MPHY3892 Radiobiology

Lecture 1
Radiation Chemistry.
DNA Damage and Repair.
Chromosome Aberrations

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Radiobiology

- Radiobiology is needed
  - To understand correlations between initial ionisation events and final tissue expression (in tumours and in surrounding normal tissues)
  - To address mechanisms of radiation damage and repair and suggest hypotheses that may be tested
  - To suggest changes to existing radiotherapy protocols

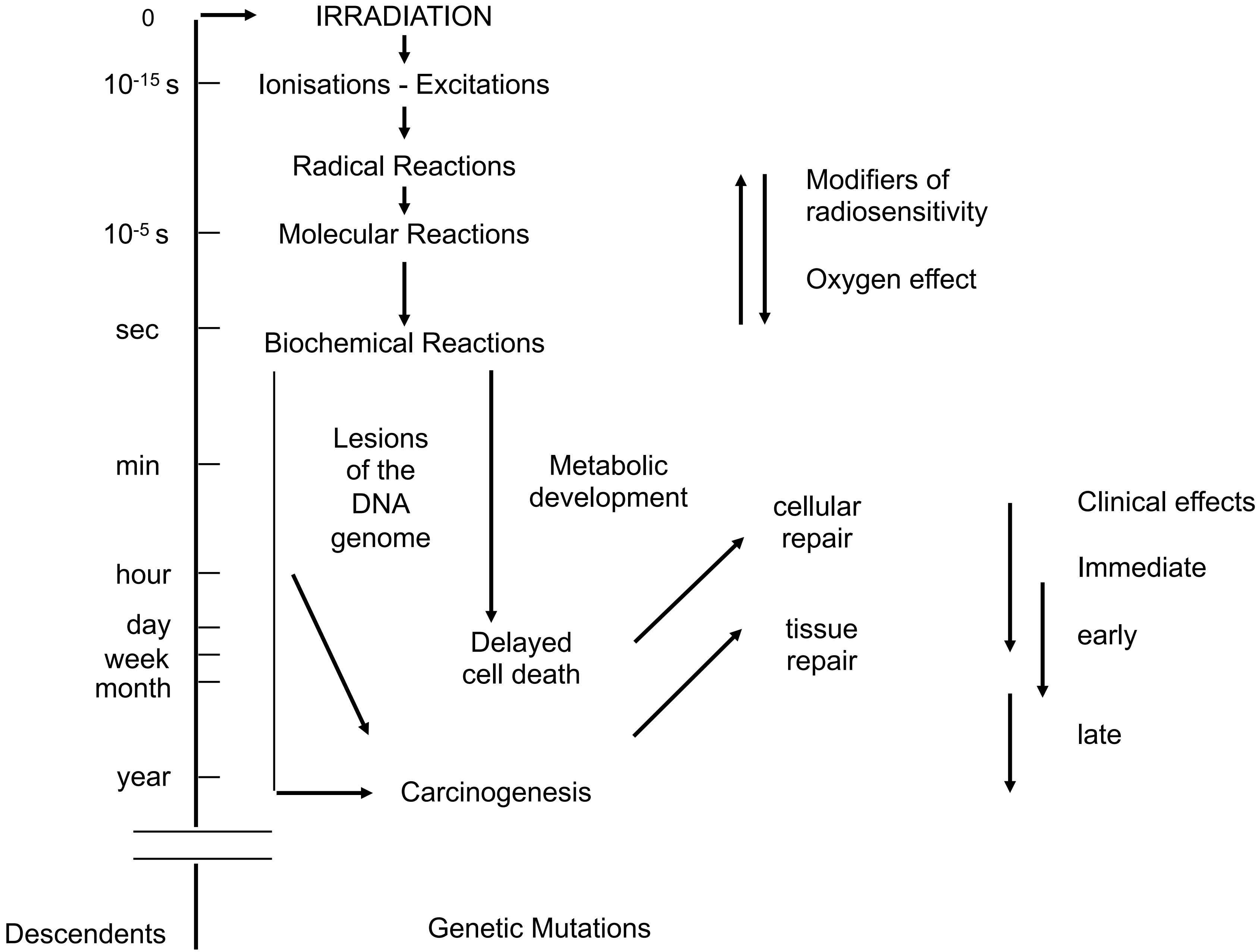
Course Programme

- Radiation Chemistry, DNA Damage and Repair
- Cellular Effects, Target Theories and Survival Curves
- Factors Affecting Radiation Effects
- Effects of Radiation on Tissues
- Epidemiology, Radiation Carcinogenesis

Course Reading

- **"Introduction to Radiobiology"**
  M. Tubiana, J. Dutreix, A. Wambersie (translated by D. Bewley)
  Taylor & Francis 1990. ISBN 0-85066-763-1
- "Radiobiology for the Radiologist"
  E. Hall
  Lippincott 1994. ISBN 0-397-51248-1
- "Biological Effects of Radiation"
  J. Coggle
  Taylor & Francis 1983. ISBN 0-85066-238-9

Timescale for Radiation Damage



Particles Used in Radiobiology

Particles		Energy range
Alpha-particles	$^{4}He^{2+}$	1MeV - 200MeV
Electrons	e^{-}	0.01MeV - 15MeV
Neutrons	n^{0}	0.025eV - 20MeV
Protons	$^{1}H^{+}$	1MeV - 30GeV
Deuterons	$^{2}H^{+}$	1MeV - 200MeV
Heavy ions		1MeV - 250MeV

