Chromosomal Rearrangements - Just Because Repair has Happened, Does That Mean the Cell Got it Right? So What for the Fate of the Cell?

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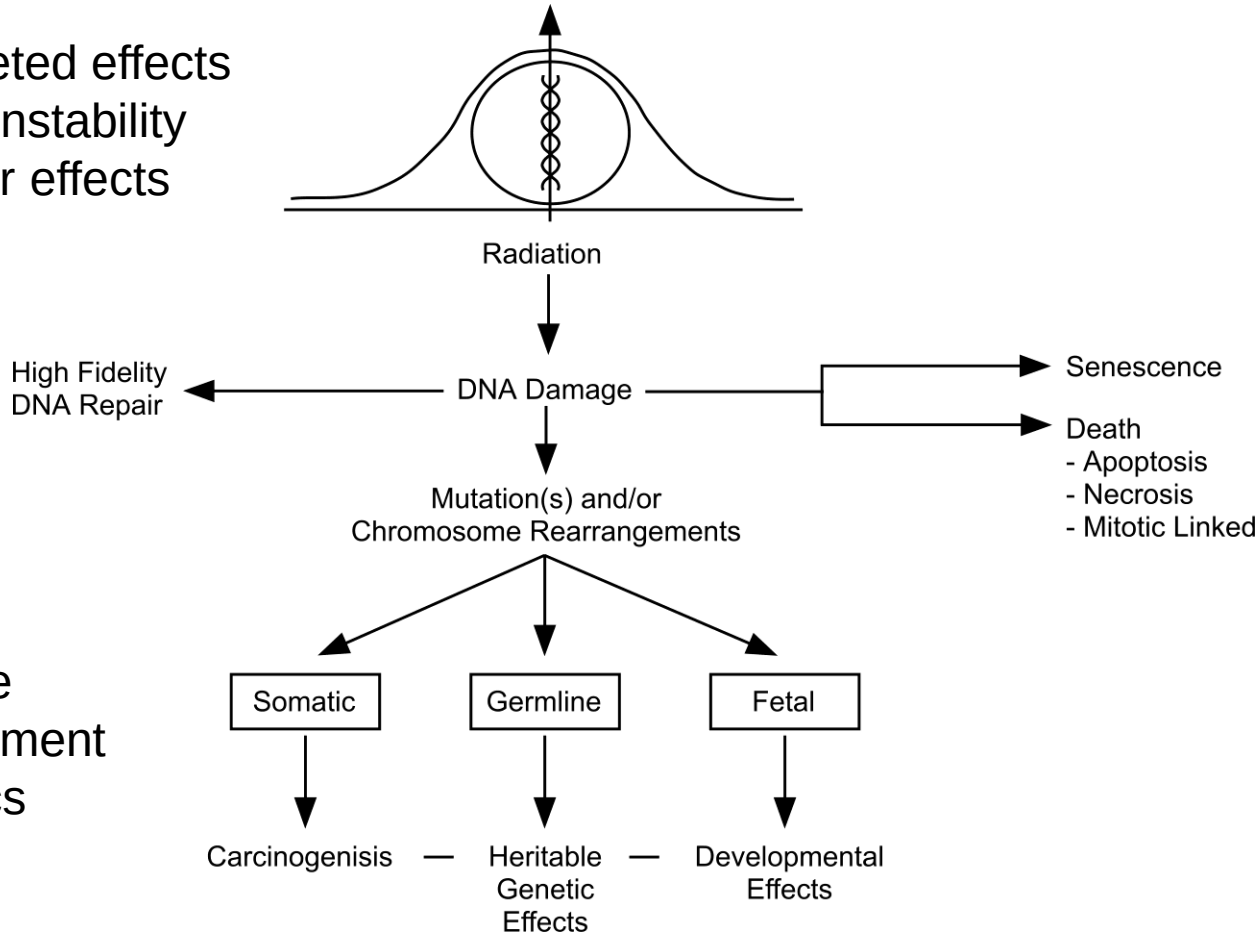
Long thought radiation had to deposit its energy in the cell nucleus and that **DNA was the target** for the observed biological effects.

Non targeted effects
Delayed instability
Bystander effects



Lifestyle
Environment
Genetics

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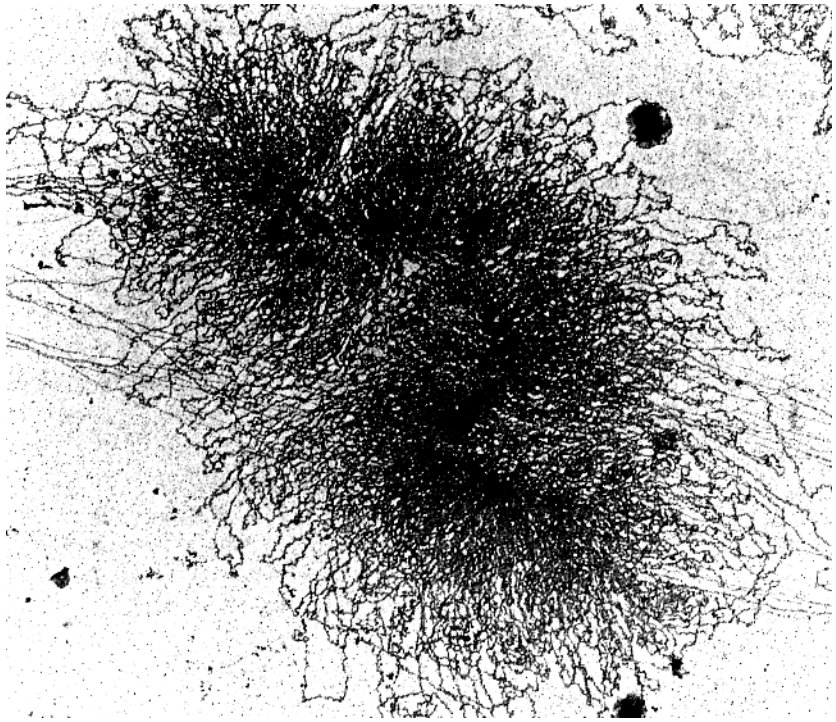
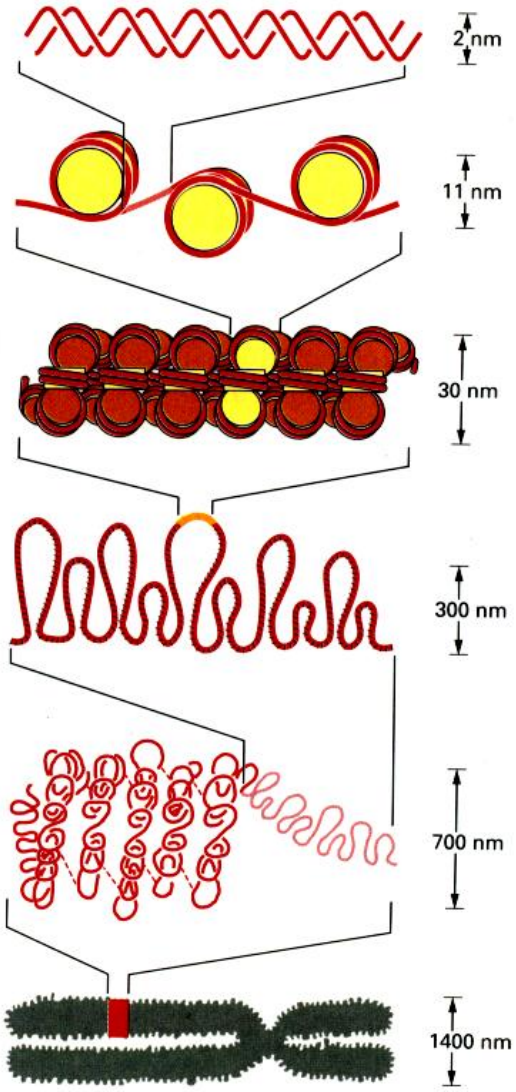
Yield of radiation-induced damage affected by macromolecular organization of DNA





From: Watson et al. “Mol. Biol. of the Cell”

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DNA damage is the result of direct and indirect effects

All 4 bases subject to damage;
~9eV sufficient to break DNA backbone
SSB correlates poorly with lethality

DSB most important lesion

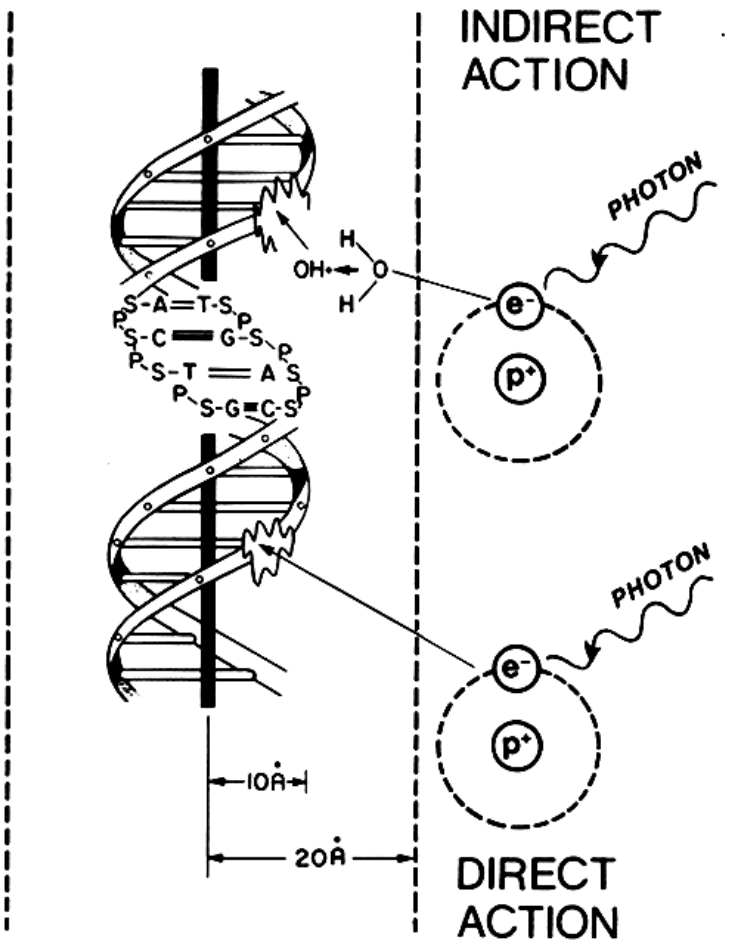
Damage / Gy of X-rays:

40 DSBs

150 DNA crosslinks

1,000 SSB

2,500 base damages



From: Hall, “Radiobiology for the Radiologist”

Methods for “evaluating” DNA double-strand breaks

- Nucleoid sedimentation
- Neutral elution
  - **Alkaline elution - SSB**
- Neutral comets
  - **Alkaline comets - SSB**
- Pulsed field gel electrophoresis
- Foci formation
  - **γH2AX, p53bp1**

Double-strand break primary lesion leading to gross chromosomal rearrangements

- Chromosome aberrations
- Premature chromosome condensation
- Micronuclei

Methods for “evaluating” DNA double-strand breaks

Nucleoid sedimentation (very high radiation doses only)

Neutral elution (elute DNA through a filter)

Alkaline elution - SSB

Neutral comets (electrophorese DNA from a cell)

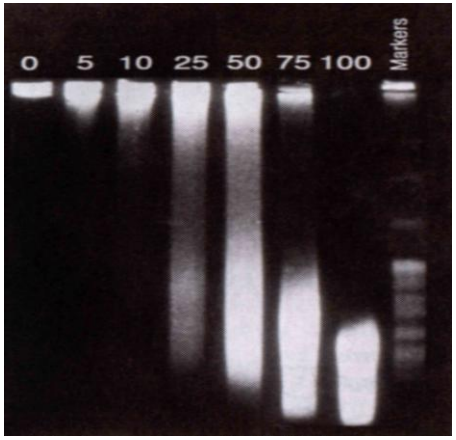
Alkaline comets - SSB

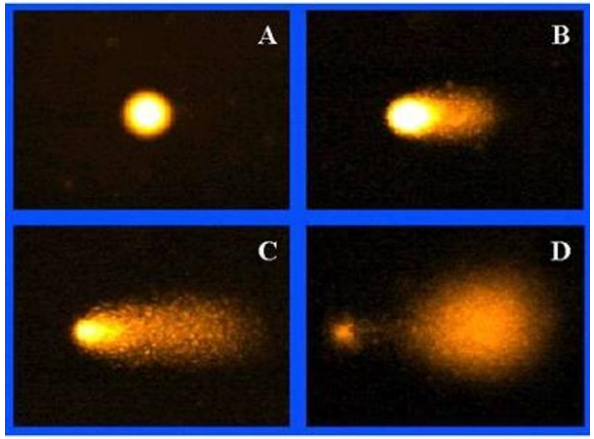
Pulsed field gel electrophoresis

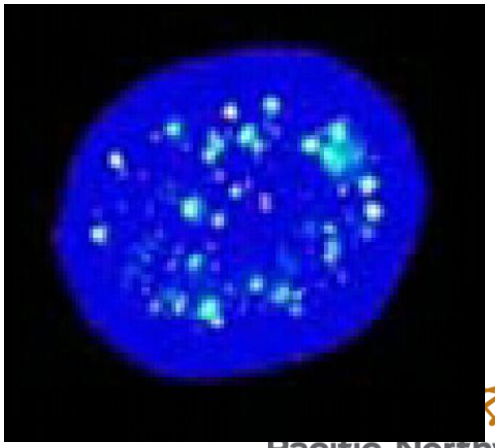
Foci formation (repair complexes at sites of breakage)

γH2AX, mre11

Double-strand break primary lesion leading to gross chromosomal rearrangements and ultimately lethality

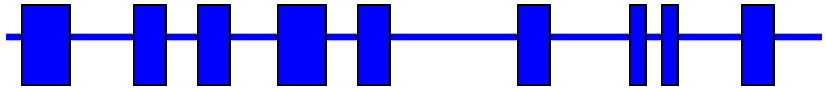




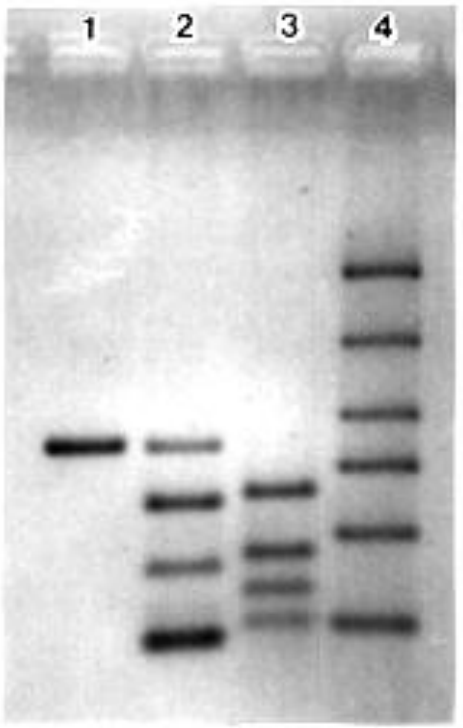


Specific locus mutations

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



X chromosome
Positive selection
(6-Thioguanine)
Mutants survive
frequency
Molecular analysis
Southern blots
Multiplex PCR



Signature of damage?

