The Legacy of Radiation Emerging Concepts

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GOAL:

- Provide overview of the long- term health effects of radiation, and their underlying radiobiology

Lethal effects (radiation syndromes)

Late effects: stochastic v deterministic

Effects on embryo and fetus

Genetic effects

Carcinogenesis

- RT patients, accidental, medical, occupational, bomb survivors

Acute versus protracted exposures

Direct and non targeted effects

- genomic instability, bystander and heritable effects

Sequence of events by which ionizing radiation affects living systems

Energy absorption (10^{-17} sec)
Ionization and excitation (10^{-5} sec)
Molecular changes (secs)
Biochemical changes (secs - hrs)
Physiologic & anatomic changes (min - hrs)
Biological effects (hrs - yrs)
Death of organism

We live in a radiation environment

Air travel

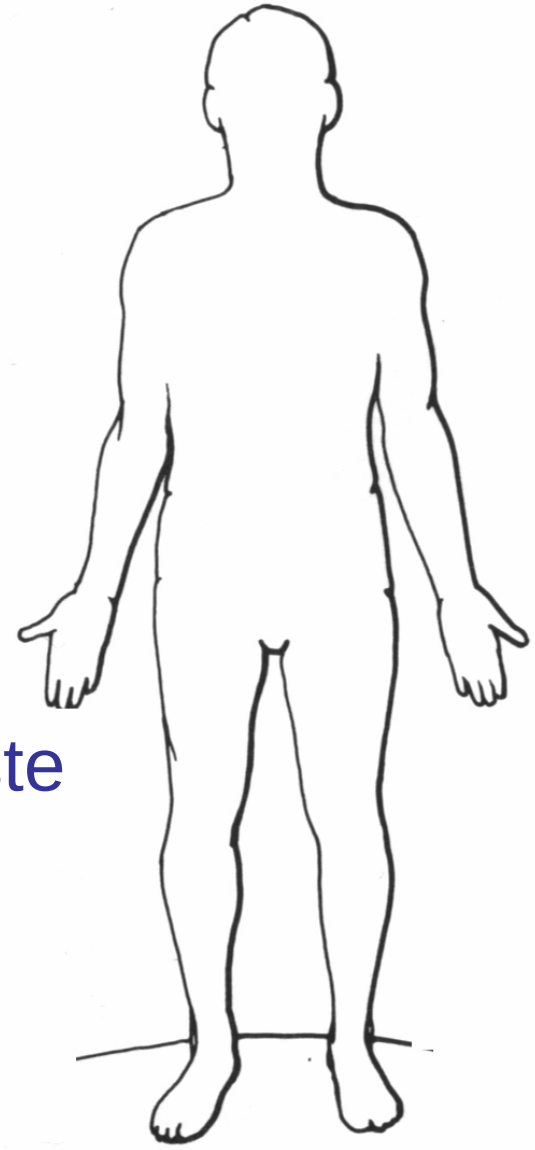
Testing fallout / contamination

TV & luminous watches

Nuclear power plants (20%)

Radioactive waste

Diagnostic & therapeutic radiation*



Cosmic rays

Air (radon)

Building material

Water

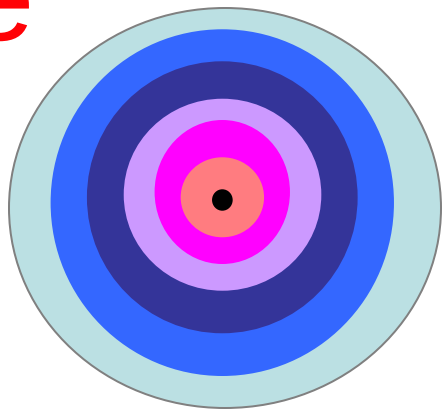
Food

Earth

*Diagnostic radiology > 200 million procedures/year

Most of our understanding of radiation effects comes following a single acute exposure

The atomic bombings of Japan: cancer incidence in survivors



Killed outright	100,000
Survivors studied	86,572
Expected cancers (no bomb)	21,643
Observed cancers	22,064
Increase	421

LETHAL EFFECTS

Survival time and mode of death dependent upon dose

Prodromal Radiation Syndrome

rapid onset

persistent

merges with other syndromes

Cerebrovascular Syndrome

high dose (>100Gy)

death rapid (24 - 48 hours)

neurologic & cardiovascular breakdown

Gastrointestinal Syndrome

Intermediate dose 5 - 12Gy

Death in days

Destruction of GI mucosa

Hematopoietic Syndrome

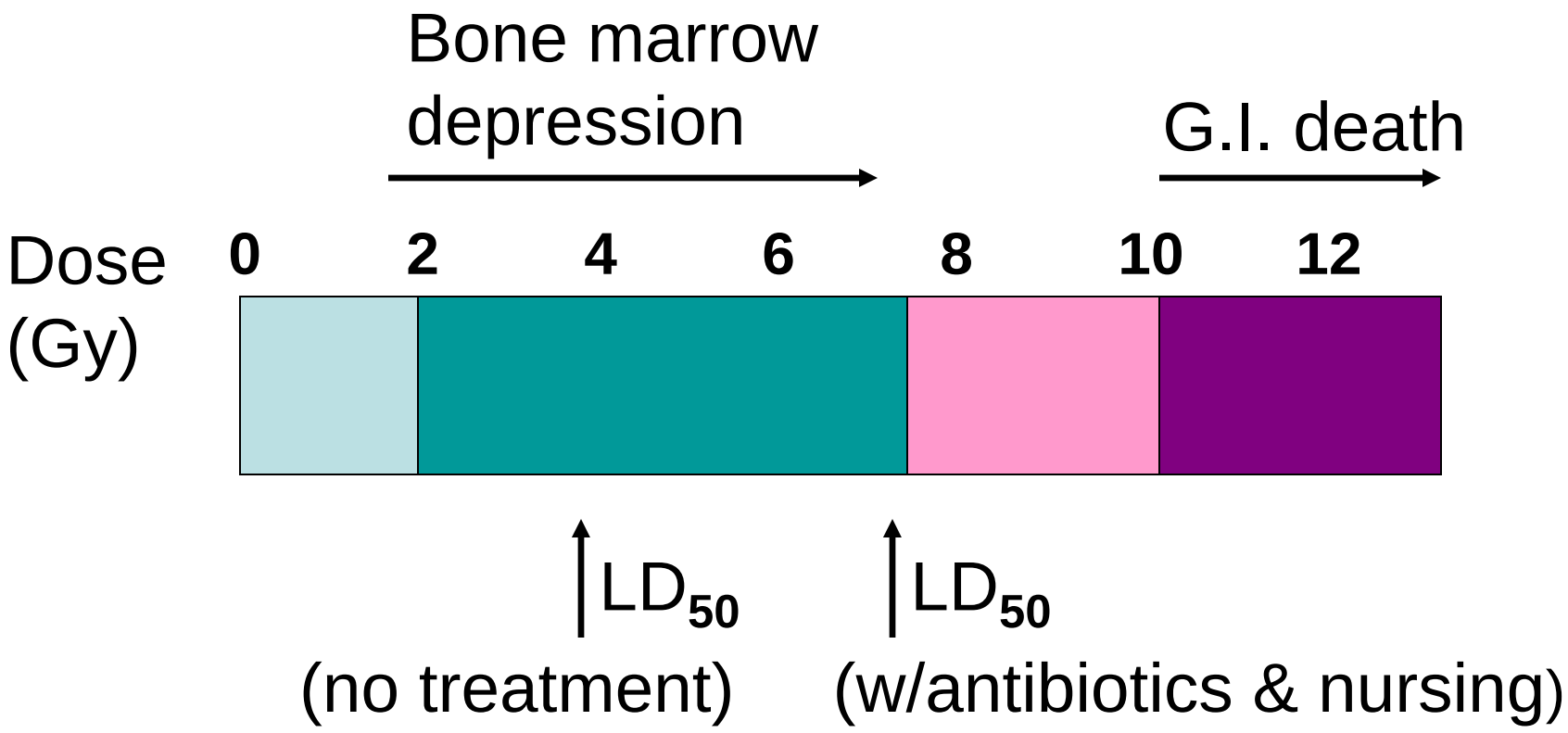
Low dose < 5Gy

Death in 3 - 4 weeks

Effect on blood forming organs

Death by depletion of stem cells of critical self-renewing tissue

LD_{50} man ~4Gy, medical care significant



No treatment

Antibiotics, transfusions, nursing

Potential for bone marrow transplants

LATE EFFECTS

If cellular damage not adequately repaired

- Germ cell - mutation in offspring
- Somatic cell - leukemia or cancer

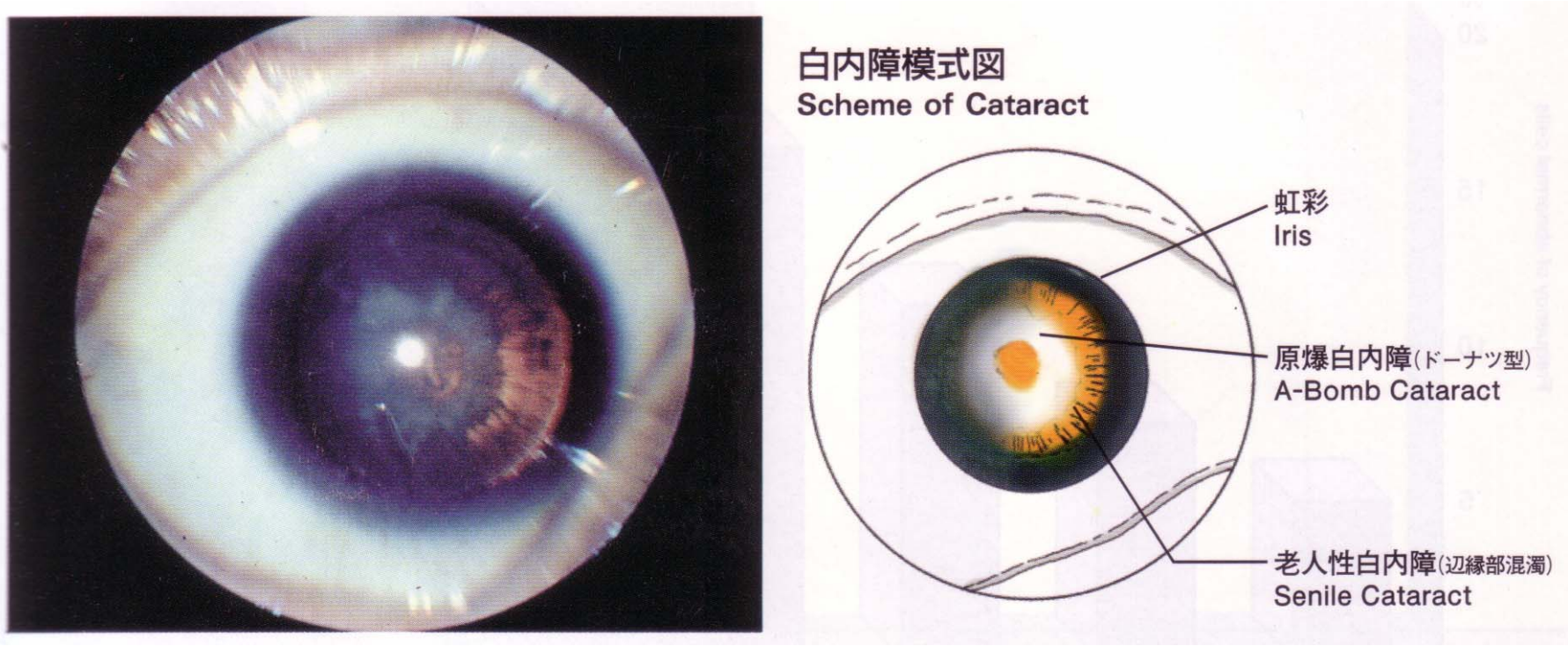
STOCHASTIC EFFECTS

- Genetic and carcinogenic effects
- No threshold with dose
- Severity independent of dose
- Probability of an effect > as dose >

DETERMINISTIC (NON-STOCHASTIC) EFFECTS

- Practical threshold e.g., cataract
- Severity dependent upon dose
- Usually requires larger doses than required to produce mutations and cancer

Radiation induced cataract



21 year old female, 0.8km from hypocenter
Cataract a donut-shaped opacity (in posterior pole of lens) appeared 3 months - 10 years after the bombing

From: Atomic Bomb Disease Institute, Nagasaki University.

