Effects of Radiation at the Cellular Level
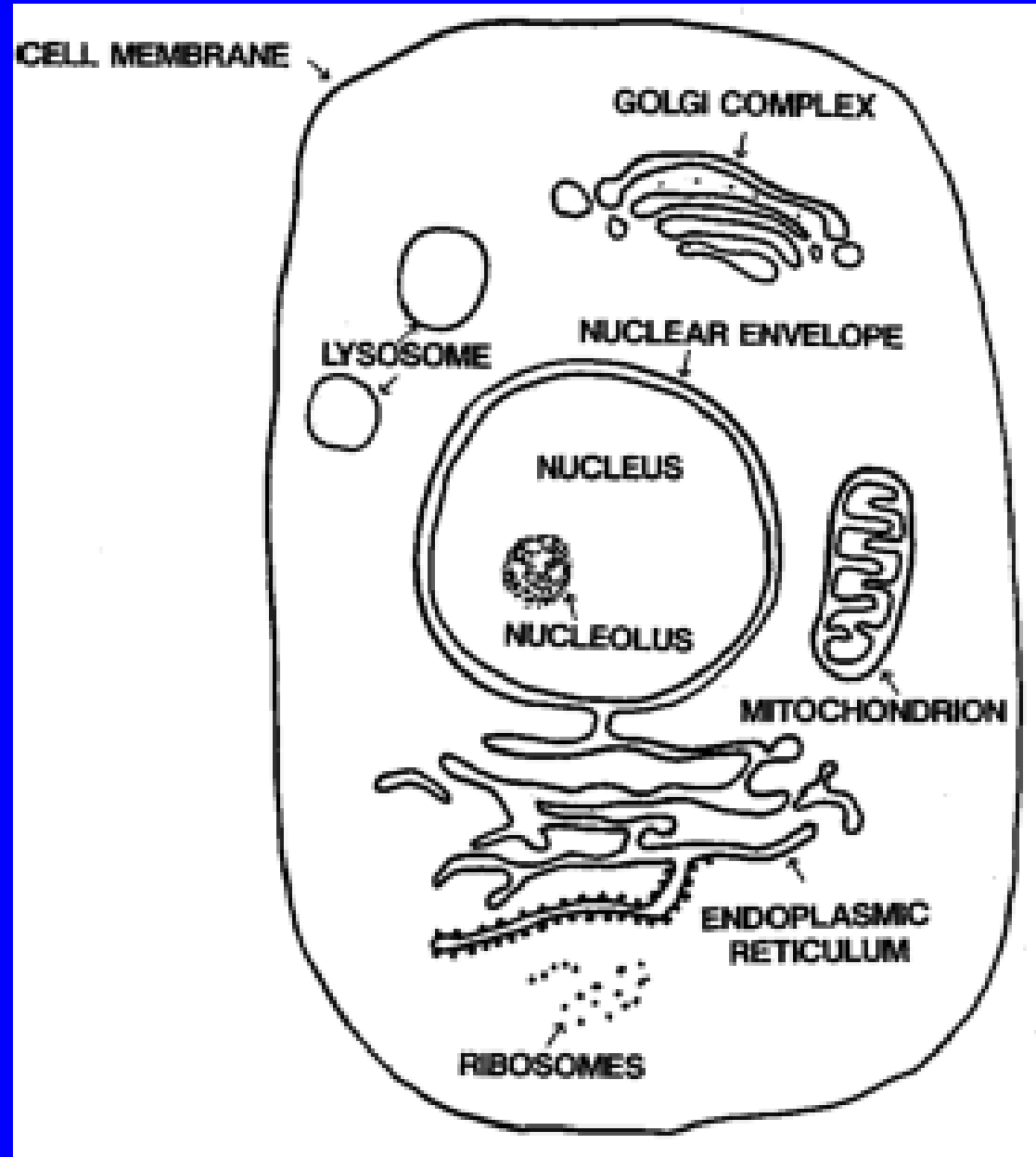
1

Table of Contents

Cellular Components

General

The cell is the basic building block of biological systems.

There are ca. 10^{14} cells in the human body.

A typical cell is 10 to 20 um in size.

Cell Membrane (plasma membrane)

A thin bilayer of phospholipids surrounding the cell.

It serves as a passive barrier impenetrable to most polar molecules (water is an exception).

Proteins distributed throughout the membrane actively control the movement of many ions and molecules into and out of the cell

Proteins

Organic molecules consisting of many amino acids.

Complex three-dimensional shapes.

Serve a structural role in the cell (e.g., filaments).

Enzymes, a particular type of protein, serve a metabolic role. They control the rate of biochemical reactions.

Nucleus

Usually the most visible cell component.

Dense and granular in appearance.

Surrounded by a double membrane (envelope).

Contains the chromosomes.

Site of RNA synthesis (transcription).
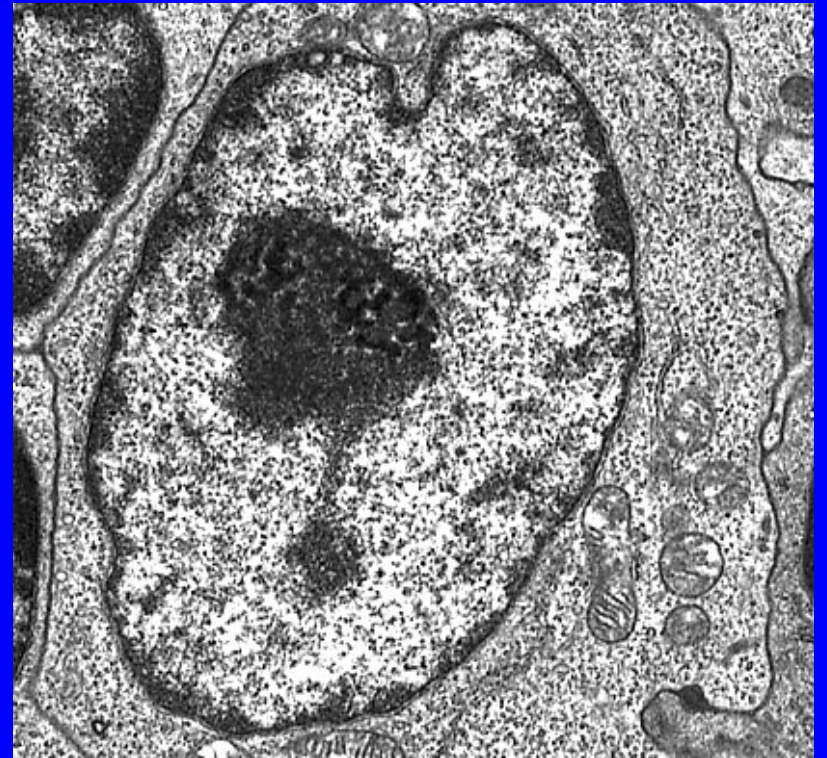
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Cytoplasm