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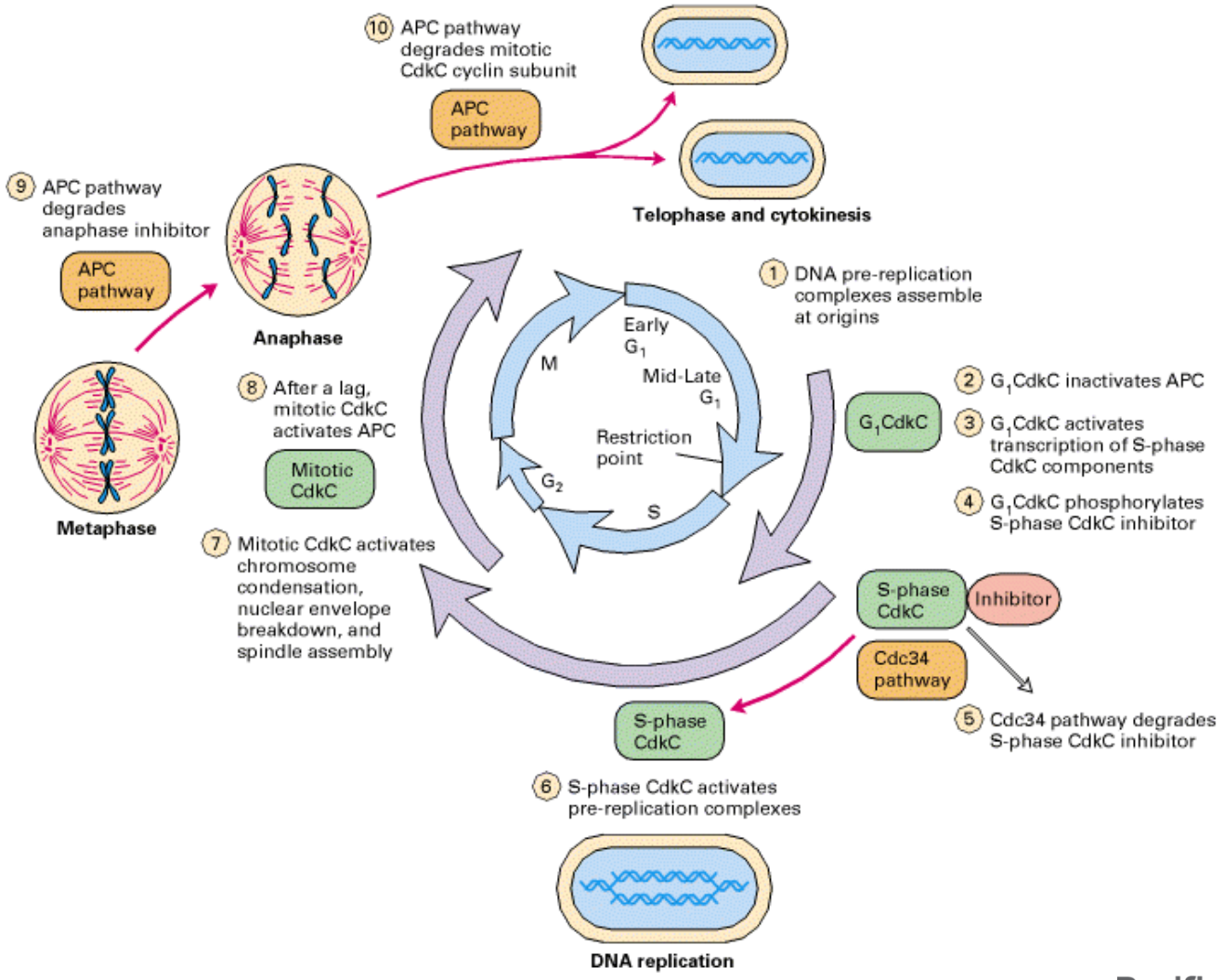
THE CELL CYCLE



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Type of cytogenetic damage observed depends upon where in the cell cycle irradiation occurs

CHROMOSOME ABERRATIONS

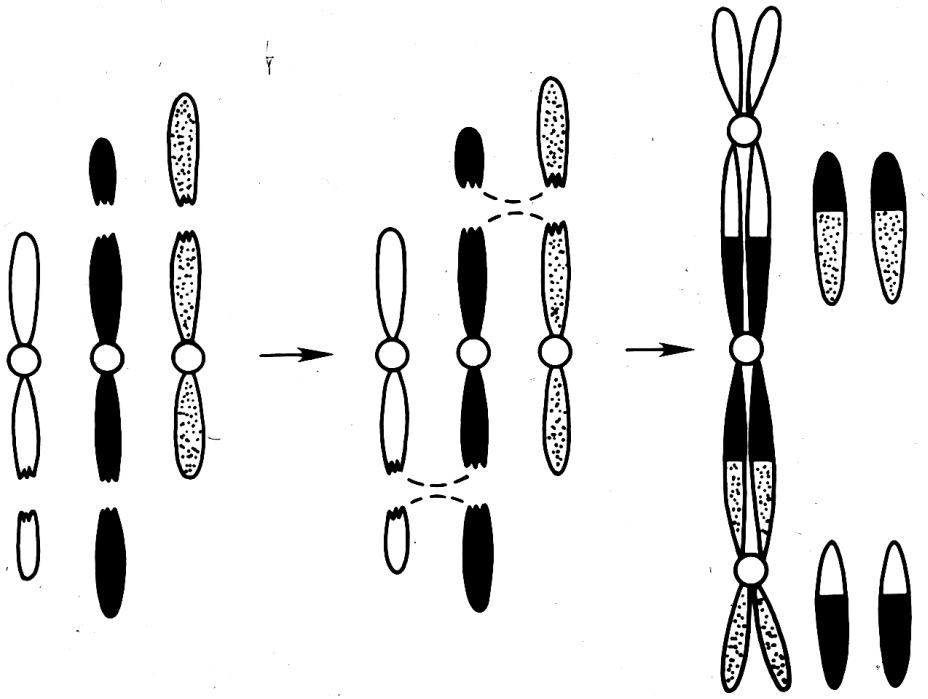
- G_1 irradiation
- Both sister chromatids involved

CHROMATID ABERRATIONS

- S or G_2 irradiation
- Usually only 1 chromatid involved

Cellular Responses to Irradiation

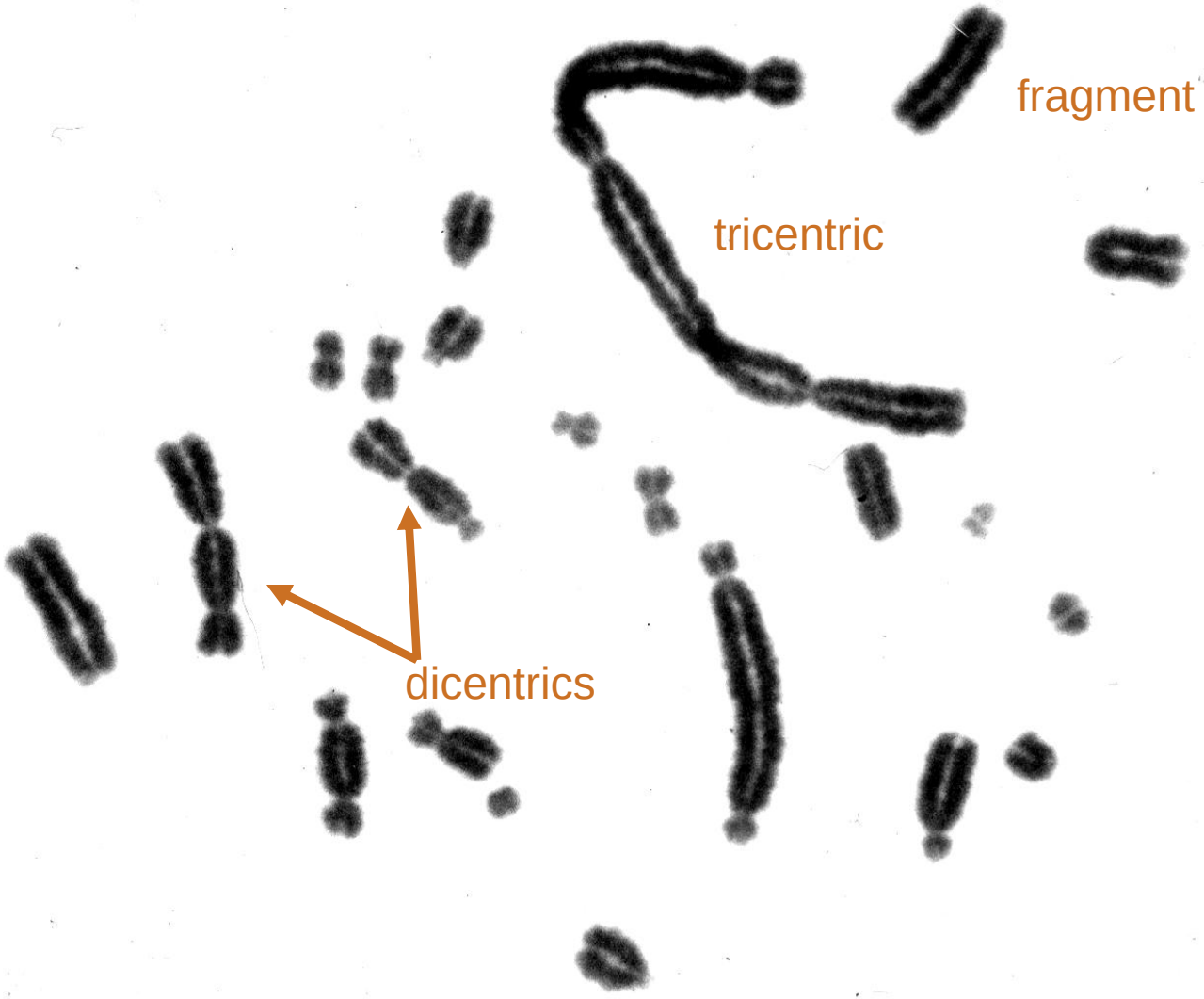
"DNA repair"
- faithful repair
- mis-repair
- mis-rejoining
- no repair



Cell cycle arrest

Signal transduction cascades

Multiple mis-rejoining events occurring in CHO chromosomes after G1 irradiation



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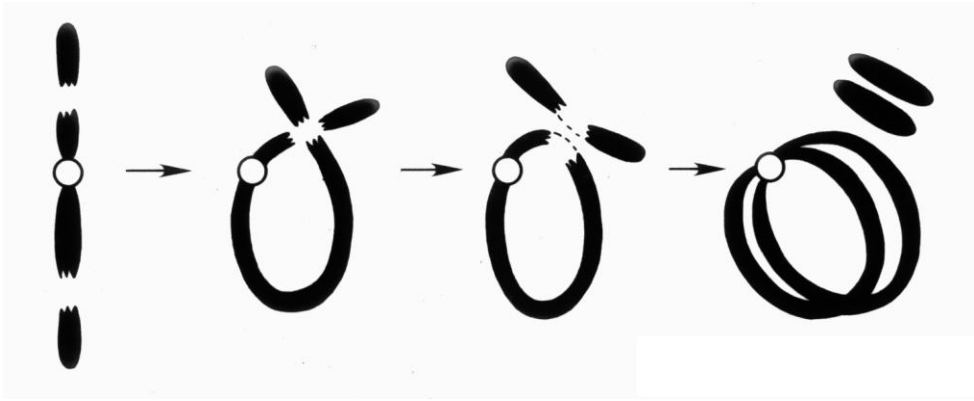
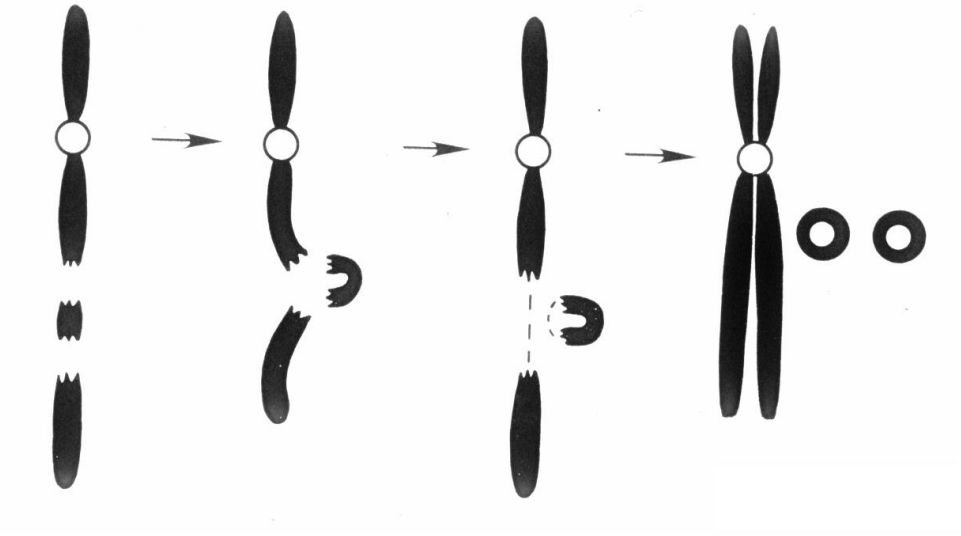
Mis-rejoining of 2 breaks on one chromatid after G_1 irradiation