GENETIC CHANGES

Gene Mutation

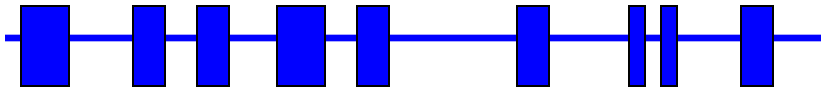
- Changes in DNA structure
- e.g., loss or gain of DNA bases
- Permanent
- No threshold

Determined by:

- Whole animals e.g., *Drosophila*
- Megamouse project (7 x 10^6 mice)
  - Mutation induction varies with loci
  - Dose rate effects
  - Sex differences (males > sensitivity)
  - Strain differences
- Specific locus mutation in cell culture

Specific locus mutations

HPRT: 34kb gene, cDNA 654bp, 9 exons



X chromosome

Positive selection (6-Thioguanine)

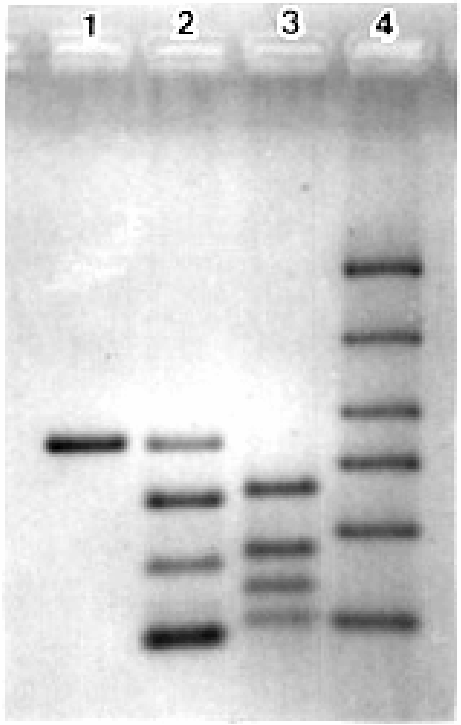
Mutants survive

frequency

Molecular analysis

Southern blots

Multiplex PCR



Induced mutations primarily deletions

Radiation-induced mutations not unique, radiation increases incidence of the same mutations that occur spontaneously

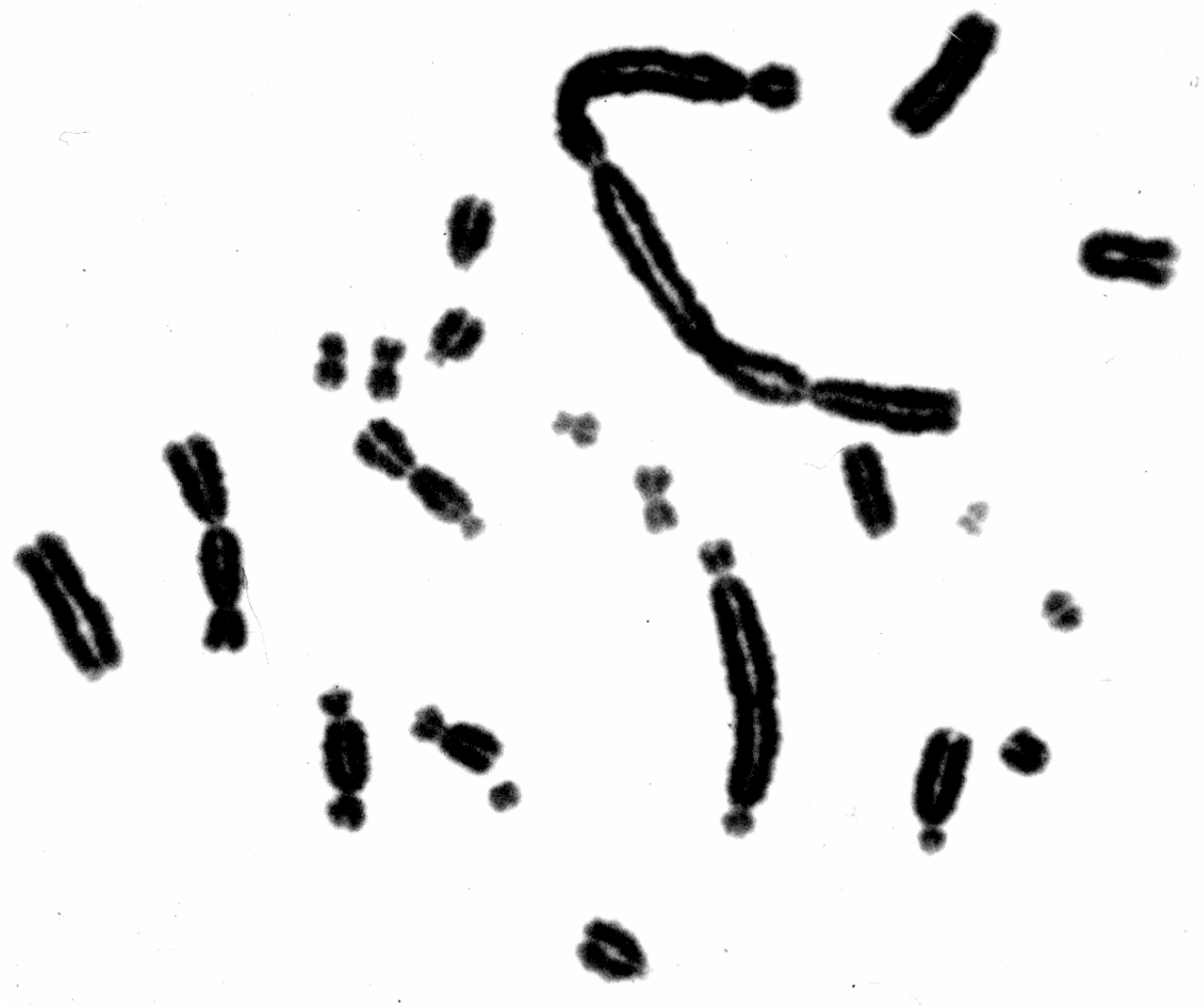
Doubling Dose

Dose required to double spontaneous mutation incidence

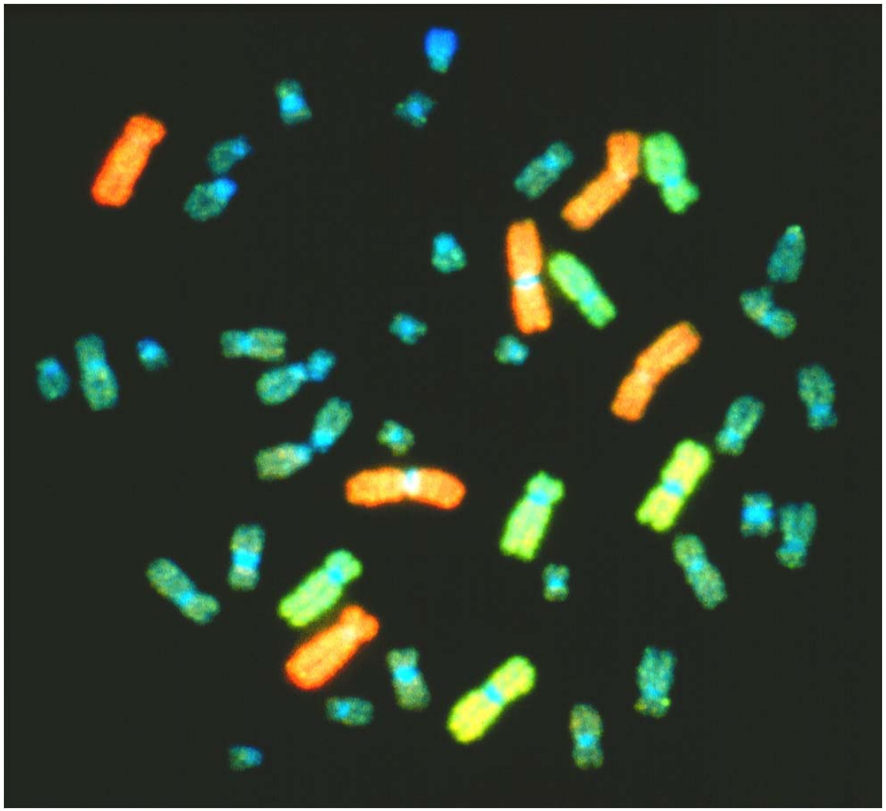
BEIR III (1980) estimate: 0.5 - 2.0Gy

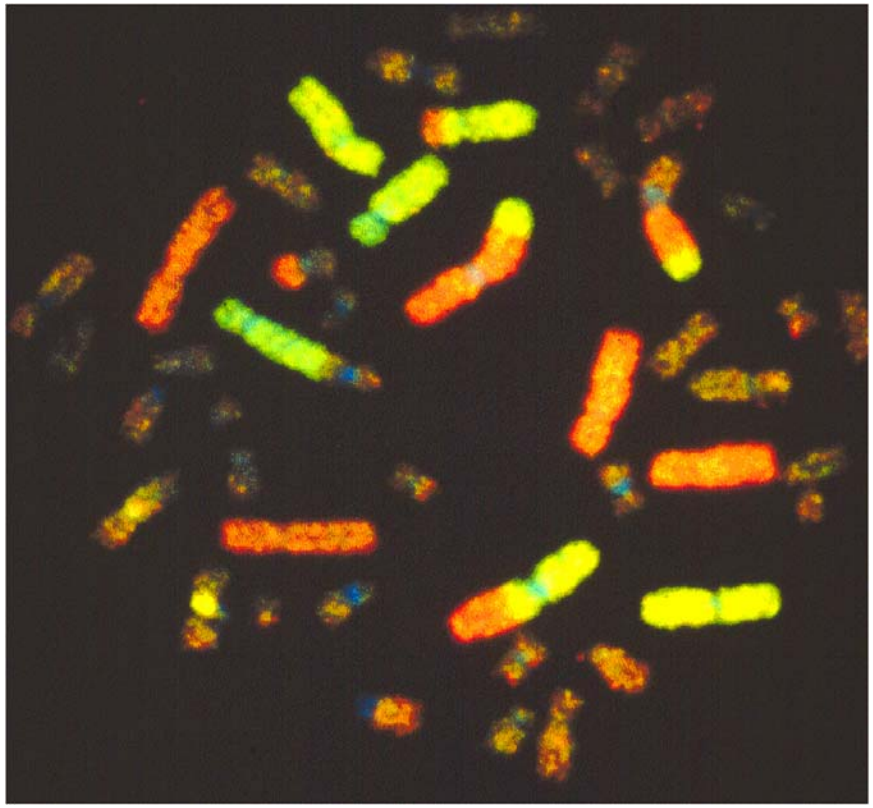
UNSCEAR (1986) estimate: ~1.0Gy

Radiation-induced chromosomal rearrangements accurate and reliable dosimeter of exposure.
Better dosimeters needed at high doses (>8Gy)



FISH enables detection of symmetric chromosomal rearrangements





From Dr. J.D. Tucker

RADIATION CARCINOGENESIS

Cancer is the most important somatic effect of radiation

Latency

Leukemia ~5 years

Solid tumors >20 years

Factors affecting carcinogenesis

Dose

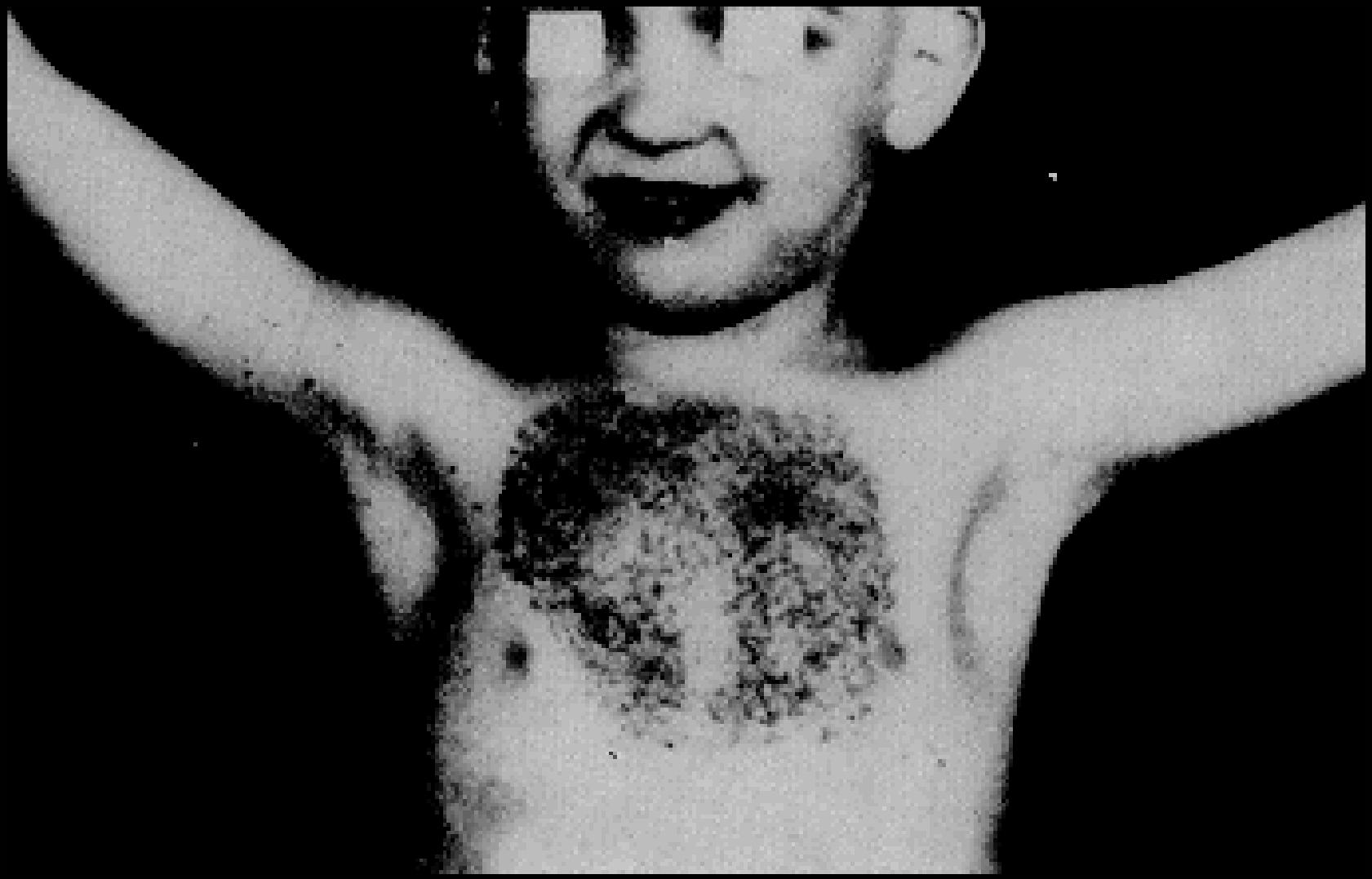
Dose rate (DREF or DDREF)

