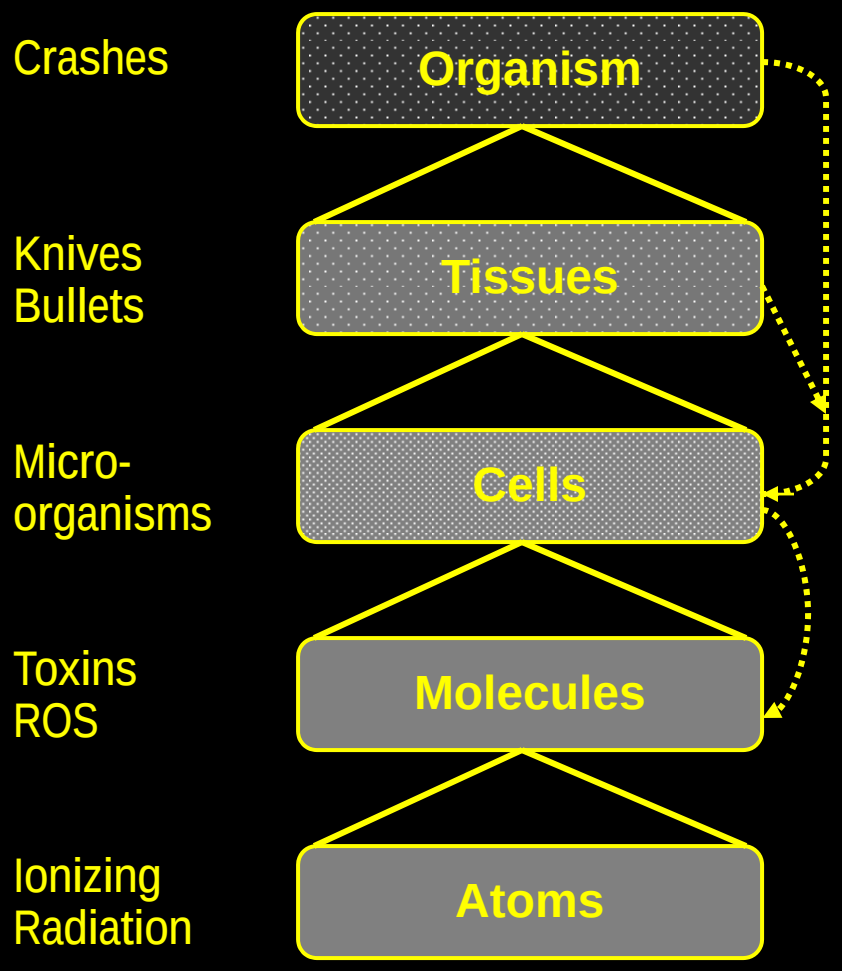


Barriers Prevent Damage from Causing Disease

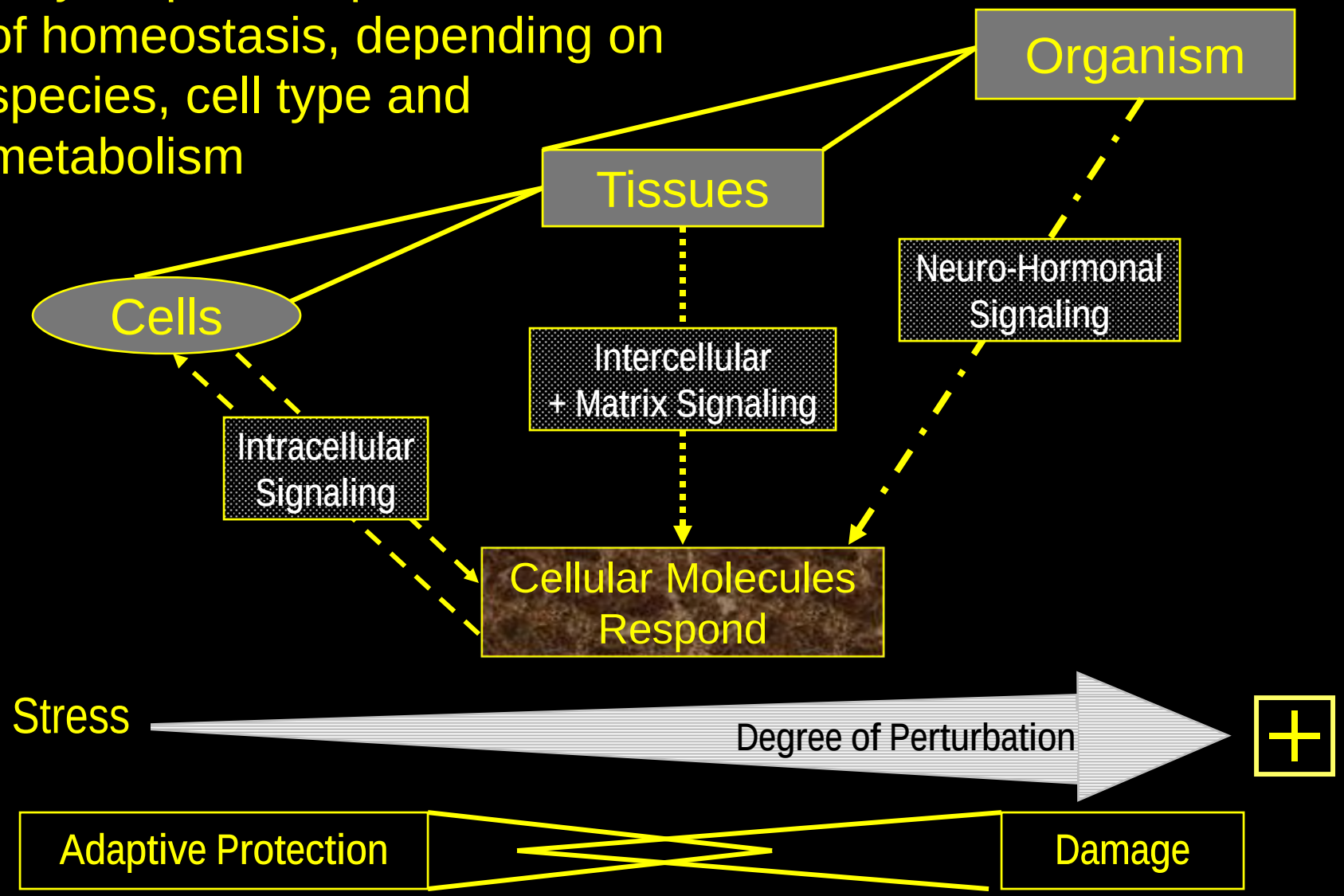
Disease

- **Multiorgan Failure**
- **Pathology**
- **Cancer**

- **Immune Response**
- **Cell Differentiation**
- **Apoptosis**
- **DNA Repair**
- **Defense, Scavenging**

Biological Systems are Complex and Adapting

they respond to perturbations of homeostasis, depending on species, cell type and metabolism



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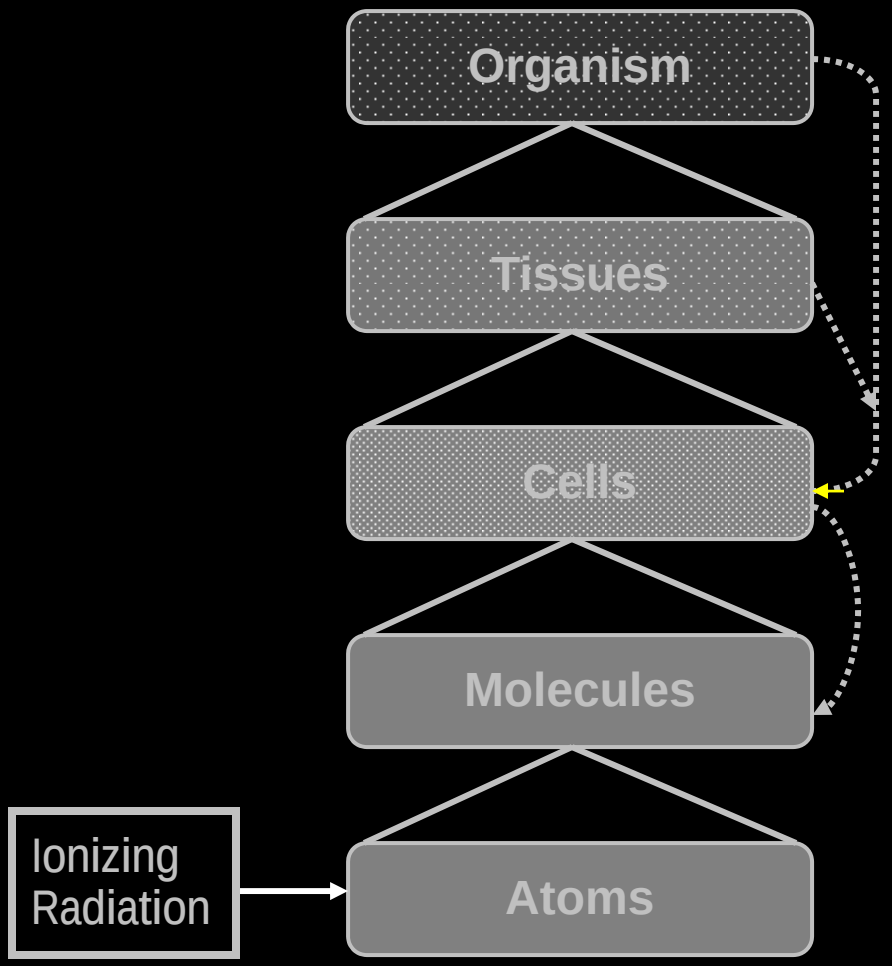


Physiological Barriers Against Disease

Disease

- ***Multiorgan Failure***
- ***Pathology Cancer***

- **Immune Response**
- **Cell Differentiation**
  Apoptosis
- **DNA Repair**
  <u>DEFENSE</u>

Low-Dose Induced ↑ of G-SH - ROS Detoxification

mouse spleen cells assayed in vitro
at various times after 0.5 Gy WB γ-irradiation

Total Glutathione (G-SH + GSSG))

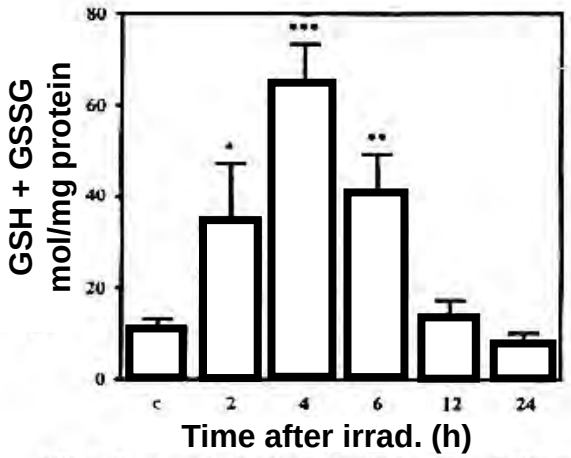


FIG. 1. Changes in levels of total glutathione (GSH + GSSG) in mouse splenocytes after 0.5 Gy of γ radiation. Each bar is the mean ± SD for four mice. *, **, ***Significantly different from the group unirradiated control (c) at $P < 0.05$, $P < 0.01$ and $P < 0.005$, respectively.

Natural Killer (NK) Cells

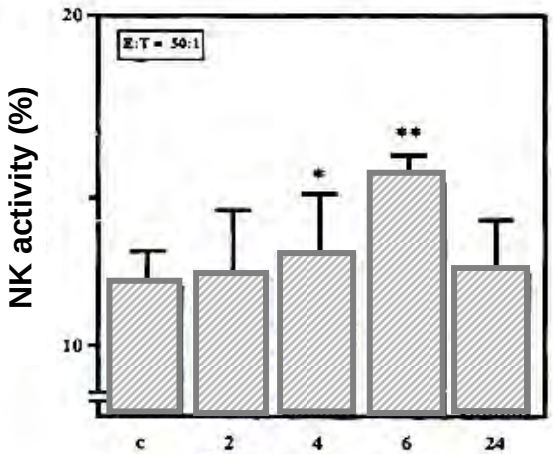
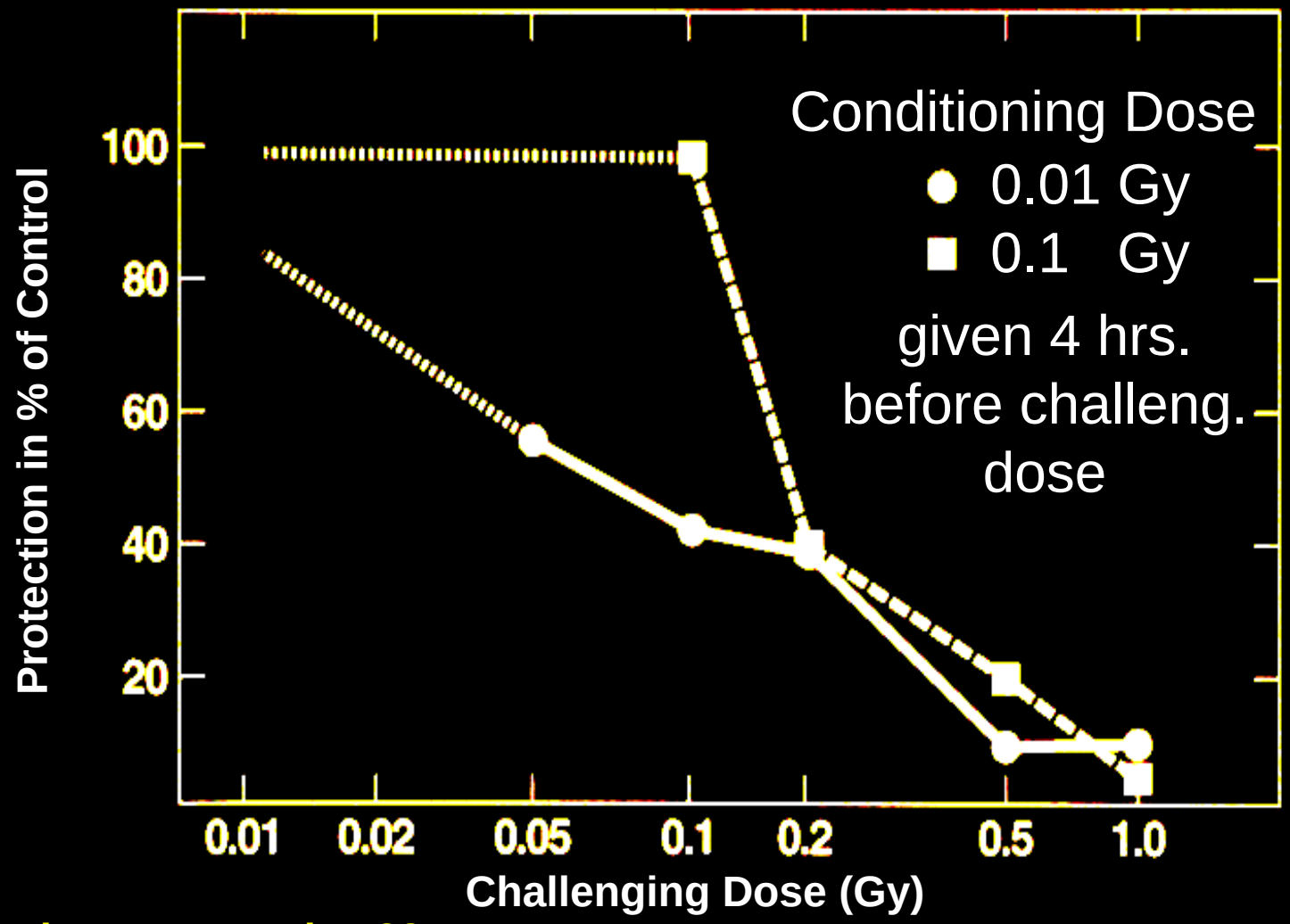


FIG. 2. Effect of low-dose whole-body γ irradiation (0.5 Gy) on the NK activity of mouse splenocytes. Each bar is the mean ± SD four mice. *, **Significantly different from the unirradiated control group (c) at $P < 0.05$ and $P < 0.01$, respectively.