Centric ring
+ fragment



Acentric ring
or interstitial
deletion



Ring chromosome

Acentric ring or interstitial deletion

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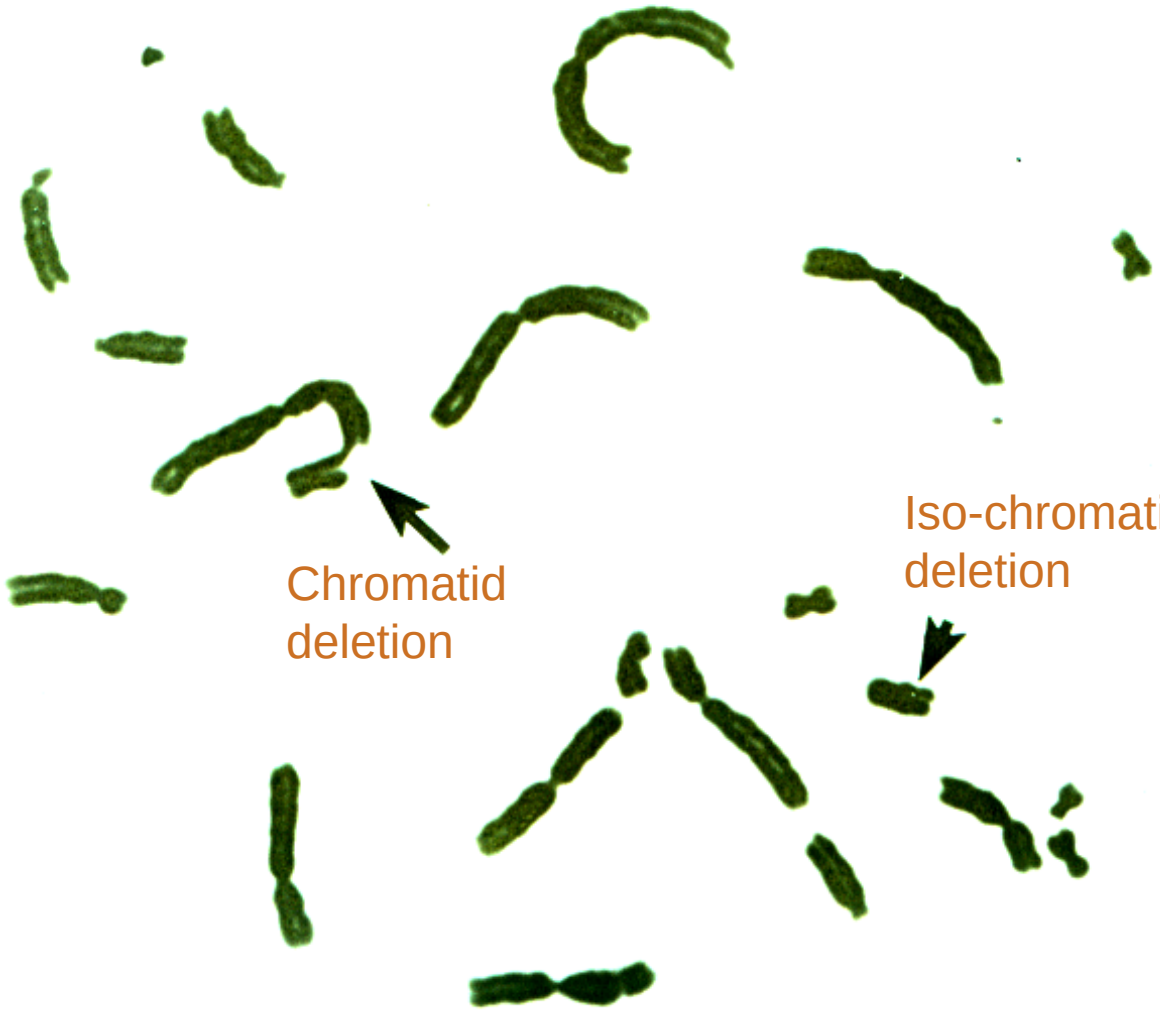
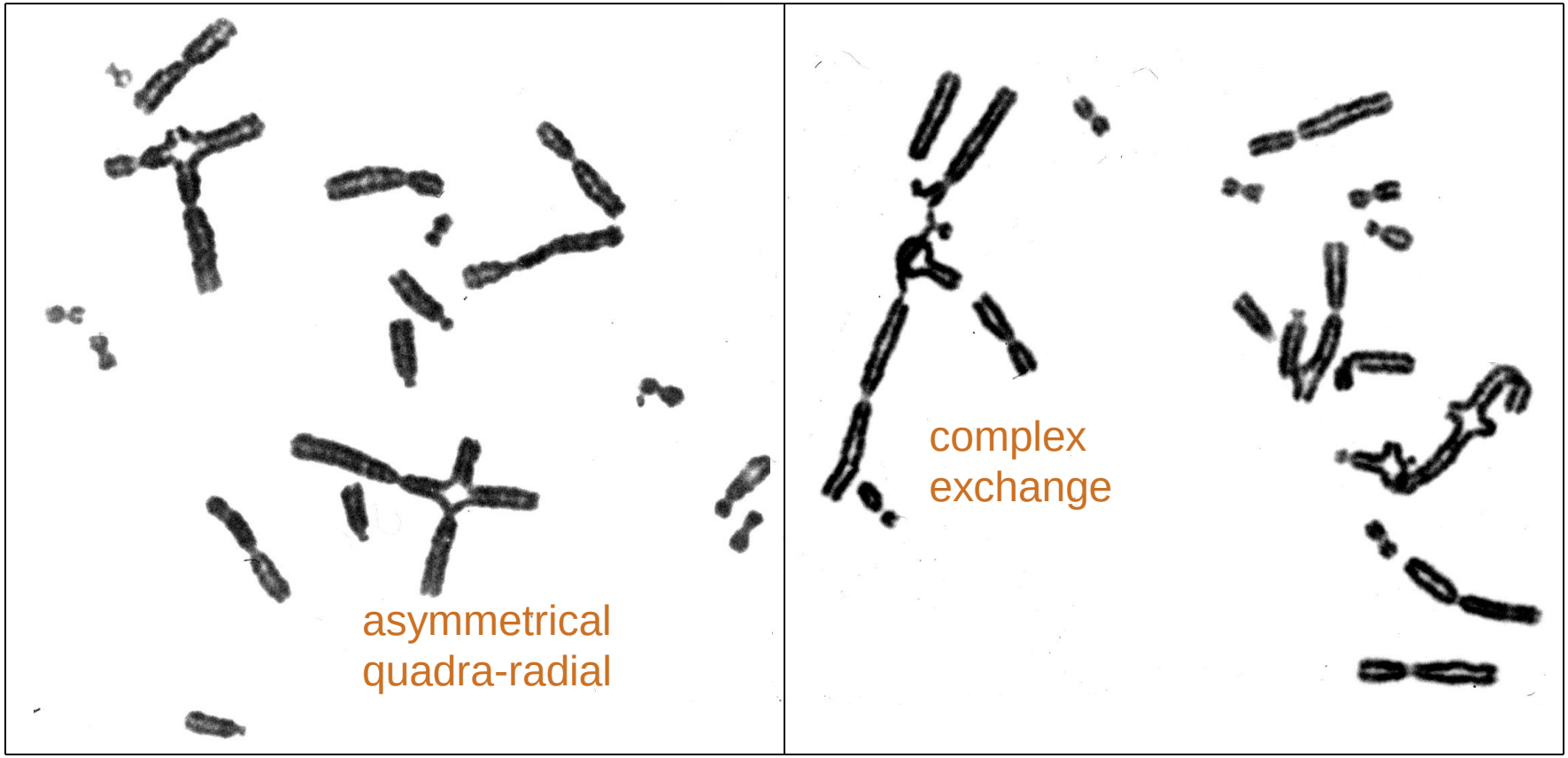
Chromatid deletions in CHO chromosomes after irradiation in S or G_2



Chromatid exchanges in CHO chromosomes after irradiation in S or G_2



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Note: the cell has responded to the induced damage. Cell cycle checkpoints are activated and DNA repair processes have recognized and responded to induced double-strand breaks.

Wrong ends are rejoined!

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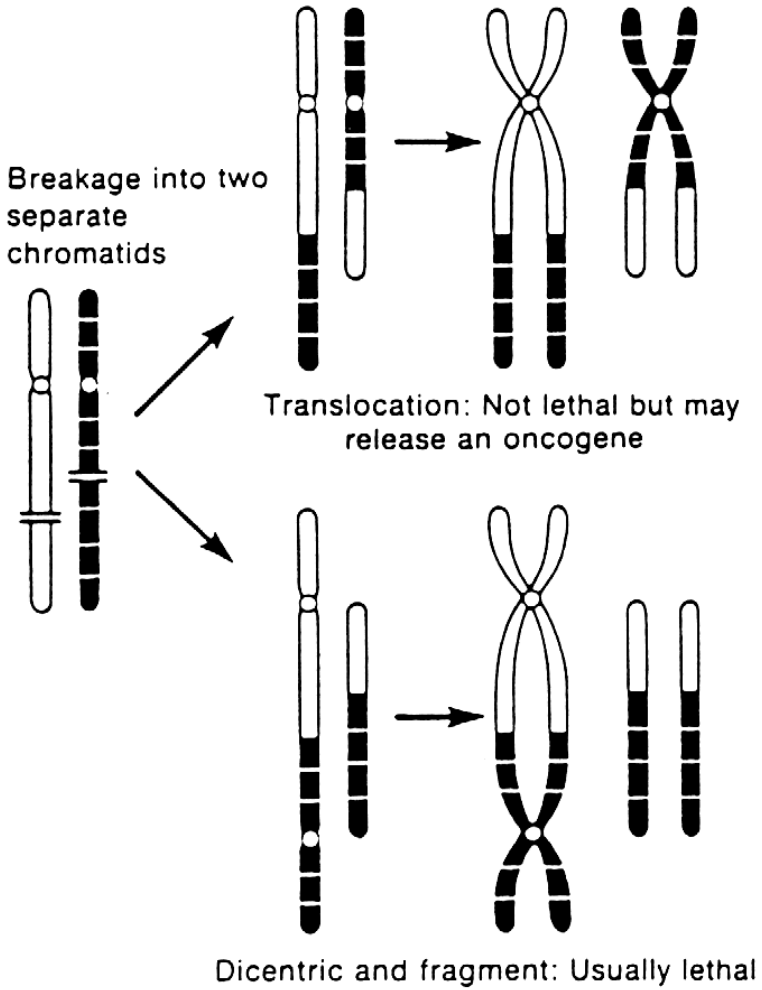
FATE OF REARRANGED CHROMOSOMES

Exchange-type rearrangements



Symmetrical (balanced) gene rearrangements

Asymmetrical (not balanced) fragment usually lost

Polycentric chromosomes

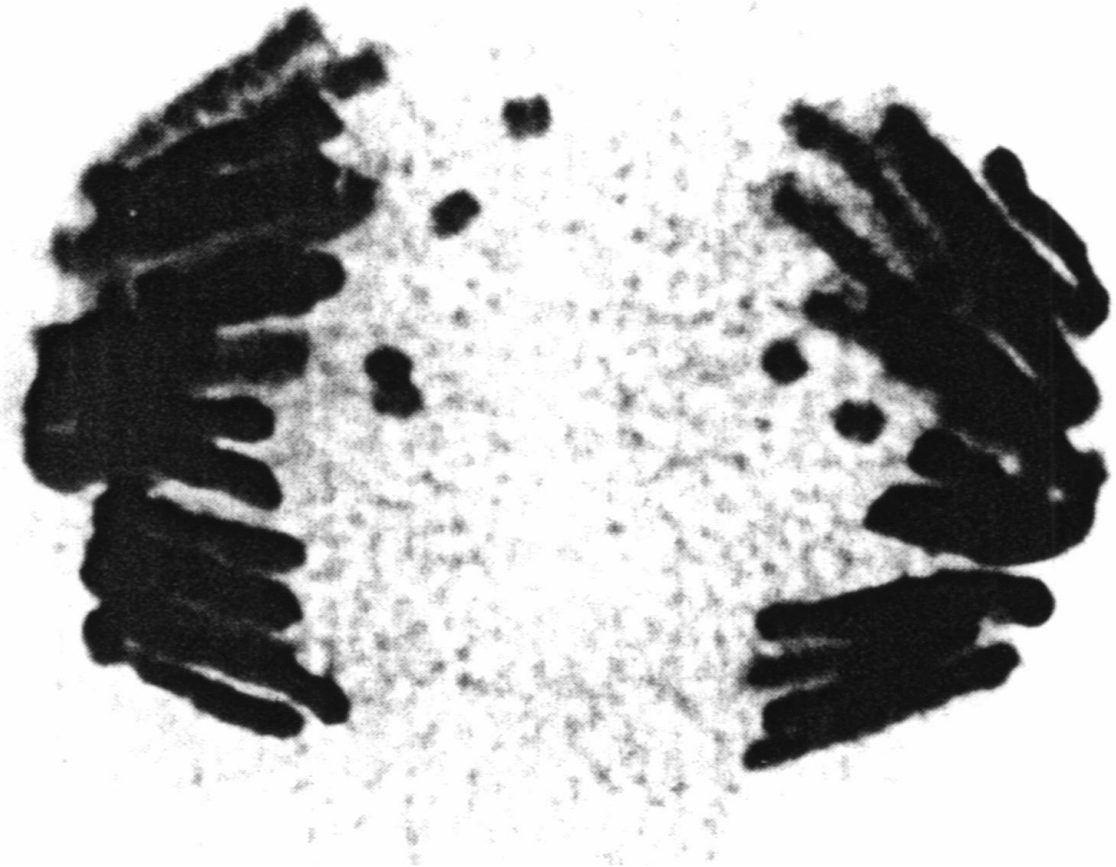
- bridge-breakage-fusion
- fail mitosis
  - cell death
  - aneuploidy

From: Hall “Radiobiology for the Radiologist”

FATE OF REARRANGED CHROMOSOMES

Deletions (acentric fragments)

Lag at anaphase and lost at mitosis



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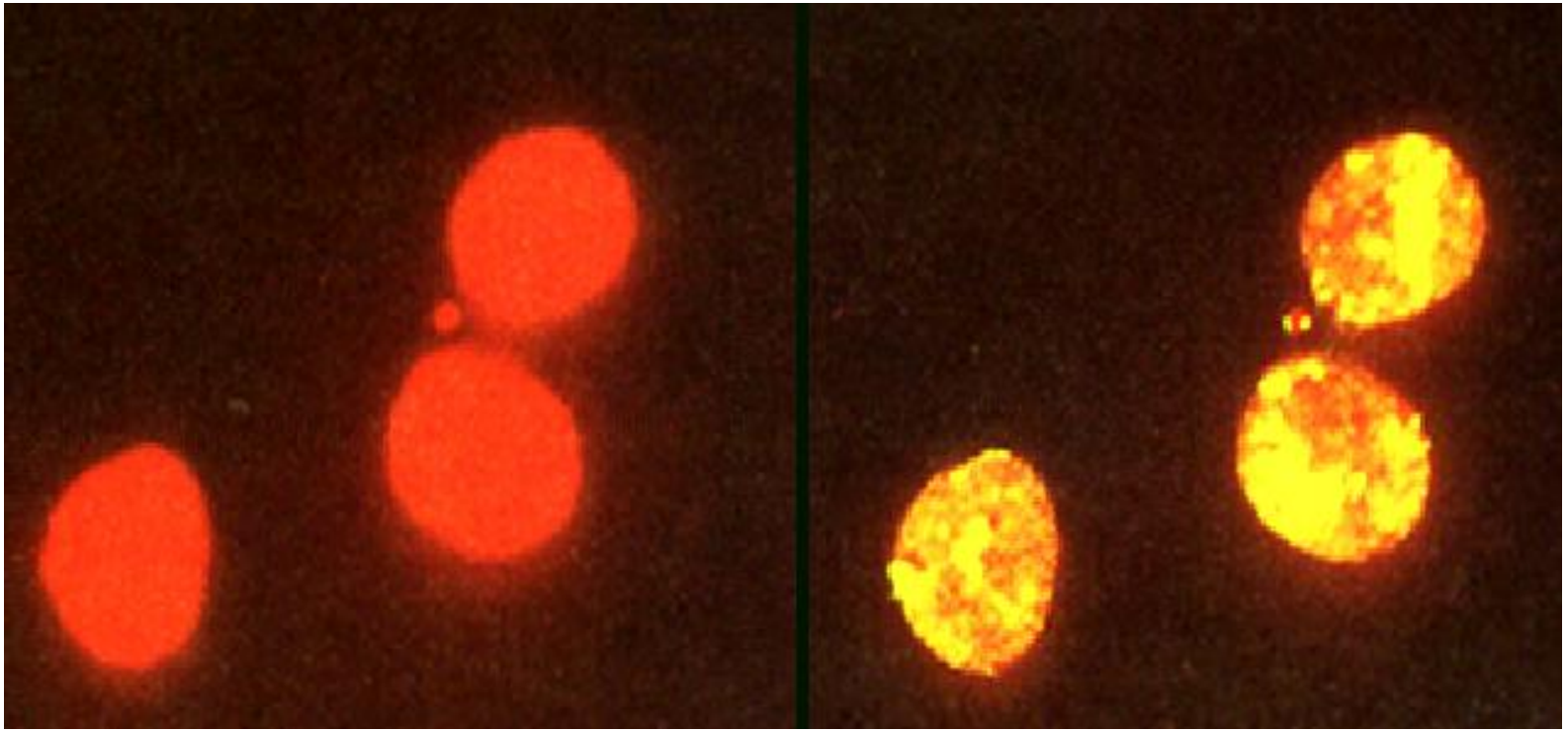
Deletions are lost at mitosis Visualized as micronuclei (analysis automated)