Gender

Age at exposure

Time since exposure

Gorlin’s Syndrome: (Nevoid basal cell carcinoma syndrome)



High frequency, rapid onset

Carcinogenesis as a multistage process:

Initiation

- Necessary but not sufficient
- Irreversible e.g., mutation in a stem cell

Promotion

- Clonal expansion
- Mediated by growth factors

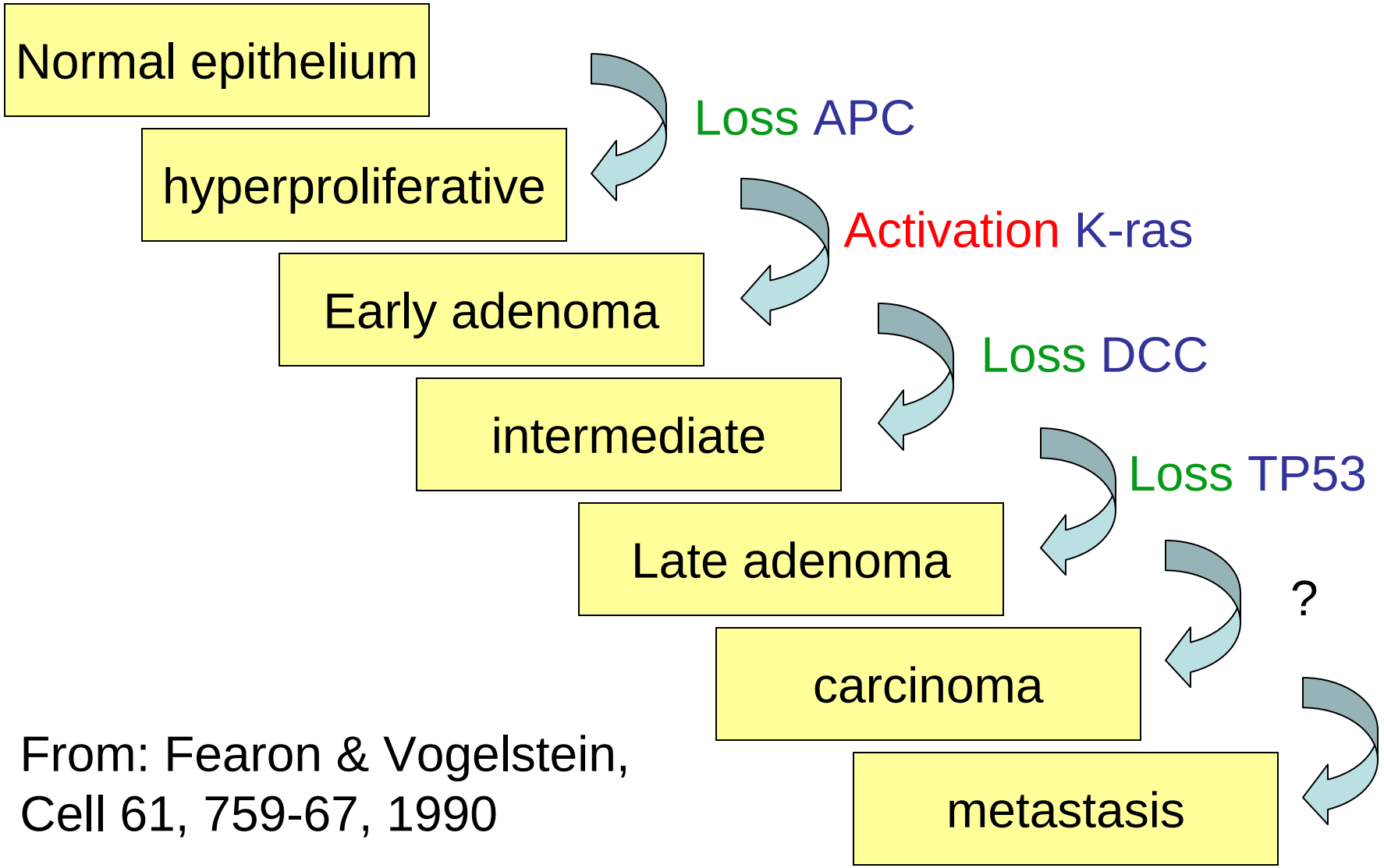
Progression

- Development of neoplastic characteristics
- Increased growth rate
- Invasive, metastatic potential

Metastasis

- May involve further genetic changes

Typical sequence of genetic changes underlying the development of a colorectal carcinoma



From: Fearon & Vogelstein,
Cell 61, 759-67, 1990

RADIATION CARCINOGENESIS

Occupational Exposures

- Radium dial painters
- Uranium miners
- Accelerator engineers & physicists

Medical Exposures

- UK ankylosing spondylitis patients
- Children irradiated for enlarged thymus
- Children epilated for *tinea capitis*
- Tuberculosis patients fluoroscoped during artificial pneumothorax

Second malignancies in RT patients

- Controversial
- “Patient selection”
- Suitable control group
- Limited patient numbers
  - statistical limitations
- Sufficient follow up period (latency)

Large studies

- Small, but statistically significant increase in relative risk

Accidental Exposures

Violation of elementary safety rules for medical sources, severe contamination, human consequences

1983 Juarez (Mexico) ^{60}Cobalt

1987 Goiania (Brazil)^{137}Cesium

Reactor Accidents

1957 Windscale (UK)

1979 Three Mile Island (USA)

No human consequences!

1957 Cheliabinsk (former USSR)

1986 Chernobyl (former USSR)

Radiation effects on the embryo and fetus

After cancer, developmental effects are of greatest concern.

Lethal effects:

embryonic, fetal, or neonatal death

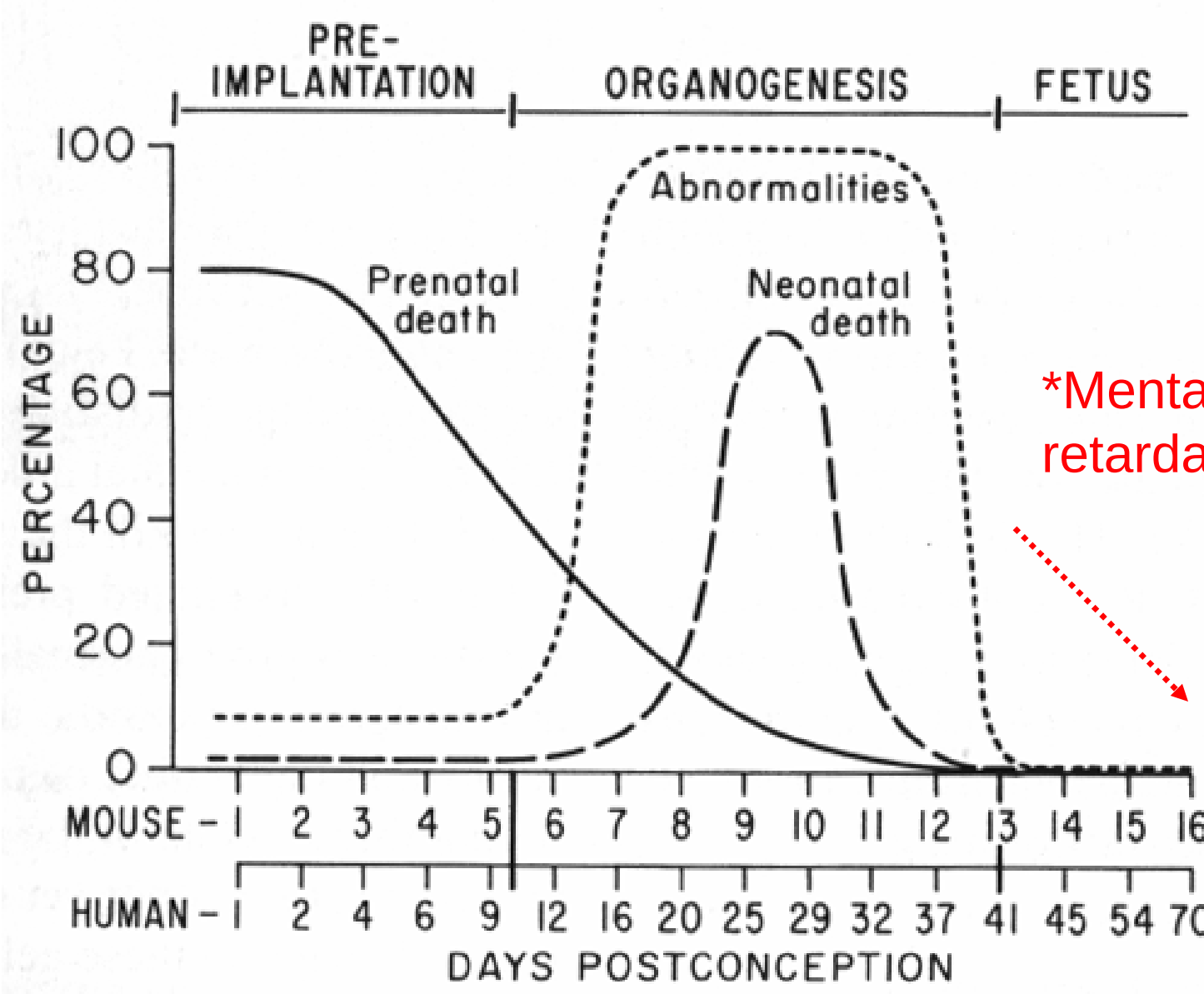
Congenital malformations:

High frequency - organogenesis

Growth disturbances:

Mental retardation, no malformation

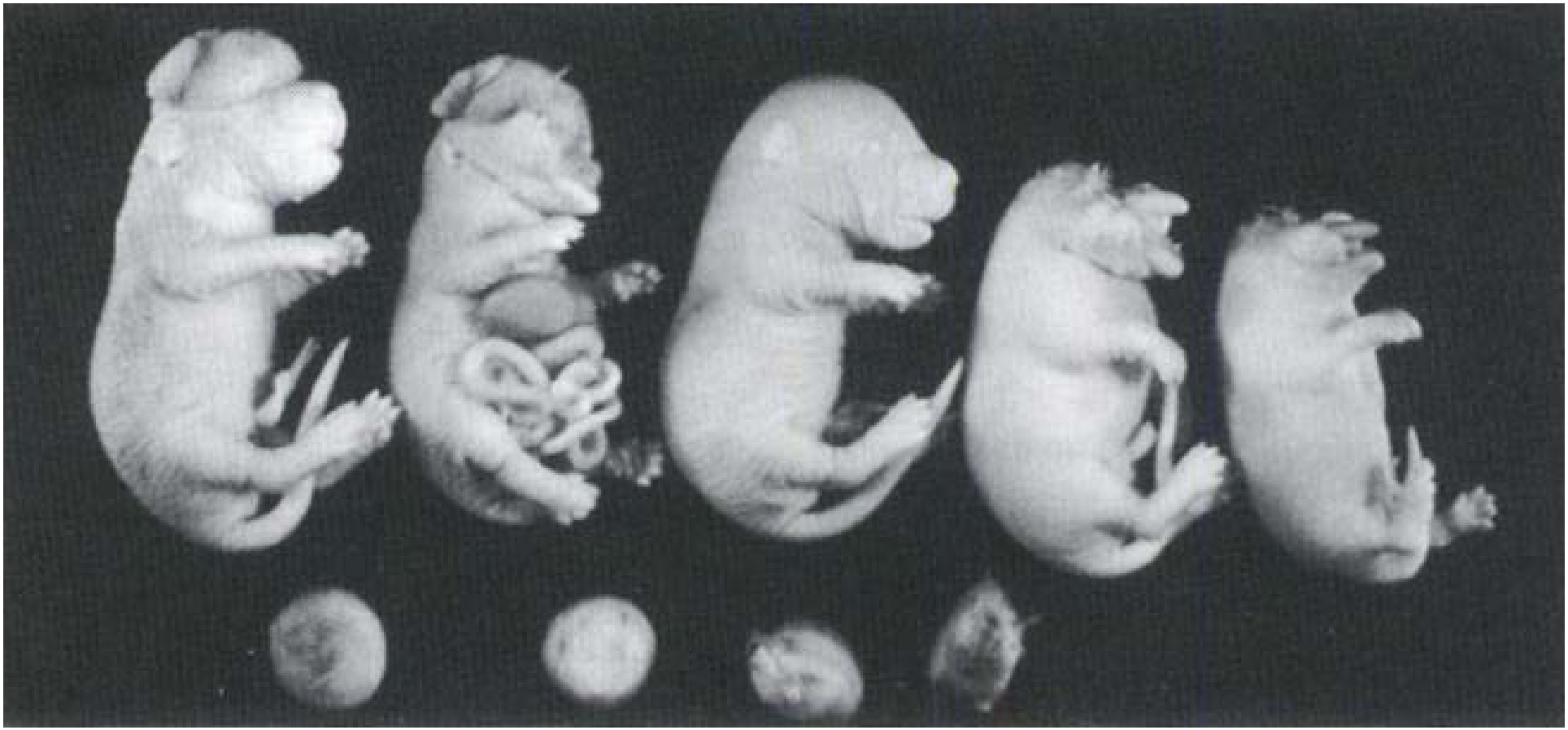
2Gy at various times after fertilization



*Mental retardation

From: Hall, “Radiobiology for the Radiobiologist”

Female mouse irradiated and sacrificed at 19 days



Exencephaly, evsceration, anencephaly, stunting

From: Hall, “Radiobiology for the Radiobiologist”

Microcephaly

(head circumference ~2x smaller)
Relatively frequent after in uterus exposure (<16 weeks gestation).