250 MeV proton synchrotron for therapy

Proton therapy

Electromagnetic Radiation Used in Radiobiology

Photon Energy	Properties
100eV - 10keV	"Soft" x-rays
10keV - 130keV	Diagnostic x-rays & superficial therapy
130keV - 1.3MeV	Deep therapy x-rays & γ-rays from ^{60}Co etc
6 MeV - 35 MeV	Radiation from linear accelerators
100MeV	Radiation from large betatrons
1GeV	Radiation from large synchrotrons


Philips 4MV linac, 1953


Co-60 therapy unit


35MV linac, 2006

Radiation Chemistry

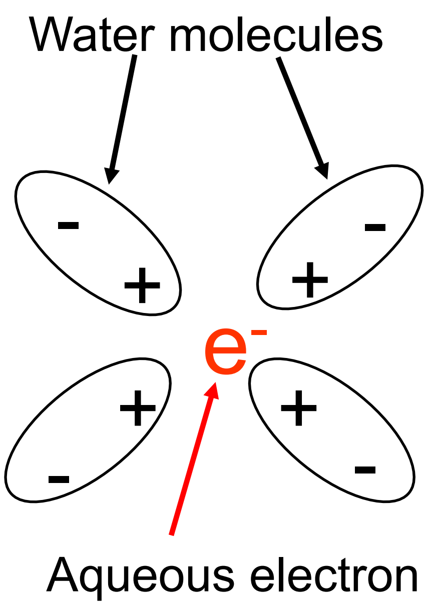
- Physical effects produced by a charged particle occur during a very short period of its passage (timescale: 10^{-24} - 10^{-14} s)
- **Free radicals**:
  - are atoms/molecules containing unpaired electron in the outer shell
  - can be neutral or charged (radical ions)
  - are very reactive and have a great tendency to pair the odd electron with a similar one in another radical or to eliminate the odd electron by an electron transfer reaction
    - electron acceptors (oxidising agents)
    - electron donors (reducing agents)
- Tissue: 70 - 90% water ⇒ radiation chemistry of water

Radical Decomposition of Water

- Ionisation of water (requires energy of 13 eV):

$$H_2O \xrightarrow{\text{radiation}} H_2O^+ + e^- \rightarrow OH^\bullet + H^+ + e^-$$

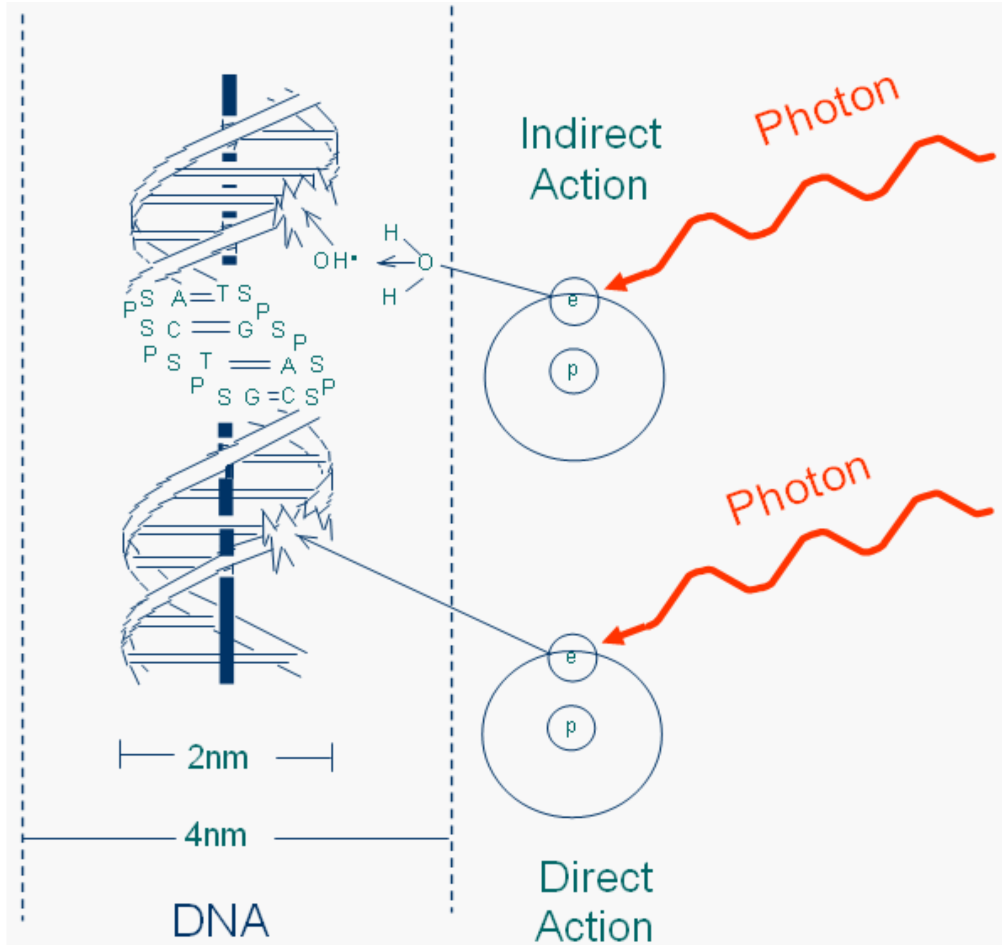
- Electron detached by ionisation, having slowed down in collisions, is captured by molecules of water ⇒ it is called aqueous electron e^-_{aq} and is a powerful reducing agent
- Some molecules of water lying close to aqueous electrons dissociate into $H^\bullet$ and $OH^\bullet$ radicals