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Analysis of chromosomal dicentrics the best biomarker of radiation exposure

- sensitive (100 mGy)
- reliable
- large data-base

Application of new techniques to cytogenetic analysis:

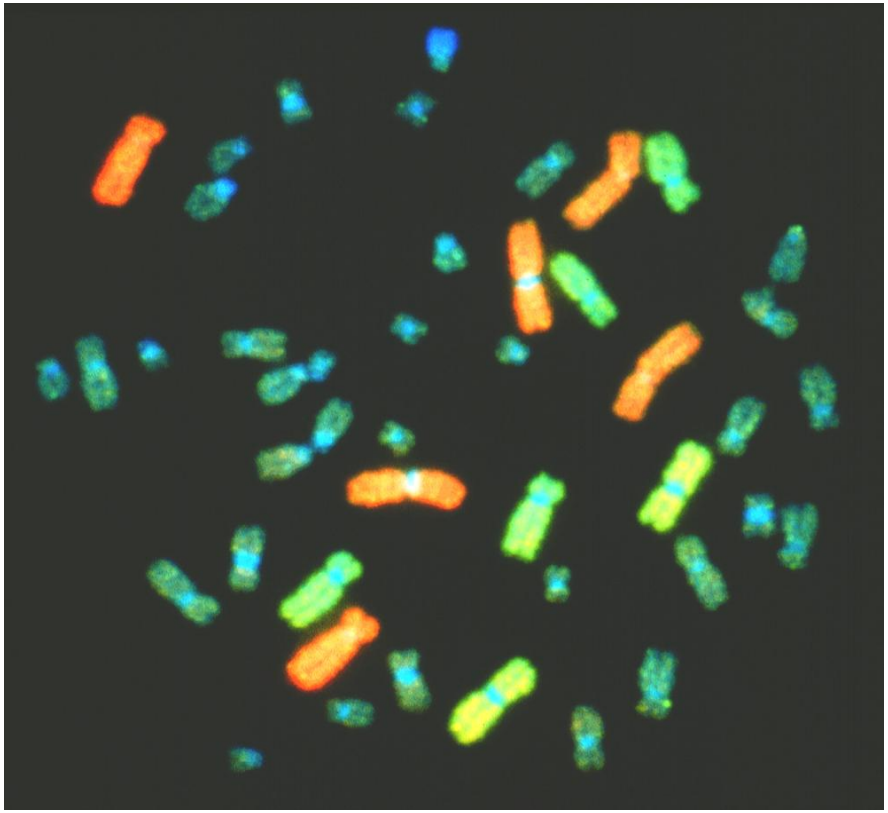
Fluorescence *In Situ* Hybridization (FISH)

Combinatorial "painting" (SKY and mFISH)

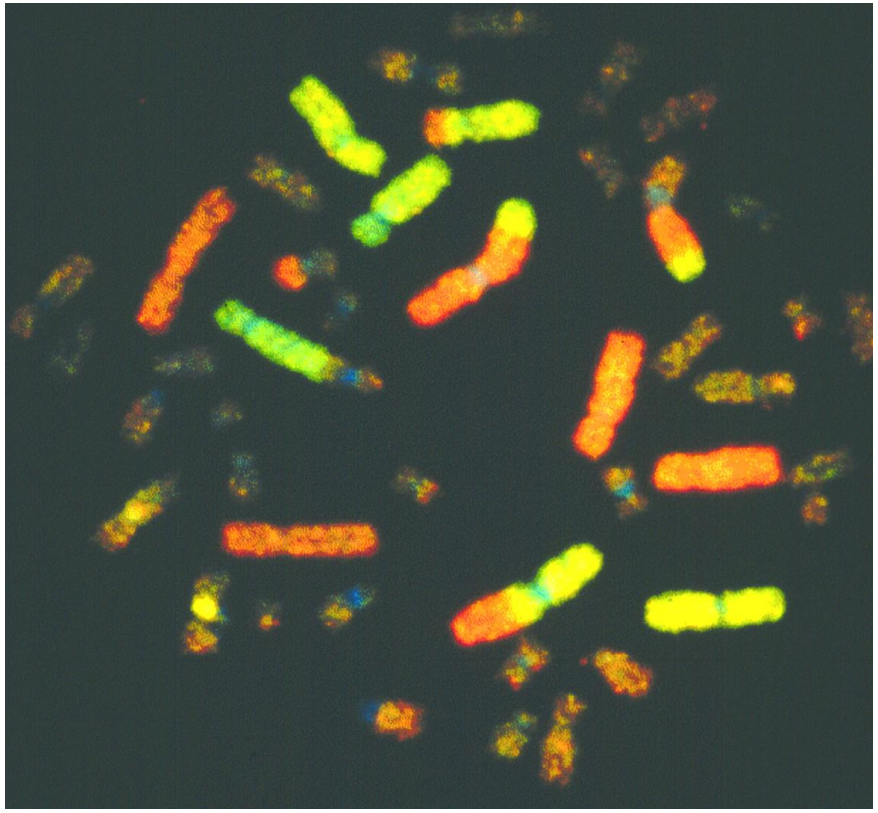
mBAND (homologous chromosomes)

Multi-color FISH in human lymphocyte chromosomes

Non-irradiated



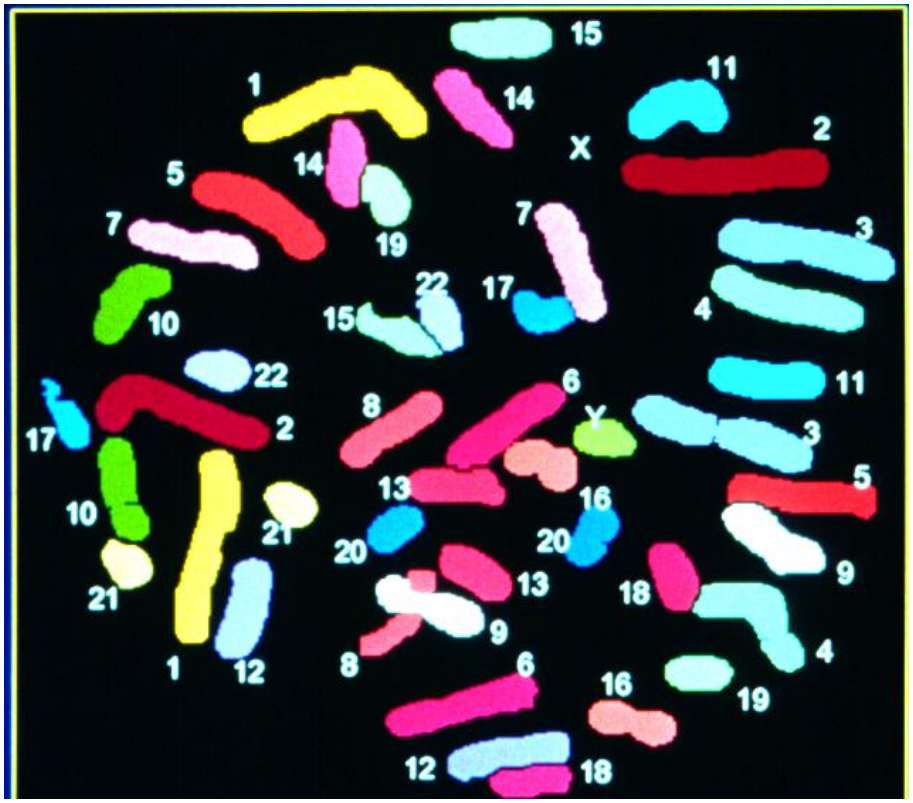
Irradiated



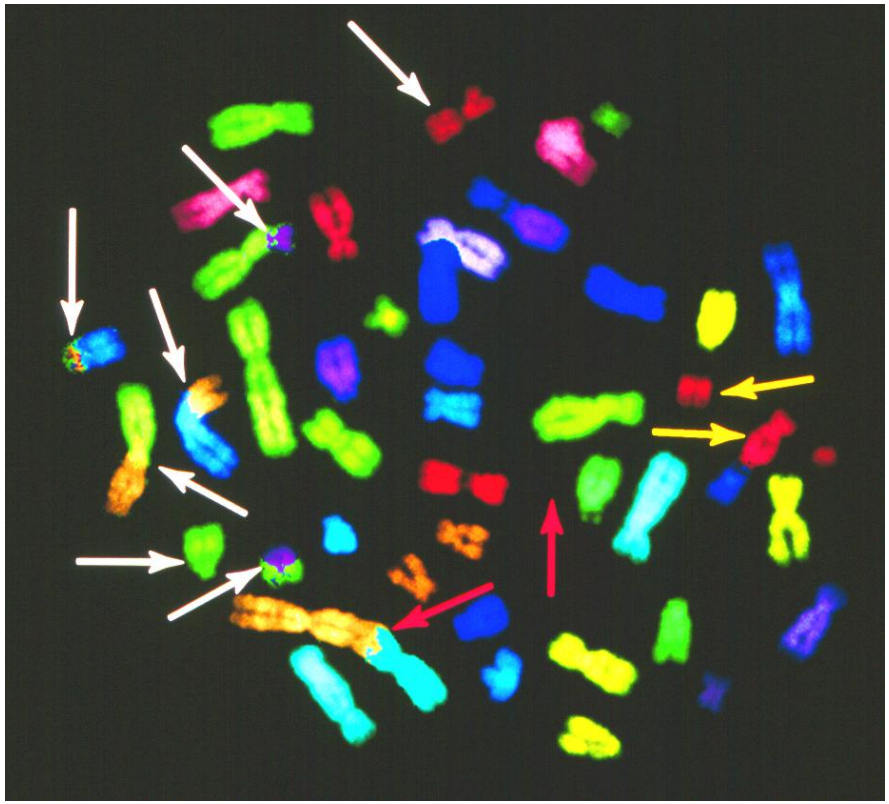
From: Dr. J.D. Tucker

Combinatorial “painting” - limited use for rare events

Spectral karyotying (Sky)



m-FISH after irradiation



From: Dr. M. Cornforth

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mBAND probe sets available for all chromosomes
Complete chromosome coverage

~ 550 band resolution in G banding (XCyte)

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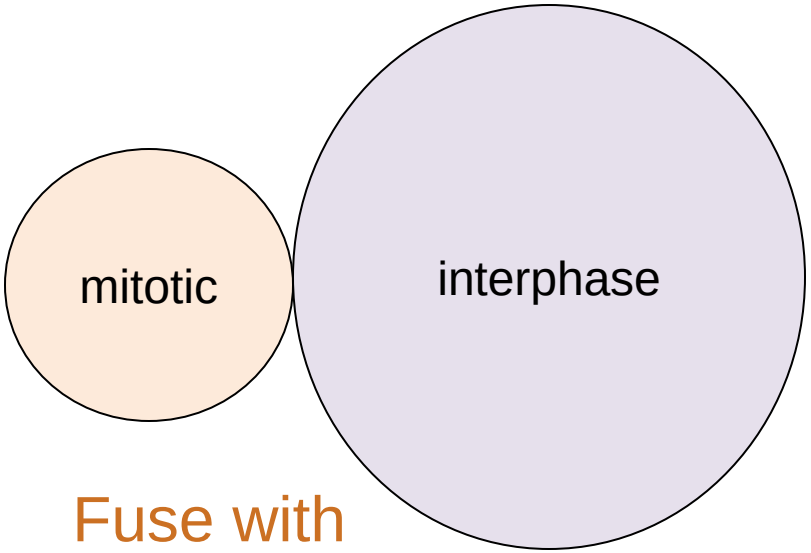
Cytogenetic damage without requiring that cells reach metaphase: **P**REMATURE **C**HROMOSOME **C**ONDENSATION **(PCC)**