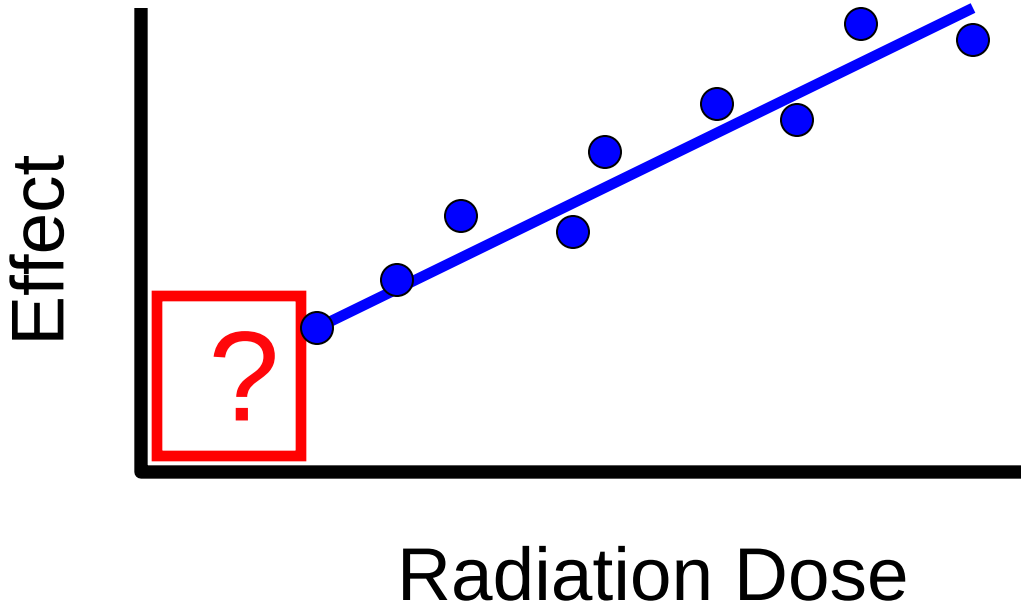
A. Normal 12 year old
B. 15 year old, exposed *in utero* 1.2km from hypocenter.

From: Atomic Bomb Disease Institute, Nagasaki University.

Radiation Effects Research Foundation (Hiroshima) a unique resource

~ 50% survivors still alive

In the laboratory acute exposures practical and convenient



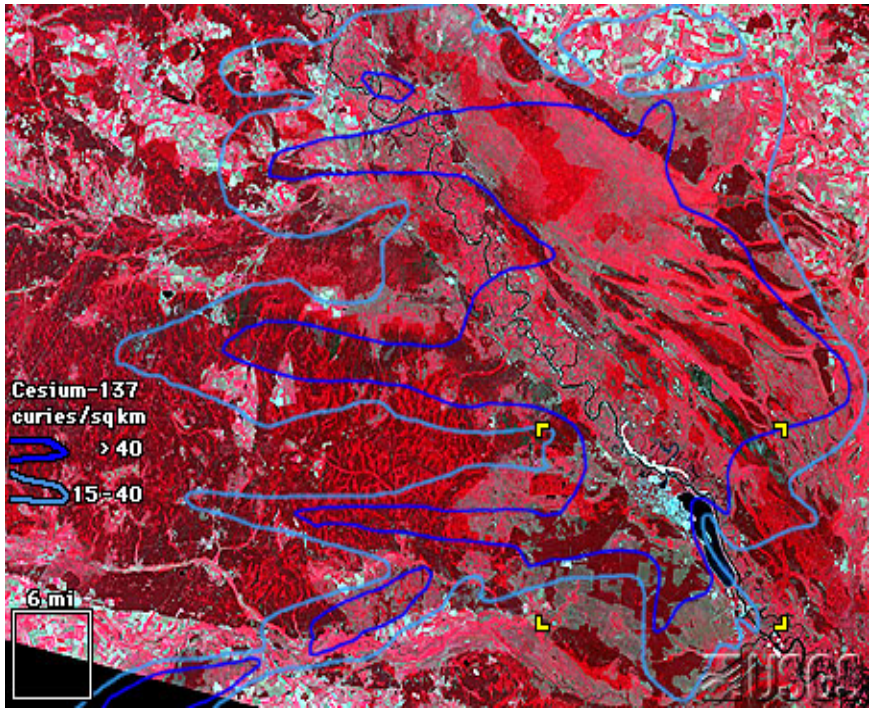
Questions:

How to extrapolate to effects at low doses where there is insufficient data?

Are extrapolations from “high dose” acute exposures appropriate when human exposure is primarily chronic low dose exposure?

Chernobyl, 1986

Protracted exposure(contamination)



Questions:

What can be done in the laboratory to investigate how low dose protracted radiation exposure leads to cancer?

Mutations (doubling dose ~ 1Gy)
Chromosomal rearrangements

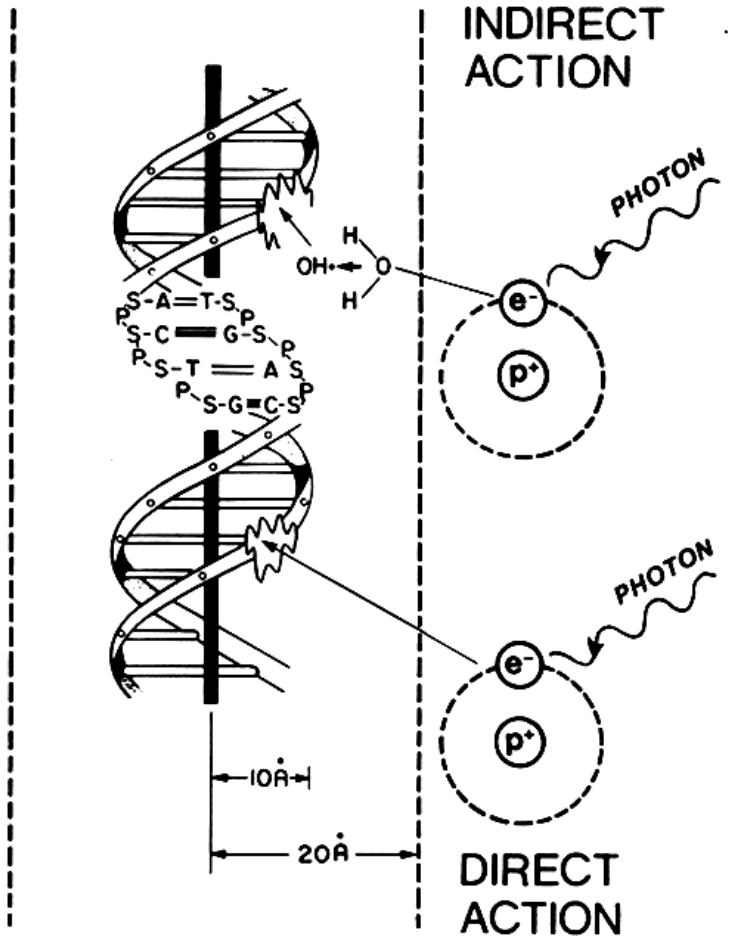
Are all effects direct effects?

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

- All 4 bases subject to damage; ~9eV sufficient to break DNA backbone
- SSB correlates poorly with lethality
- DSB most important lesion

Damage / Gy of X-rays:

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



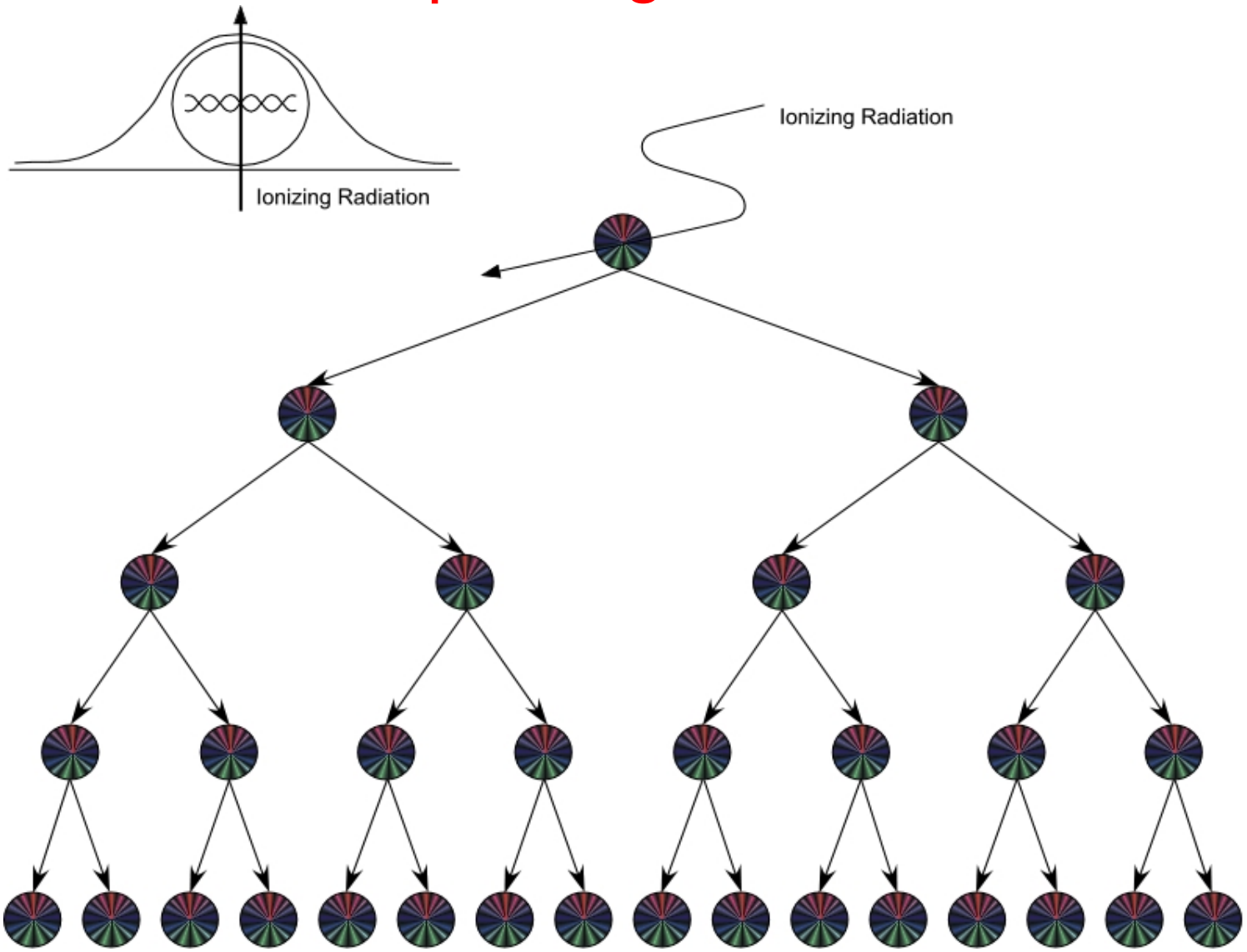
From: Hall, “Radiobiology for the Radiologist”

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

Evidence?