The cell contents (excepting the nucleus)

Components: organelles
              cytosol
              misc. inclusions (e.g., pigments)

Cytosol

The cell fluid

Components: water (makes up the bulk of the cell)
              salts
              organic molecules (e.g., proteins)

Ribosomes

Small particles found in the cytoplasm

Some are attached to the surface of endoplasmic reticulum (rough ER)

They consist of two subunits

Each subunit is made up of ribosomal RNA

They organize the transfer and messenger RNA into the proper geometry for the production of protein

Endoplasmic Reticulum (ER)

Convoluted system of membranous passages in the cytoplasm

Two types: rough (ribosomes on surface)

smooth (no ribosomes on surface)

Involved in: protein and lipid production

calcium sequestration

moving proteins and carbohydrates to the plasma membrane, lysosomes and Golgi apparatus

Endoplasmic Reticulum (ER)
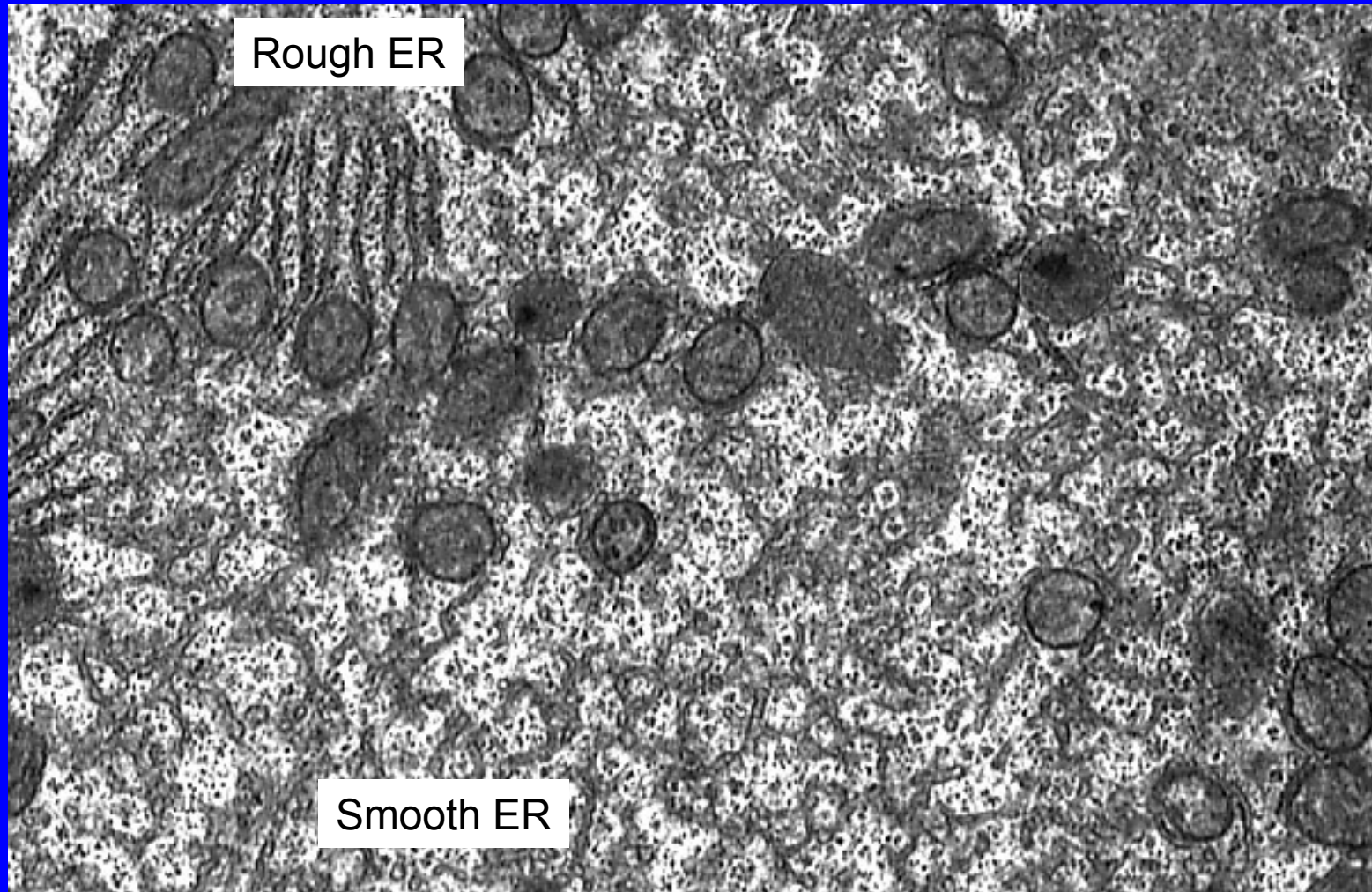
Rough ER

Smooth ER

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Golgi Complex

Organelle consisting of stacks of flattened membrane-bound sacs (cisternae)