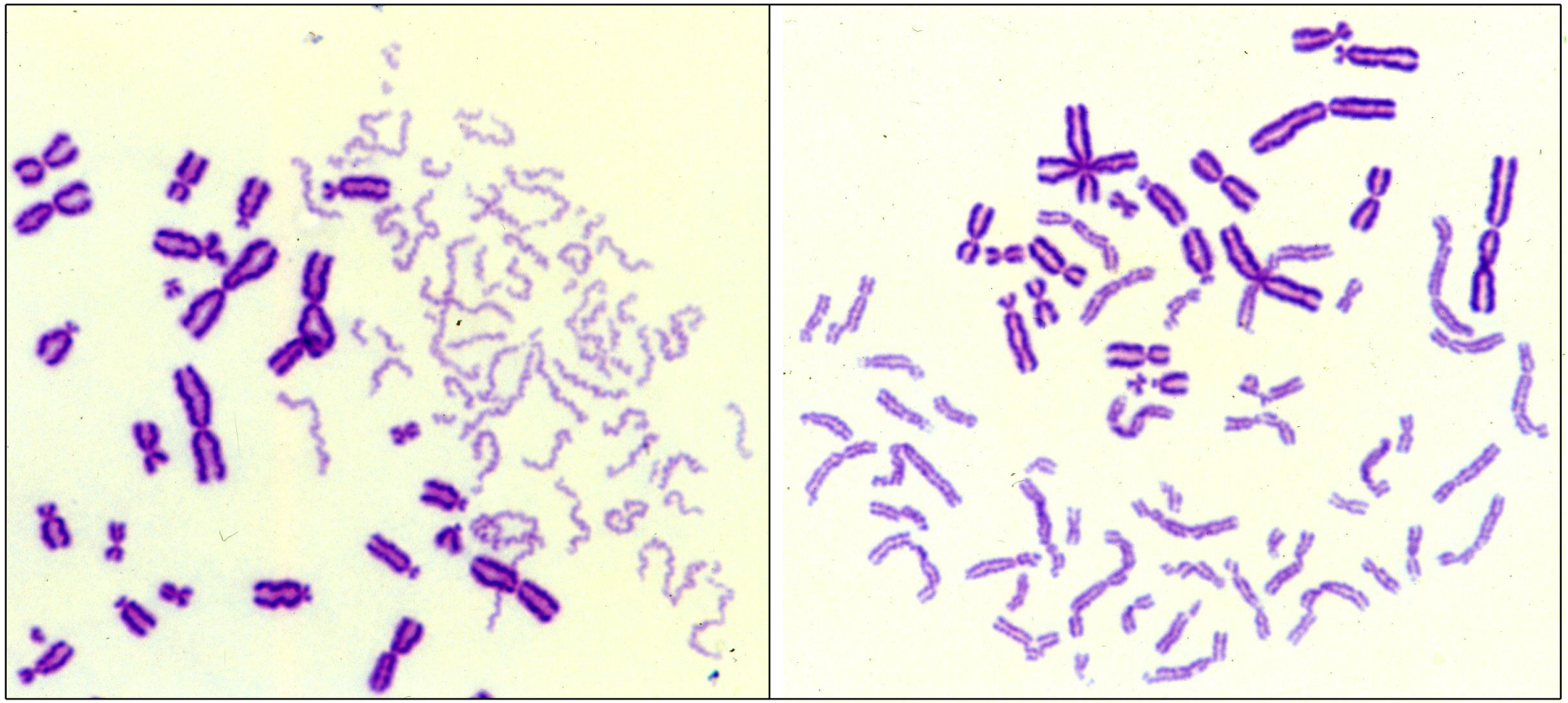
Fuse with
Sendai virus or PEG

Mitotic factors cause interphase chromatin to prematurely condense

Mitotic CHO cells fused with human lymphocytes

G_0 lymphocytes

Irradiated G_2 lymphocytes



SCE frequency significantly increased after exposure to many DNA damaging agents, particularly alkylating agents, not after ionizing radiation

Control (untreated)

Mitomycin C treated

Yield of radiation-induced chromosome damage

1. Deletions

Terminal deletion = 1 hit
Chromatid deletion = 1 hit
Interstitial deletion = 2 hits

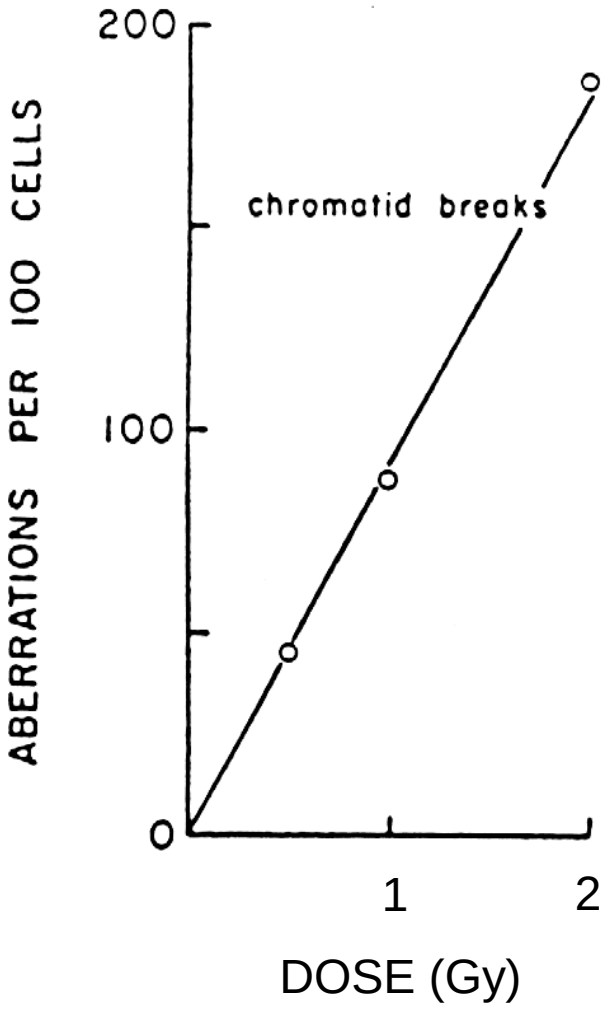
Yield (Y) ~ linear

$$Y = k + \alpha D$$

k = background
α = proportionality

Fate:

Deletions lost at mitosis



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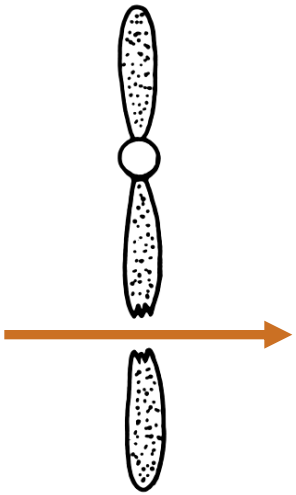
Yield of radiation-induced chromosome damage

2. Exchange-type rearrangements

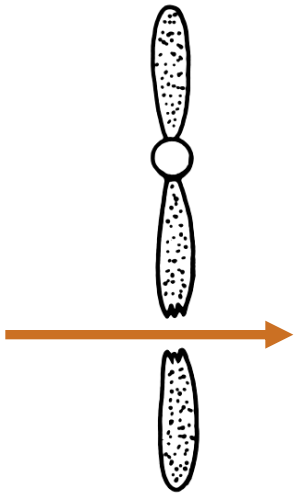
≥ 2 hits required; dependent upon:

SPACE = proximity



P α D

TIME = interaction

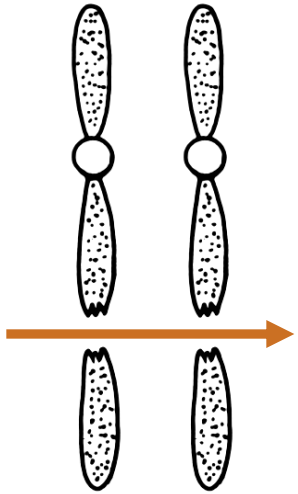


P α D

$$P\ (2\ hits) = D \times D = D^2$$

$$Y\ (yield) = k + D^2$$

$$Y = k + \beta D^2$$



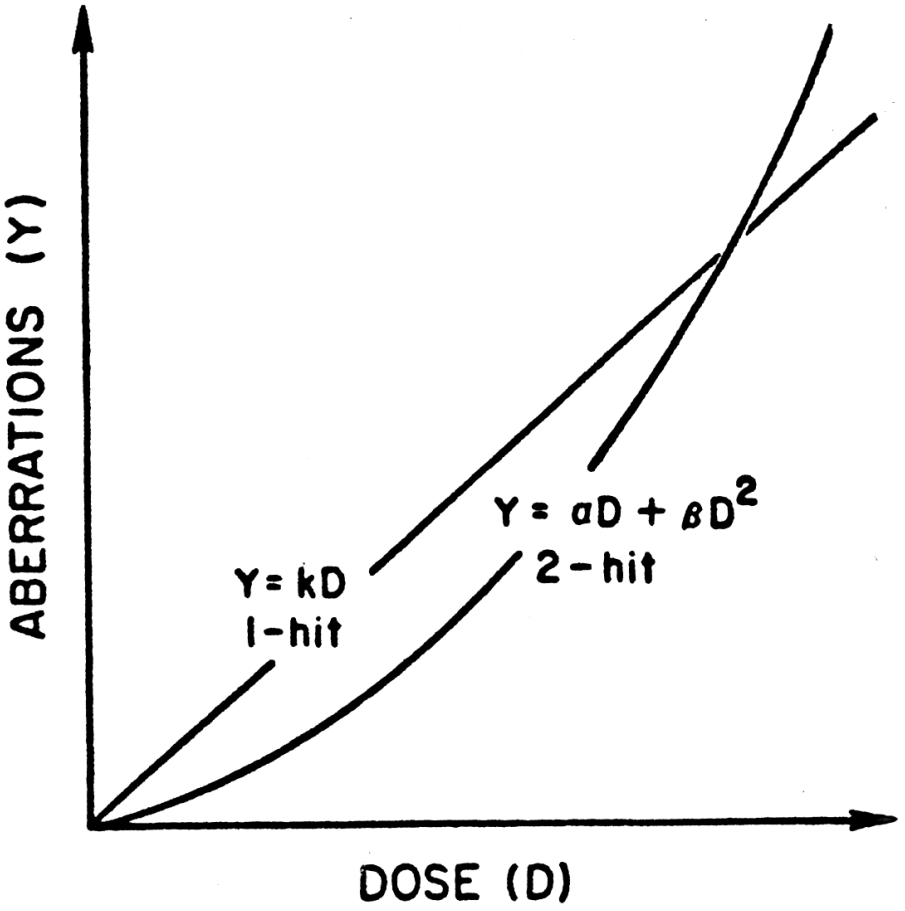
Linear component

$Y = \alpha D$

Linear + quadratic

For exchanges

$Y = k + \alpha D + \beta D^2$



Low dose dominated by linear component assume k (background) constant

$$Y = k + \alpha D + \beta D^2$$

$$Y(0.1Gy) = 0.1 + (0.1)^2 = 0.11$$
$$Y(0.2Gy) = 0.2 + (0.2)^2 = 0.24$$

$$Y(1.0Gy) = 1.0 + (1.0)^2 = 2.00$$
$$Y(2.0Gy) = 2.0 + (2.0)^2 = 6.00$$
$$Y(3.0Gy) = 3.0 + (3.0)^2 = 12.00$$

$$Y(6.0Gy) = 6.0 + (6.0)^2 = 42.00$$

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DOSE RATE EFFECTS

One hit aberrations - no dose rate effect

Two hit aberrations - dose and fractionation effect

As dose rate <

aberrations <

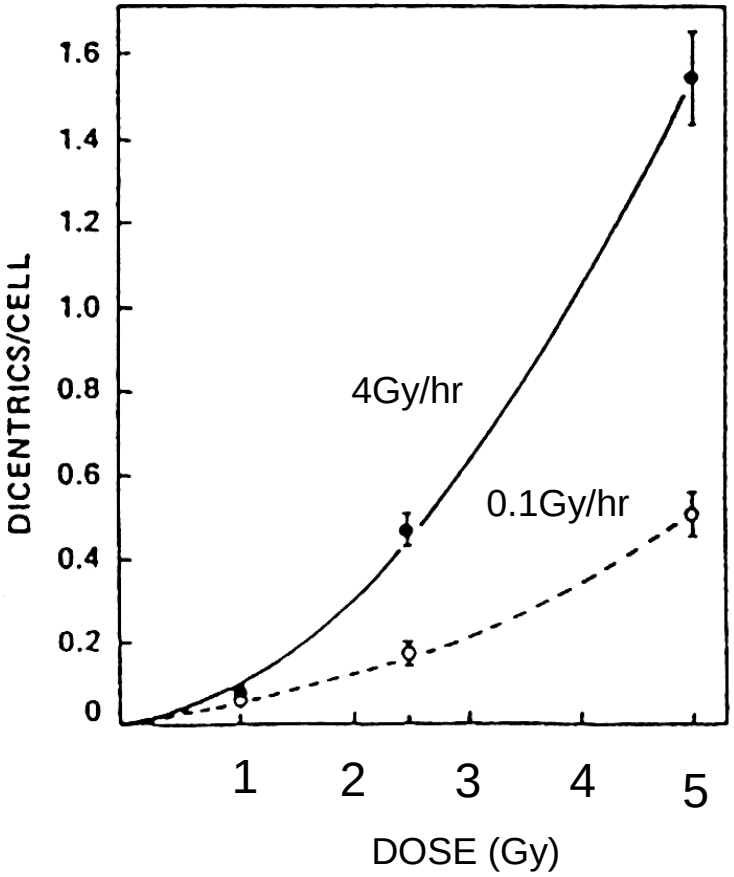
WHY?

Repair

Breaks rejoined

thus unavailable for

further interaction



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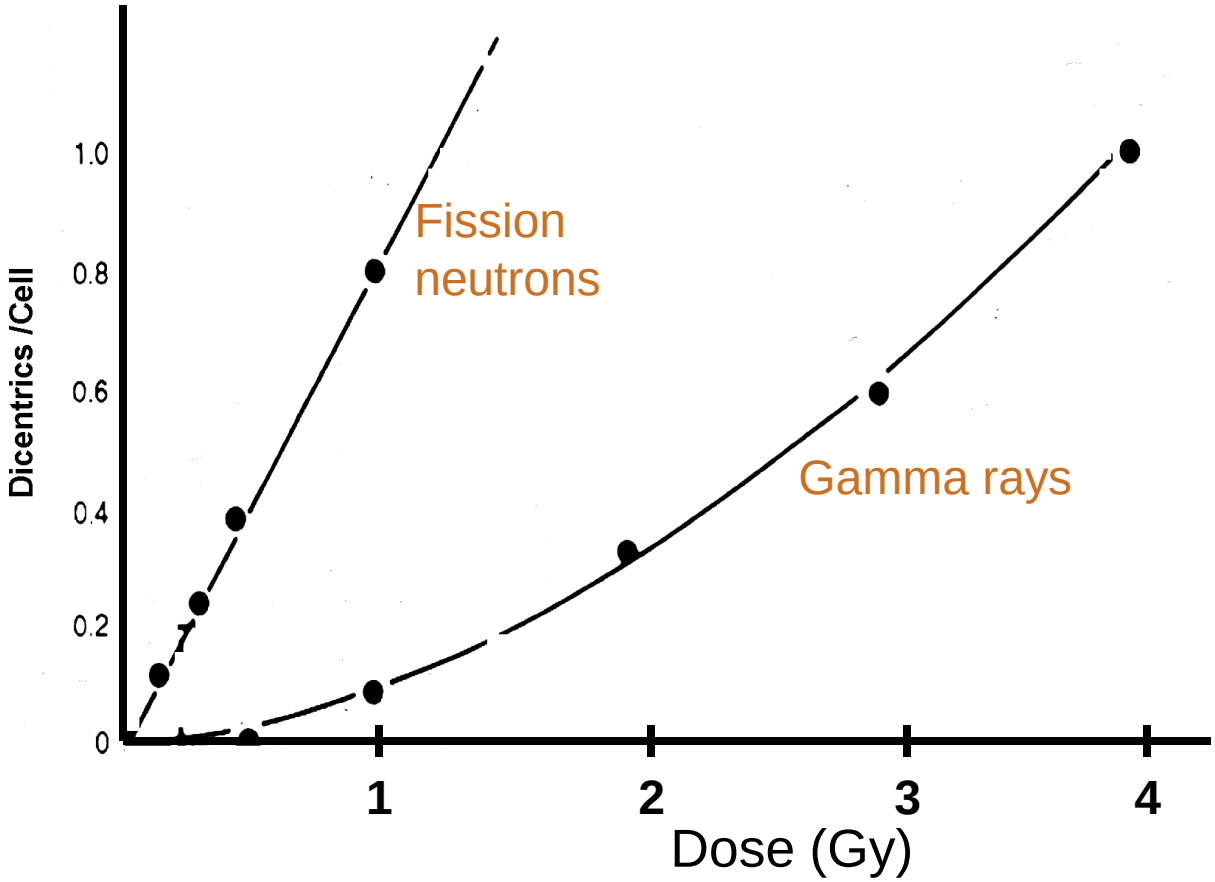
LET EFFECTS