^{3}H-Tdr - short range β
BrdUrd - radiosensitization
plutonium needle experiments

Conventional paradigm for radiation effects

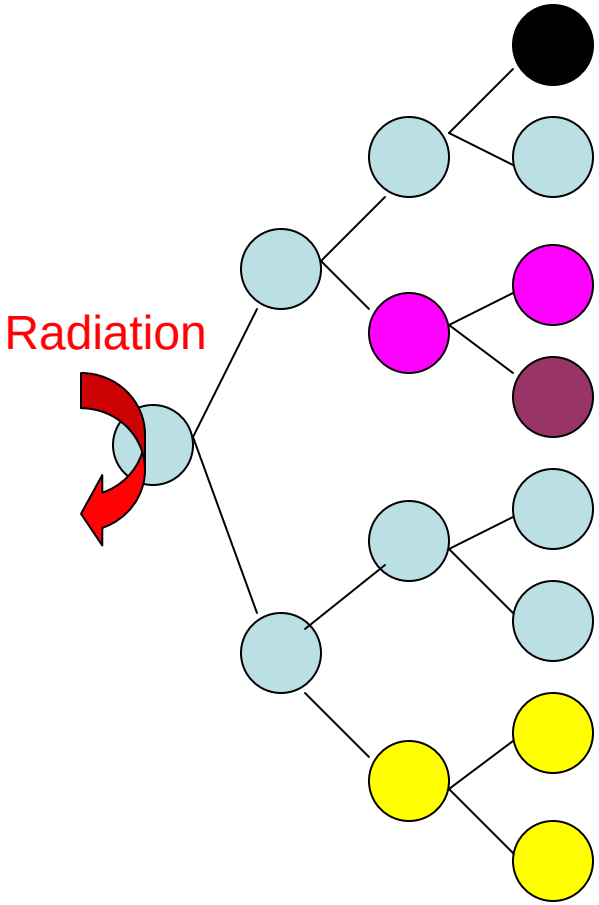


Also consider “non-targeted” effects of ionizing radiation

- Genomic instability
  - chromosomal change, mutation, gene amplification, aneuploidy, micronuclei, transformation, cell kill (reduced PE)
- Bystander effects
- Clastogenic factors
- Death inducing effect
- Transgenerational effects

RADIATION-INDUCED GENOMIC INSTABILITY

Increased rate of genomic alterations in the progeny of irradiated cells



Manifests in progeny as:

chromosomal rearrangements

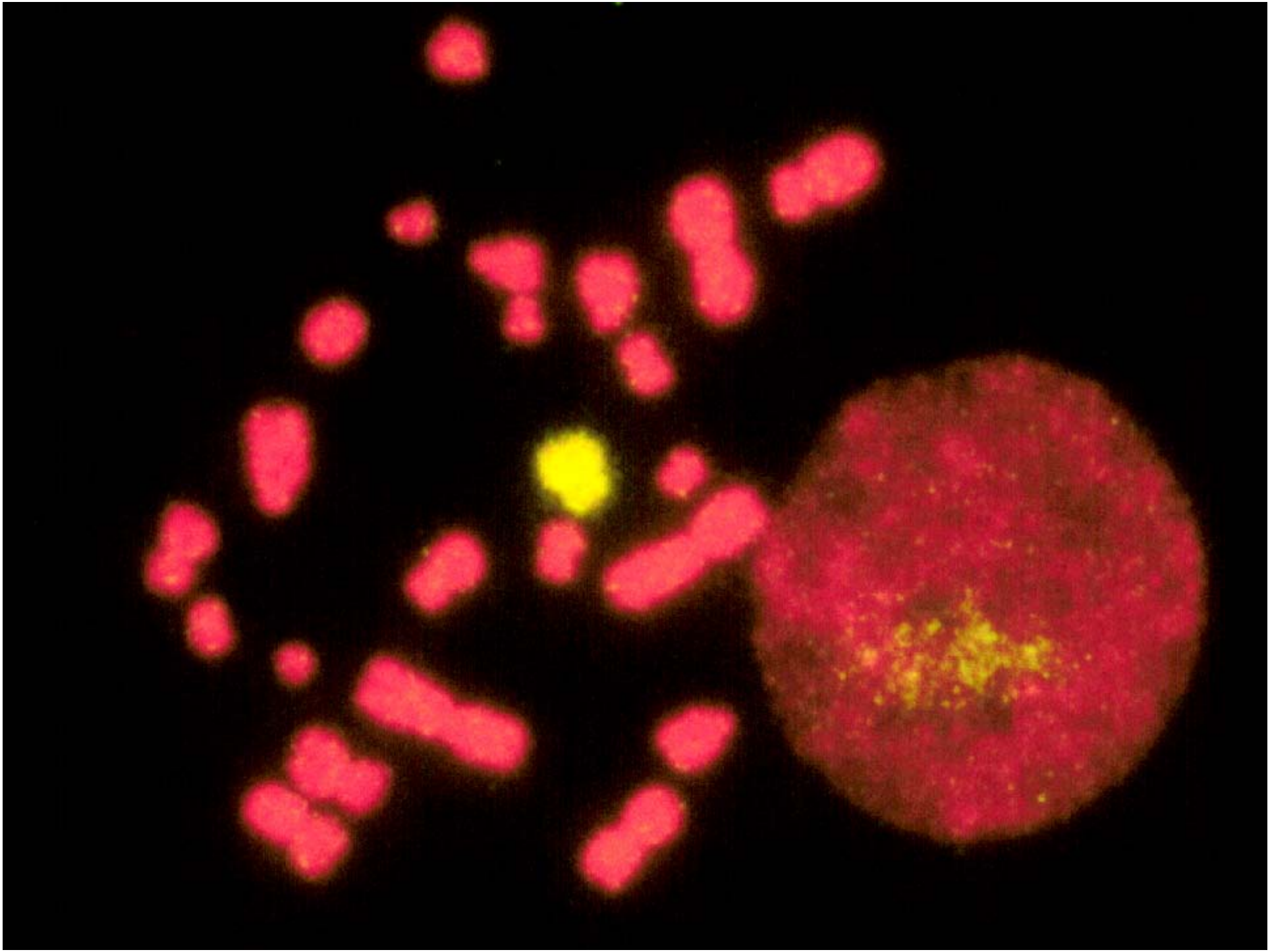
micronuclei

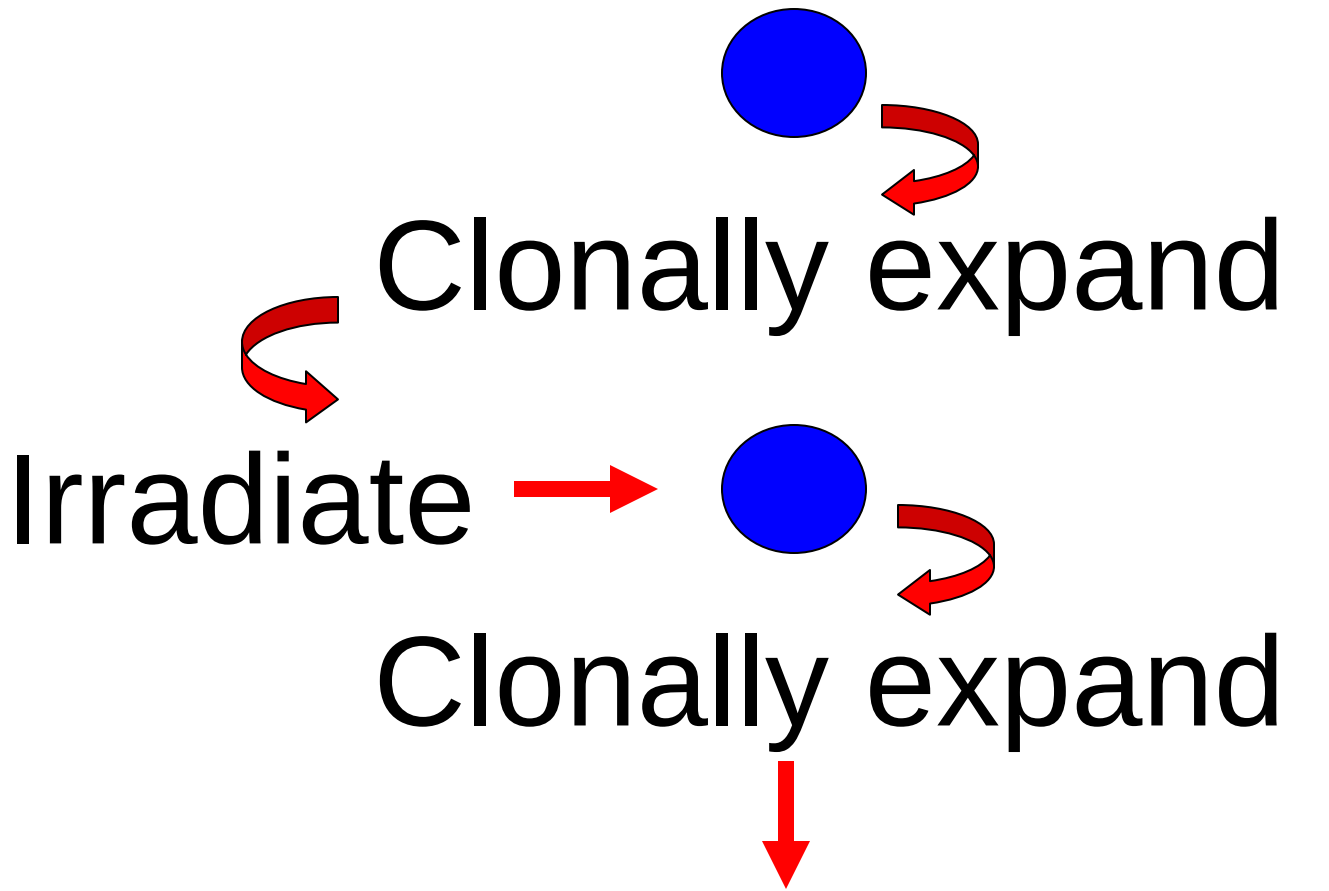
aneuploidy

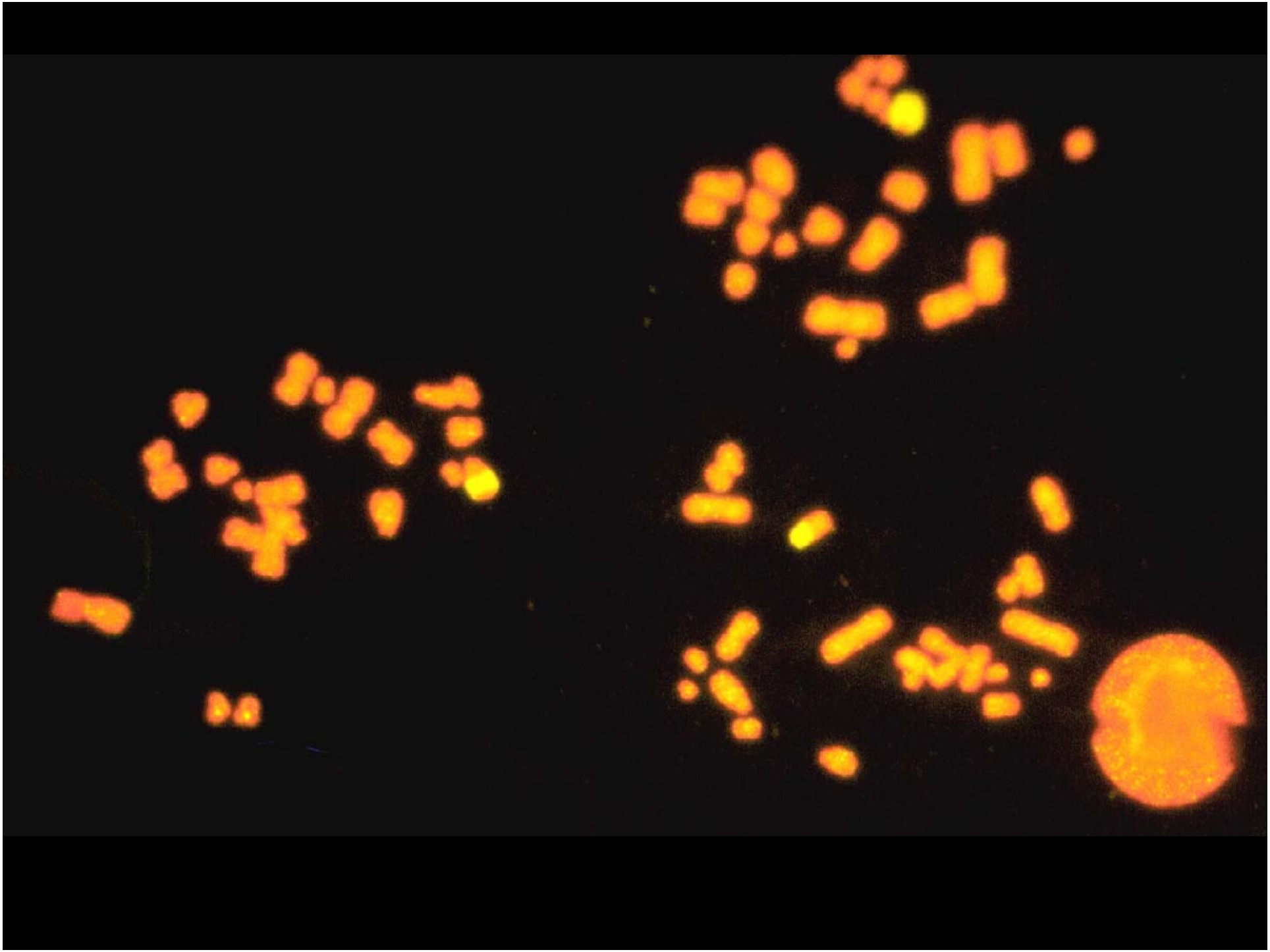
delayed mutation (spectrum)