Kojima S et al., Radiat. Res., 2002

Low-Dose Induced Protection ↓ with Dose

γ-radiation-induced TdR-K-inhibition, mouse BM cells



Feinendegen LE et al., 1995

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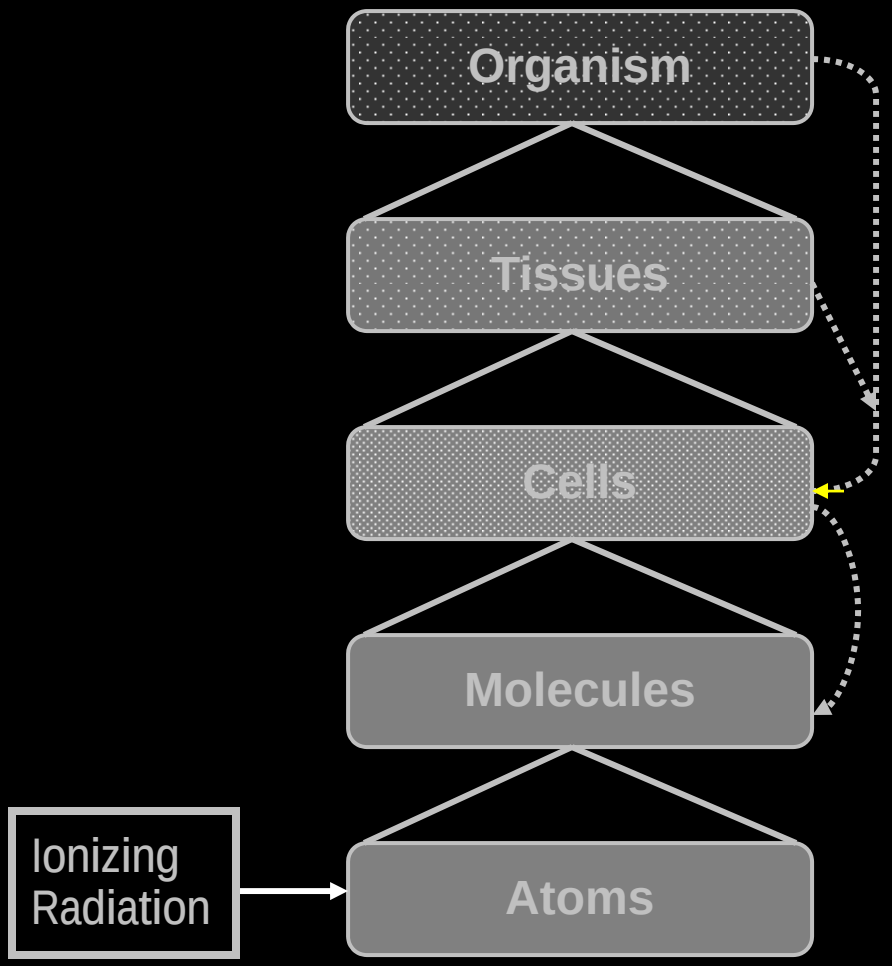

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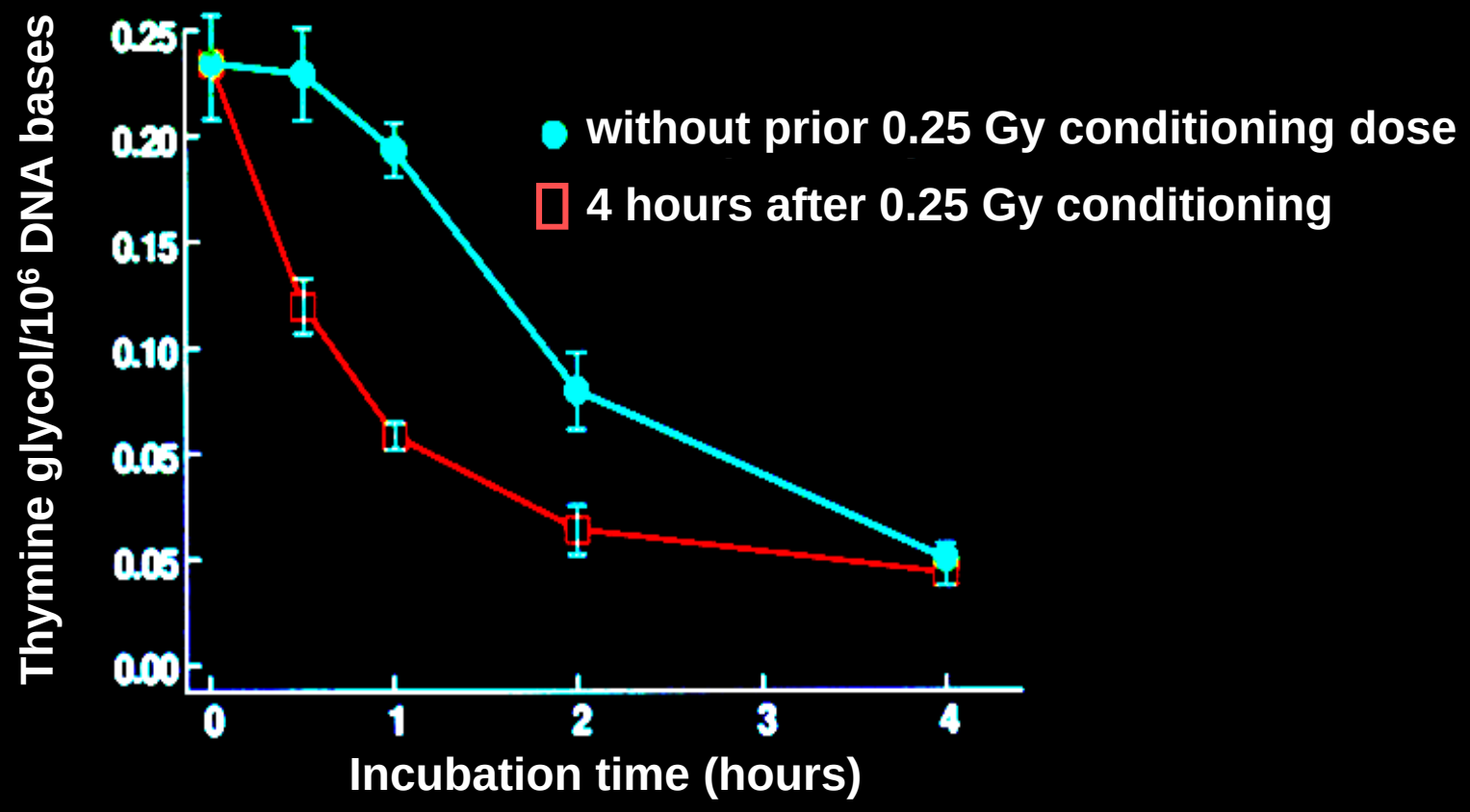
- ***Pathology Cancer***

- **Immune Response**
- **Cell Differentiation**
  Apoptosis
- **<u>DNA REPAIR</u>**
  Defense, Scavenging

Low-Dose Induced ↑ of DNA Repair

human lung cancer cells (A-549) in culture
removal of thymine glycol after 2 Gy γ -radiation

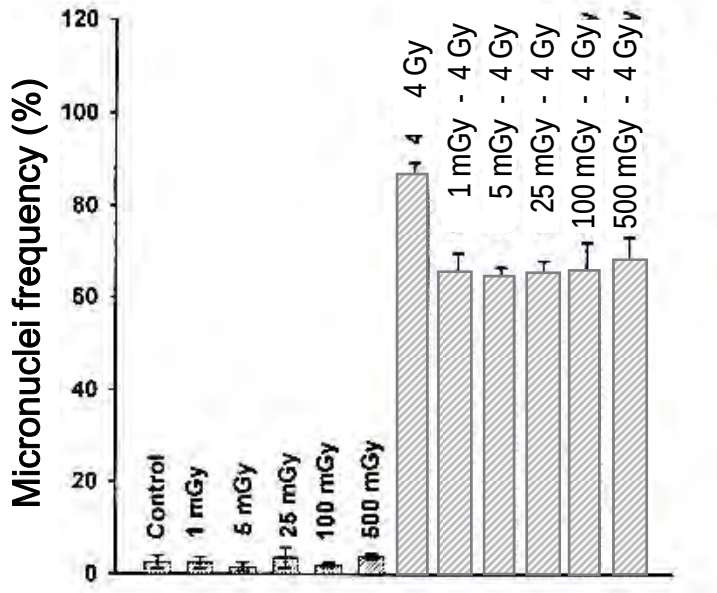


Le X et al., Science, 1996

Low-Dose Induced ↓ of DNA Damage

human fibroblasts micronucleus assay after ^{60}Co γ-irradiation

Conditioning doses: 1 – 500 mGy. Challenging dose 3 hrs later: 4 Gy



Low-dose induced adaptive protection as measured by micronucleus frequency is highly significant - compared to cell response to 4 Gy without adapting dose.

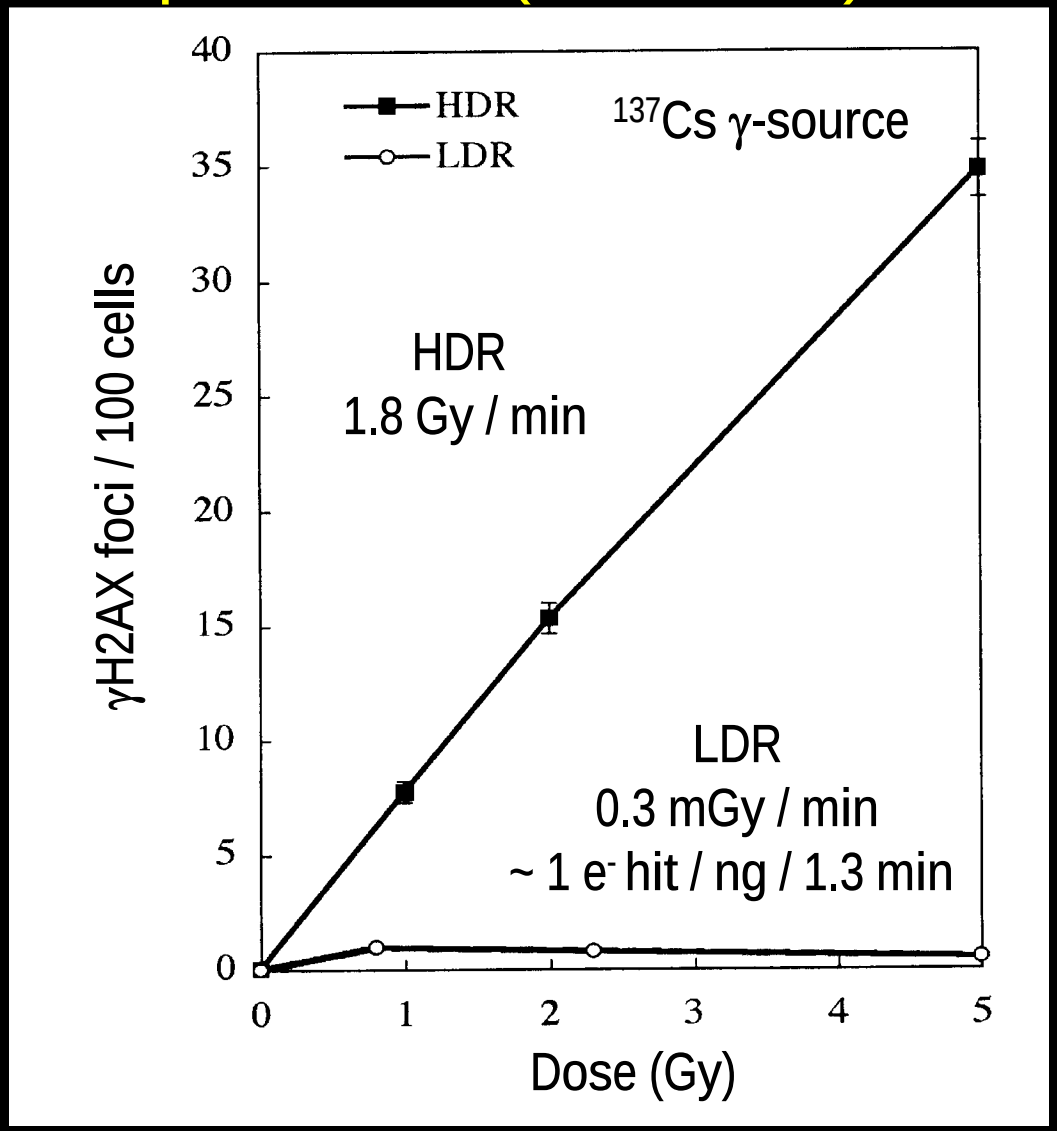
- $p < 0.05$
- *Mean results are given ± std. dev.* - $n = 3$

~25% protection by < 100 mGy seen at 4 Gy

No damage seen < 100 mGy

Broome EJ, Brown DL, Mitchel REJ, Radiat. Res., 2002

Low Dose Rate Induced DNA Repair
human diploid cells (SuSa/T-n) in culture



Ishizaki K et al.
J. Radiat. Res.
45: 521-525, 2005

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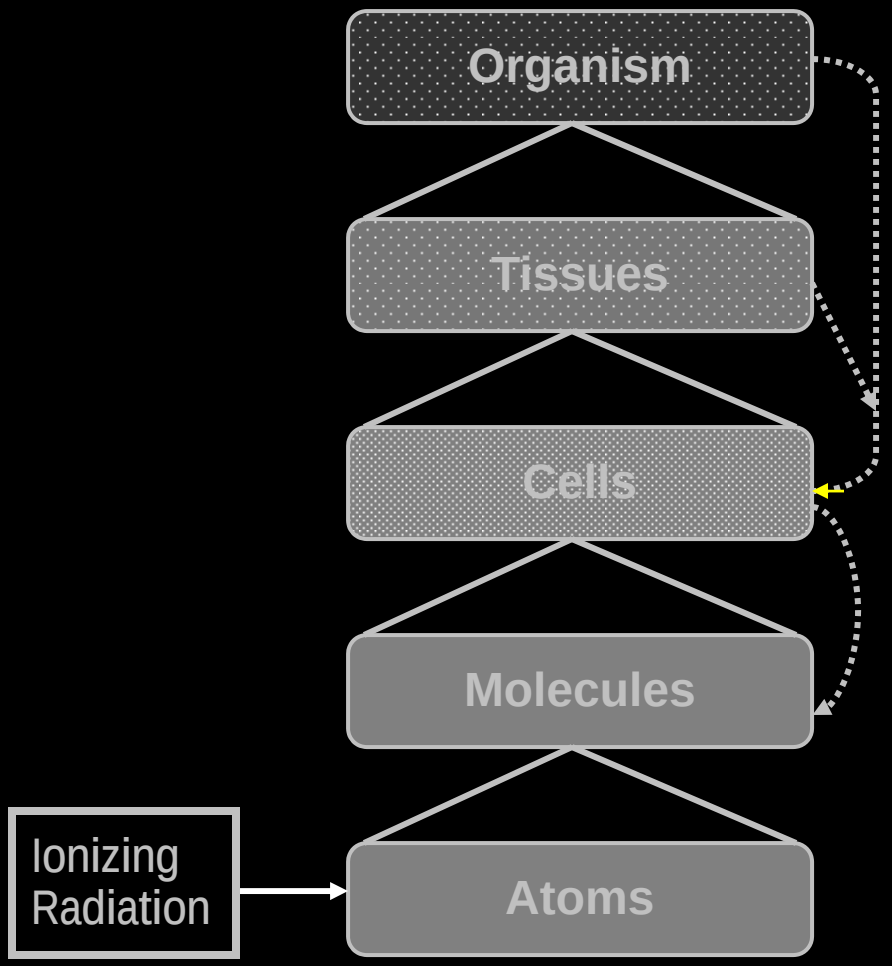