- Excitation of water (requires energy of 5 eV):

$$H_2O \xrightarrow{\text{radiation}} H_2O^* \rightarrow OH^\bullet + H^\bullet$$

Direct and Indirect Action



- Radiation can ionise organic molecules RH directly
- The indirect effect results from the interaction between the products of radical decomposition of water and the molecules contained in the aqueous solution.

Oxygen Effect

Radical + Oxygen → Peroxide Radicals:

$$O_2 + H^{\bullet} \rightarrow HO_2^{\bullet}$$

$$O_2 + e_{aq}^{-} \rightarrow O_2^{-}$$

$$O_2^{-} + H^{+} \rightarrow HO_2^{\bullet}$$

$$2HO_2^{\bullet} \rightarrow H_2O_2 + O_2$$

Peroxide radical $HO_2^{\bullet}$ is a less powerful oxidising agent than $OH^{\bullet}$ but has considerably longer lifespan.

Peroxide radicals are shown to be biologically damaging.

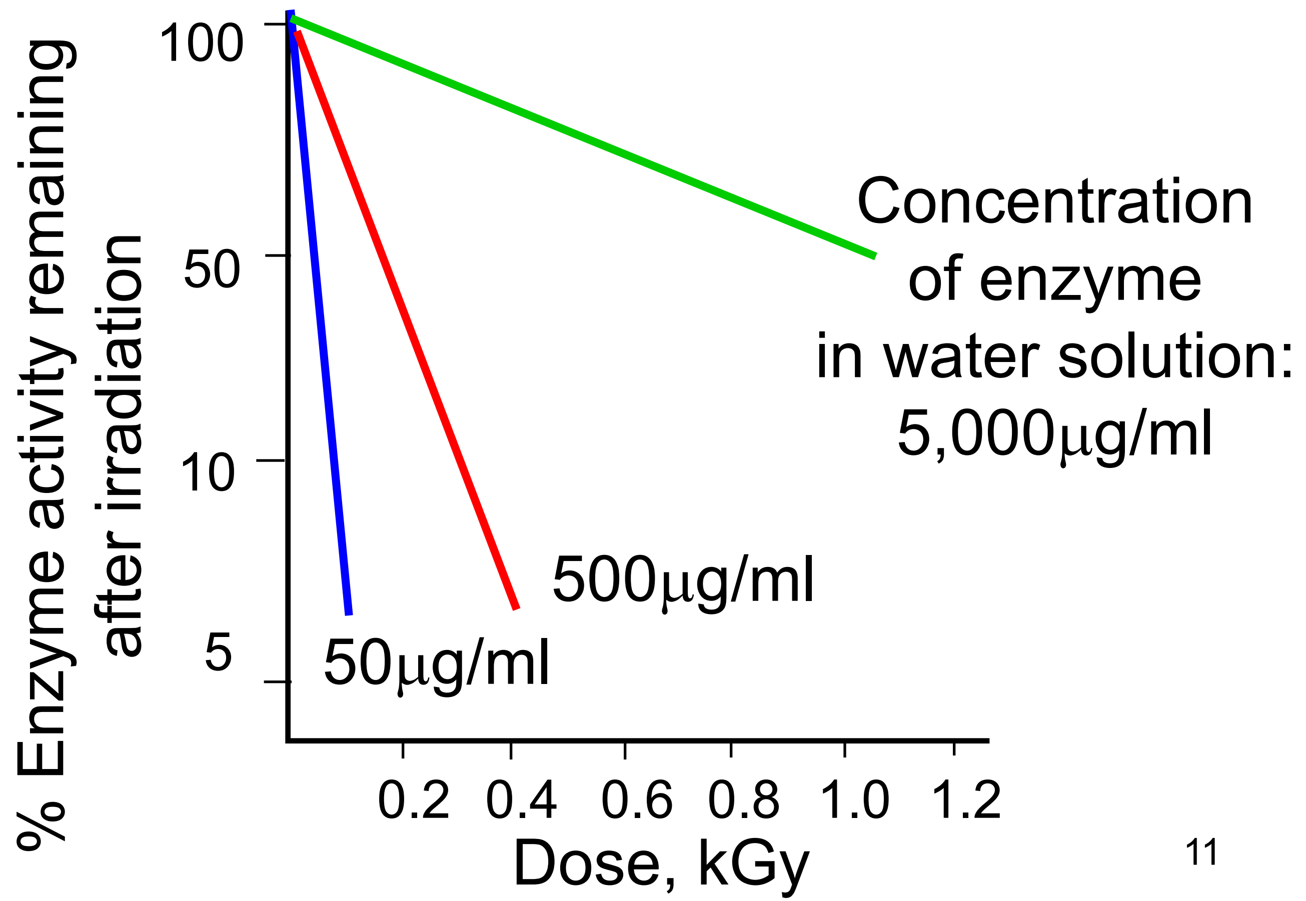
Capture of $H^{\bullet}$ radicals also increases the number of $OH^{\bullet}$ radicals available by preventing recombination reaction $H^{\bullet} + OH^{\bullet} \rightarrow H_2O$.