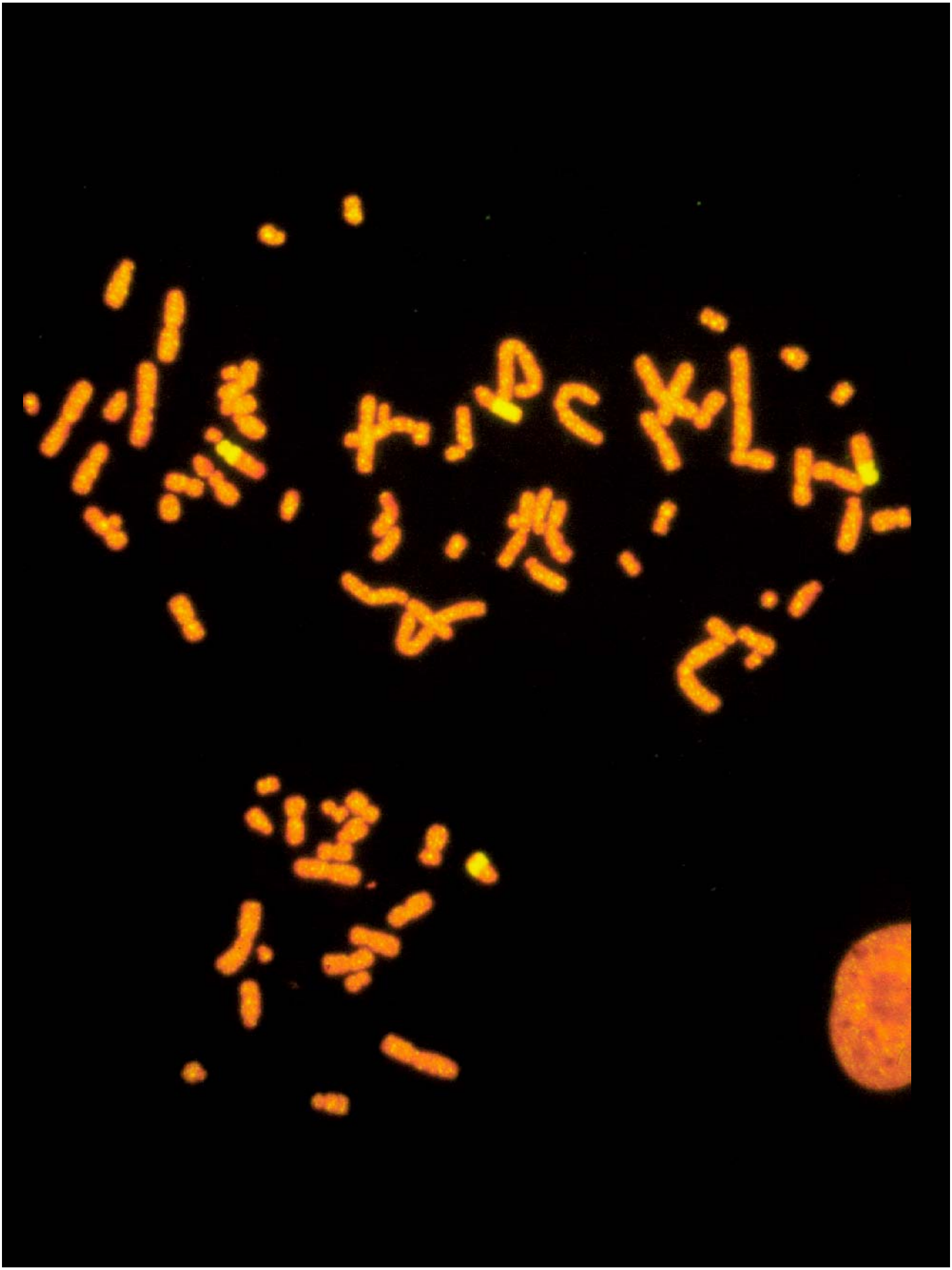
gene amplification

cell killing




Clonally expand
Irradiate
Clonally expand
Metaphase analysis of
clonally expanded cells





Questions:

Does radiation-induced instability provide requisite plasticity to drive the stepwise progression of genetic changes required for neoplastic progression?

Frequent event, probably not a mutation

Bystander Effects

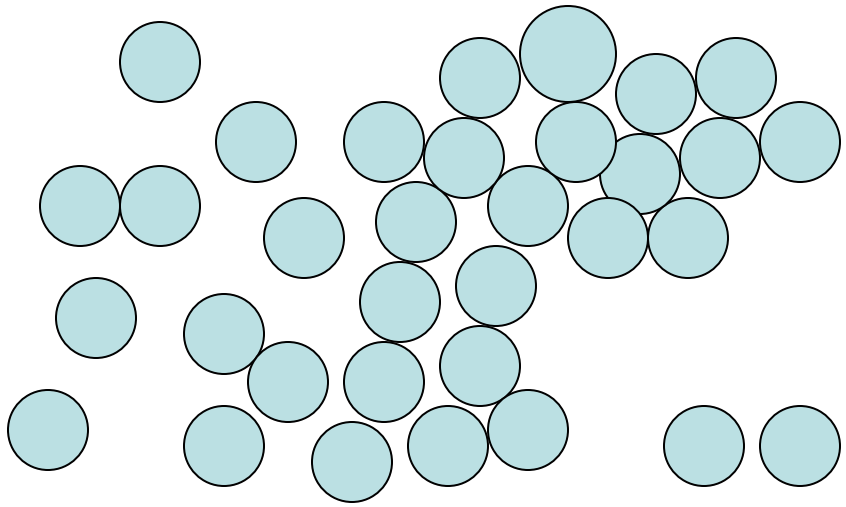
Effects observed in cells that were not irradiated but were “bystanders” at the time of irradiation

Low fluences of α-particles
(In radiation environment but “not hit”)
Single cell microbeam irradiation
Medium transfer experiments

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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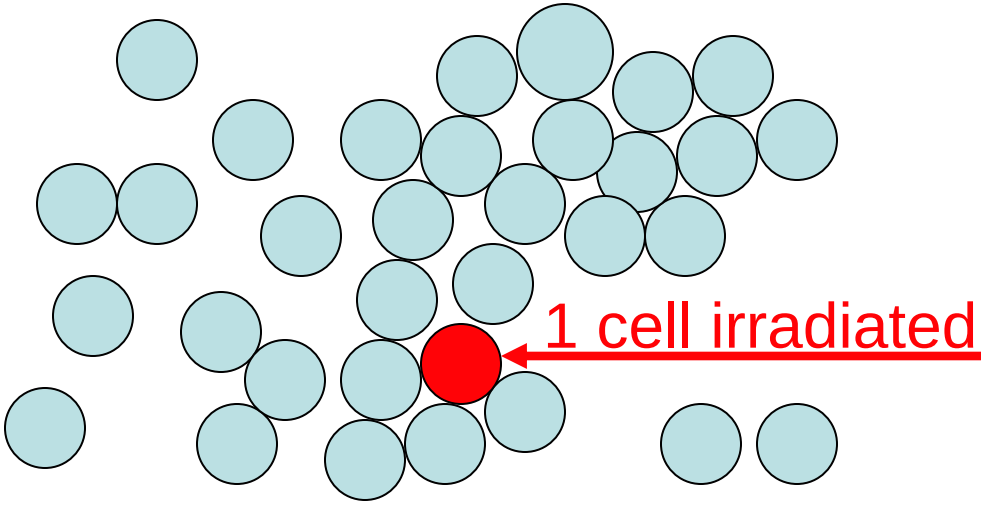
Single cell microbeam irradiation



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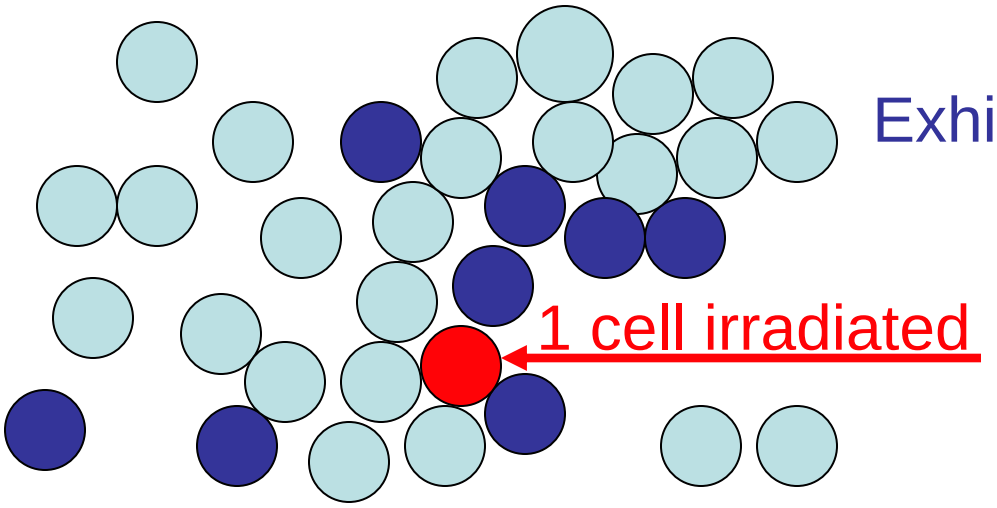
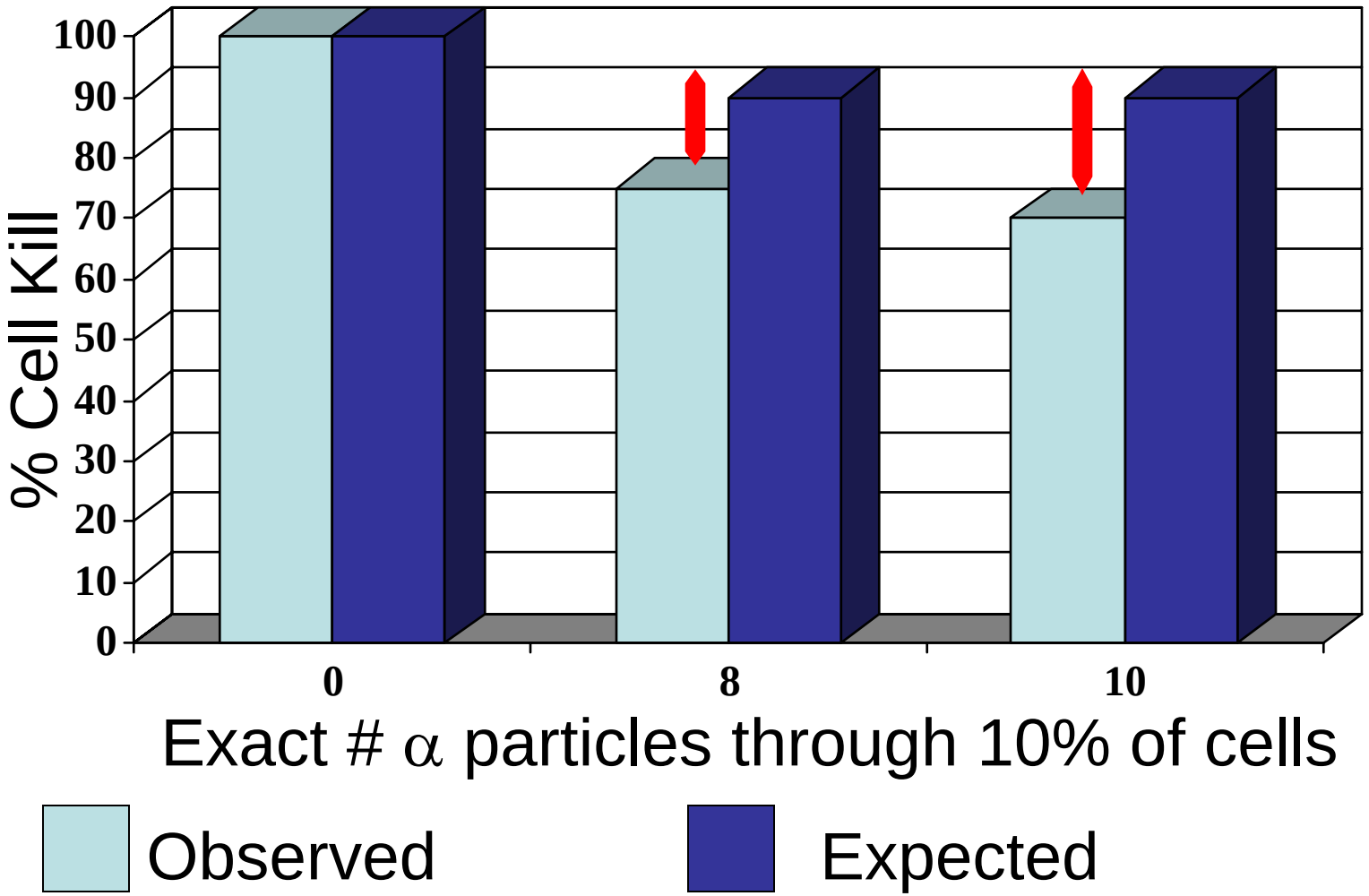