Vesicles bud off the cisternae

Involved in the processing and packaging of organic molecules, e.g., carbohydrates
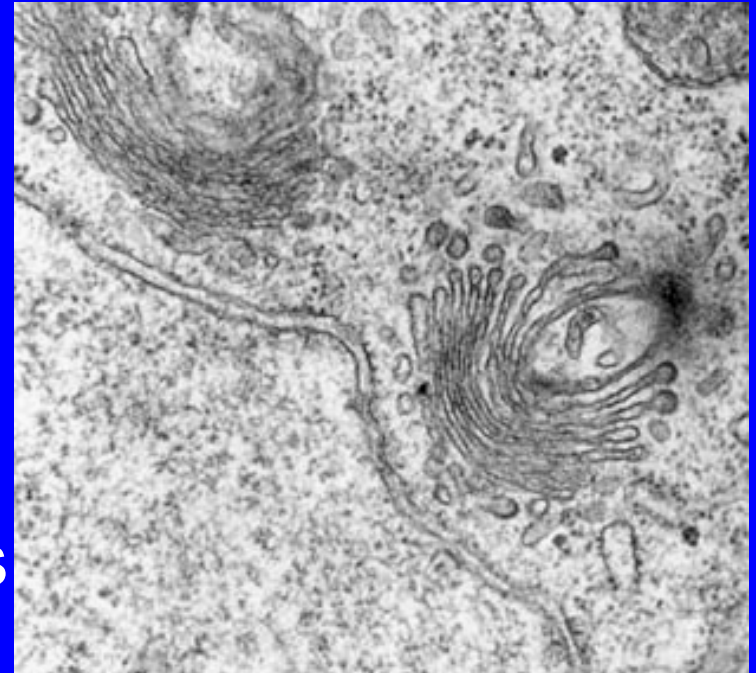
Package organic molecules in membranes for internal use or exocytosis (excretion)

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Lysosomes

Small vesicles in cytoplasm bounded by a single membrane

No discernible internal structure

Contain a variety of digestive enzymes

Lysosomes can fuse with and digest the contents of endosomes (vesicles formed by endocytosis that contain material from outside the cell).

Lysosomes can carry undigested material to cell membrane for expulsion from the cell.

Sometimes referred to as "suicide bags", a rupturing of their membrane can lead to cell death.

Mitochondria

Organelle bounded by double membrane (envelope)

© U.L.B. Louis De Vos

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Mitochondria

High degree of internal organization

Contain their own complement of DNA and ribosomes

Involved in respiration, an oxygen fueled process by which energy is obtained from the breakdown of organic molecules into water and carbon dioxide

Play an important role in apoptosis

Cytoskeleton

Cytoplasmic component maintaining the cell's structure and shape

The cellular "skeleton"

Components: filaments

               microtubules

Chromosomes, DNA and RNA

Chromosomes

Usually found inside the nucleus

Each human somatic cell has 46 chromosomes (23 from each parent)

A chromosome consists of a single molecule of DNA (or two identical molecules of DNA) and histones, a type of protein

Chromosomes

Chromosomes are usually amorphous, i.e., they have no recognizable structure

When they do have structure (during mitosis) they look like either of the following

No matter which form they take, they have a centromere (a region of repetitive DNA that appears as a constriction)

CENTROMERE

SISTER CHROMATIDS
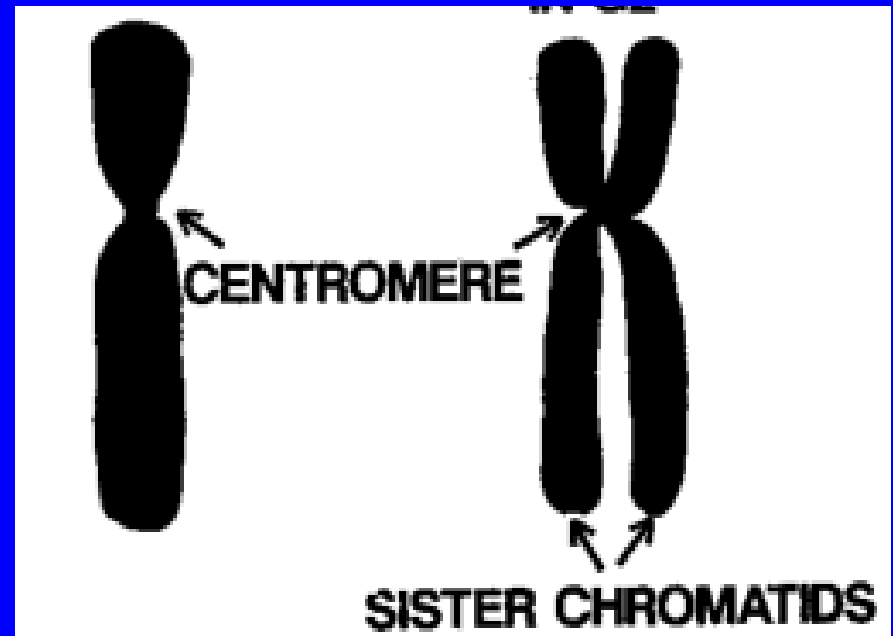
DNA

Deoxyribonucleic acid

In 3-D, a "double helix"

In 2-D, a ladder

The sides of the ladder are made of an alternating sequence of sugar and phosphate groups

The ladder's rungs join the sugar groups

Each rung consists of two nitrogenous bases

DNA

Four types of
nitrogenous bases:

Adenine
Thymine
Cytosine
Guanine

```
  |        |
S—AT— S         S   Sugar
  |        |     P   Phosphate
P        P       A   Adenine
  |        |     T   Thymine
S—CG—S          C   Cytosine
  |        |     G   Guanine
P        P
  |        |
S—GC—S
  |        |
P        P
  |        |
S—TA— S
  |        |
```