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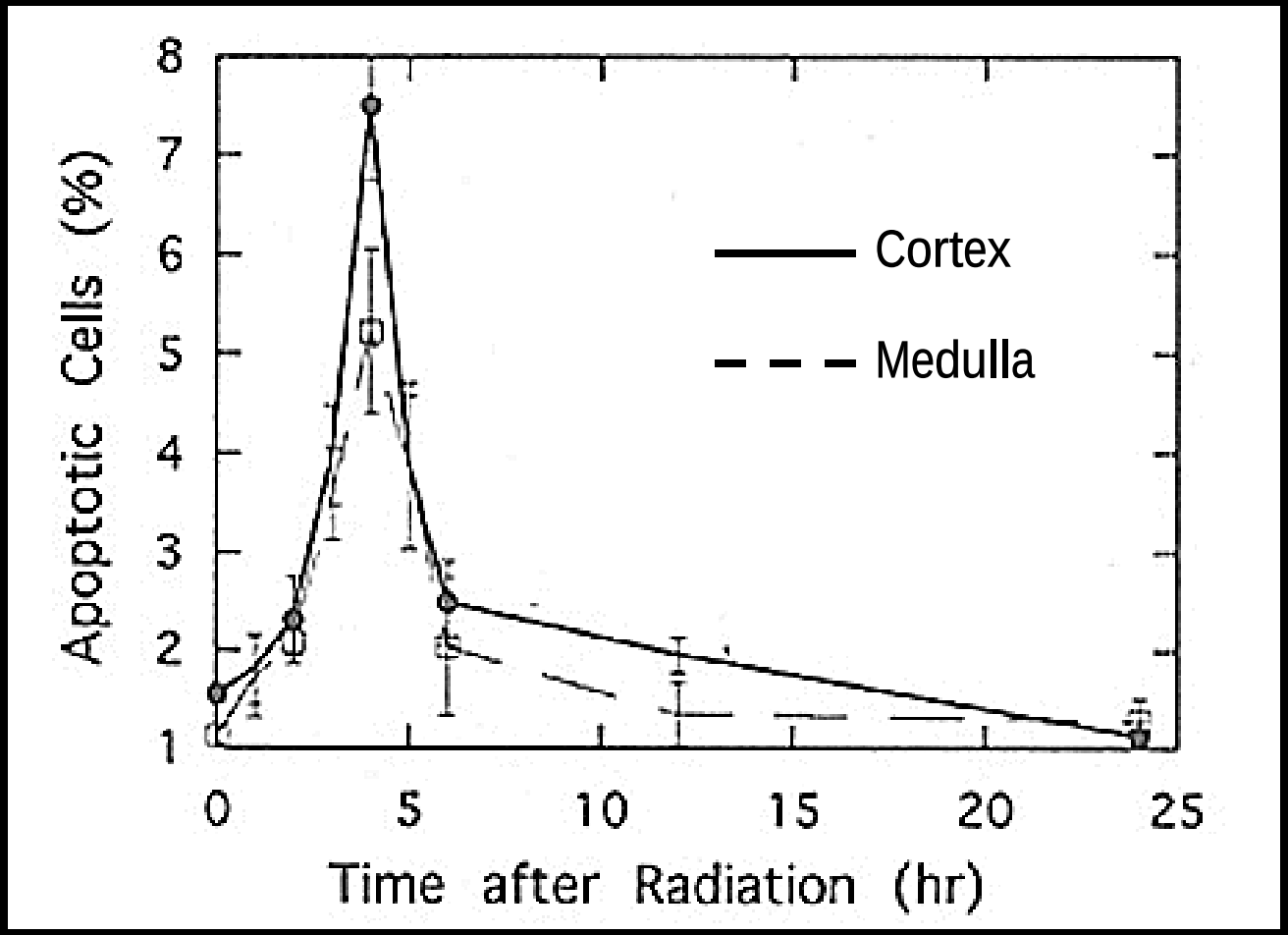
- ***Pathology Cancer***

- **Immune Response**
- **Cell Differentiation**
  <u>APOPTOSIS</u>
- **DNA Repair**
  Defense, Scavenging

Radiation-Induced ↑ of Apoptosis

0.5 Gy WB γ-irradiation mice, thymus, *in vivo*



Fujita et al., in Apoptosis, Business Ctr. Acad. Soc. Japan, 1998

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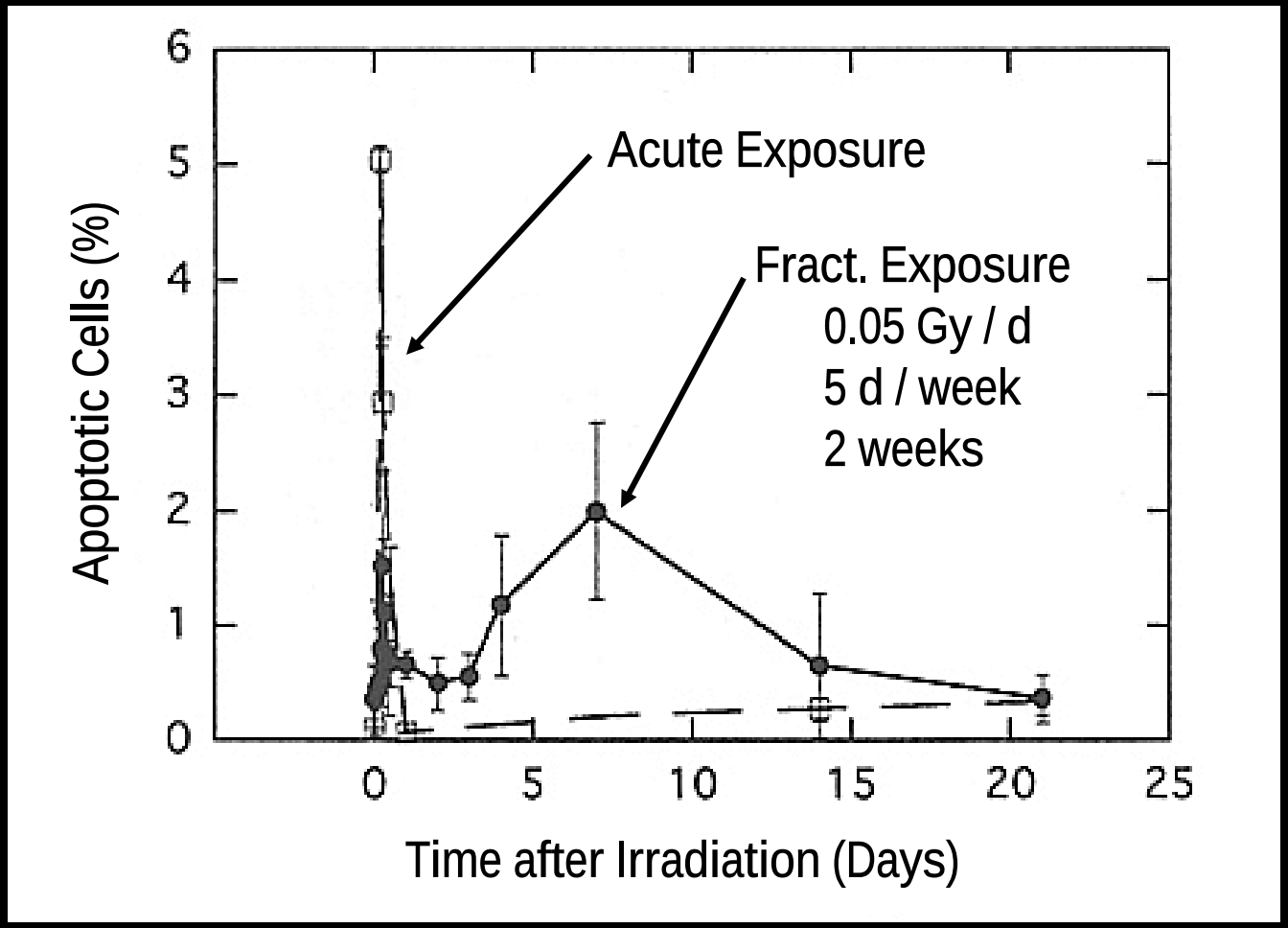


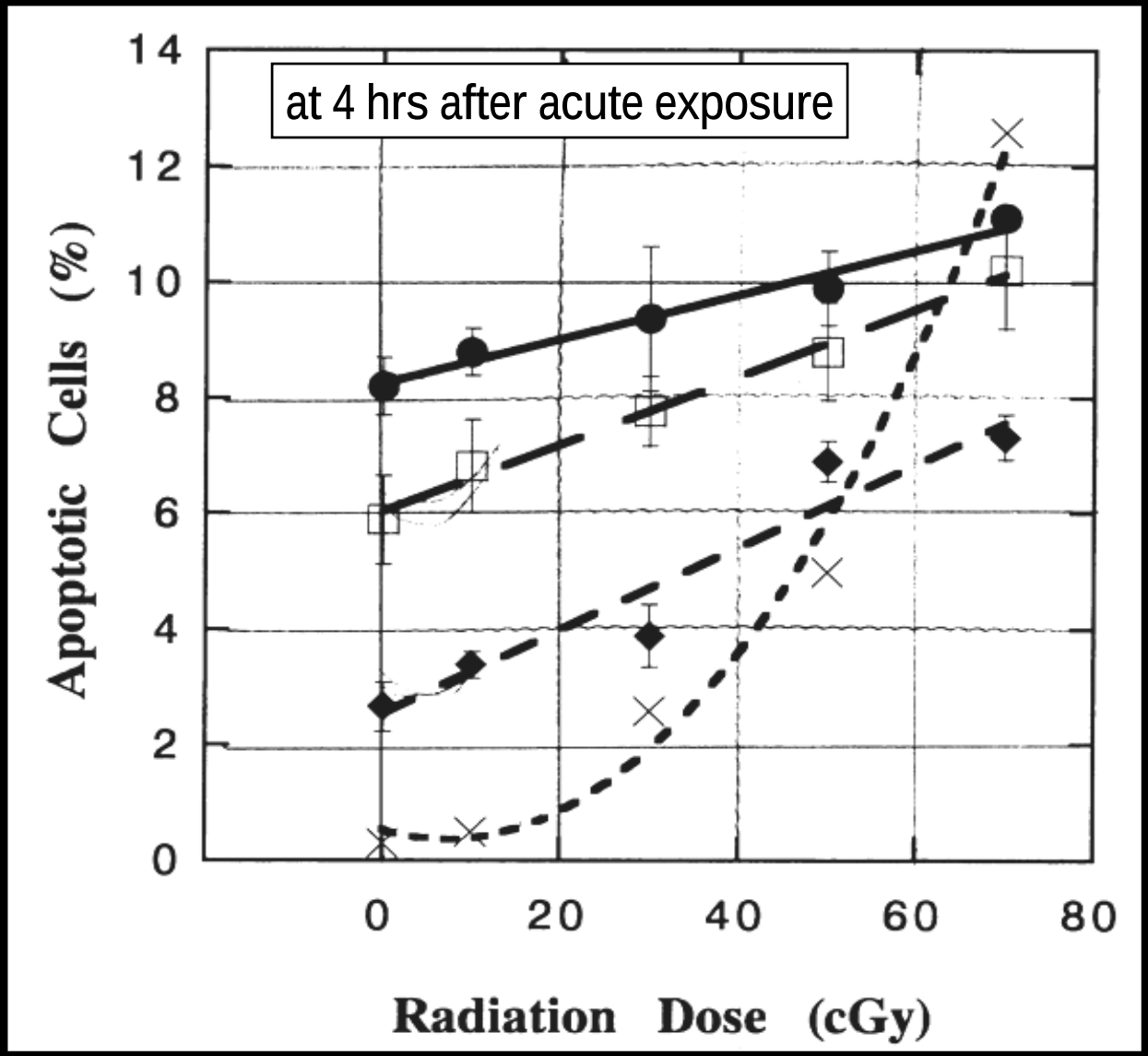
Fujita et al., in Apoptosis, Business Ctr. Acad. Soc. Japan, 1998

Apoptosis Incidence ↑ with Dose

Mouse
Thymus
(WB γ-irrad.)

Detection methods:

- ● TUNEL (TdT)
- □ ISEL
- ◆ HE Stain
- X Flow Cytometry



Ohyama H, Yamada T, in Apoptosis, Business Ctr. Acad. Soc. Japan, 1998

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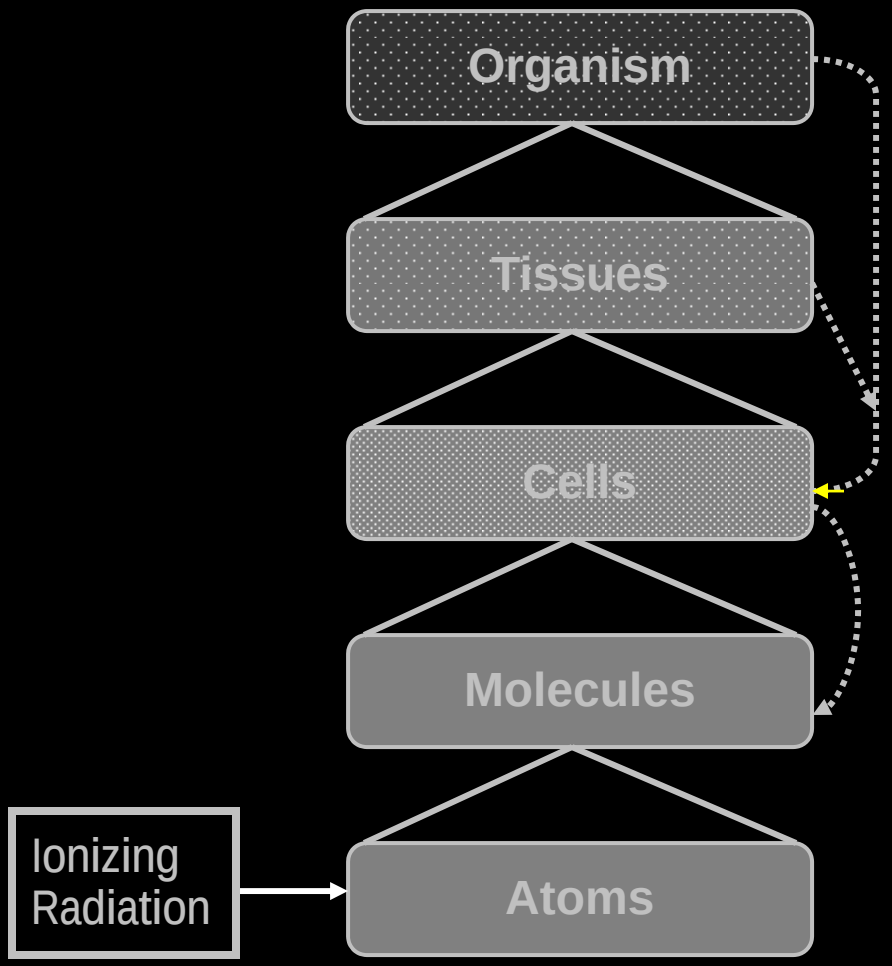

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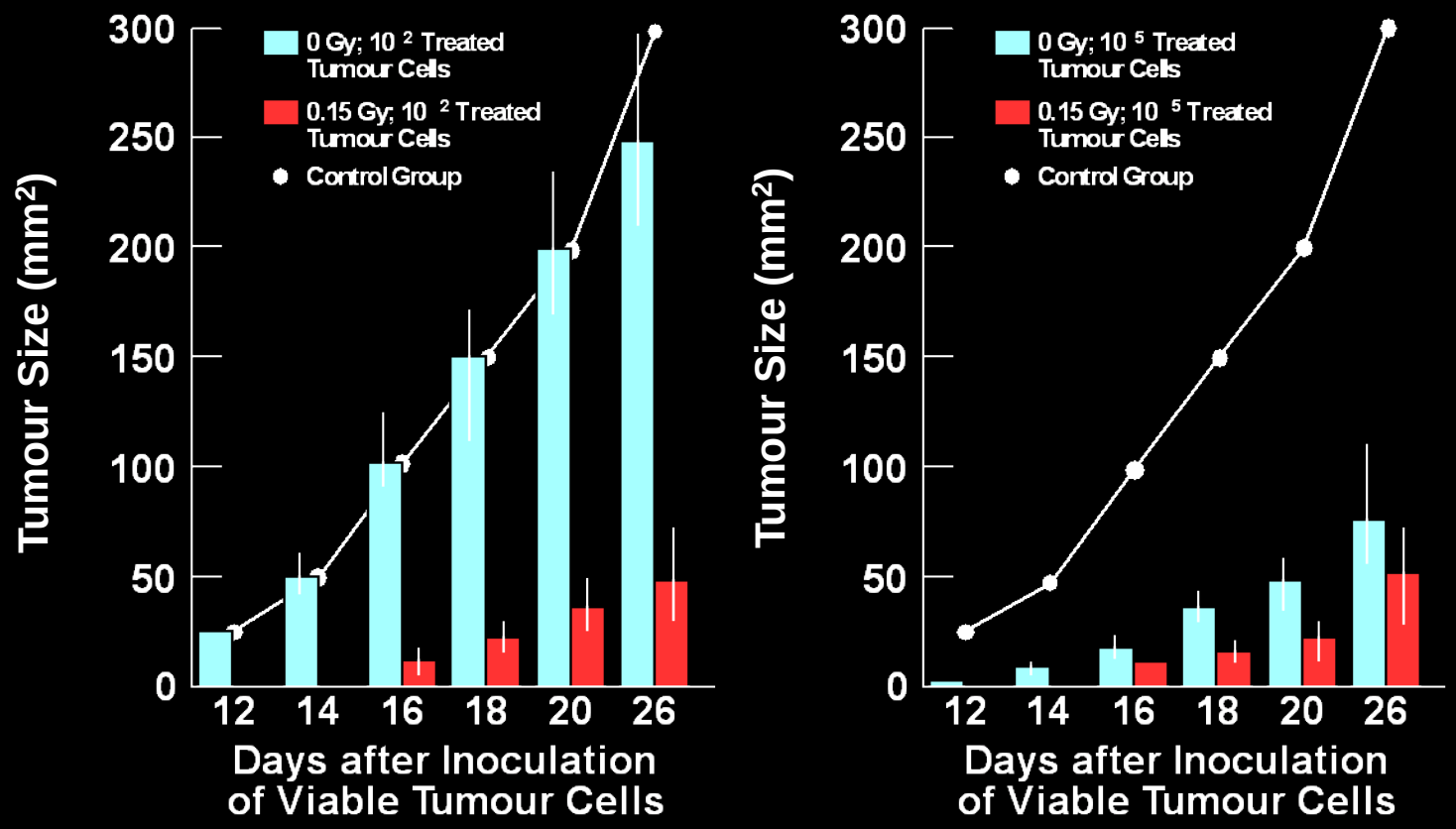
Disease