Exhibit bystander effect

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

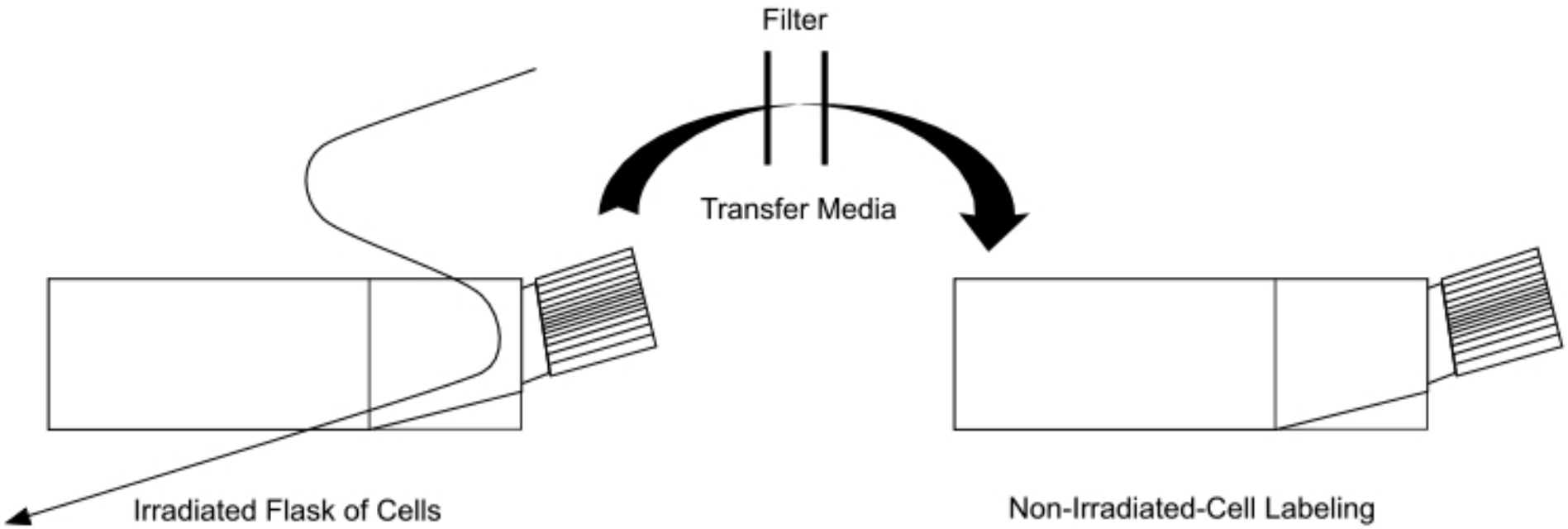
Bystander effect for cell survival



Cell kill due to bystander effect

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

Bystander effect after medium transfer
Mothersill and colleagues



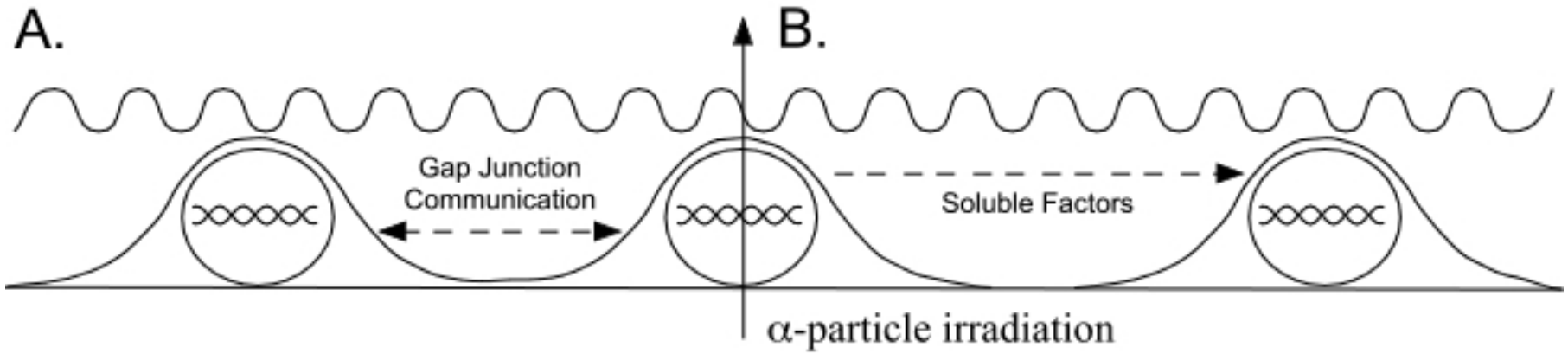
Reduced plating efficiency in cells that have never been exposed to ionizing radiation

Bystander effect after low fluences of α-particles

Little and colleagues,

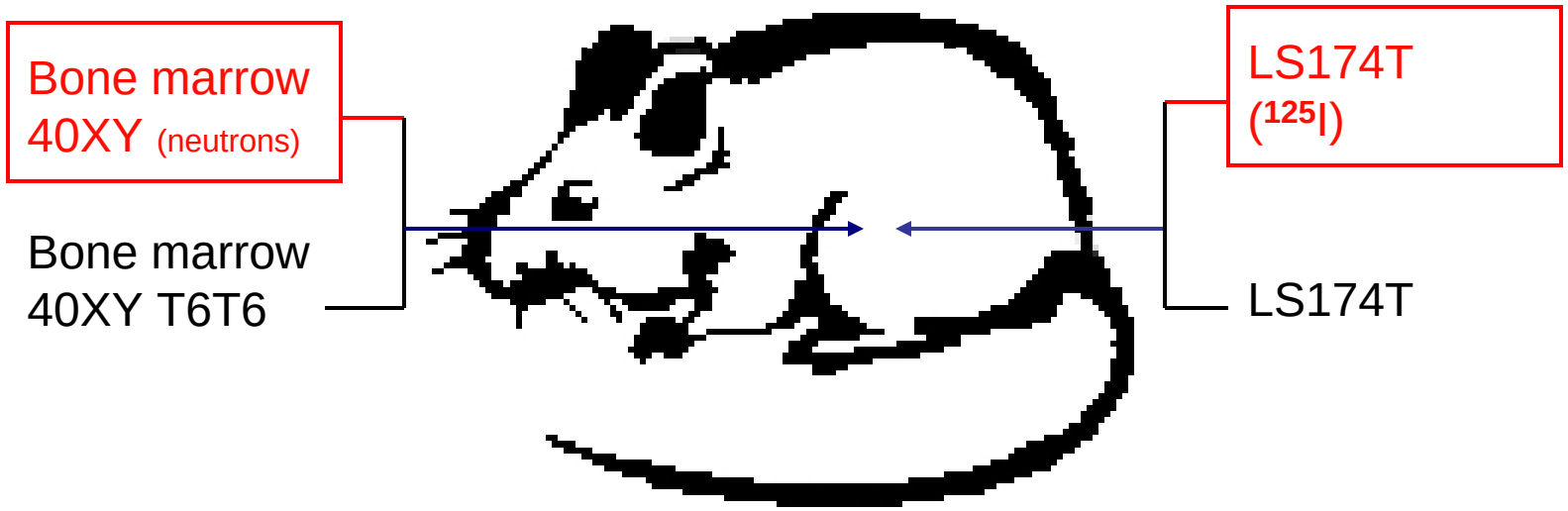
or targeted microbeam irradiation

Columbia group, Gray Laboratory



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

Bystander effects *in vivo*



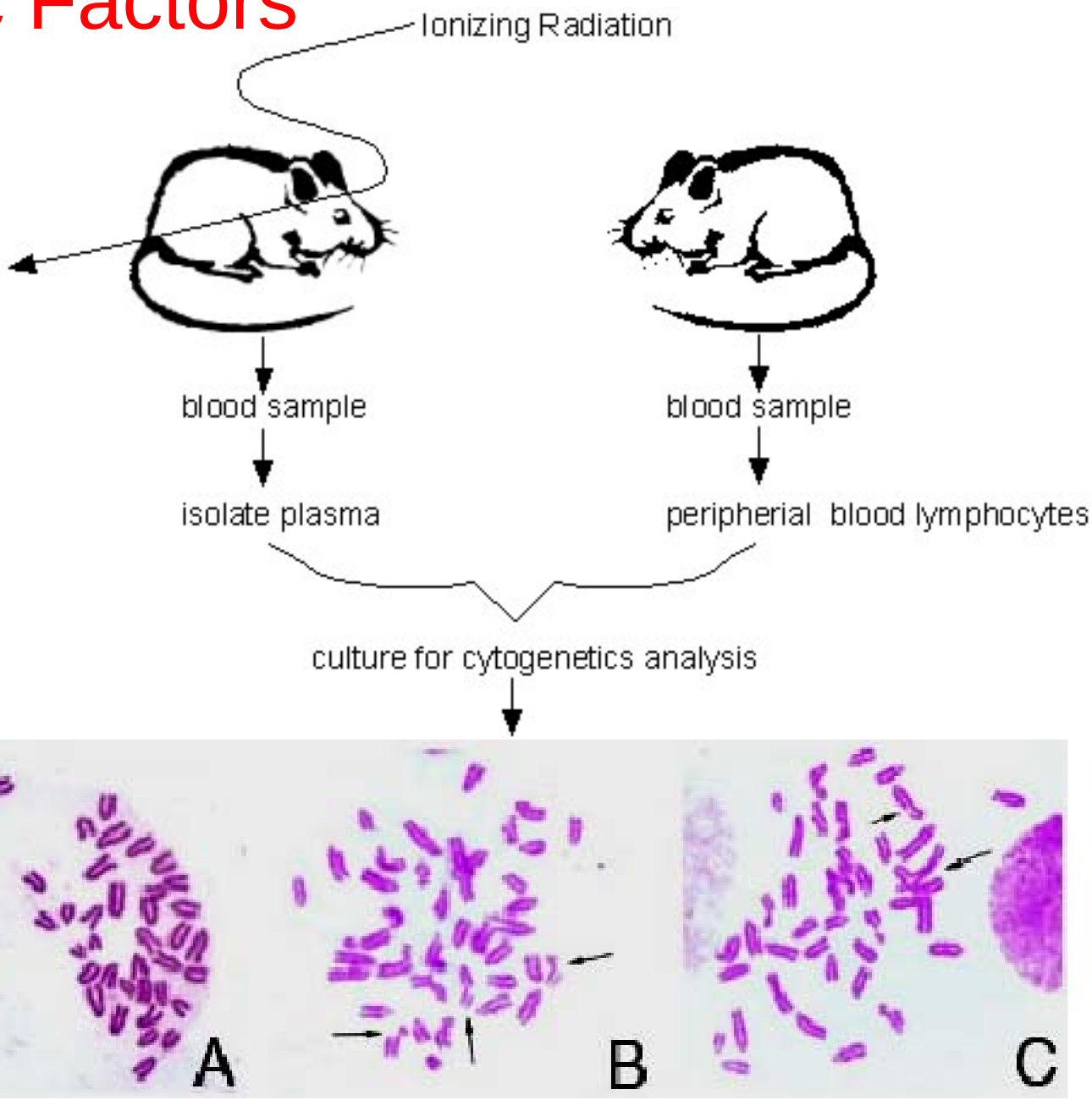
Chromosomal instability in progeny of non-irradiated hemopoietic stem cells

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, Exp. Biol. Med. 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)

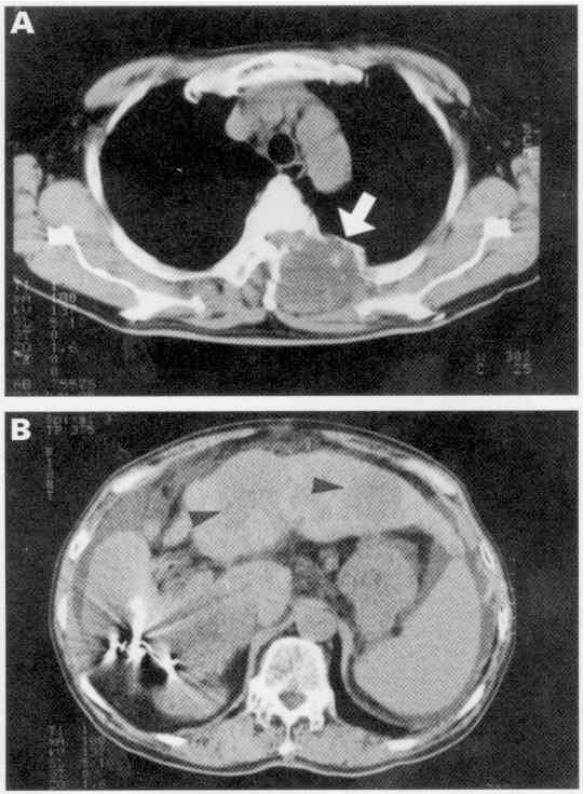
CF-Nelson rats

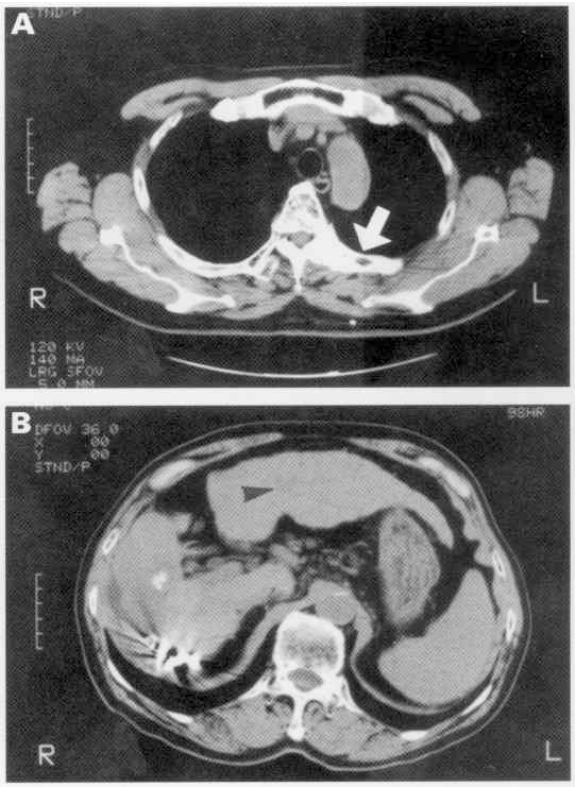
Fagnet et al., Cancer Genet. Cytogenet. 12, 73-83 (1984)