DNA DOUBLE HELIX
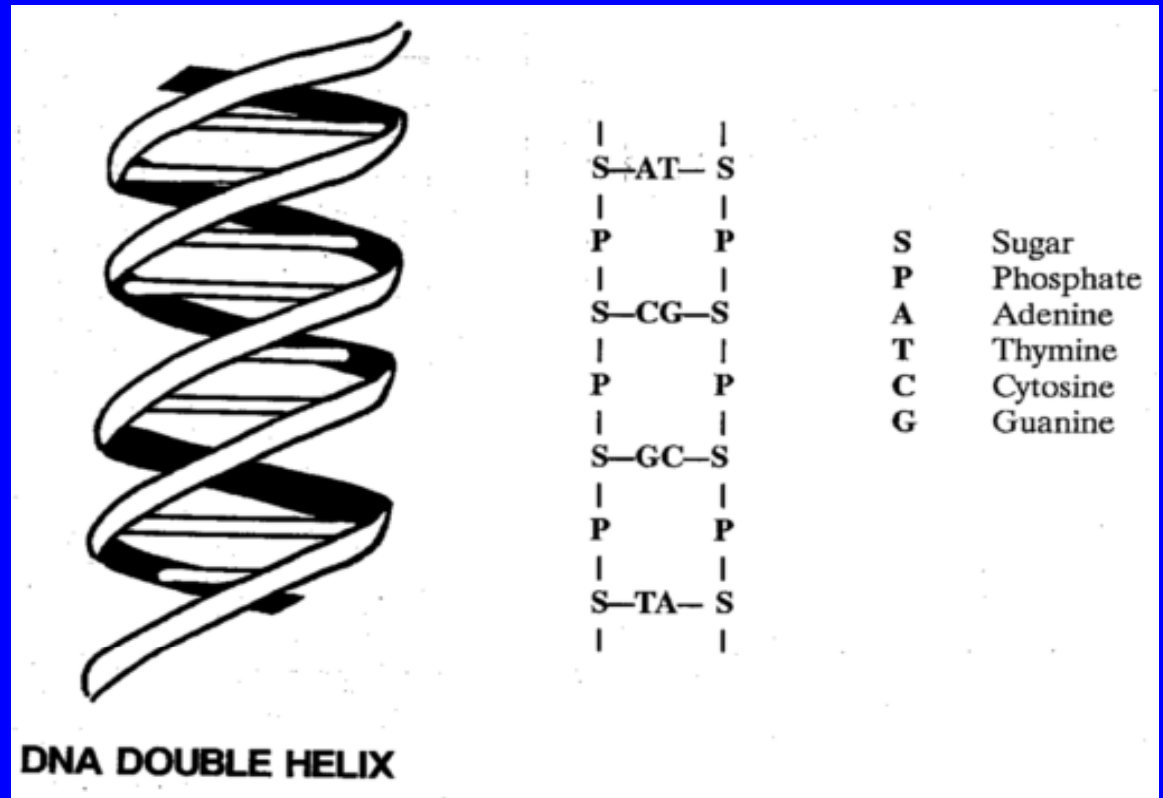
Adenine and thymine always pair up

Cytosine and guanine always pair up

DNA

"Controls" the cell by controlling protein production

The sequence of the nitrogenous bases along one side of the DNA molecule determines which types of RNA are produced

The type of RNA that is produced determines the type of protein that is produced

The sequence of nitrogenous bases that codes for a particular protein is called a "gene"

The DNA is a "master blueprint." Only one copy exists.

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RNA

Ribonucleic acid

Produced (transcribed) from DNA

Similar to DNA but single stranded

Three types: transfer RNA
                messenger RNA
                ribosomal RNA

The three types come together in the cytoplasm to produce proteins (transcription)
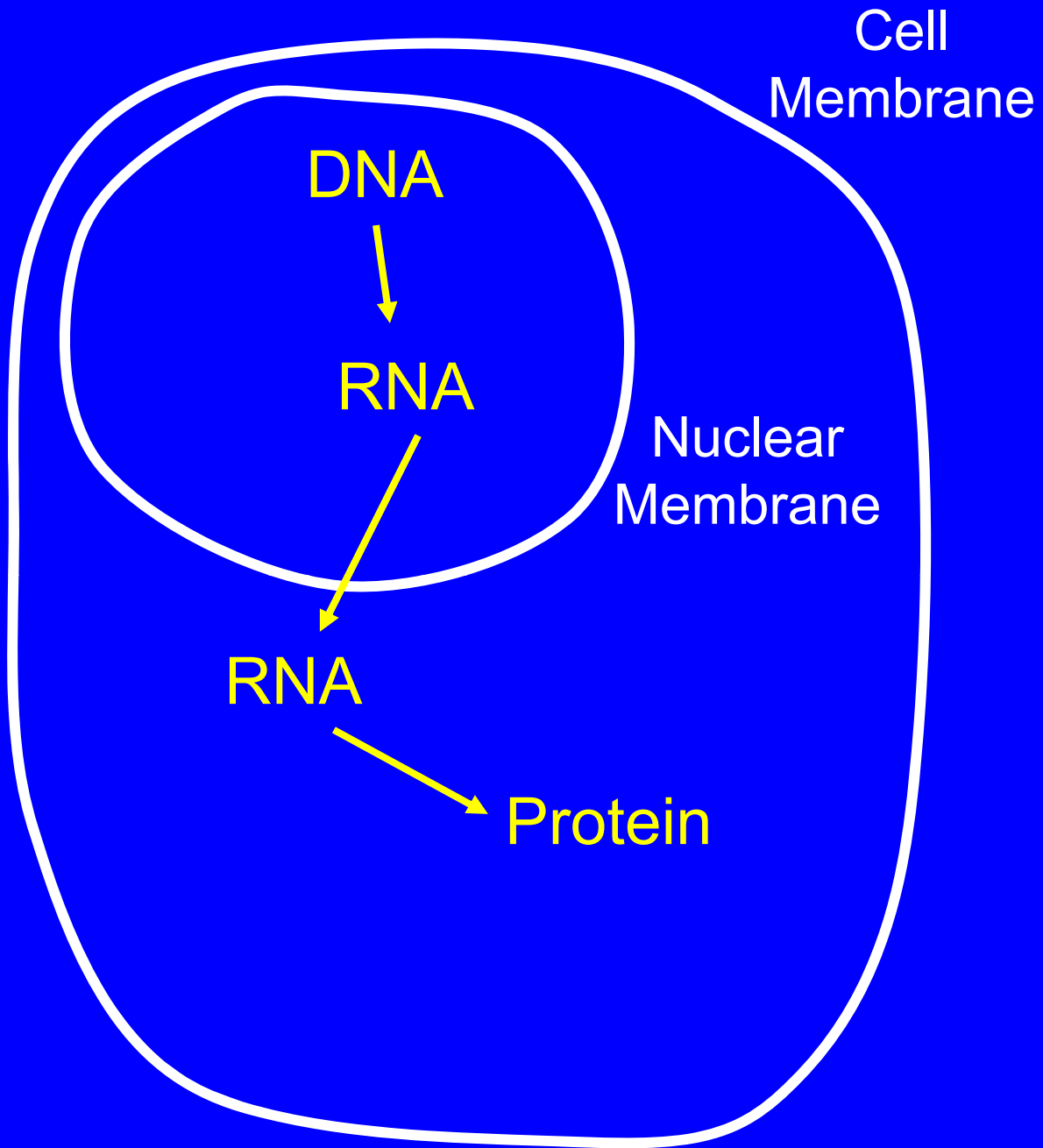
Apoptosis