- ***Multiorgan Failure***
- ***Pathology Cancer***

- **IMMUNE RESPONSE**
- **Cell Differentiation**
  Apoptosis
- **DNA Repair**
  Defense, Scavenging

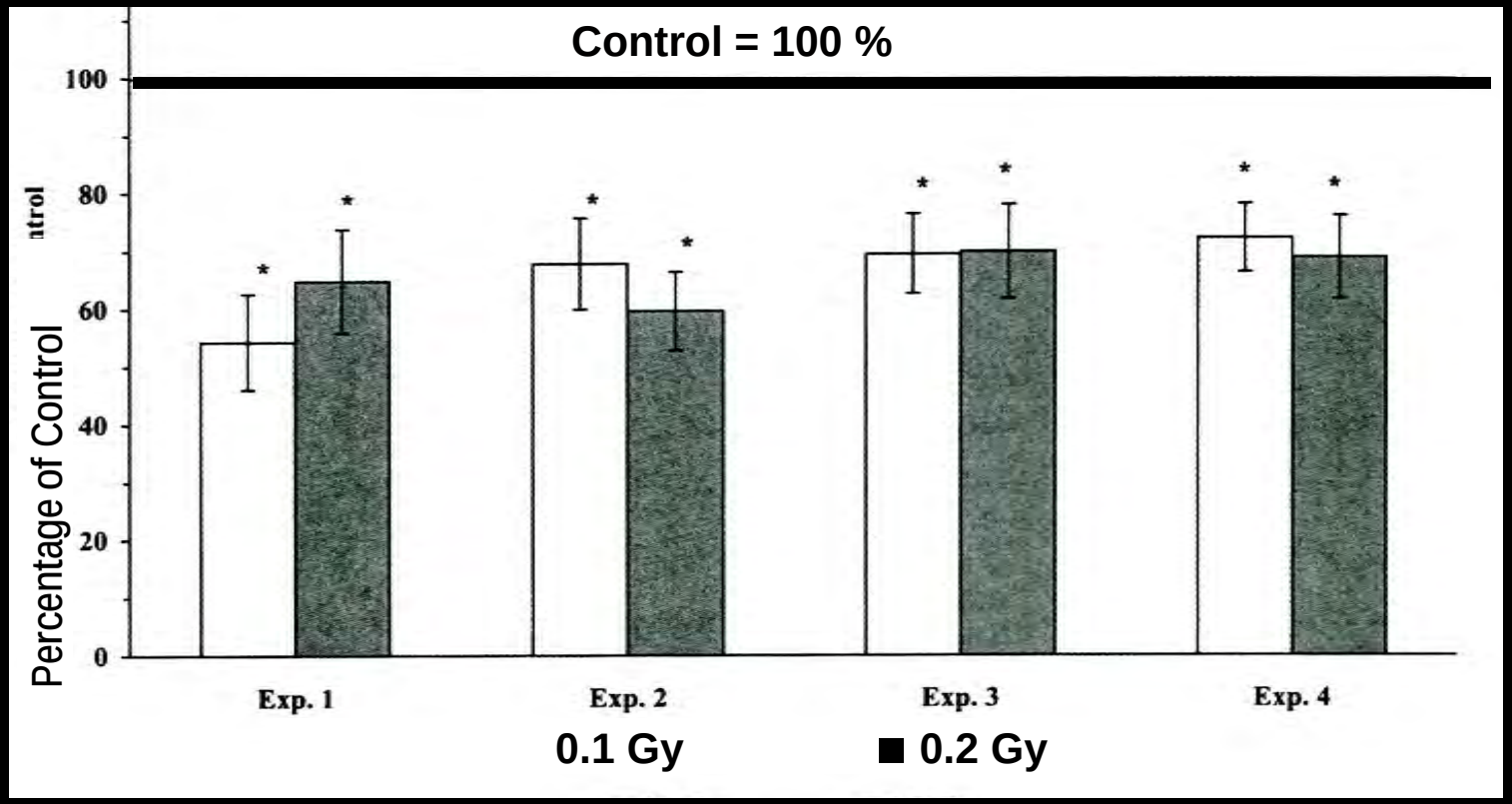
Low-Dose Induced ↑ of Immune Response Mice



A/J mice received s.c. either 10^2 or 10^5 non-viable fibrosarcoma (Sal) cells with or without WB γ-irradiation with 0.15 Gy → 3 wks: 10^4 viable Sal cells transpl. → for 26 days: tumor sizes measured - (n = 60 mice / group)

Anderson et al., 1986; UNSCEAR, 1994

Low-Dose Induced ↓ of Lung Metastases Mice



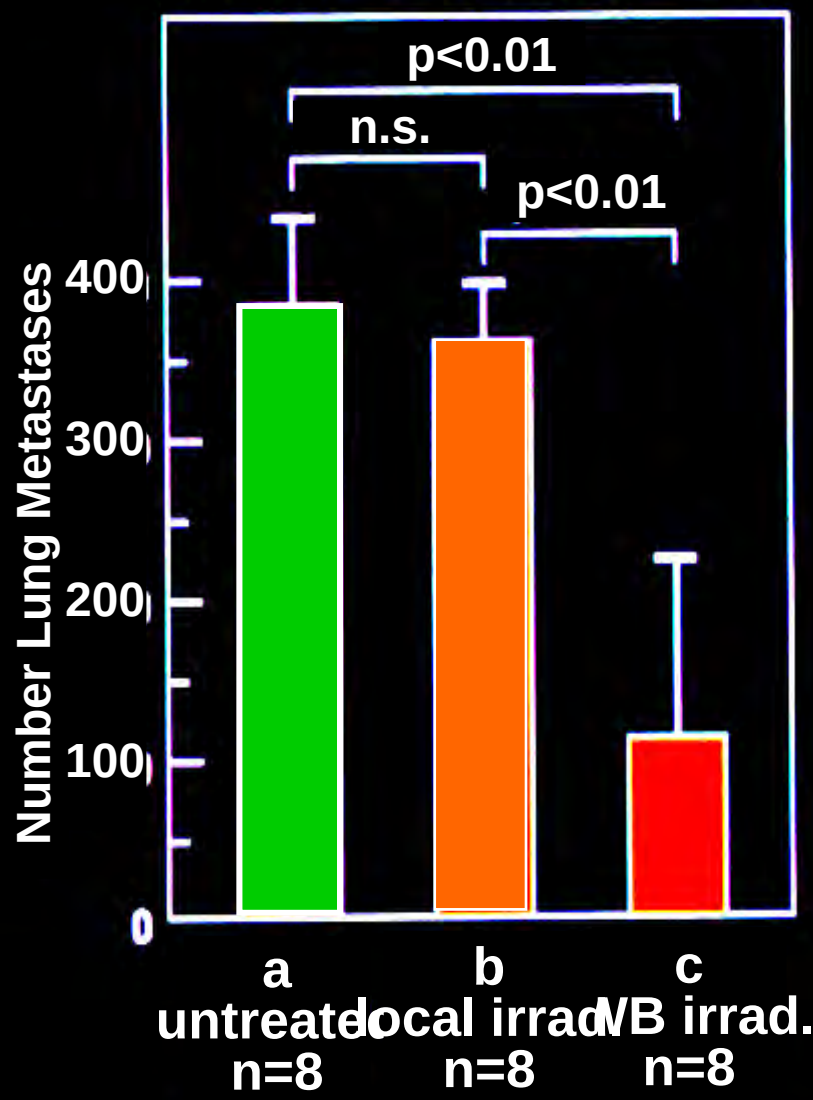
BALB/c mice received WB x-irradiation with 0.1 or 0.2 Gy
→ 2 hrs.: sarcoma cell transpl. → at 2 wks: lung nodes count - (n = 12 mice / group)

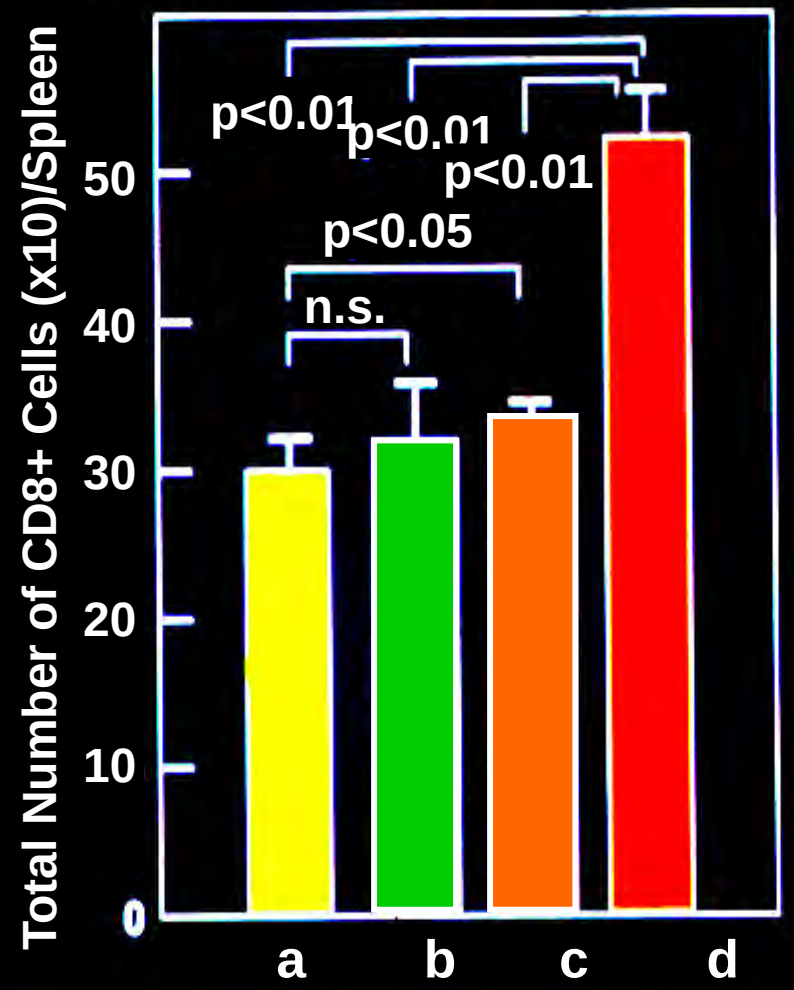
L 1 sarcoma cells are nonimmunogenic for BALB/c mice

Cheda A et al., Radiat. Res. 161: 335-340 (2004)

Low-Dose Induced ↑ of Immune Response Rats

hepatoma impl.→ 2 weeks: 0.2 γ-irrad.→ 4 weeks: counts





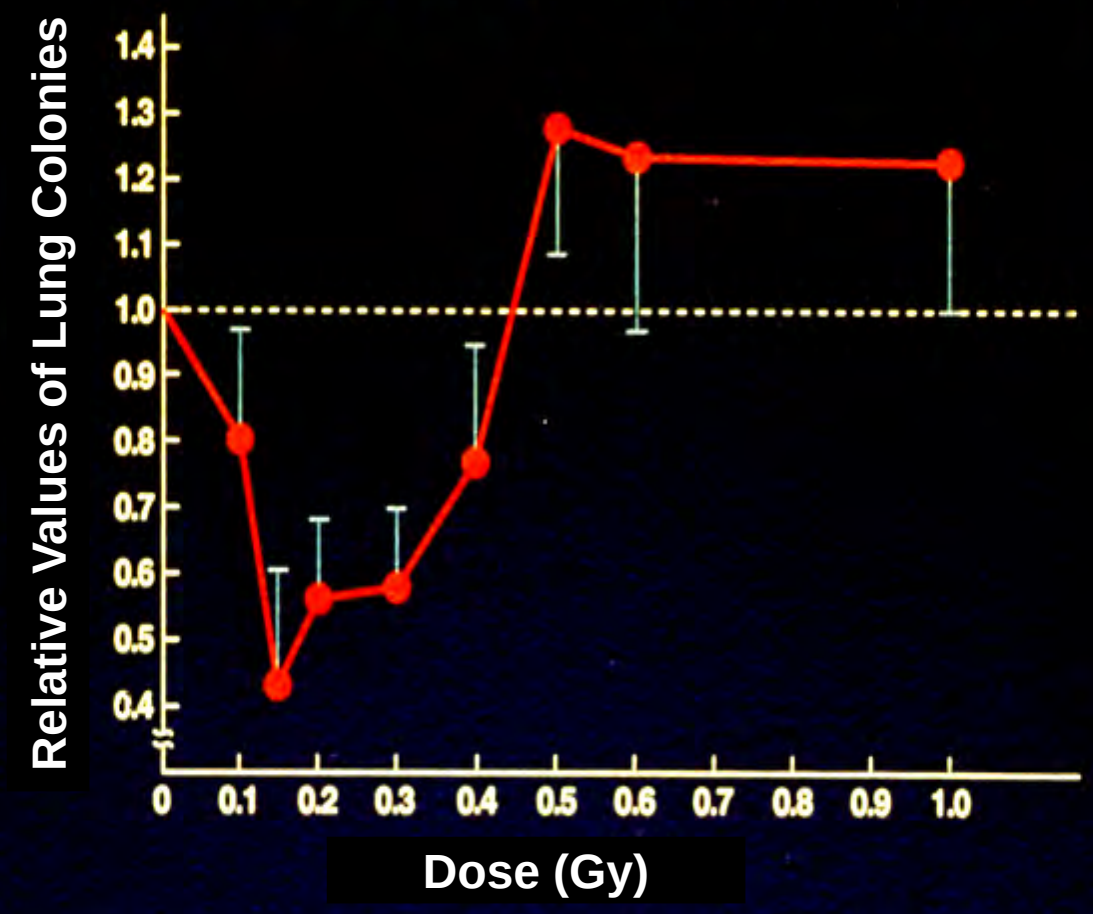
a): normal rat; b) tu: not treated; c): tu: local irrad.; d) WB irrad.