The presence of Oxygen increases the effect of radiation.

Effects of Radiation on Proteins

Proteins act as structural components in cells and as organic catalysts in biochemical reactions (enzymes)

- **Physico-chemical damage:** chain fragmentation, amino acid destruction, disorders of structure
- **Biochemical damage:** loss of enzyme function



Data for DNAase enzyme in vitro

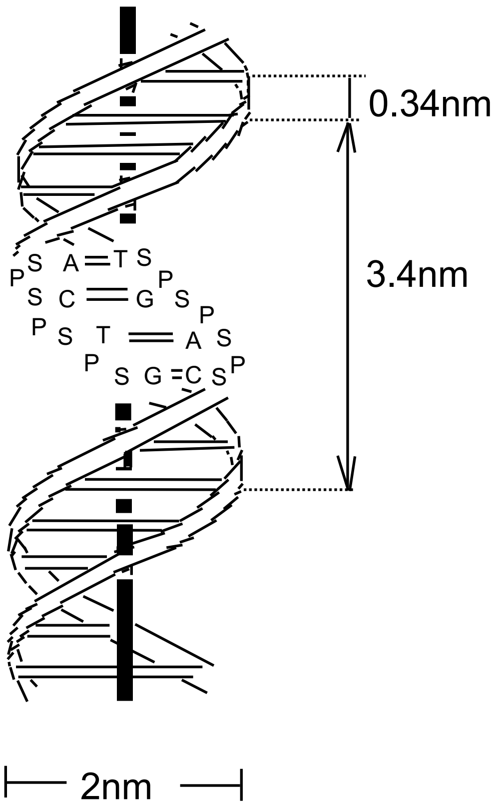
Inactivation of Enzymes *in vitro*

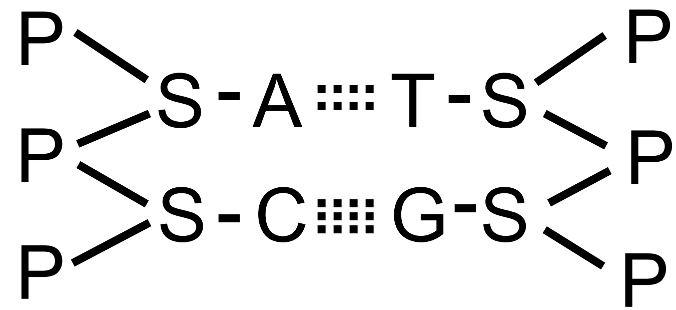
- Percentage of active molecules of enzyme decreases with dose
- When concentration of enzyme in water falls, the relative number of water molecules increases and it is easier for the radiation to inactivate enzyme molecules
- At low concentrations of the enzyme, the damage to the enzyme is caused by the diffusion of free radicals of water
- At a very high concentration of the enzyme, the majority of the radiation effect is due to direct interaction of radiation with the enzyme
- *In vivo* enzymes are present in great quantities and are continuously produced by the cells ⇒ a loss of a sizeable fraction of enzymes may be of no consequence to the cell

Deoxyribonucleic Acid (DNA)

- Double stranded molecule twisted into a double helix
- Is the main constituent of chromosomes
- Transfer of genetic information to daughter cells
- Damage to DNA is the main cause of lethality in cells after radiation
- Two functions of DNA - replication (creating a copy of itself) and transcription (expressing its genetic information by the formation of messenger mRNA to specify the sequence of amino acids during the synthesis of proteins
- Replication is more radiosensitive than transcription
- A single molecule of DNA forms the backbone of a chromosome, it extends continuously from one end to the other
- Functional integrity of chromosome depends on the continuity of the DNA

DNA Structure





P - phosphate
S - sugar
A - adenine
G - guanine
} Purine bases
C - cytosine
T - thymine
} Pyrimidine bases

Two complementary strands are linked by hydrogen bonds between the bases

Chromosome Structure

DNA and Genes

- The nucleus of a human diploid cell has 1m DNA in 46 chromosomes
- About 1000 base pairs are needed to code 1 protein
- About 10^5 proteins are coded in the genome of the mammalian cell
- Genes are zones of DNA that code the synthesis of proteins