As LET > yield of aberrations >



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Dicentric chromosomes and reciprocal chromosomal translocations:

A. Are lethal events
B. Are formed by irradiating cells in G1
C. Are formed by irradiation cells in G2 / S
D. Can be measured by alkaline elution
E. Can be observed in the S phase of the cell cycle

A normal diploid cell has 46 chromosomes. If radiation induced polyploidy, this means that the chromosome number in the cell is:

A. 23
B. Less than 46 but greater than 23
C. 92
D. Any number greater than 46
E. None of the above

Following a radiological incident, the best cytogenetic biodosimeter is:

A. Analysis of chromatid aberrations
B. Sister chromatid exchange
C. Spectral karyotyping
D. Genomic instability
E. Analysis of dicentric chromosomes

Chromosomal aberrations incompatible with cell survival in normal cells ($Y = k + \alpha D + \beta D^2$)

Relationship to the survival curve:

$SF = k + \alpha D + \beta D^2$

Dose deposition after irradiation RANDOM

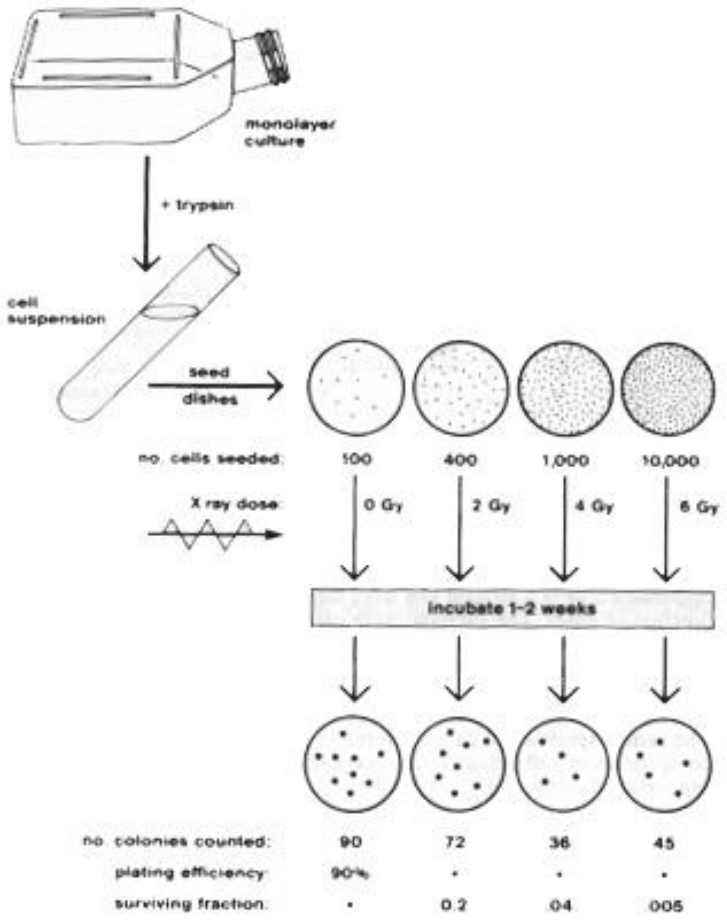
Same proportion NOT same number of cells killed with a given increment of dose

RESULT: a proportional decline in unaffected cells with a linear increase in dose => Exponential decline

Generating a Cell Survival Curve

Survival curve:

Quantitative way of relating absorbed dose with surviving fraction. *In vitro* survival curves artificial but the parameters of dose-response relationships similar to those *in vivo*



From: Hall, "Radiobiology for the Radiologist"

$$SF = \frac{\text{colonies counted}}{\text{cells seeded} \times PE}$$

The Shape of the Cell Survival Curve

Surviving fraction (SF)

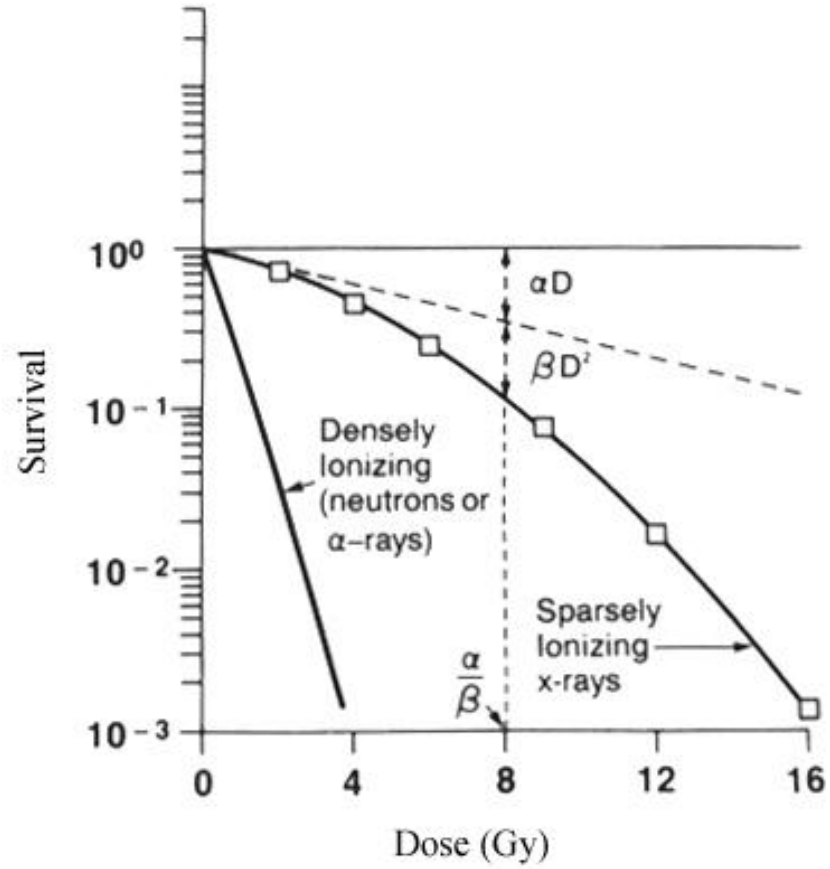
Logarithmic

Dose (D)

Linear

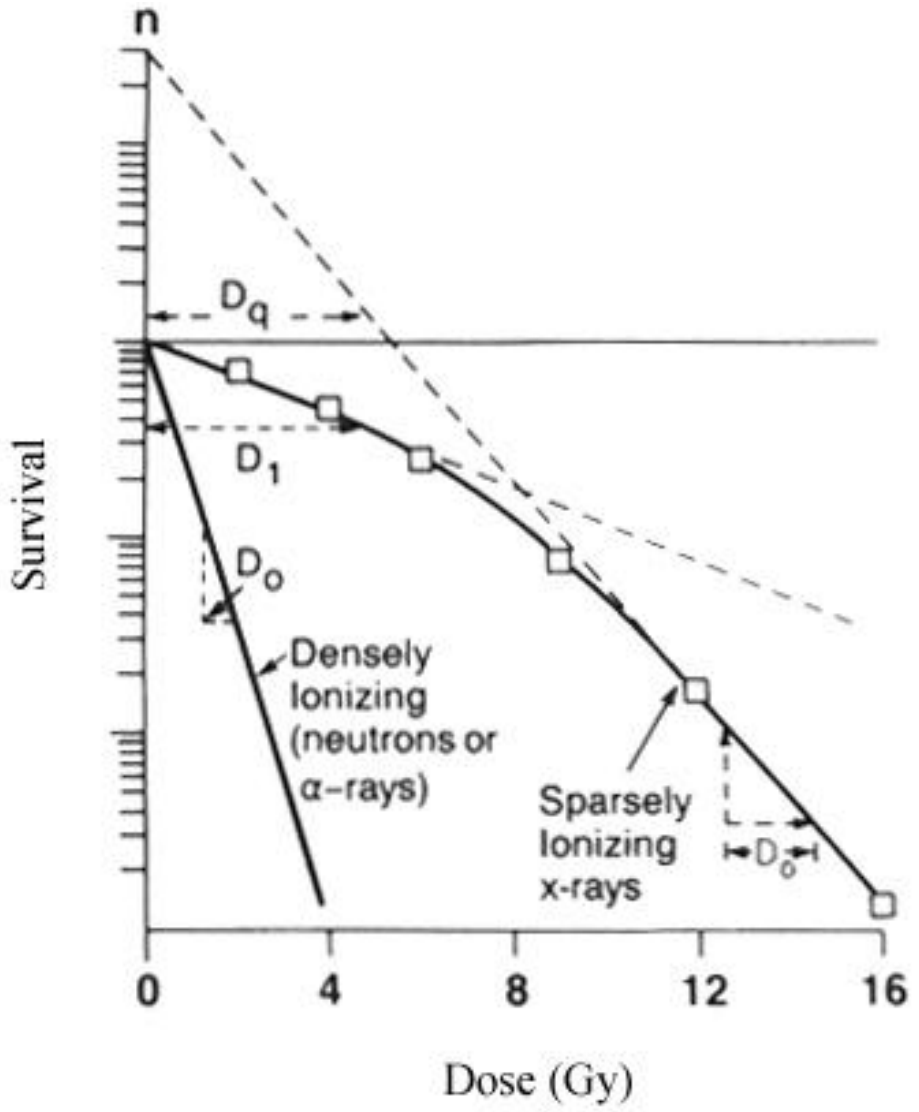
SF_2

Surviving fraction after 2Gy



From: Hall, "Radiobiology for the Radiologist"

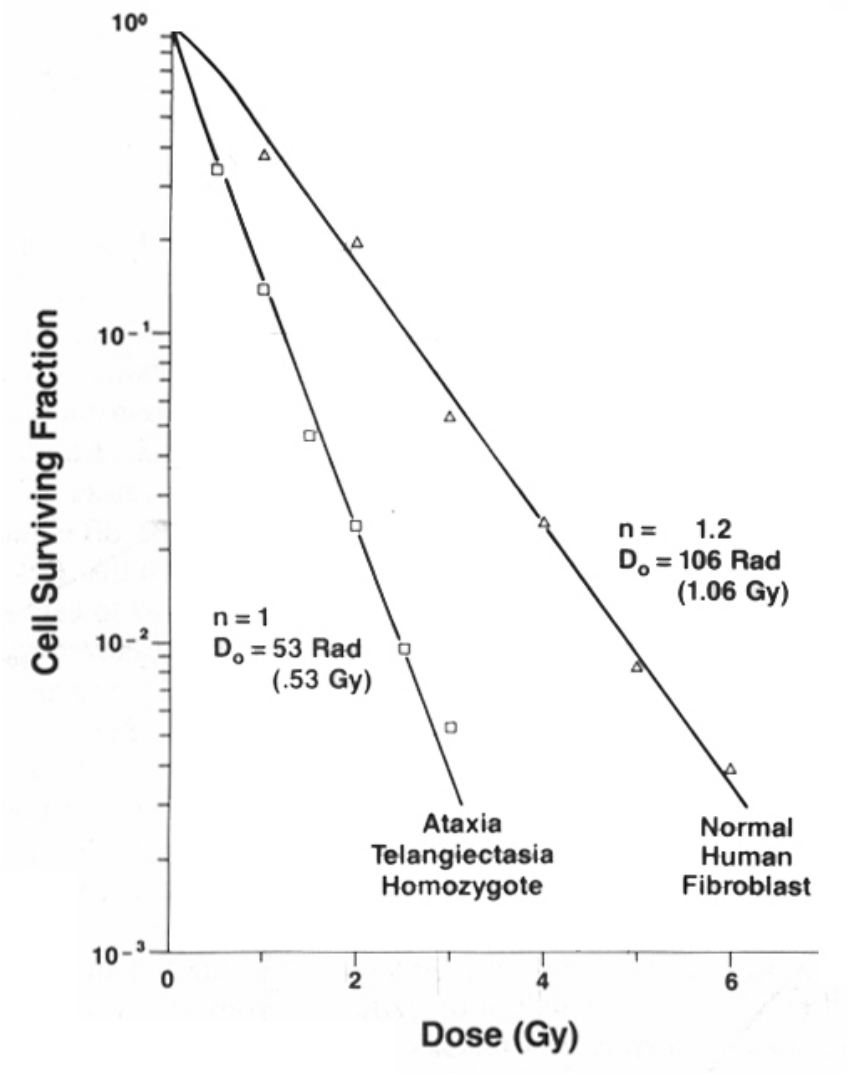
Describing the Cell Survival Curve



From: Hall, "Radiobiology for the Radiologist"

Initial slope **(D_1)**
Dose to < SF to 37% on initial portion of the curve
Final slope **(D_0)**
Dose to < SF 37% on straight line portion of the curve
Extrapolation number **(n)**
Guesstimate of width of the shoulder
Quasi threshold **(D_q)**
***Almost* a threshold dose, dose below which radiation purportedly has no effect**