Hashimoto S et al., Radiat. Res., 1999

Low-Dose Induced ↓ of Lung Metastases Mice

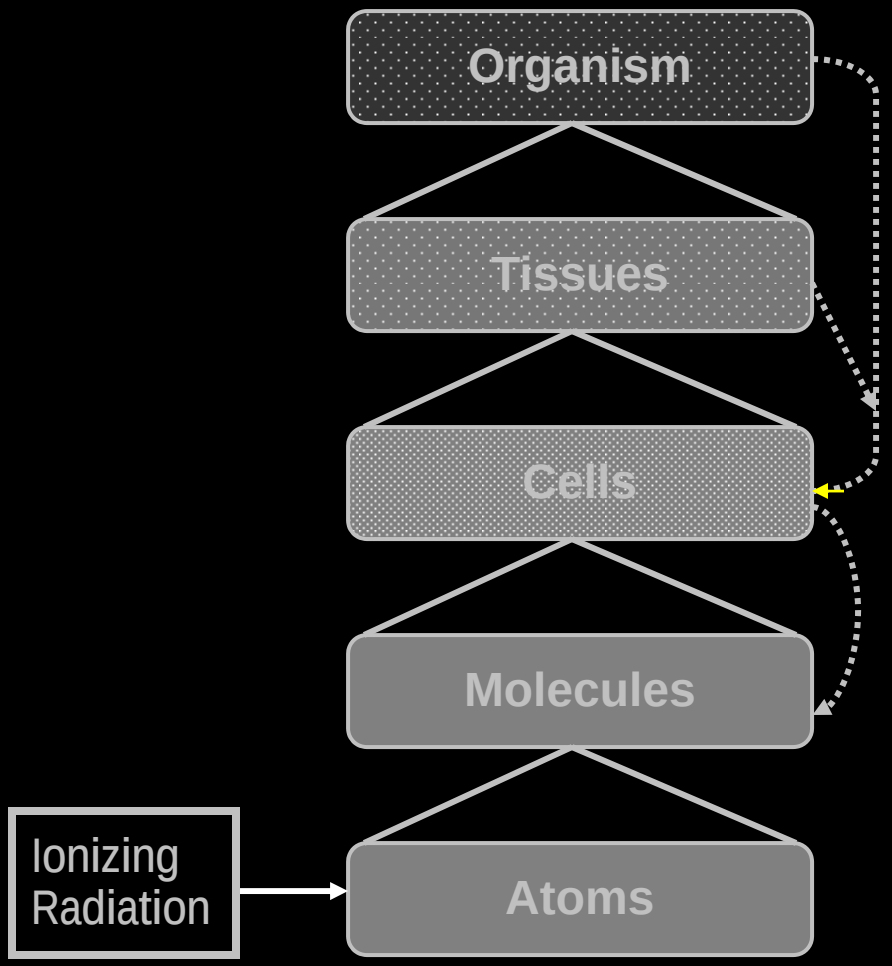
tumor cell transplantation into axilla →

12 days: WB γ-irradiation → 20 days: lung nodes count



Adapted from Sakamoto et al., Jpn. Soc. Ther. Radiol. Oncol., 1997

Complex Adaptive Systems at Various Levels



Physiological Barriers Against Disease

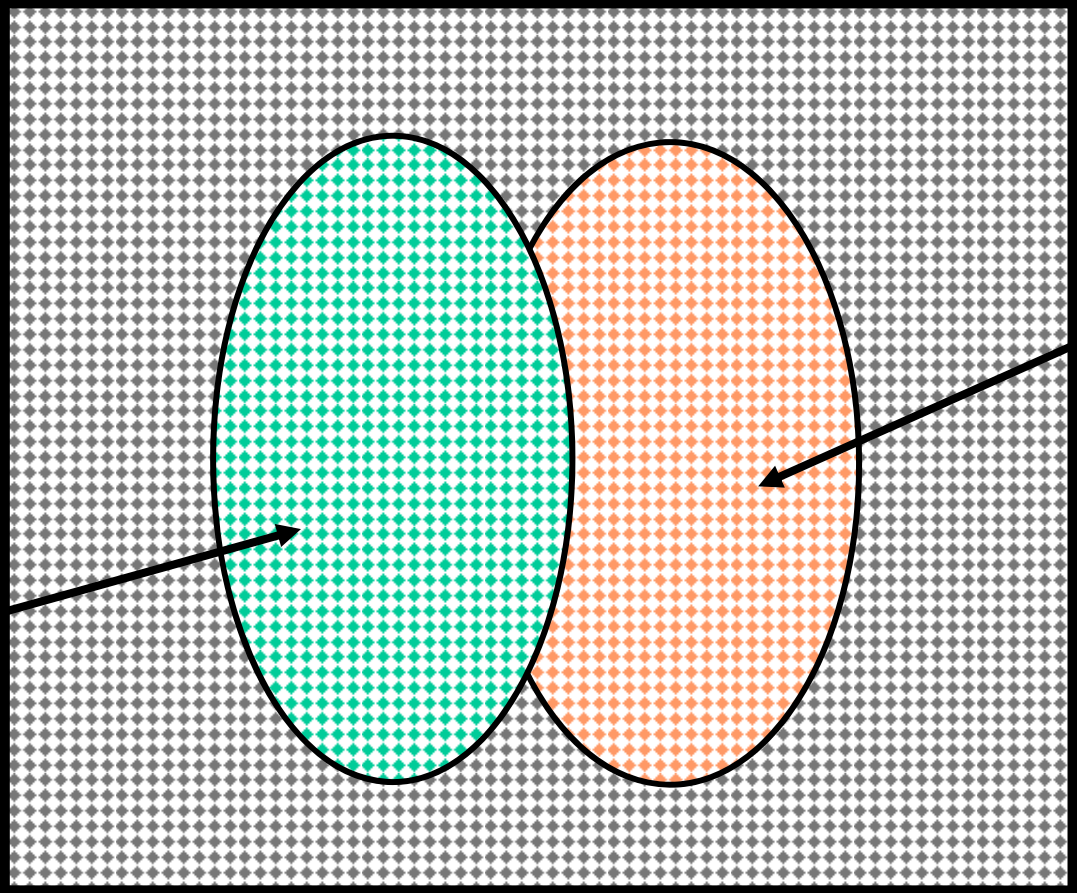
Disease

- ***Multiorgan Failure***
- ***Pathology Cancer***

- **Immune Response**
- **Cell Differentiation**
  Apoptosis
- **DNA Repair**
  Defense, Scavenging

Gene Expression in cDNA Microarray Analysis in low and high dosed normal human skin fibroblasts

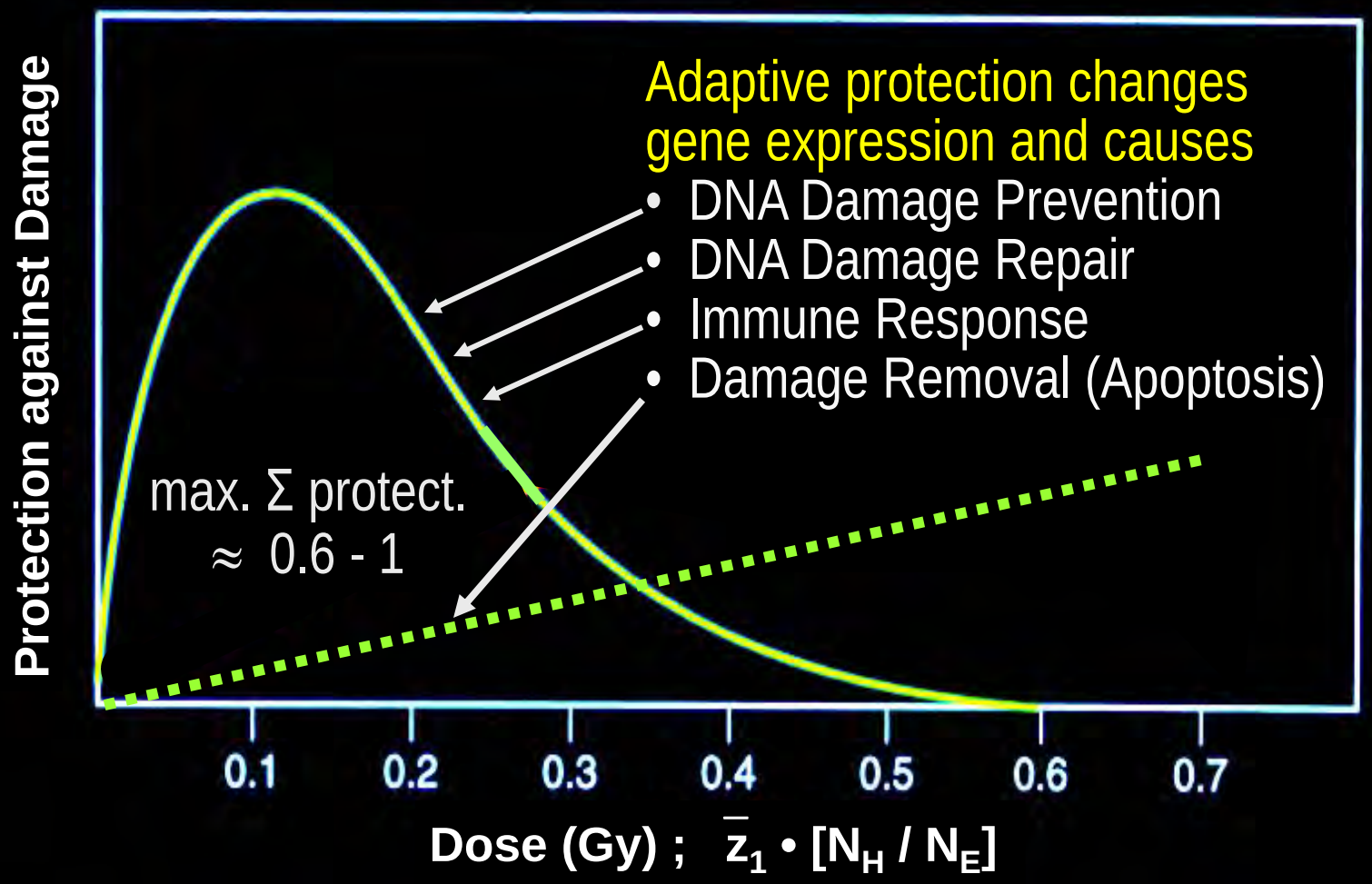
~ 2 hours after γ-irradiation



Golder-Novoselsky E et al., DOE Workshop, 2002
Yin E et al., Int. J. Radiat. Biol. , 2003

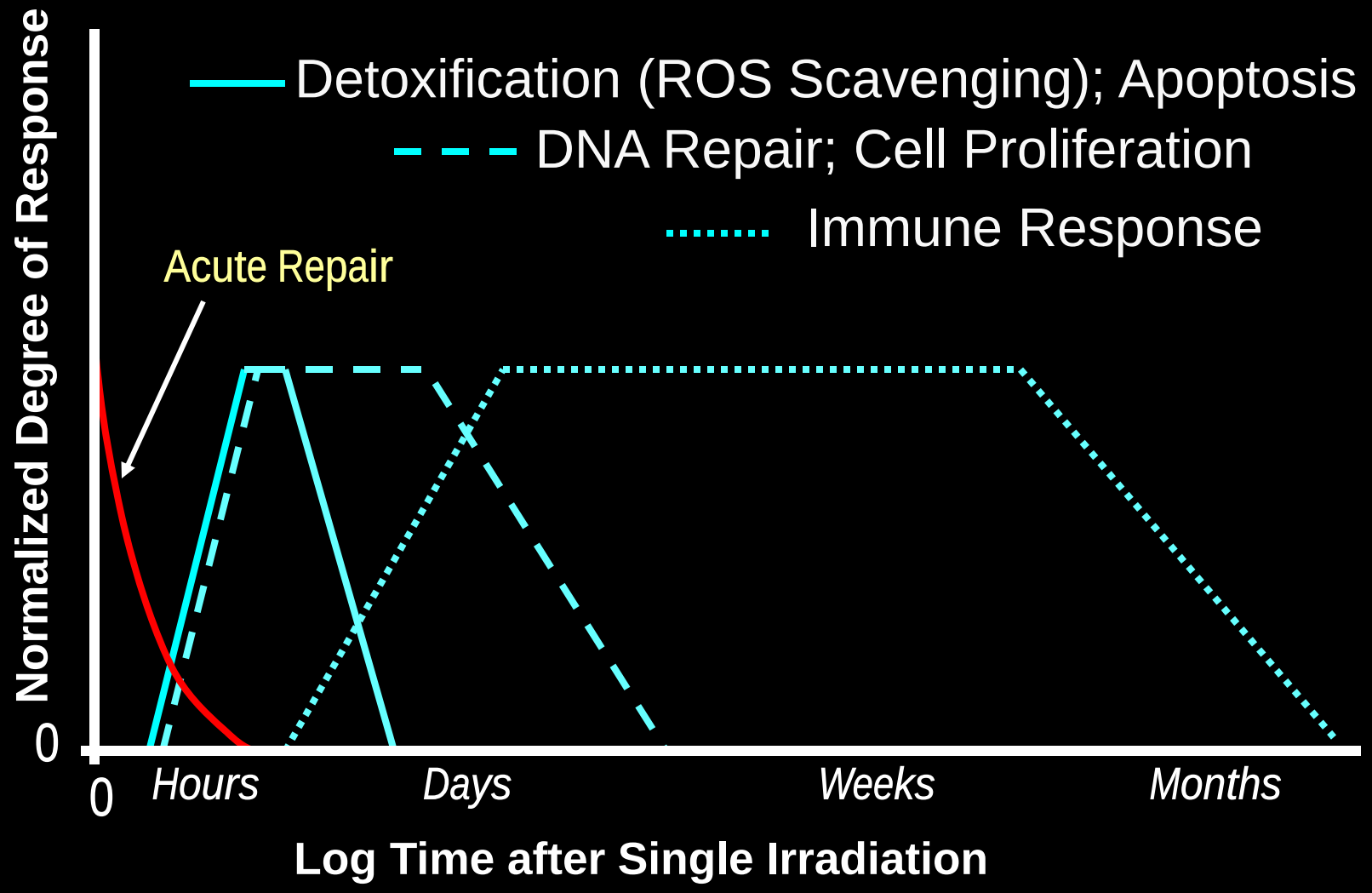
Low-Dose (Low-LET) Induced Adaptive Protection

scheme of dose-response functions



Low-Dose (Low-LET) Induced Adaptive Protection

scheme of durations of protection (t_p)



Agenda

Dose	1. Dose and energy deposition events
	2. Choice of primary target
Effects	3. Damaging effects
	4. Adaptive Protection
	5. Projected net effect on cancer
Analysis	6. A Model

Cancer develops

on the basis of DNA damage.

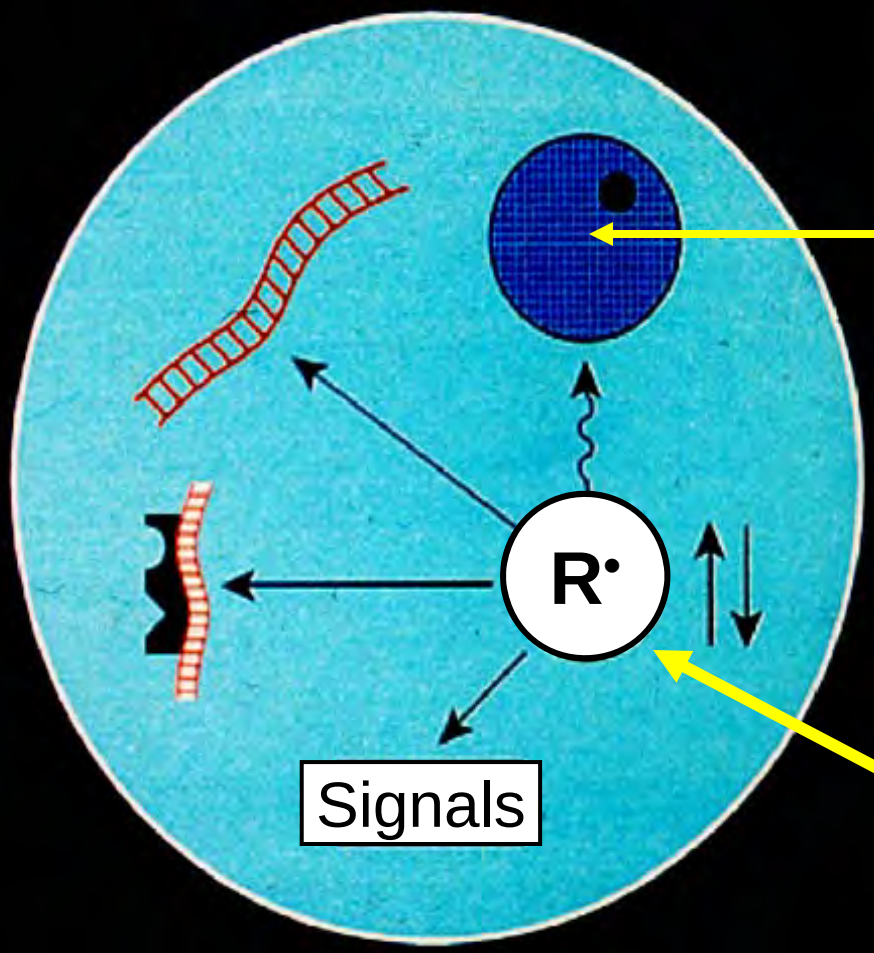
Most of this damage comes from

endogenous sources,

largely through

oxygen metabolism.