Cell Cycle

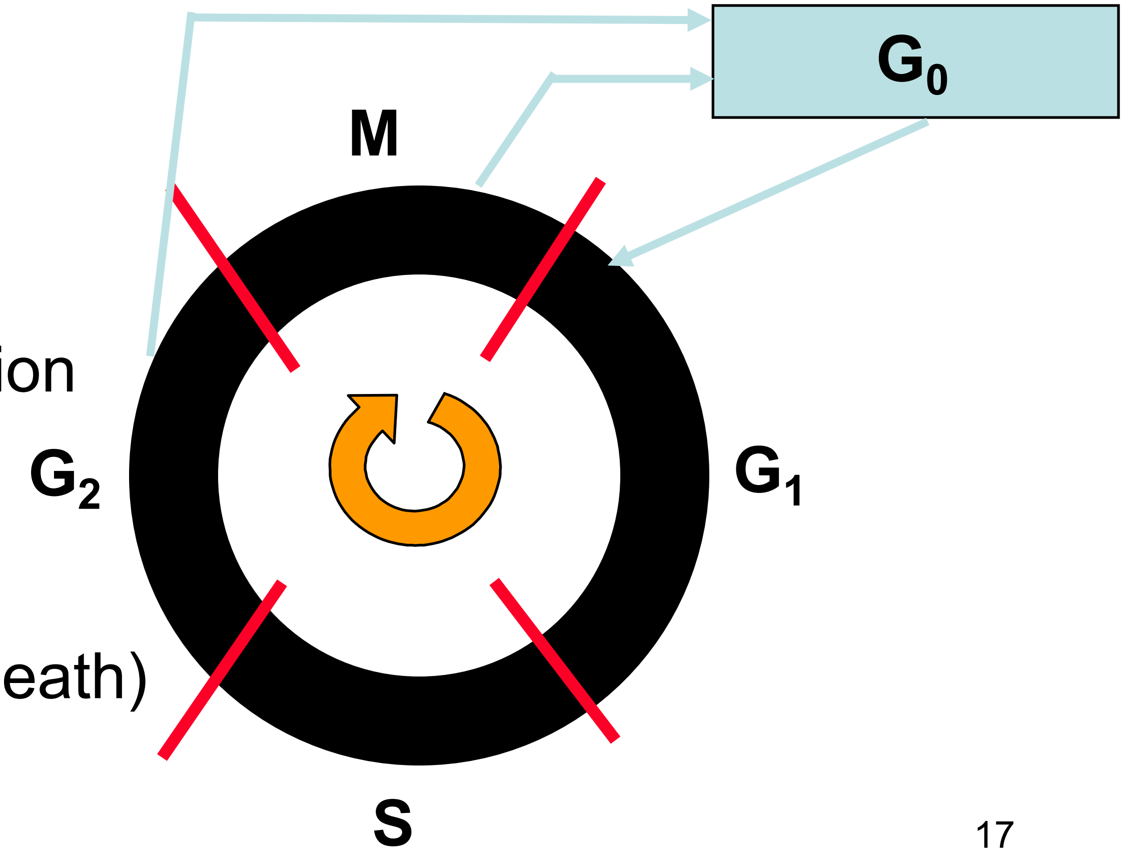
M mitosis (cell division) (1 hr)

G_1 pre-synthesis gap (8-12 hr)

S DNA duplication (9-12 hr)

G_2 post-synthesis gap preparation for division (4-6 hr)

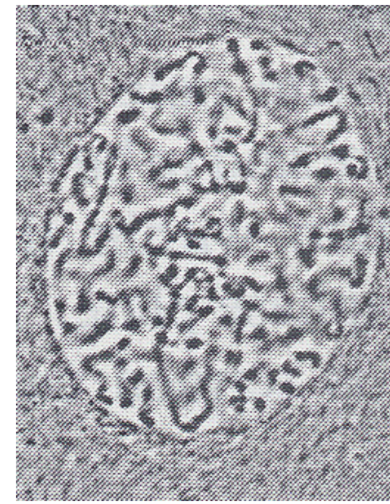
G_0 time out of cycle (e.g. differentiation, premitotic block or death)

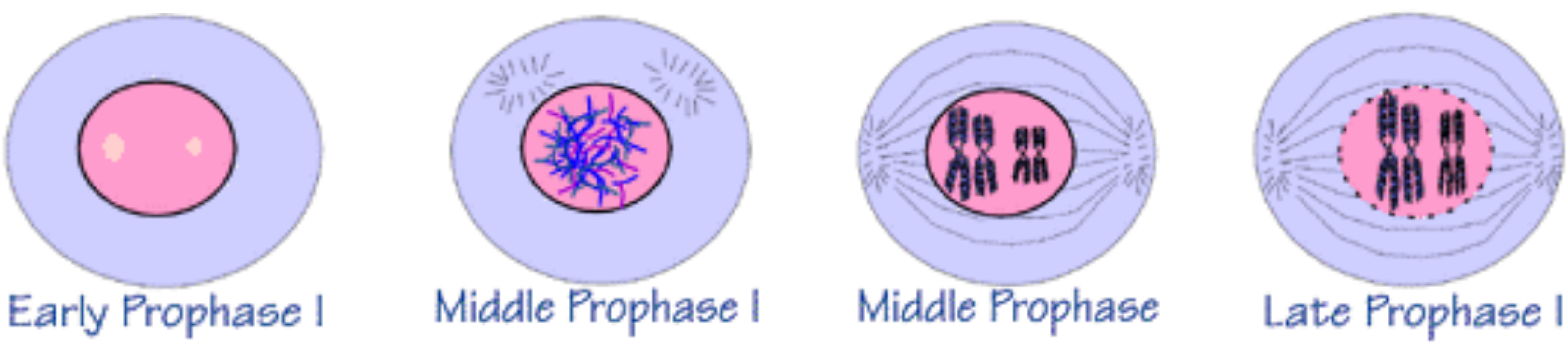


Mitosis (Cell Division)