Purpose of Apoptosis

Apoptosis (aka programmed cell death) is a means by which an organism induces cells that might pose a threat to commit suicide.

By destroying specific cell types, it also serves as a selective tool that guides development of the embryo.

Apoptosis can be induced by radiation. This is generally a good thing because dead cells don't become cancerous.

Failure of Apoptosis

If radiation damages the genes that induce apoptosis, there is an increased possibility that any malfunctioning cancerous cells will proliferate.

As a "survival mechanism," some cancer cells produce chemicals that suppress apoptosis. In fact, the ability to suppress apoptosis seems to be characteristic of all cancers.

Role of mitochondria in apoptosis

When the cell is stressed by radiation, pro-apoptosis bcl-2 proteins (e.g., Bad) in the cytosol can move to the surface of the mitochondria.

Here they interfere with anti-apoptosis bcl-2 proteins. The result is that pores are formed in the mitochondrial envelope.

Cytochrome C and other pro-apoptosis molecules leave the mitochondria and go into the cytosol.

Role of mitochondria in apoptosis

In the cytosol, the cytochrome C interacts with the proteins Apaf-1 and caspase 9 to form a complex known as the apoptosome.

This complex activates the capase enzymes, the "executioner" enzymes responsible for breaking down proteins.

Role of mitochondria in apoptosis

The cells undergoing apoptosis then:

shrink

degrade and fragment

are engulfed (eaten) by phagocytes

Cell's Life Cycle

Cell Life Cycle

There are four phases to the cell's life cycle:

- G_1 when each chromosome contains a single molecule of DNA

- S (synthesis) when the DNA molecule of each chromosome is perfectly (almost) replicated

- G_2 when each chromosome has two nearly identical molecules of DNA joined at the centromere. The pair of molecules form what is called a pair of sister chromatids.

- M (mitosis) when the cell divides
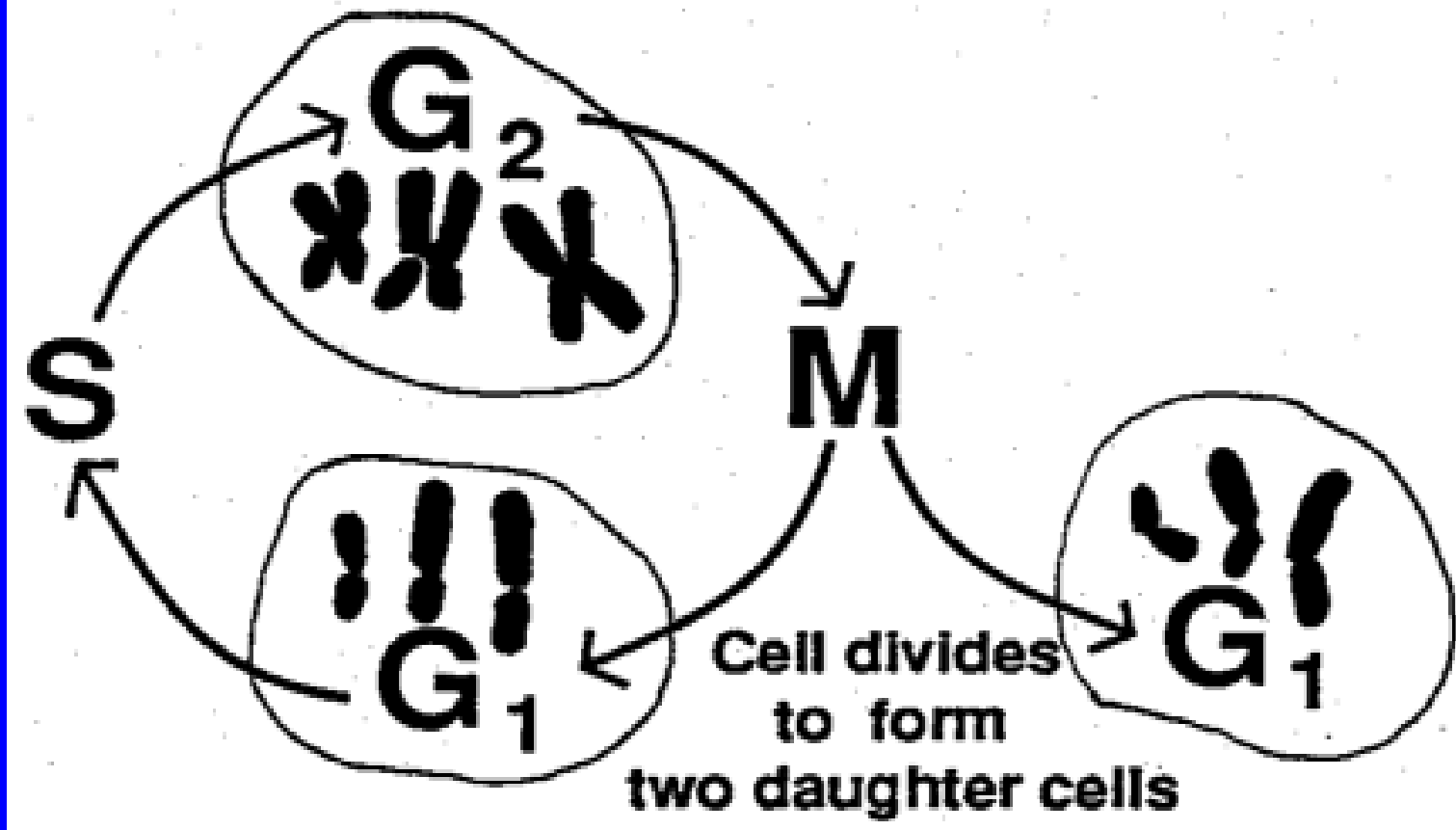
Chromosomes during G_1 and G_2 don't look like they are shown here. They do look like this during mitosis (M).