Reactive Oxygen Species (ROS) by Metabolism



Normal Cell

~ 10^9 ROS ($R^{\bullet}$)
per cell per day →
~ 10^6 DNA alterations,
with ~ 0.1 DSB

Lipid peroxidation

Protein-carbonylation

Cytoskeletal disruption

Perturbs Ca^{2+} homeostasis

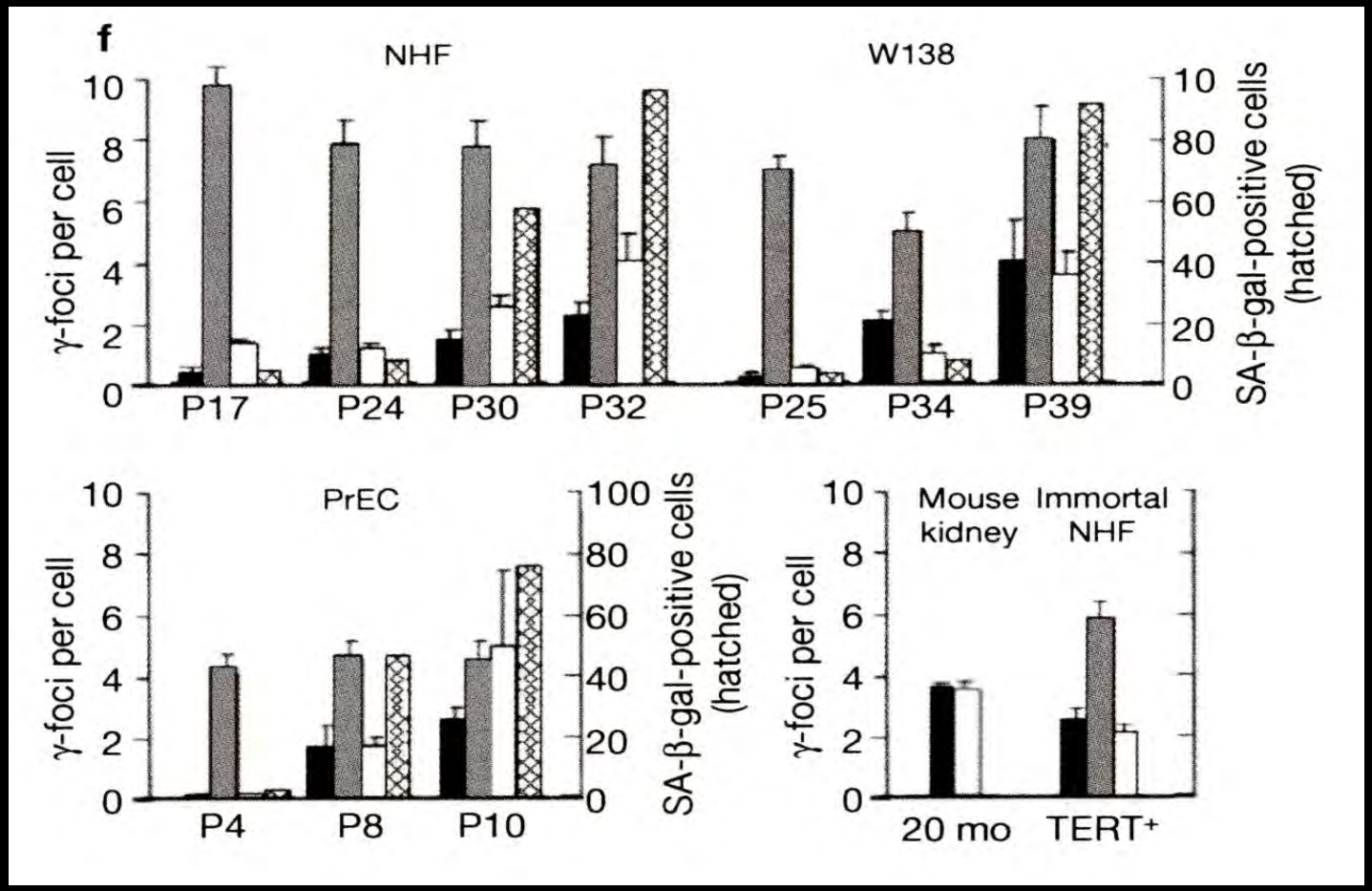
Interfers with cell signaling

Activates apoptosis

Pollycove M, Feinendegen LE, 2003

Orrenius S et al., 2000

Occurrence of γ-H2AX Foci (DSB) ↑ with Age



Black bars: Non-irradiated cells

White bars: 24 hrs after irradiation

Shaded bars: 30 min after 0.6 Gy γ-irradiation

Hatched bars: Fraction of SA-β-gal-pos. cells (indicator of senescence)

Sedelnikova OA et al., Nature Cell Biol., 2004

Ratio of DNA Alterations per Cell per Day

E = alterations from endogenous sources
(mainly reactive oxygen species, ROS)

R = total alterations from background radiation
(~1 mGy/year x-rays):

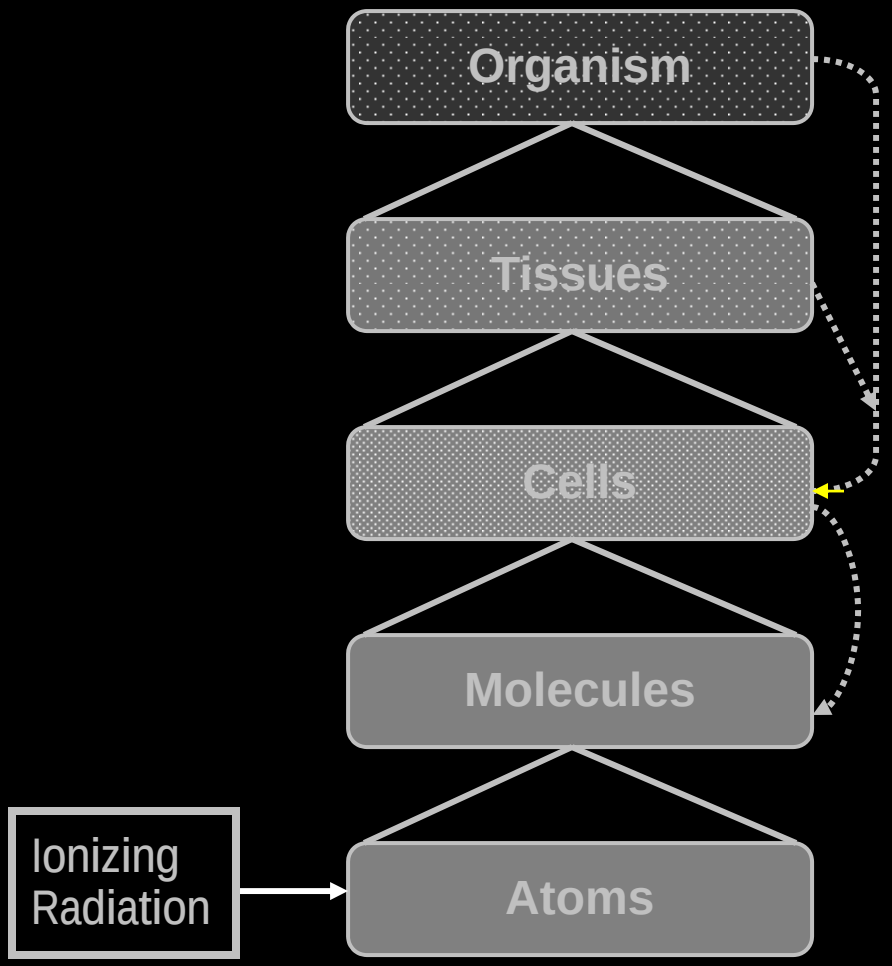
E/R: Total alterations ~ 10^7

E/R: Total DSB > 10^3

More than 97 % of human cancers are non-radiogenic

Pollycove M, Feinendegen LE, 2003; Vilenchik MM, Knudson AG, 2003

Complex Adaptive Systems at Various Levels



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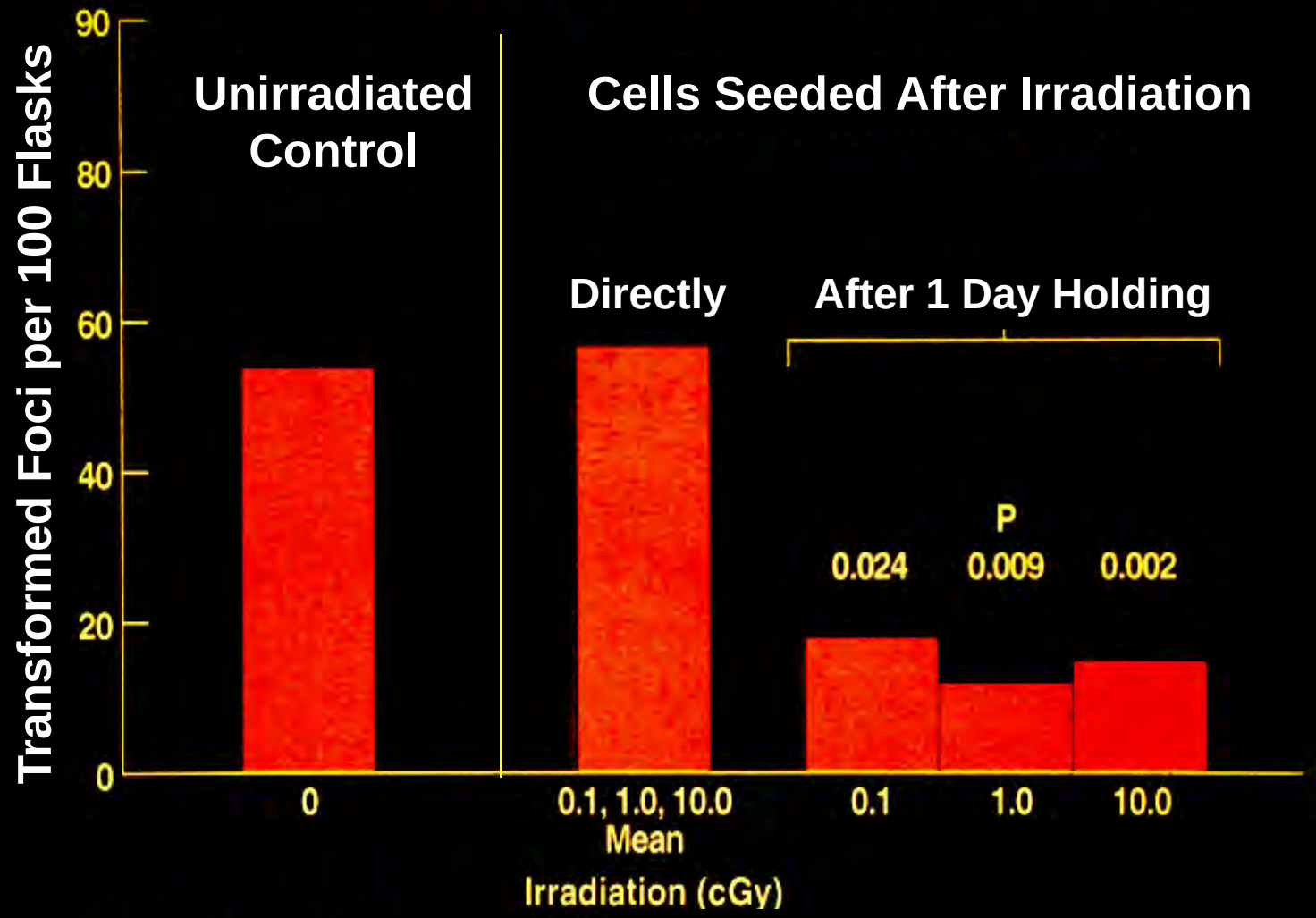
- ***Multiorgan Failure***
- ***Pathology***
- ***Cancer***

- **Immune Response**
- **Cell Differentiation**
  Apoptosis
- **DNA Repair**
  Defense, Scavenging

Low-Dose Induced ↓ of Spontaneous Transformation

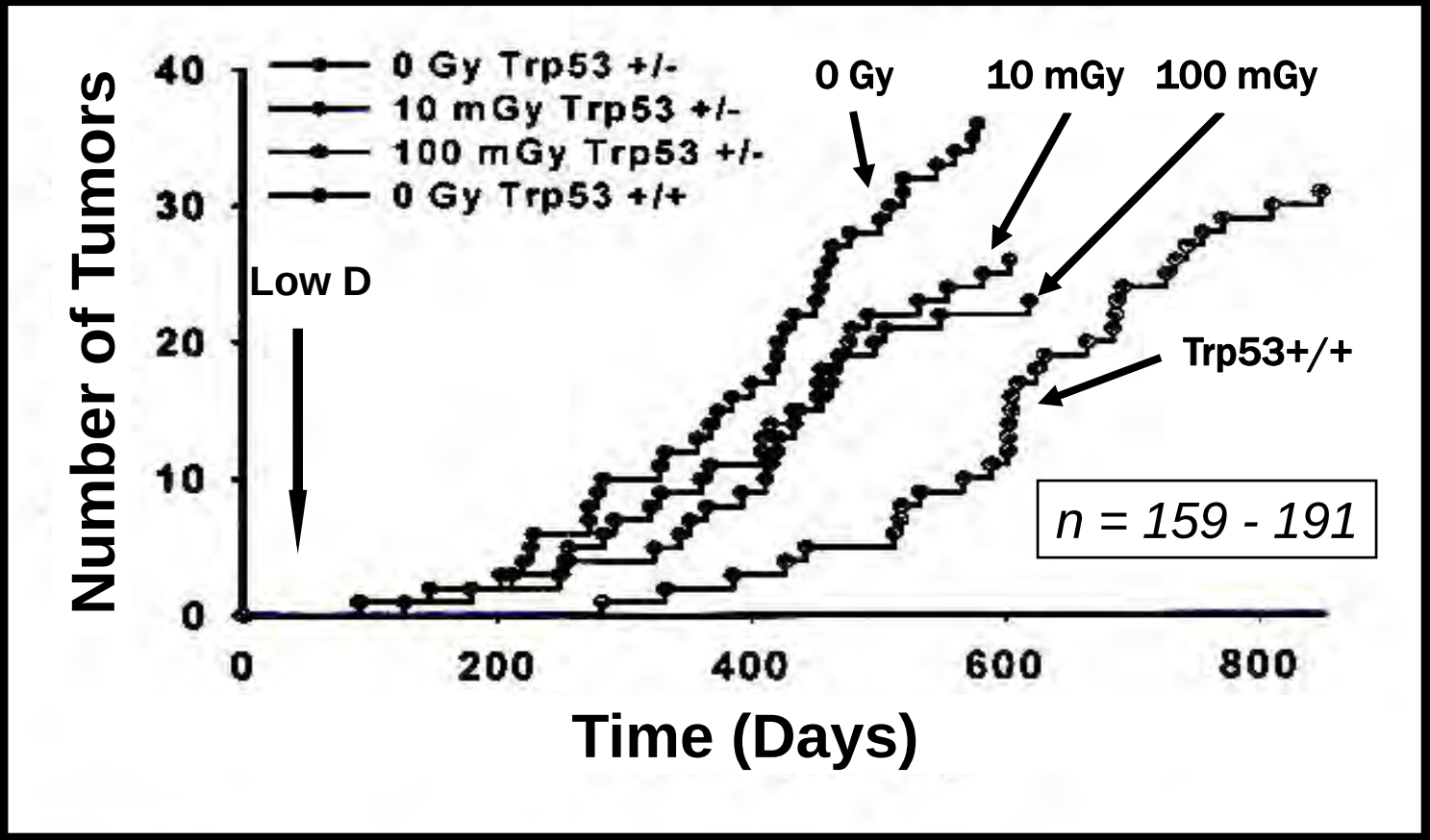
C3H10 T ½ cells after ^{60}Co γ-irrad. with 0.1, 1.0, 10 cGy, vs. control