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)

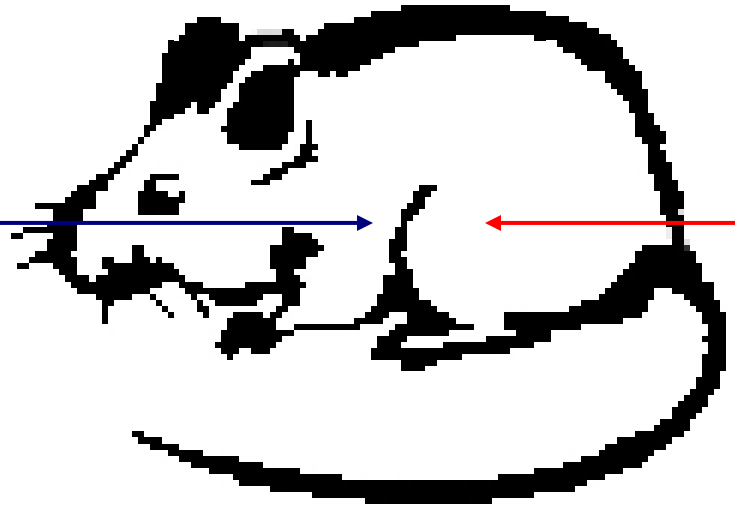




Retrospective analysis of serum concentrations of IL-1 beta, IL-2, IL-4, IL-6, HGF, and TNF-alpha

Abscopal antitumor effects *in vivo*

Implanted LLC cells

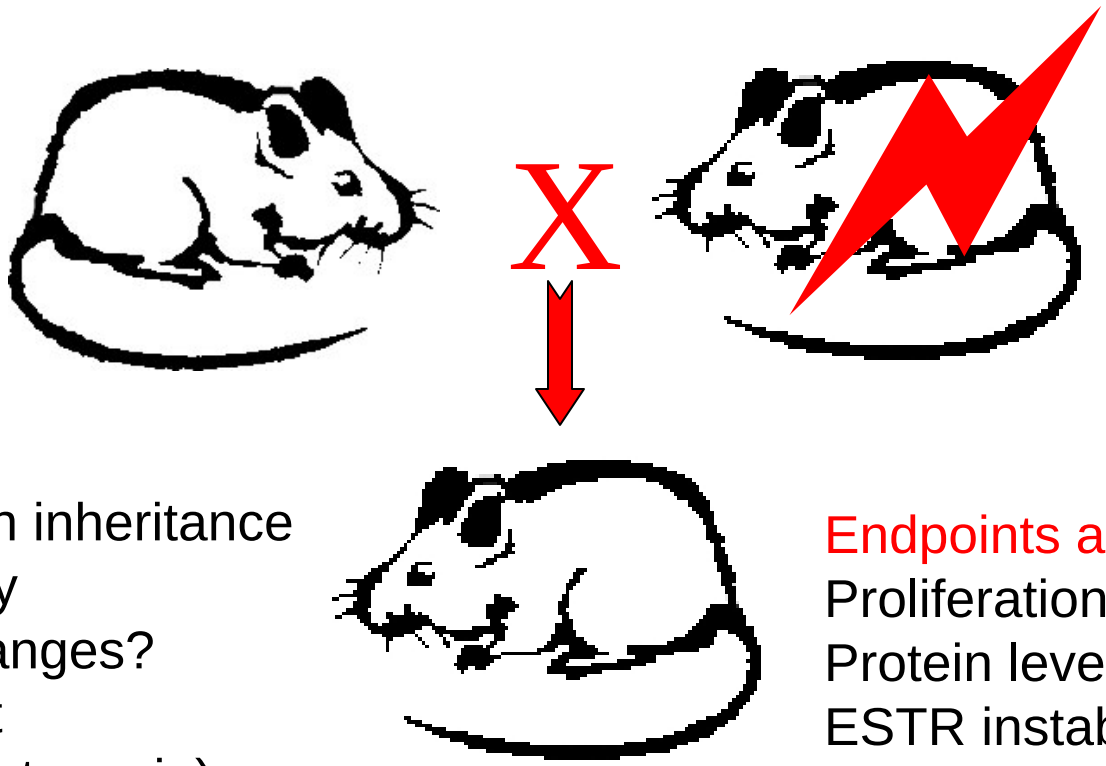


5 x 10Gy
12 x 2Gy
fractionated

Significant delay in implanted tumor cell growth. P53 mediated

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

Trans-generational (heritable) effects after preconception paternal irradiation



Non Mendelian inheritance
High frequency
Epigenetic changes?
Paternal effect
(type B spermatogonia)

Endpoints affected:
Proliferation rates
Protein levels & activity
ESTR instability
DNA damage (comets)
Microsatellite instability

Trans-generational effects

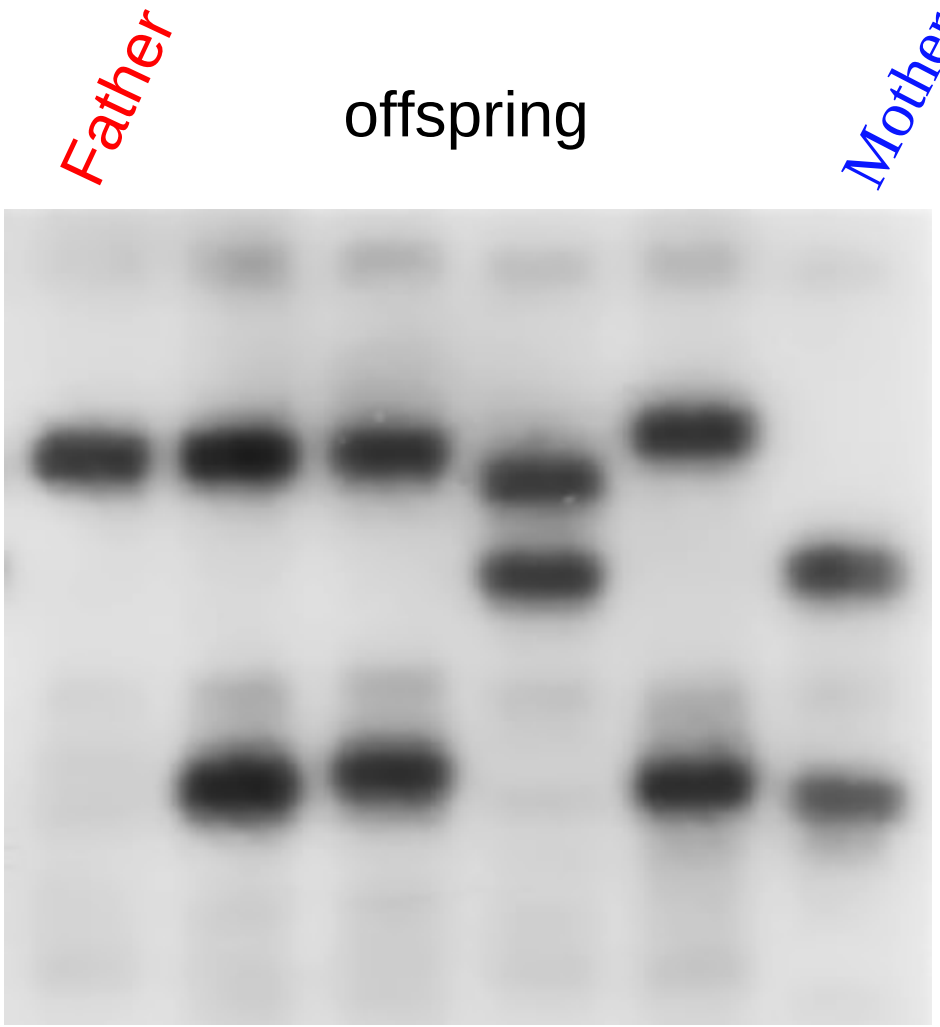
Children of A-bomb survivors showed no transmitted genetic effects

Kodaira et al. Am. J. Hum. Gen. 57, 1275-83 (1995)
Satoh et al. Env. Hlth. Pers. 104, 511-510 (1996)

Two fold increase in mini-satellite mutations in the progeny of individuals living in radiation contaminated environments

Dubrova et al. Nature 380, 683-686 (1996)
Dubrova et al. PNAS 95, 6251-6255 (1998)

Minisatellite mutation analysis at the mouse ESTR Ms6-hm loci



From Yuri Dubrova

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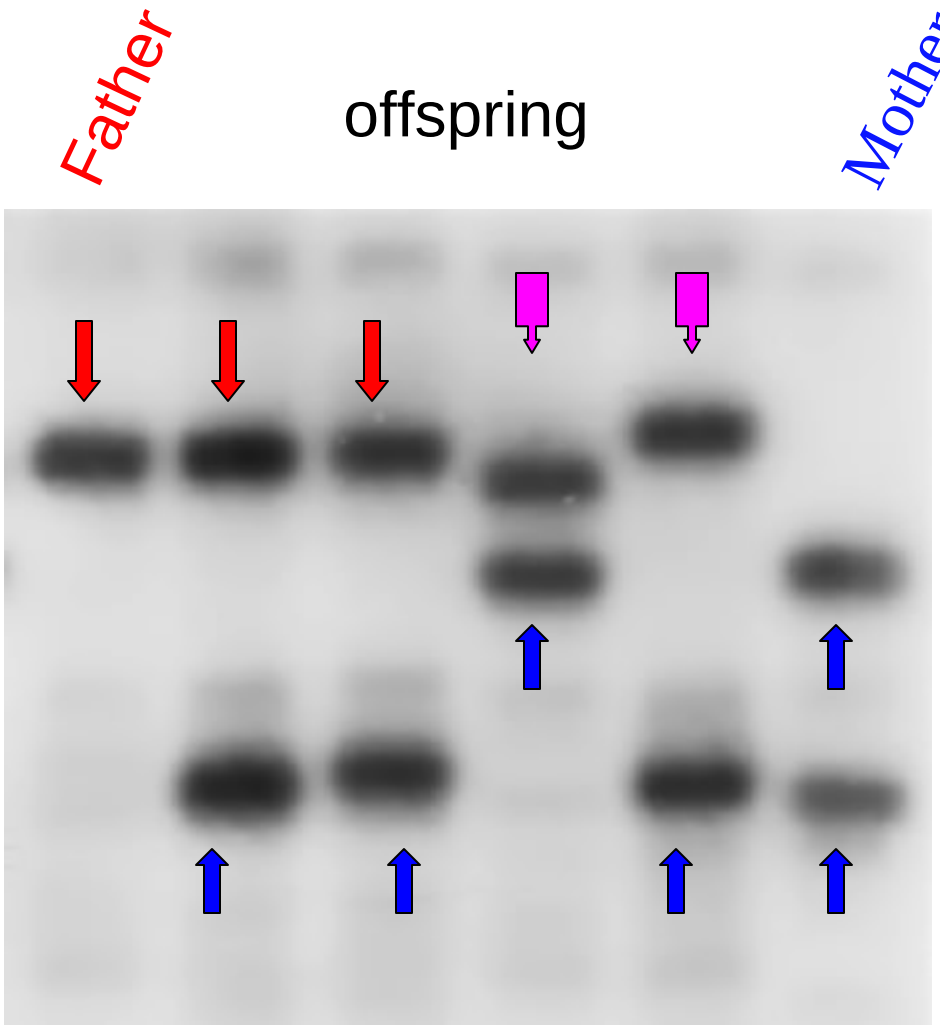

Paternal mutations

From Yuri Dubrova

Mini-satellites an efficient system for monitoring mutations in small population samples

High spontaneous frequency

Biological significance unknown

High frequency => not a direct effect of radiation

Reconciling the differences

Acute versus protracted exposure

Stage of spermatogenesis important

Chernobyl studies - often both parents exposed (female no effect)

Types of radiation

Japan: gamma and some neutrons

Chernobyl: ^{131}Iodine and ^{137}Cesium

SUMMARY

- Radiation can and does cause cancer
- Stochastic and deterministic effects
- Significant embryonic/fetal effects
- Evidence for transgenerational effects
- Acute versus protracted exposures
- Evolution in thinking of how deposition of energy results in these delayed effects

Comments and/or questions

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