Cell Division
(mitosis)
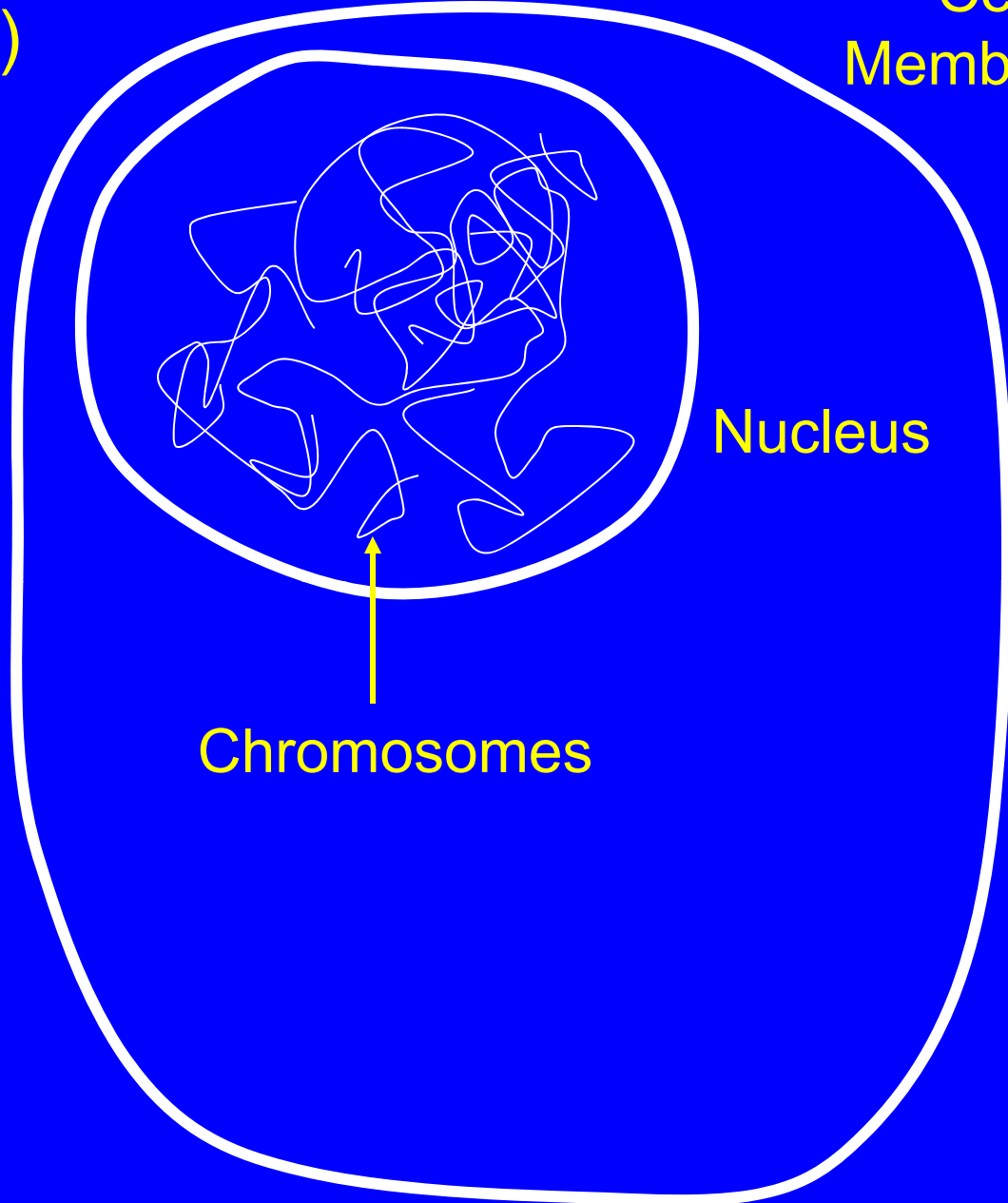
Cell Membrane

Nucleus

Chromosomes

Cell in G_2

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Cell Division
(mitosis)