The Cell Survival Curve



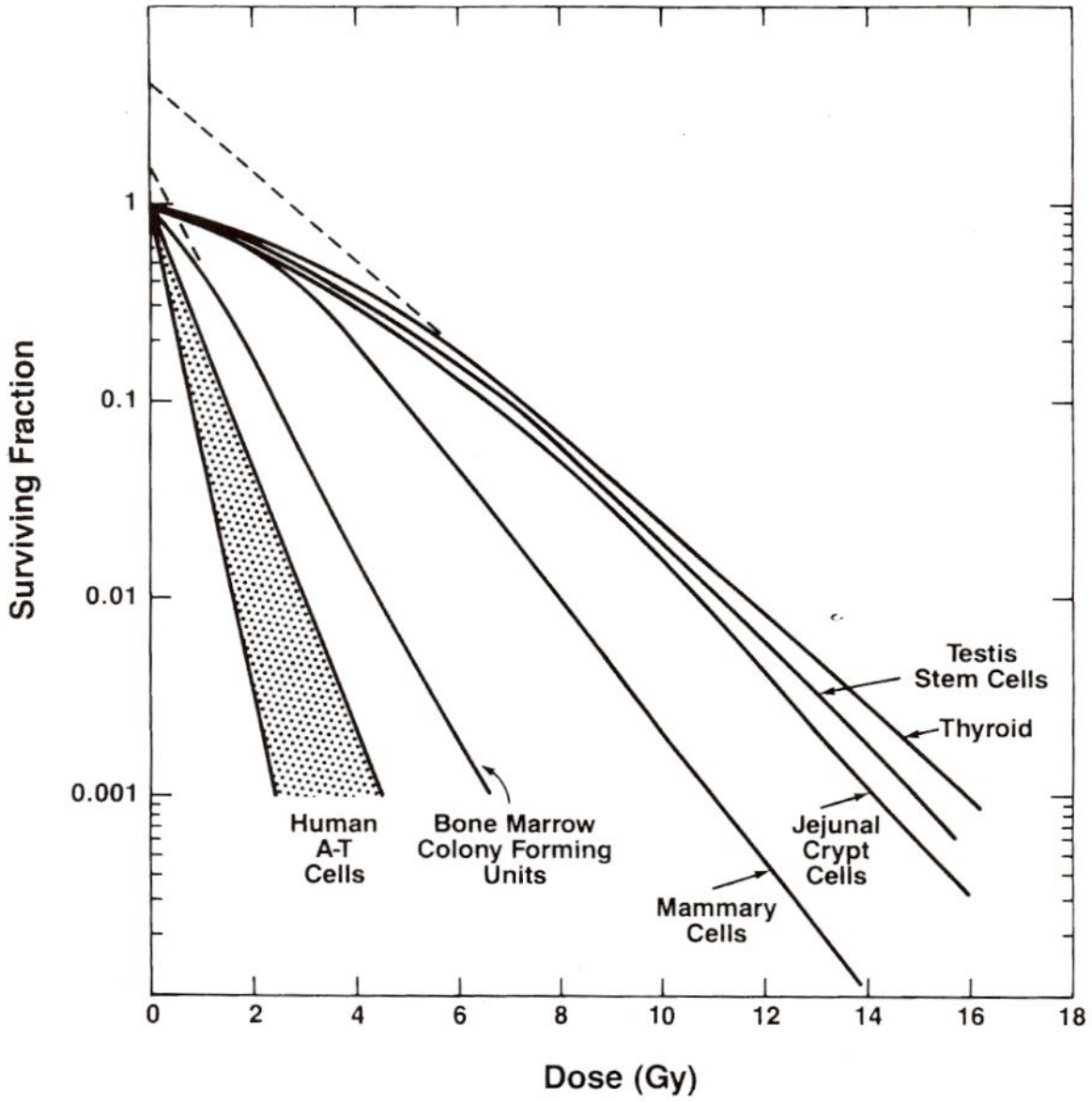
From: Hall, "Radiobiology for the Radiologist"

Remember this measures reproductive integrity not cell killing - just because a cell survives and replicates does not mean its normal: **genomic instability!**

Note: low D_0 - radiosenstive (e.g., 0.5 Gy)

high D_0 - radioresistant (e.g., > 1.2 Gy)

Inherent variation in radiation sensitivity in human cells and tissues



Human radiation sensitive disorders

Increased cancer predisposition

Ataxia telangiectasia (AT)

ATM gene mutation
kinase involved in DNA damage responses

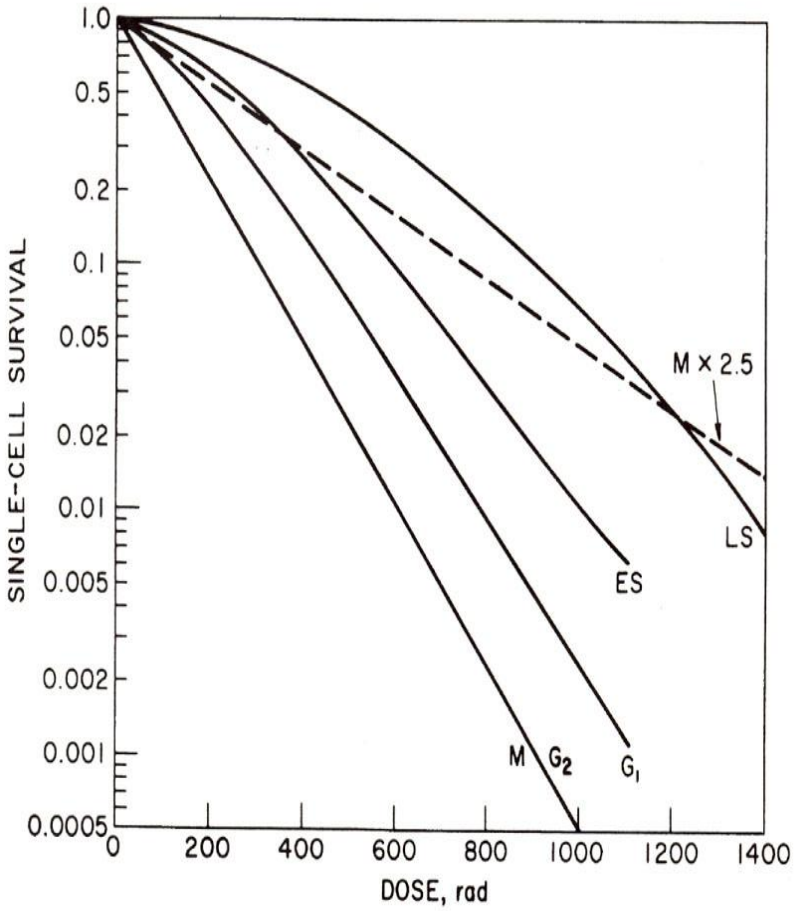
Nijmegen Breakage Syndrome (NBS)

NBS1 gene mutation
Codes for NBS/p95 protein

AT-Like Disorder (ATLD)

MRE11 gene mutation
Low level of MRE11 protein

Cell Cycle and Radio-sensitivity

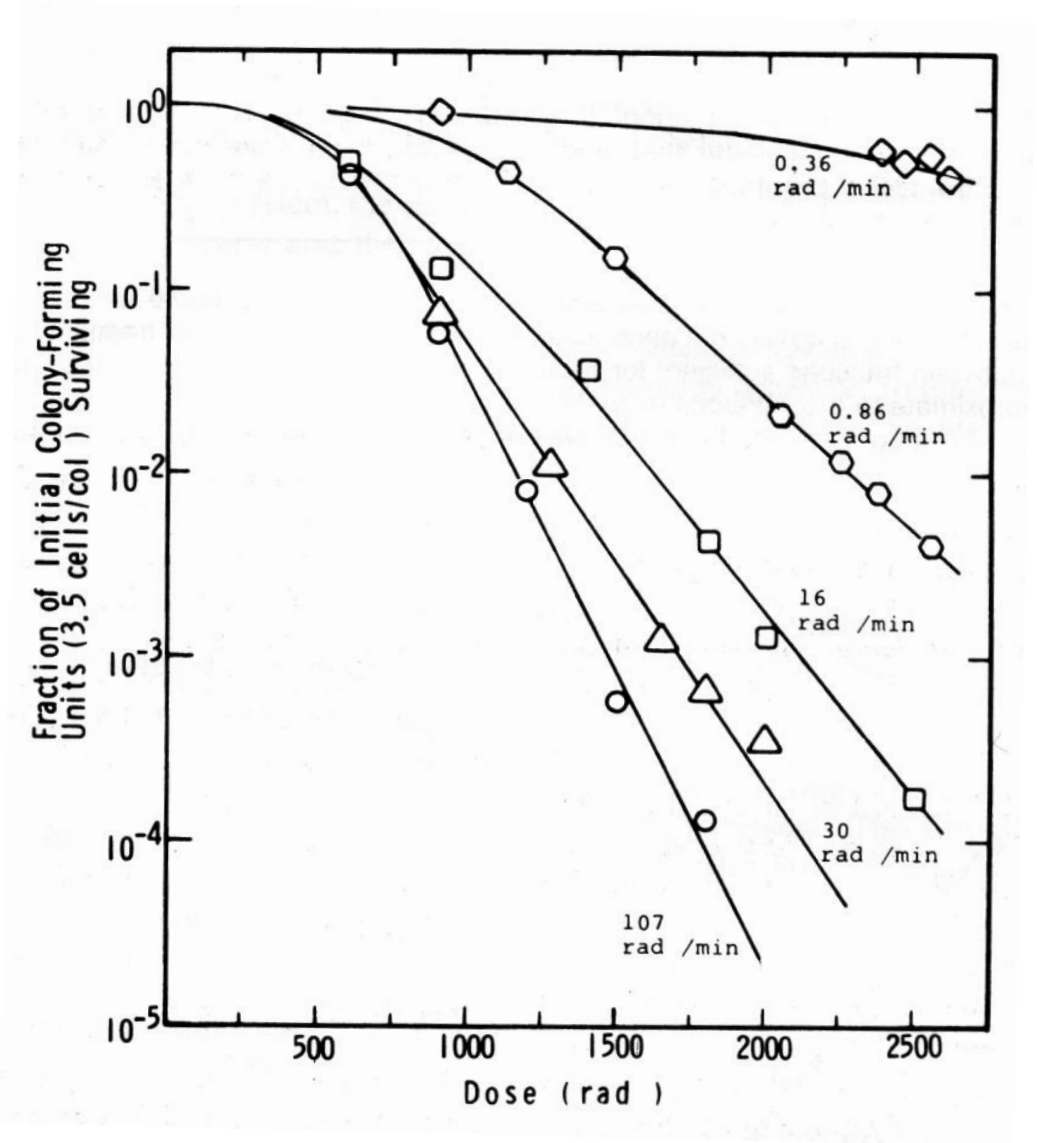


Cell cycle effects are important in radiation therapy, because fractionated irradiation can lead to sensitization by re-assortment to sensitive phase of the cell cycle.

The oxygen enhancement ratio (OER) does not vary much with the phase of the cell cycle.

High LET responses are less affected than low LET radiation.

Dose Rate Effects



Protracting the dose rate increases cell survival

- WHY? -

increases time to repair induced damage and repopulation

(E.J.H., Fig.5.10, p.76, 2000)

Modifying the survival curve

Oxygen / hypoxia

Dose rate

Radiation quality (LET)

Cells apoptotic capacity

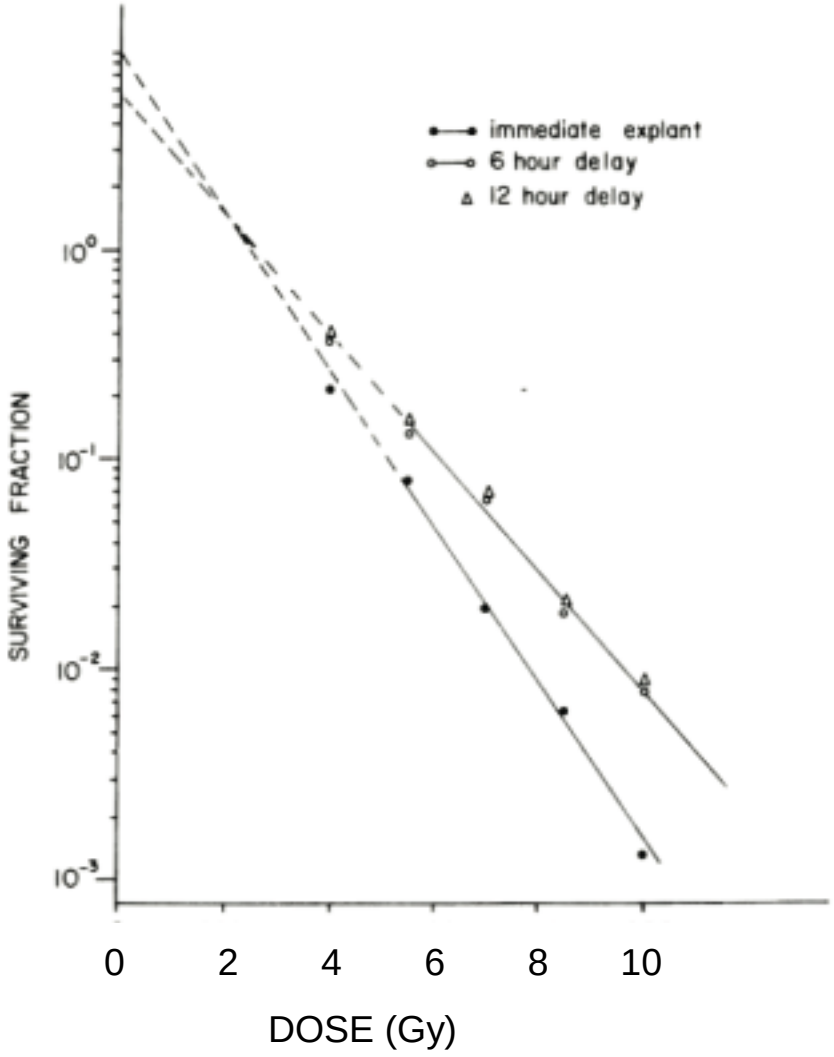
< apoptosis, > radiation resistance

Target tissue

Position in the cell cycle

Potentially Lethal Damage Repair (PLDR)