Azzam EI et al., Radiat. Res. 1996

Low-Dose Induced ↓ of Spont. Tumor

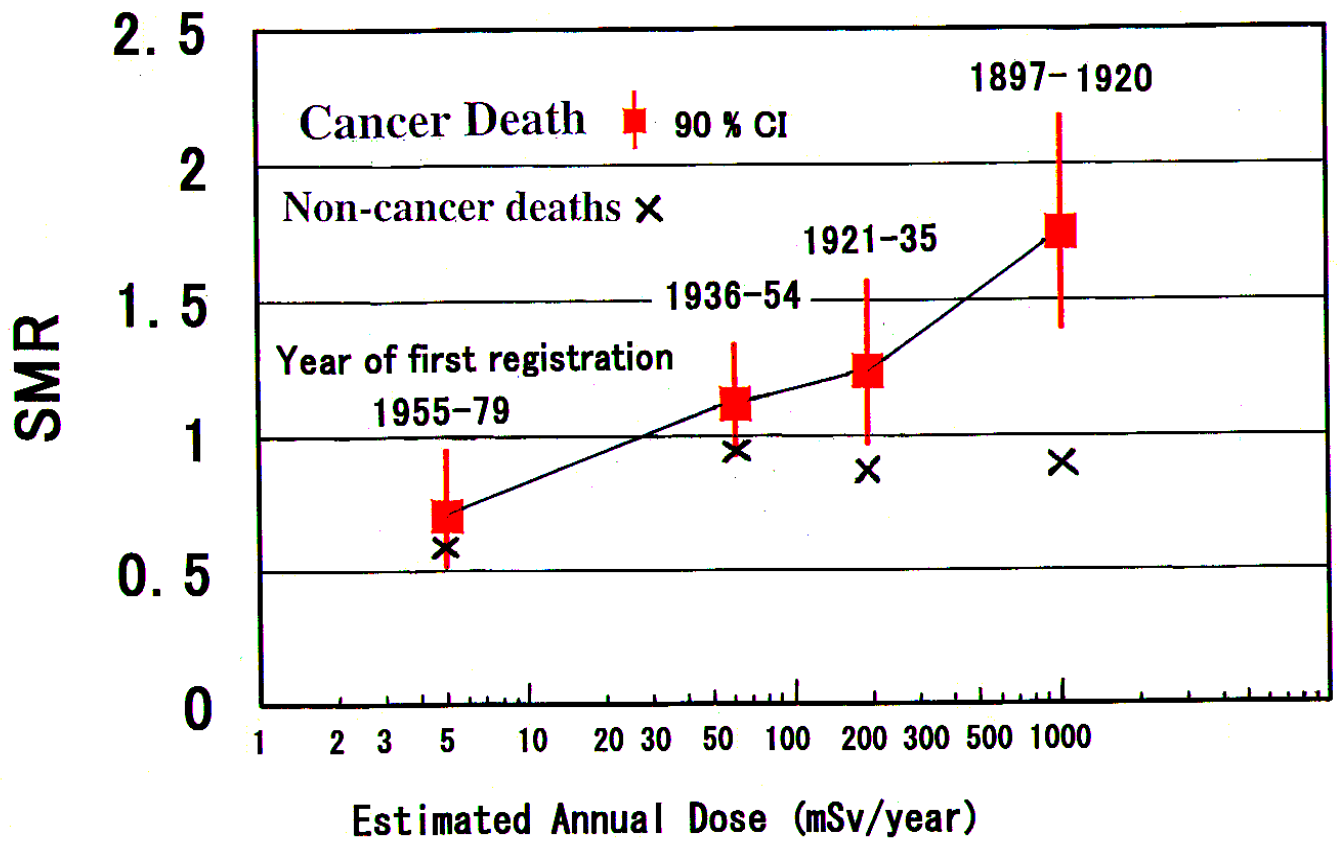
lymphoma in Trp 53 +/- mice after single WB γ-irrad. at age ~ 2 months



Mitchel REJ et al., Radiat. Res., 2003

SMR for British Radiologists (100 Years Study)

compared with medical practitioners



By Courtesy: Kaneko M, 2004; adapted from British J. Radiology, 2001

Atkinson WD et al. published in 2004 the mortality among 51 367 employees of the UK Atomic Energy Authority, from 1946-1997.

The all cancer mortality
was significantly lower for radiation workers than for non-radiation workers.

Atkinson WD et al., Occup. Environ. Med. 61: 577-585, 2004

Zablotska LB et al. in 2004 published the mortality among 45,468 Canadian nuclear power industry workers after chronic low-dose exposure to ionizing radiation:

For all solid cancers combined, the categorial analysis shows a significant reduction in risk in the 1- 49 mSv category compared to the lowest category (<1 mSv) with a relative risk of 0.699 (95% CI: 0.548, 0.892).

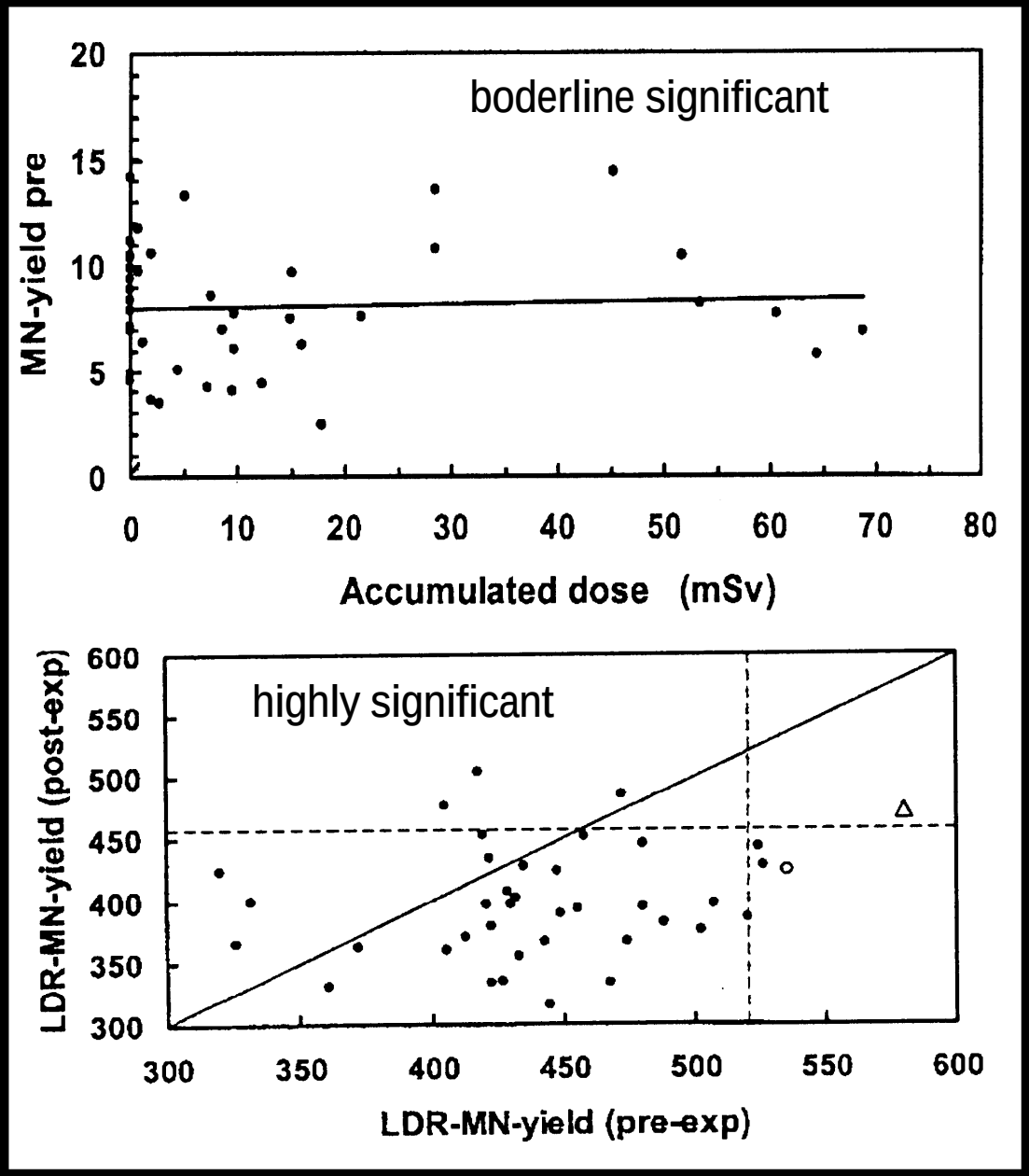
Above 100 mSv, risk appeared to increase.

Zablotska LB et al., Radiat. Res. 161: 633-641, 2004

DNA Damage
in human lymphoc. of 41 exp.workers

in vivo

Above cells show
enhanced DNA repair
upon Co^{60} irrad. in vitro
with 4 mGy / min
(1 hit / ng q ~ 4.6 sec)
accum. D = 3.5 Gy



Thierens H et al. , Int. J. Rad. Biol. 78: 1117 - 1126, 2002

Agenda

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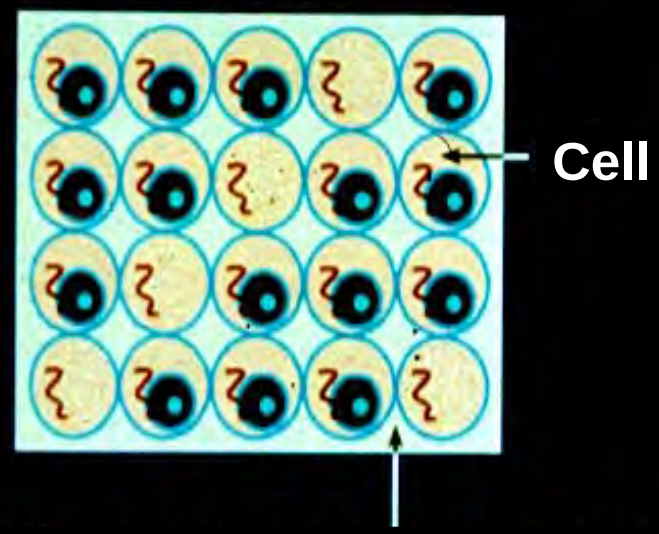
Tissue Response to Low-Dose Radiation

of a given quality (defined $\bar{z}_1$) is determined by all cell reponse probabilities, p_x, per $\bar{z}_1$



N_H: No of hits in micromasses N_E
(intra- and extracellular)

R: incidence of late effects (cancer)

p_{spo} : spont. (+) oncogenesis/cell

p_{ind} / $\bar{z}_1$: induction, constant

p_{prot} / $\bar{z}_1$: protect. ↓ with N_H;t_p

p_{apo} / $\bar{z}_1$: apoptosis, constant

Tissue Response to Low-Dose Radiation

of a given quality (with defined $\bar{z}_1$)

is the sum of all cell response probabilities.

From $R = \alpha \bullet D$

to $R = [\alpha \bullet \bar{z}_1] \bullet [N_H / N_E]$

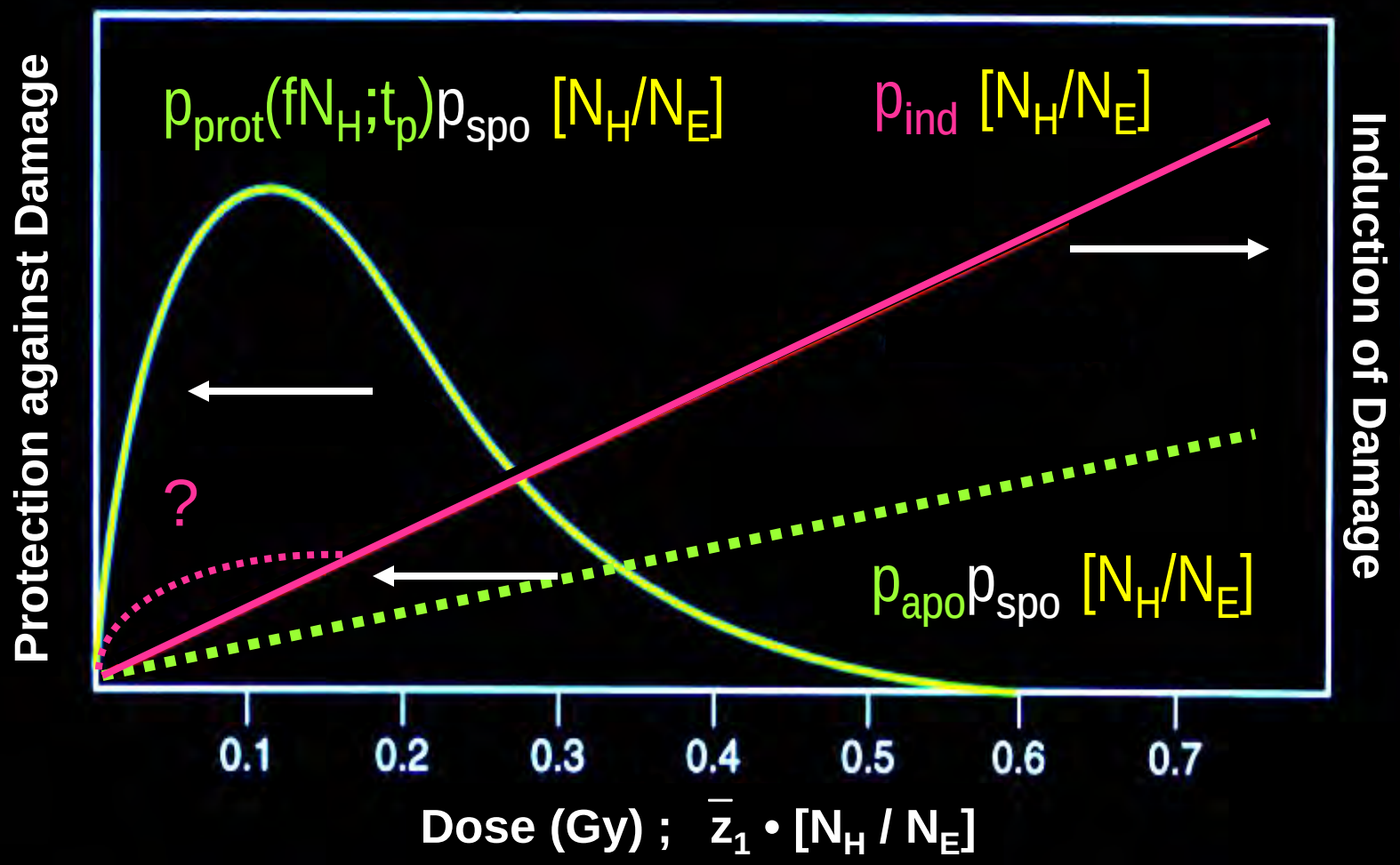
and with p-values / $\bar{z}_1$, for damage and protection

as defined, $[\alpha \bullet \bar{z}_1]$ expresses Σ of p-values:

$$R = \{ p_{ind} - [p_{prot}(fN_H;t_p) + p_{apo}]\, p_{spo} \} [N_H/N_E]$$

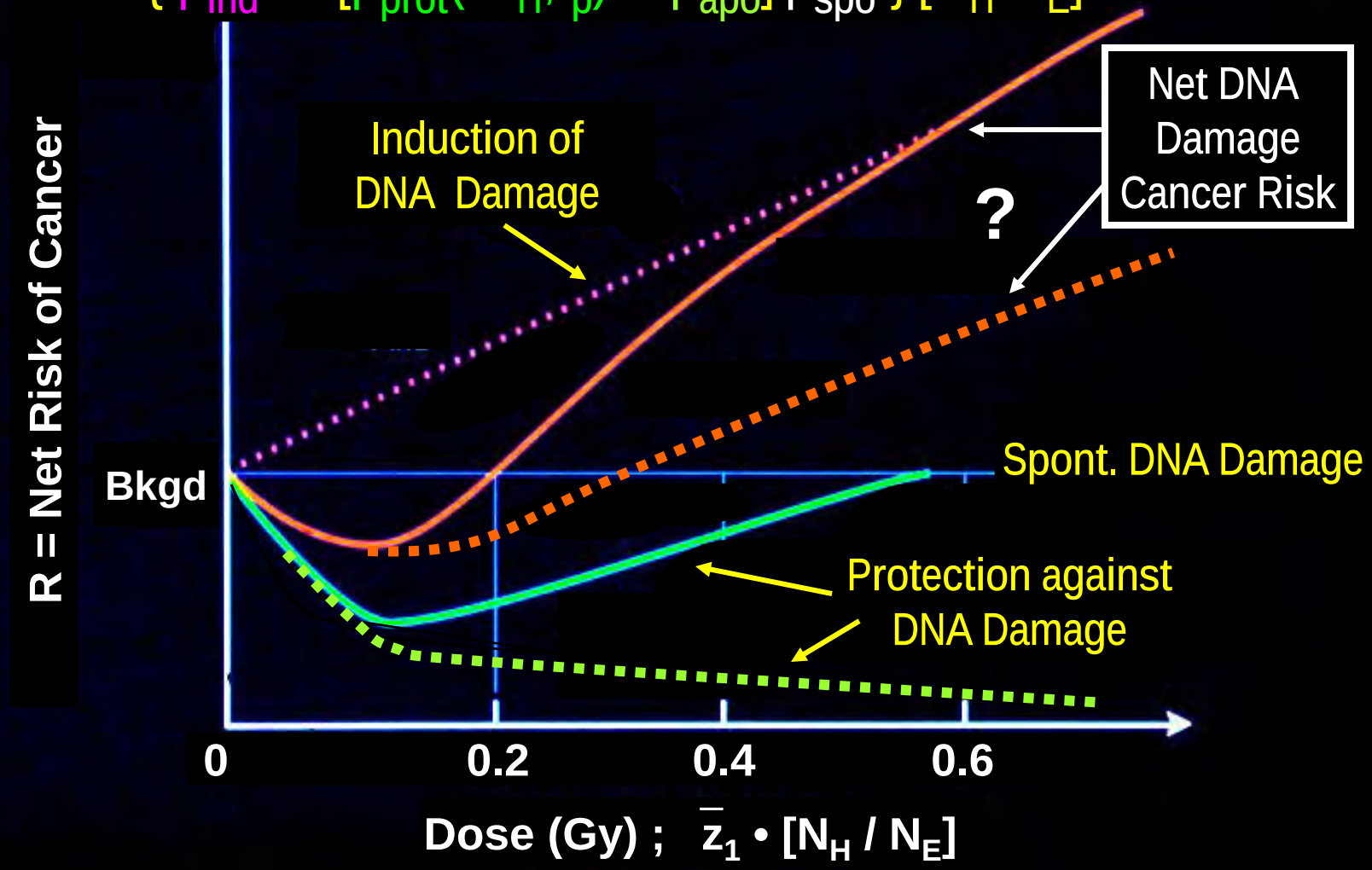
Dual Effect of Low-Dose Radiation

protection against damage ..... induction of damage

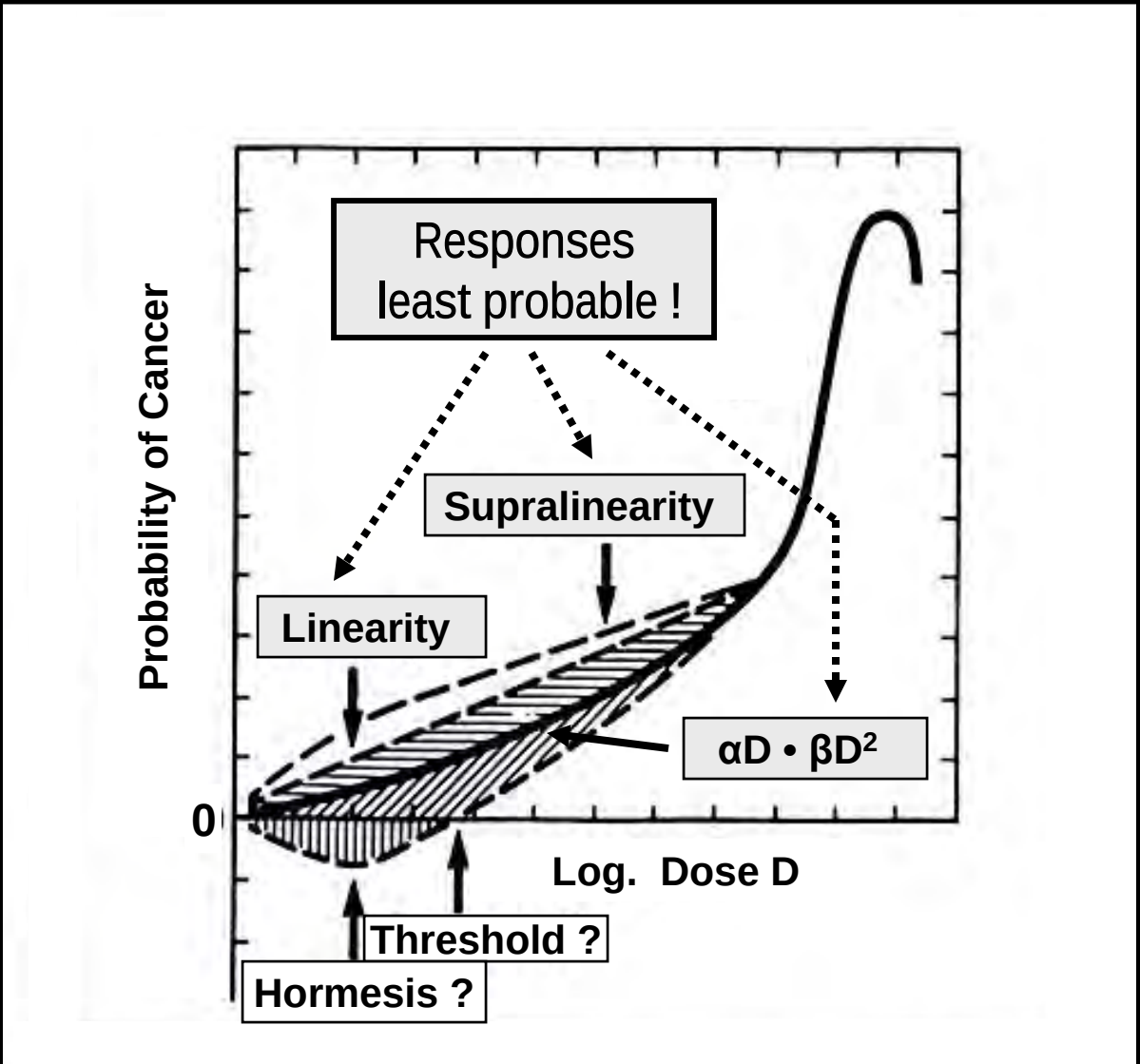


Dual Effect of Low-Dose of Ionizing Radiation

$$R = \{ p_{ind} - [p_{prot}(fN_H;t_p) + p_{apo}]\, p_{spo} \} [N_H/N_E]$$

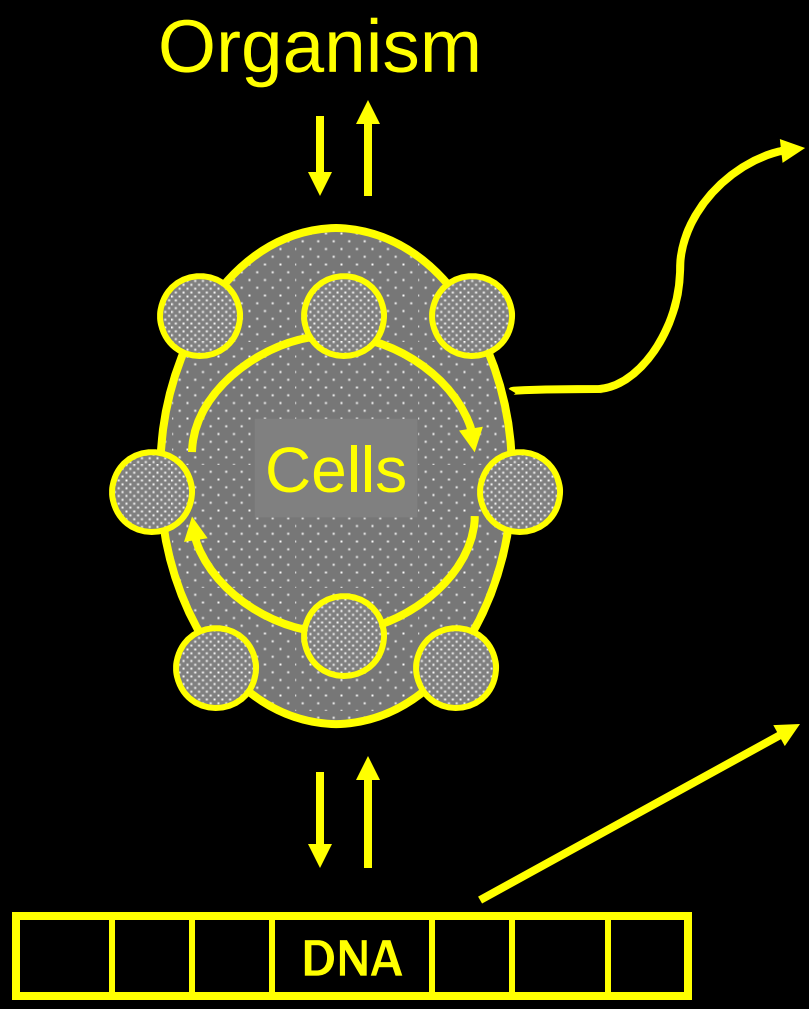


Options of Low-Dose Induced Cancer Risk



Conclusion 1

Biological systems exposed to ionizing radiation



Damage propagation
<u>and</u> its low-dose
induced reduction
<u>are not linear with dose</u>

Low-dose induced
rise in DNA damage
<u>is linear with dose</u>

Conclusion 2

An apparently simple truth

$$A + B \neq A$$

with $B \neq 0$

Linear (f) + Non-linear (f) $\neq$ Linear (f)

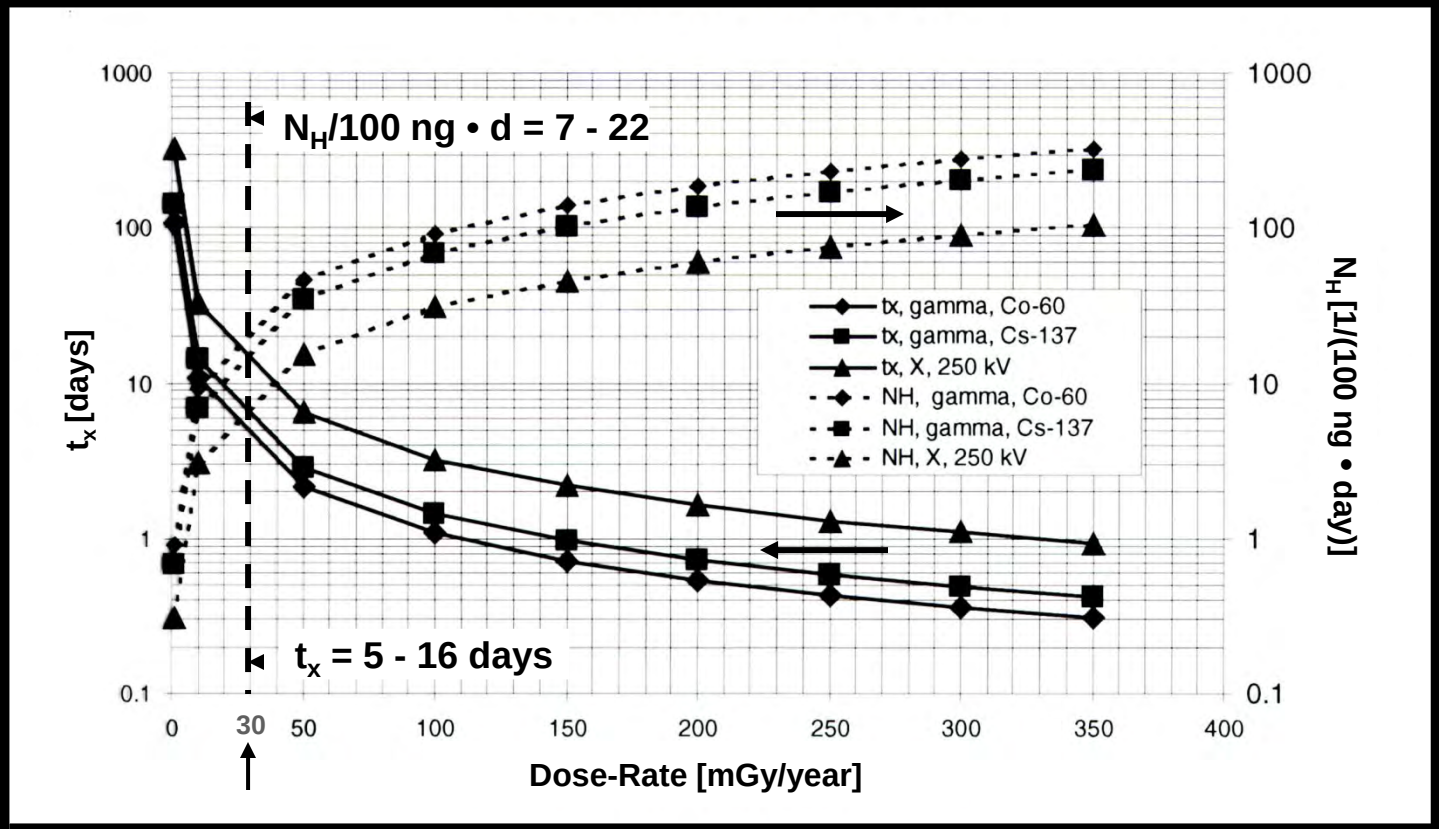
Conclusion 3

The linear-no-threshold (LNT) hypothesis does not comply with newest experimental data. The linear-dose-risk function is artificial and needs replacement by a function that includes both linear and non-linear terms. Basic research data and epidemiological data conform with threshold or hormesis in the low-dose range.

I gratefully acknowledge the fruitful collaboration and discussions over many years with

Drs. K.I. Altman, V.P. Bond, J. Booz, E.P. Cronkite, T.M. Fliedner, M. Frazier, D. Harder, R.D. Neumann, H.G. Paretzke, M. Pollycove and C.A. Sondhaus, and chief technician H. Muehlensiepen.

Dose-Rate and Microdose-Hits

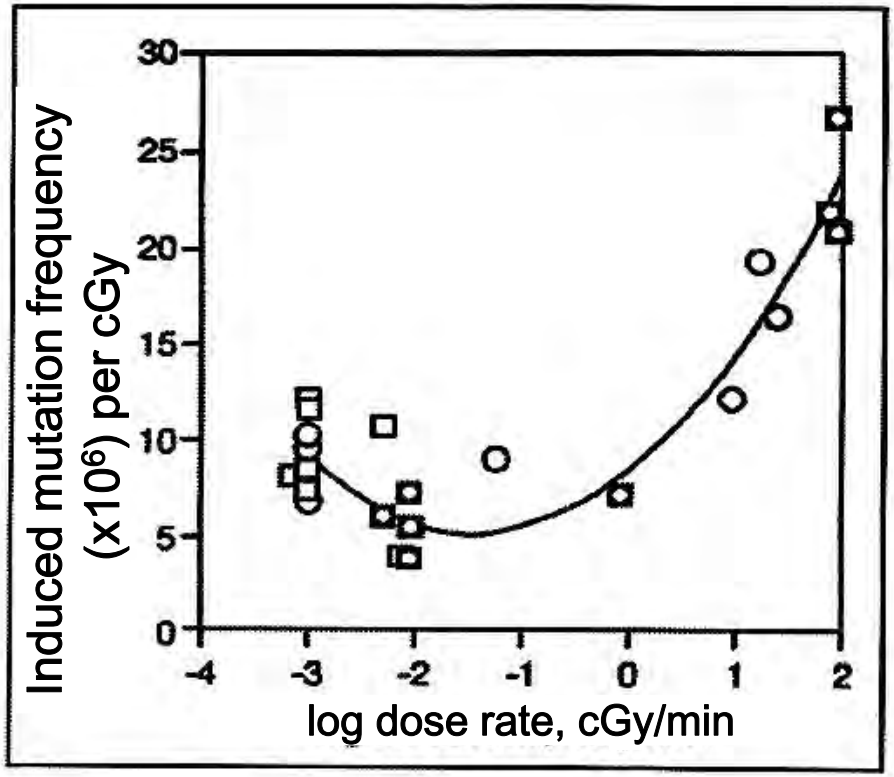


Microdoses $\bar{z}_1$ in mGy: ^{60}Co-gamma radiaton 0.3 ~ 45 ROS
^{137}Cs-gamma radiation 0.4 ~ 60 ROS
250 kV x-rays 0.9 ~ 130 ROS

Feinendegen LE, Graessle DH 2002

Low-Dose Rate Induced ↓ of Locus Mutations

In vivo low-LET irradiation, mouse spermatogonia



0.1 – 1 cGy/min ≅ 1 hit / cell q 3 – 30 sec

Vilenchik MM, Knudson AG, PNAS, 2000