Prophase

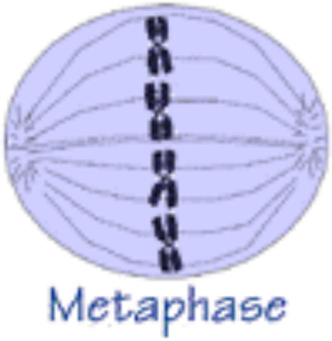
- chromatin thickens
- chromosomes become visible under light microscope
- nuclear membrane disappears
- nuclear plasm and cytoplasm mix





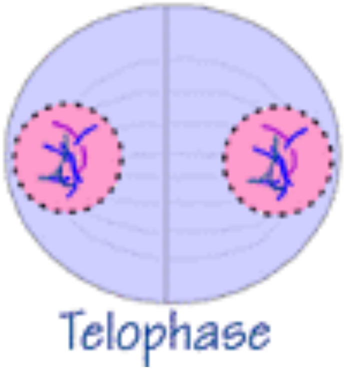
Mitosis

Metaphase
• chromosomes move to cell equator
• spindle forms pole-to-pole
• chromosomes divide at centromere

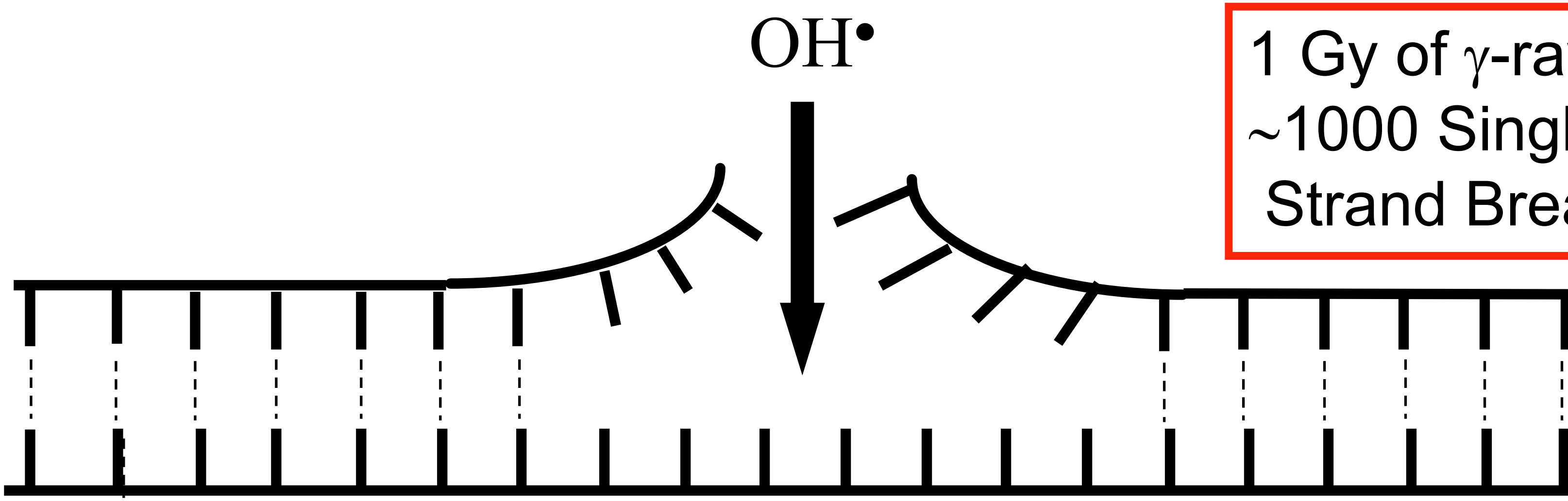


Anaphase
• chromosomes move to poles along spindle

Telophase
• chromosomes reach pole and uncoil
• nuclear membrane reappears



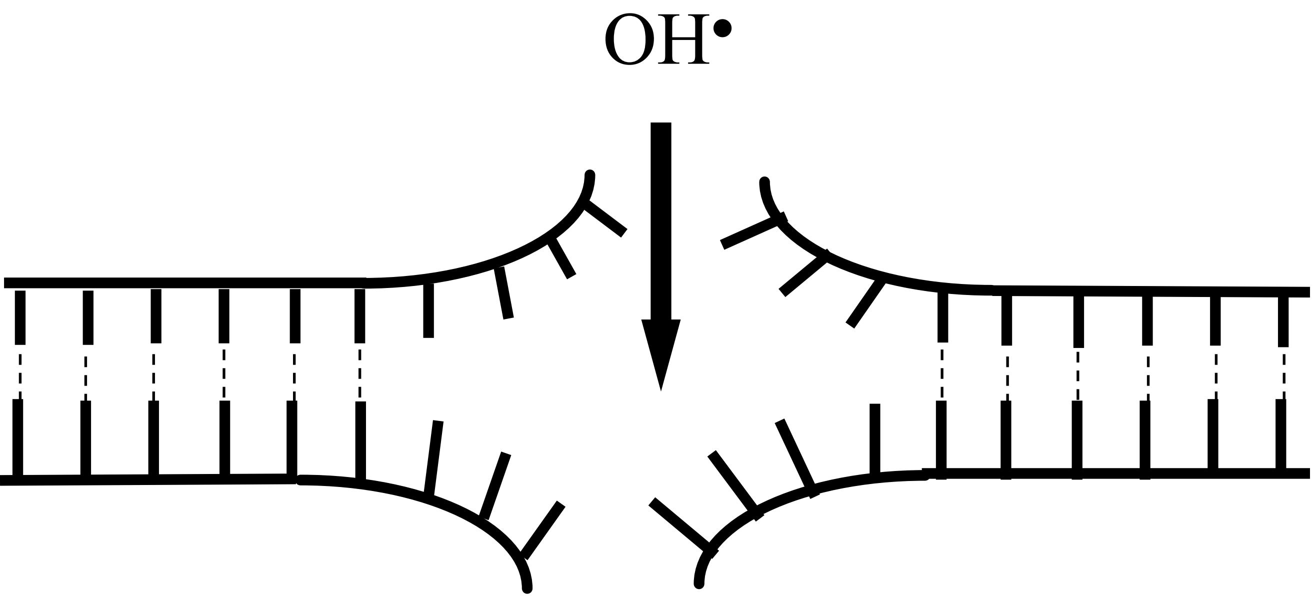
Single Strand Breaks



1 Gy of γ-rays → ~1000 Single Strand Breaks

- Number of single strand breaks is linearly related to dose
- Repair assumes existence of a complementary strand as a template
- Repair is rapid, with high fidelity, one error per 10^7 - 10^{11}, so nearly error free
- Final result - little cell killing

Double Strand Breaks



1 Gy of γ -rays
→ 50–100
Double Strand
Breaks

- Both strands break within 3 base pairs
- Complementary strand is not available as a template
- Repair is likely to give errors
- Number of double strand breaks versus dose is linear or linear-quadratic
- These are critical lesions causing radiation cell killing

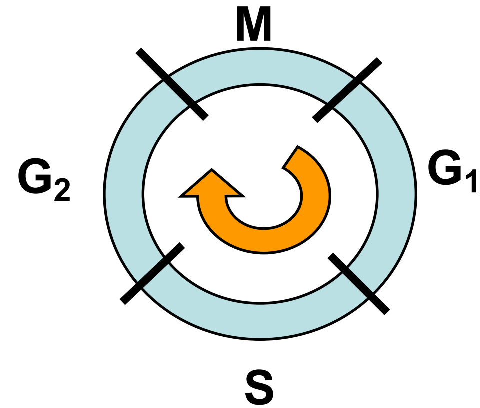