Centrosomes

Nuclear membrane breaks downs

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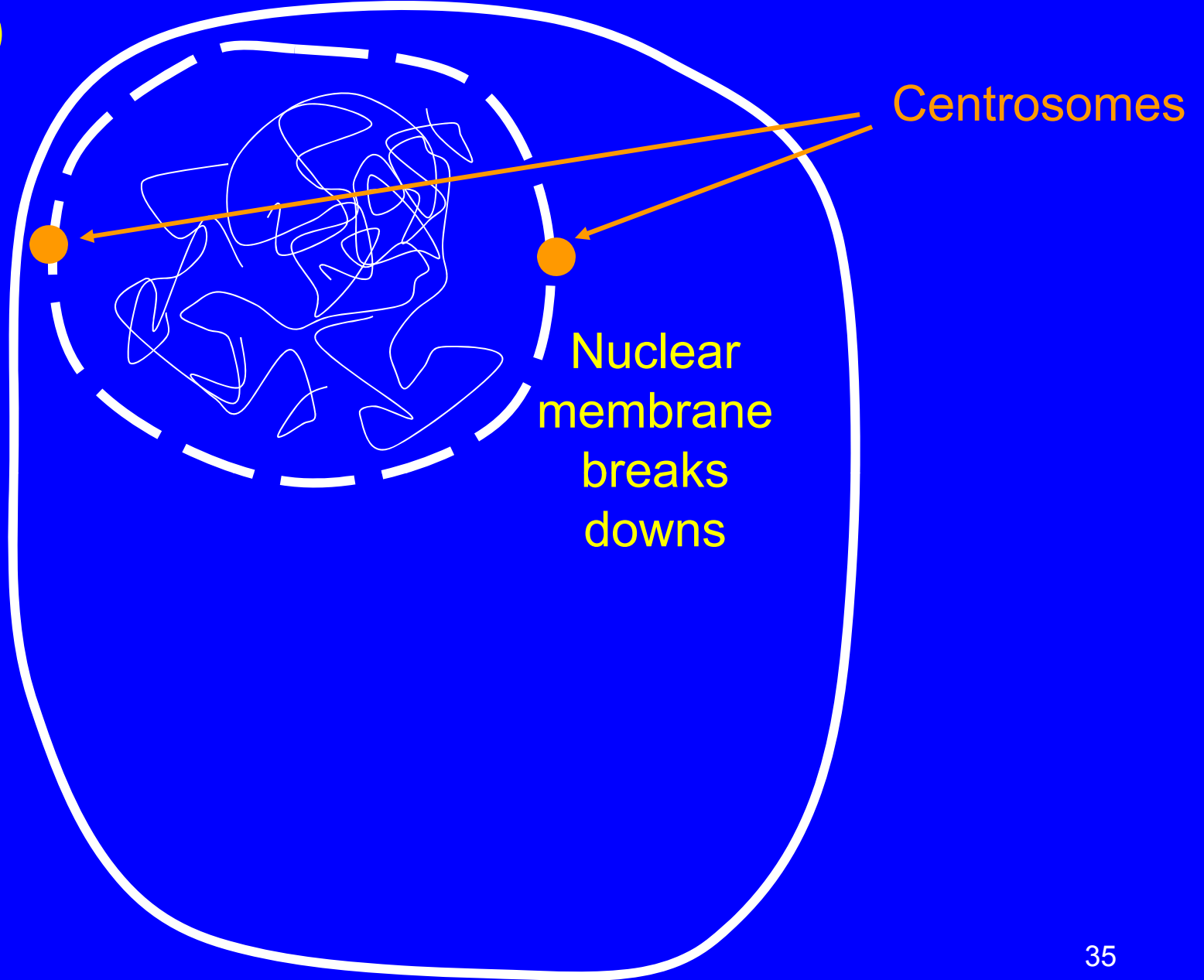
Cell Division (mitosis)

Chromosomes "condense" and line up along cell equator

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Cell Division (mitosis)
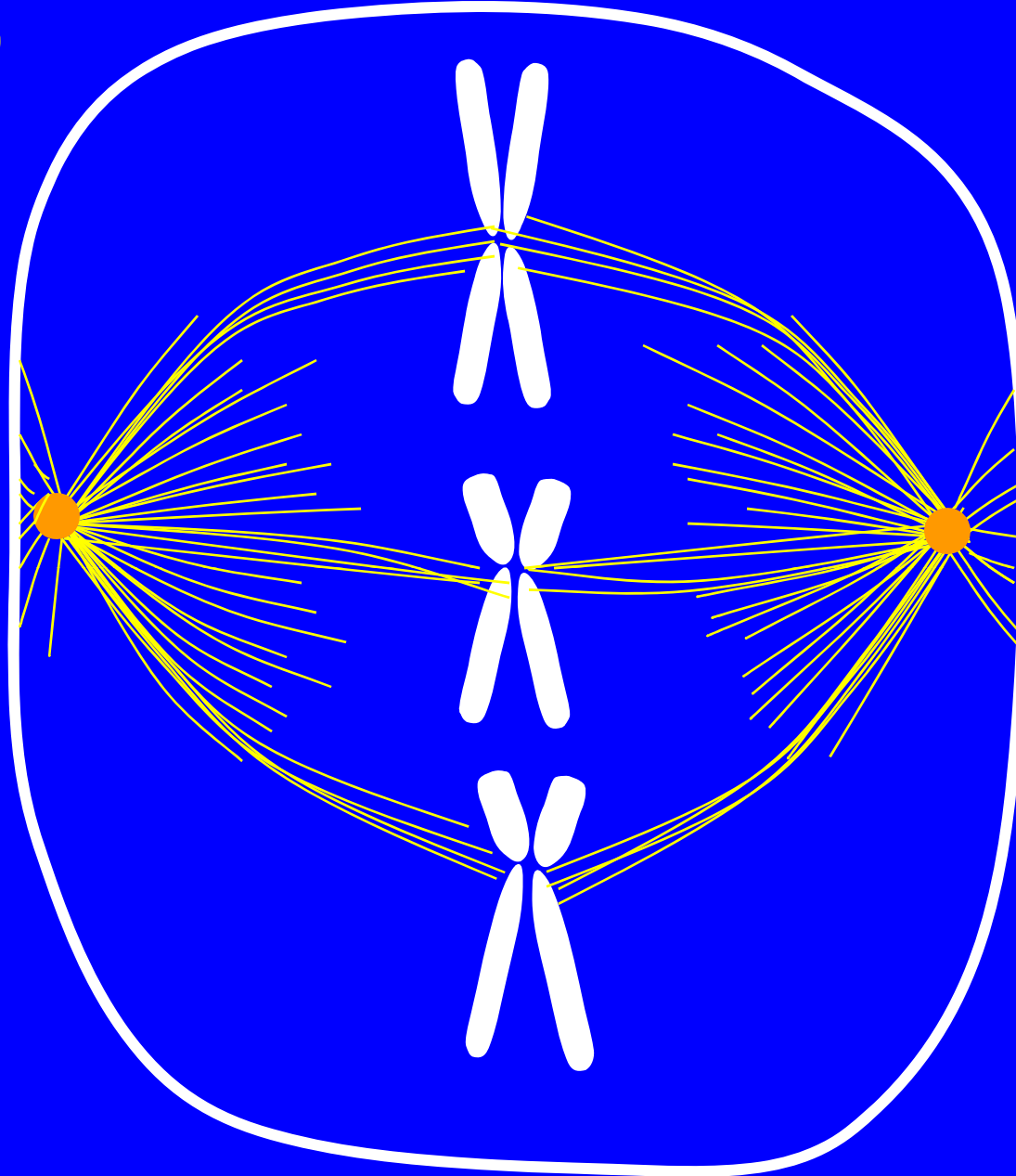
The centrosomes produce microtubules some of which attach to the chromosomes at the centromere.