Hold cells in a non-proliferative state



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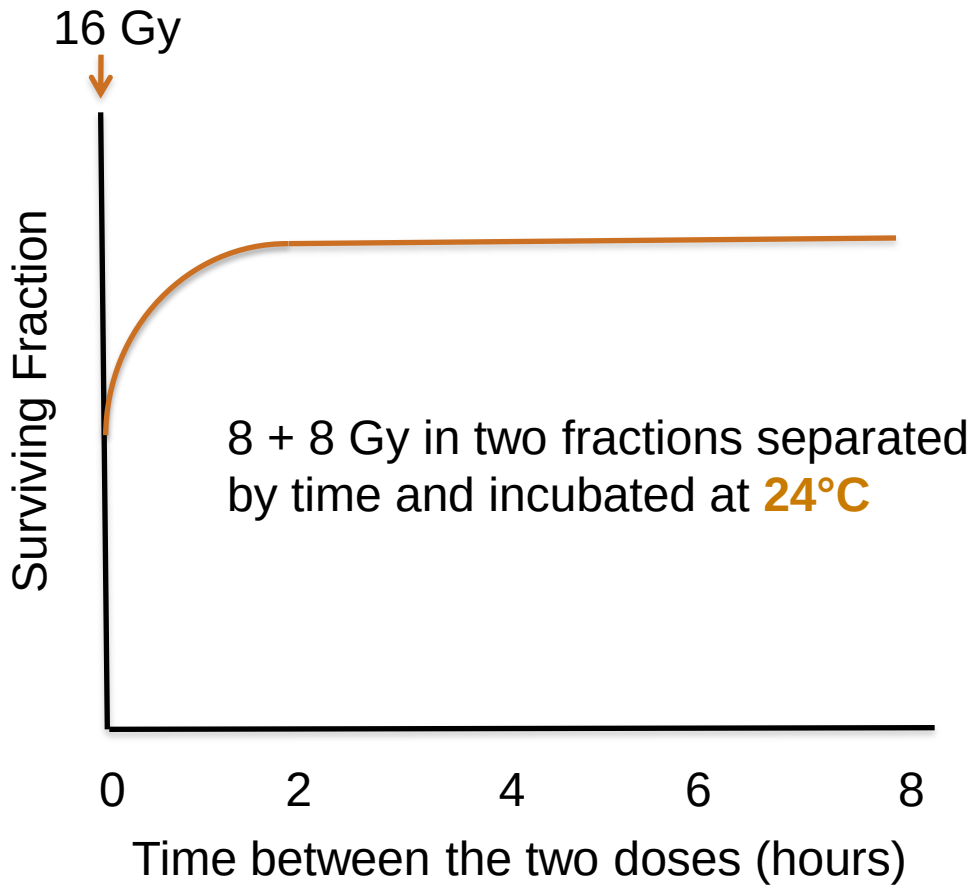
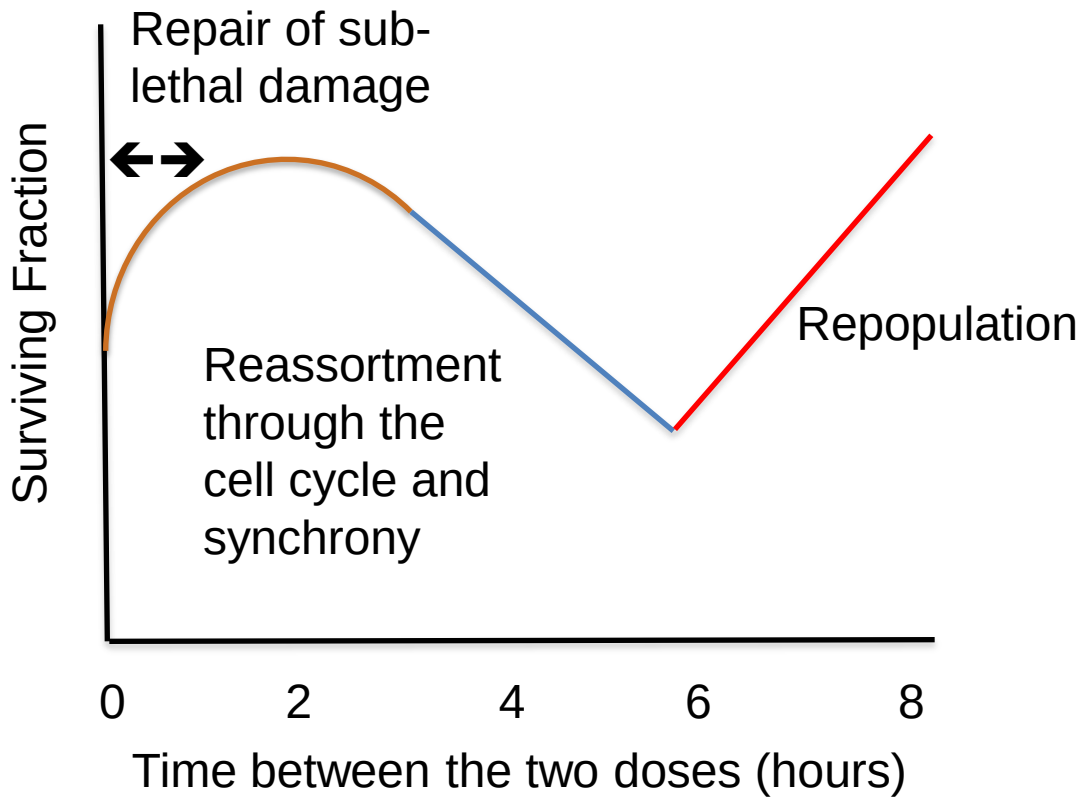
Sub-Lethal Damage Repair (SLDR)

Split dose (Elkind recovery)



Three of the R's of Radiotherapy

8 + 8 Gy in two fractions separated by time and incubated at 37°C



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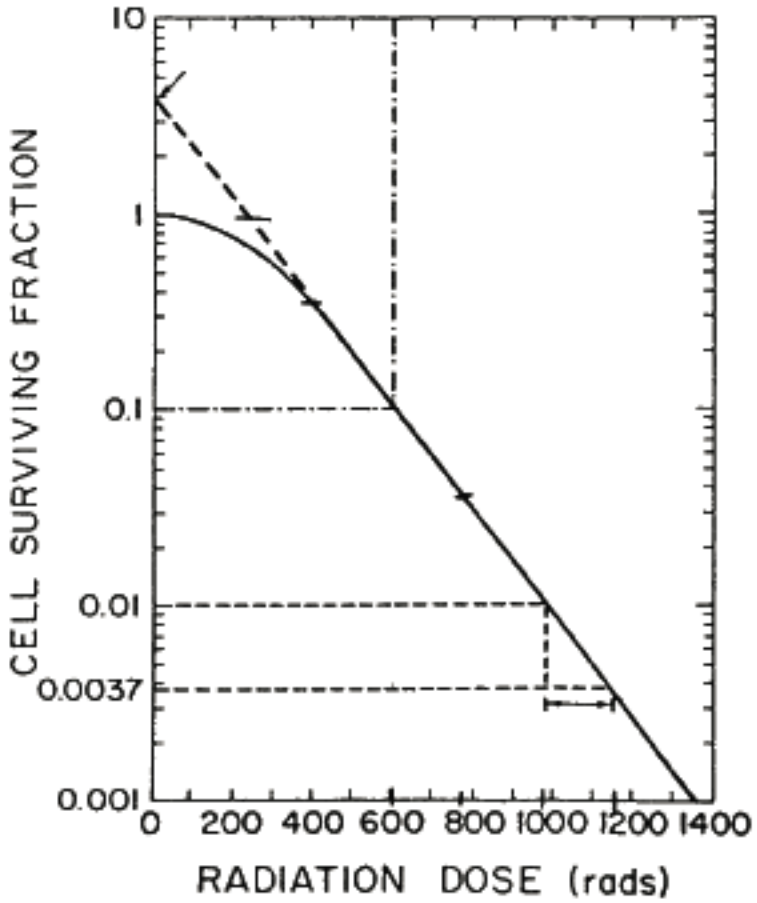
14. Figure 1 shows a typical survival curve for mammalian cells. The D_0 for these cells is:
    a) 50 rad (0.5 Gy)
    b) 100 rad (1.0 Gy)
    c) 170 rad (1.7 Gy)
    d) 220 rad (2.2 Gy)
    e) 250 rad (2.5 Gy)
15. For the cell survival curve in Figure 1, the extrapolation number is closest to:
    a) 0
    b) 1
    c) 2
    d) 3
    e) 4
16. For the cell survival curve in Figure 1, the quasi-threshold dose is closest to:
    a) 20 rad (0.2 Gy)
    b) 120 rad (1.2 Gy)
    c) 220 rad (2.2 Gy)
    d) 320 rad (3.2 Gy)
    e) 420 rad (4.2 Gy)



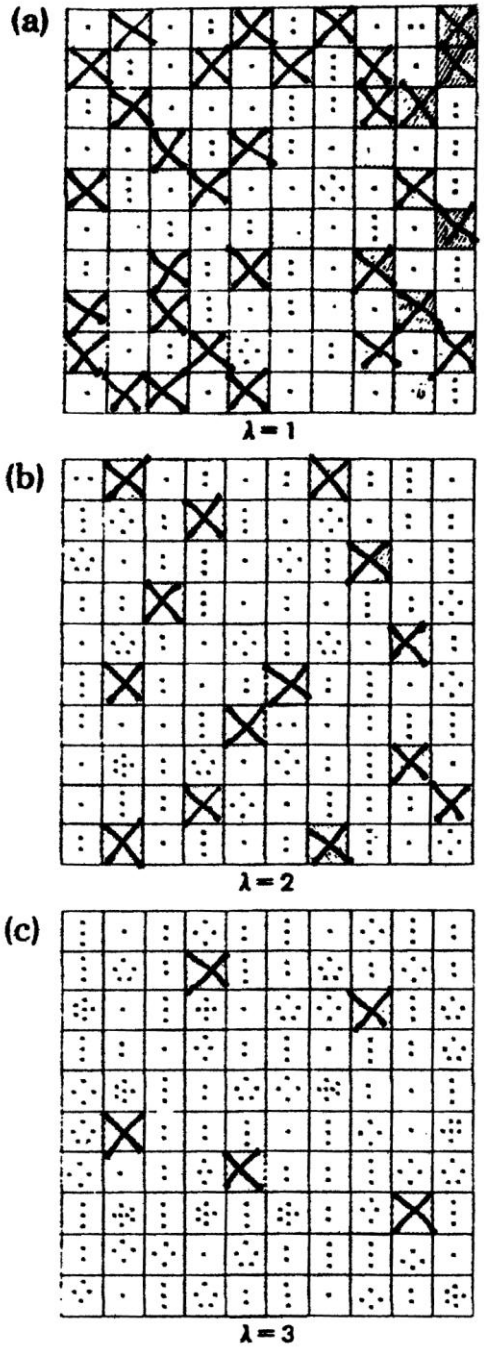
Target Theory - Thank the Physicists!

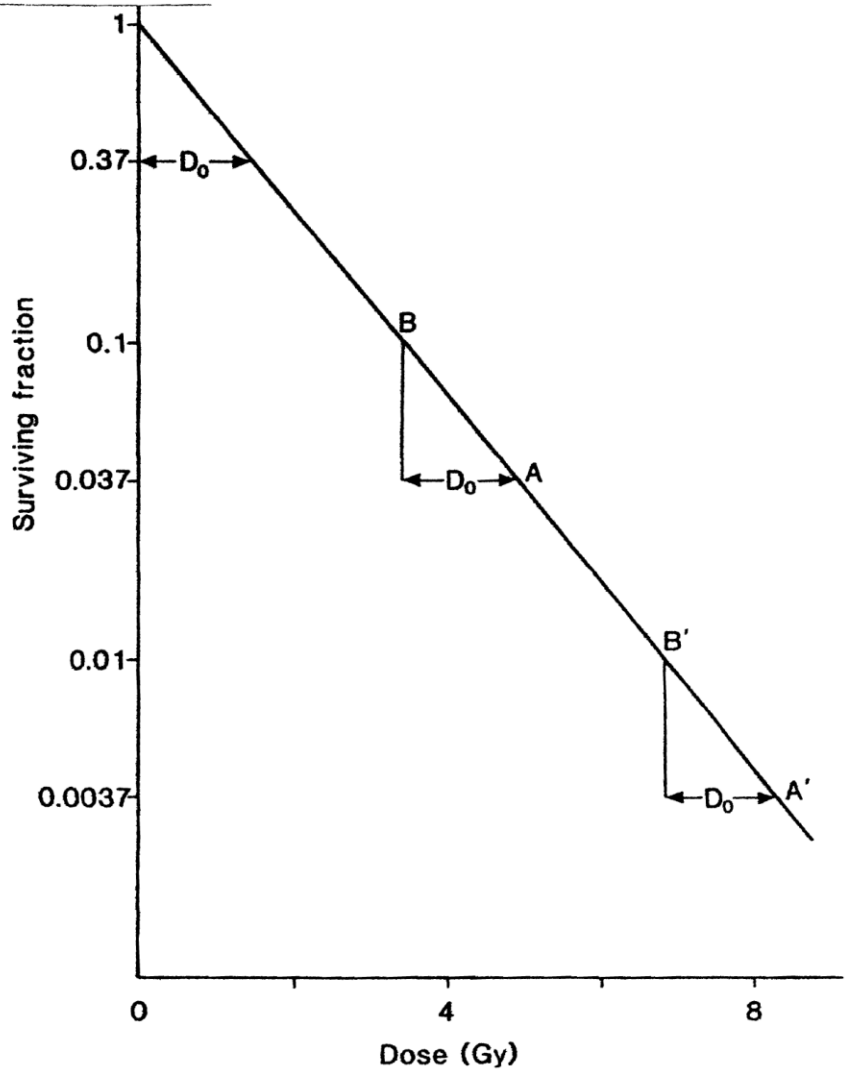
Ionizations occur randomly
One lethal event sufficient to inactivate the cell

POISSON DISTRIBUTION / STATISTICS

100 targets - 100 hits
Random distribution = ~ 63% of targets hit = dead
$\Rightarrow$ ~ 37% still alive
NB: Some targets hit more than once

200 hits - 100 targets
37% x 37% not hit = 14% survival





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$S = N/N_0 = e^{-D/D_{37}}$ or e^{-x} where $x = D/D_0$

S = surviving fraction
N = number of cells surviving a dose D
N_0 = initial number of cells
D_{37} = constant dose related to cell sensitivity (mean lethal dose)

Target theory implies a linear response

But in real life it generally requires more than one hit to inactivate a cell. **But there are a finite number of targets in the cell, so at higher doses the dose-response relationship is linear**

At 2 x D_0 ~14% of cells survive

The dose to reduce the number of cells to 10% of the starting value (D_{10}) is: $D_{10} = 2.3 \times D_0$

Example Calculations:

If 5 fractions are given and each fraction kills 50% of cells remaining, what is the fraction of remaining cells?

$(0.5)^5 = 0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5 = 0.031$

Number of surviving cells can be determined by moving down the straight line portion of the survival curve (terminal slope)

To Solve Survival Problems - 3 Formulas

1. $D_{10} = 2.3 \times D_0$
2. Total dose / D_{10} = decades of cell killing
3. Total # of cells / Decades of cell kill = survivors

From: Richard Miller

A tumor with 10^{10} clonogenic cells is treated to a total dose of 64 Gy in daily 2 Gy fractions. If the D_0 of the survival curve is 3.48 Gy, how many clonogenic tumor cells will remain at the end of treatment?

D_{10} = 2.3 x D_0: 2.3 x 3.48 = 8 Gy
64 Gy / 8 Gy = 8 decades of cell kill
10^{10} / 10^{8} decades of cell kill = 10^{2}

A tumor contains 10^{10} clonogenic cells. Using a 2 Gy/ day fractionation scheme the D_0 is 2.8 Gy. What total dose would achieve 99% tumor cure?

Require 10^{-12} decades of cell kill = 1% survival
[i.e., 10^{-10} / 10^{-12} = 10^{-2}]
Dose to achieve 1 **decade** of cell kill = D_{10}
2.3 x 2.8 = 6.44 Gy [This = D_{10}] = 1 **log** cell kill
For 12 decades of cell kill: 12 x 6.44 = 77.28 Gy

For the formula $S = 1 - (1^{-e-D/D37})^{n}$, match the symbol with the correct term

A. D
B. S
C. D_{37}
D. e
E. n

1. Surviving fraction
2. Radiation dose
3. Ordinate intercept
4. 2.71828
5. Mean lethal dose

According to classical target theory and Poisson statistics, if 1 hit were all that were required to inactivate a cell then survival after 100 cells were exposed to 300 hits, survival would be:

A. 37%
B. 14%
C. 5%
D. 1%
E. 0