Causes of Chromosome Abnormalities

- Chemical substances - the largest damage
- Ionising radiation
- Non-ionising radiation (e.g. UV, microwaves)
- Mechanical waves (e.g. ultrasound)

Chromosome Abnormalities

- Are visible under light microscope at metaphase
- Broken end of a chromosome is “sticky” and can rejoin with other “sticky” end
- Broken end cannot join with a normal, unbroken chromosome
- Breaks in chromosomes may
    - restitute
    - rejoin in wrong place
    - fail to rejoin

Chromosome Abnormalities



Chromosome Aberrations

- Lesions occur early in interphase before DNA is replicated (during pre-synthesis gap G_1)
- Lesion will be replicated
- Aberration at next mitosis
- Damage affects both chromatids

Chromatid Aberrations

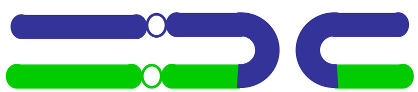
- Lesions occur late in interphase after DNA is replicated (during post-synthesis gap G_2)
- Damage may affect only one chromatid

Dicentric Aberrations

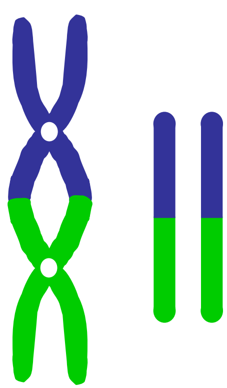
Exchange between two separate chromosomes

2 different pre-replication chromosomes

1 break in each chromosome



“Sticky” ends join incorrectly



Result after replication:

Dicentric chromosome (with two centromeres) + acentric fragments (without centromere). Acentric fragments will be lost at next mitosis

This is a lethal aberration

Rings

Pre-replication chromosome

Breaks in both arms of single chromatid early in the cell cycle

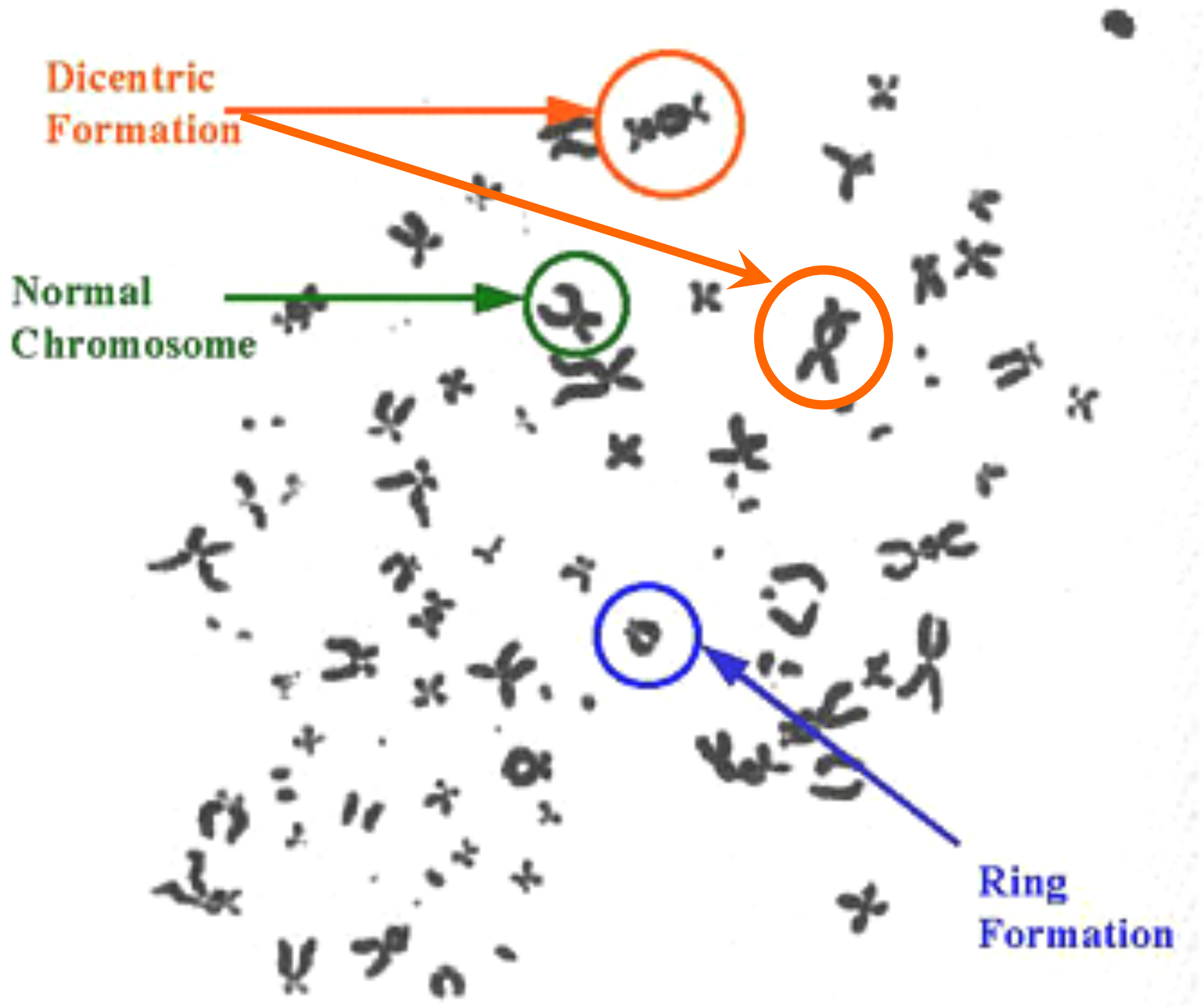
“Sticky” ends rejoin to form a ring and a fragment

Result after replication:

Overlapping rings + acentric fragments

This is a lethal aberration

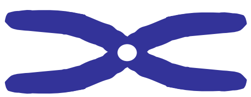
Dicentrics and Rings under Light Microscope



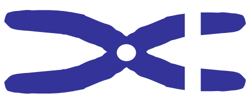
Chromosome damage following radiation exposure

Other Aberrations

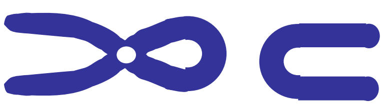
Anaphase Bridge - chromatid aberration



Post-replication chromosome

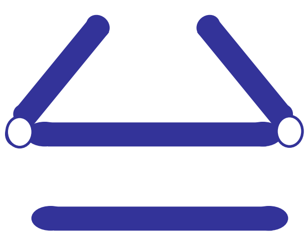


Break in each chromatid late in the cell cycle (in G_2) when chromosomes are replicated



Sister union

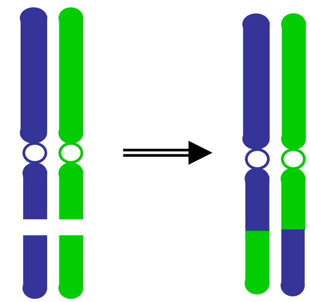
The result:



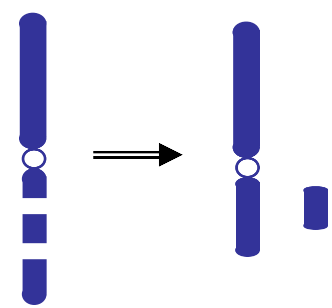
Chromatid that would not allow the cell to divide + Acentric fragment

This is a lethal aberration

Translocation



Deletion



These are not lethal aberrations

Chromosome Aberrations

- Lethal aberrations are lost at subsequent mitosis
- Non-lethal aberrations (e.g. translocation) persist for many years $\Rightarrow$ stable aberrations
- Either type of aberration may be used to estimate radiation doses (using lethal aberrations for dose estimation soon after irradiation)
- Frequency of translocations correlates with total-body dose in exposed persons. This is relevant for survivors of Hiroshima and Nagasaki A-bomb attacks even after more than 60 years