Collectively, the microtubules form the "spindle"

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Cell Division (mitosis)
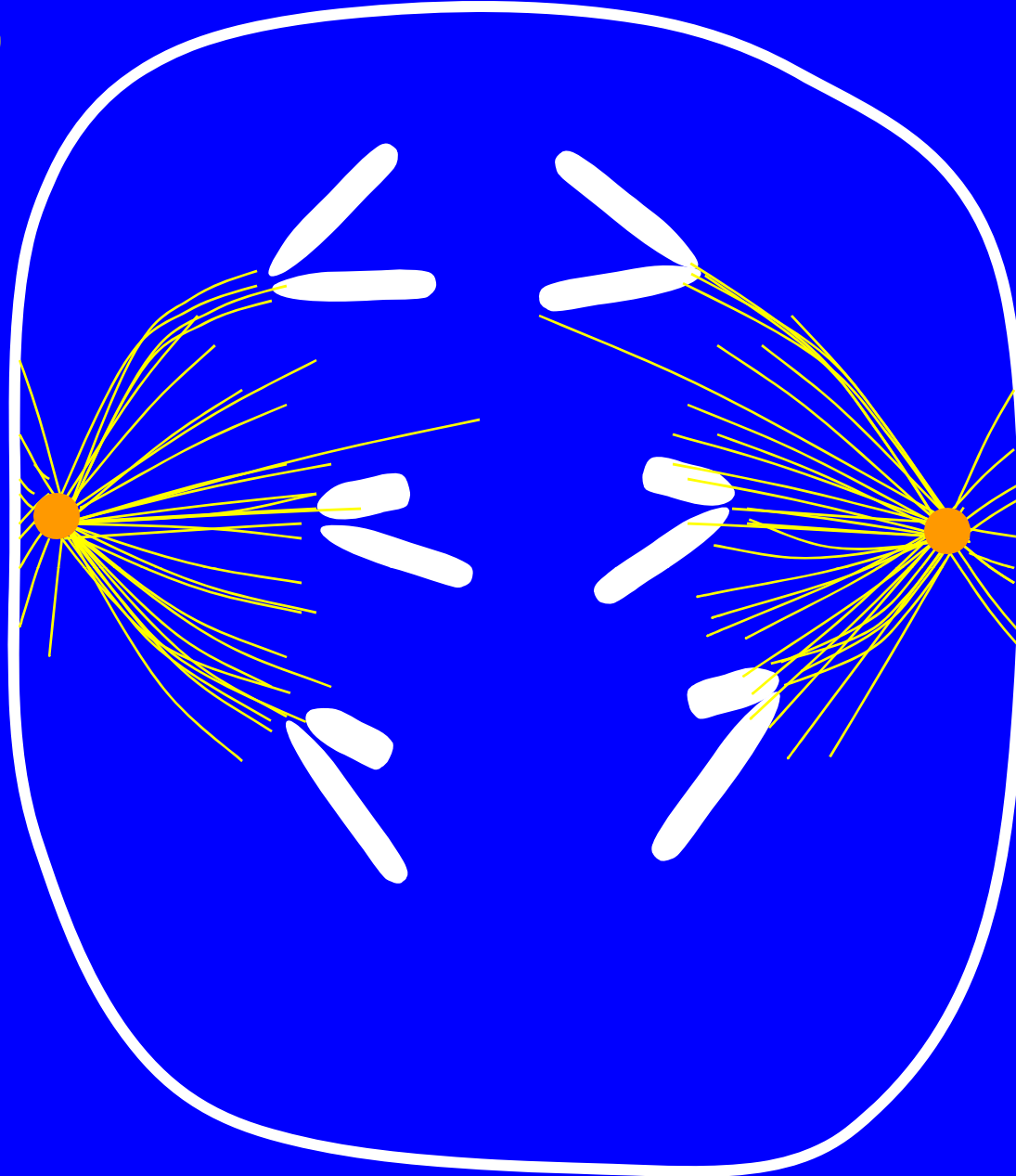
Each chromosome splits in two thus forming two identical chromosomes.

One is pulled to the right and one is pulled to the left.
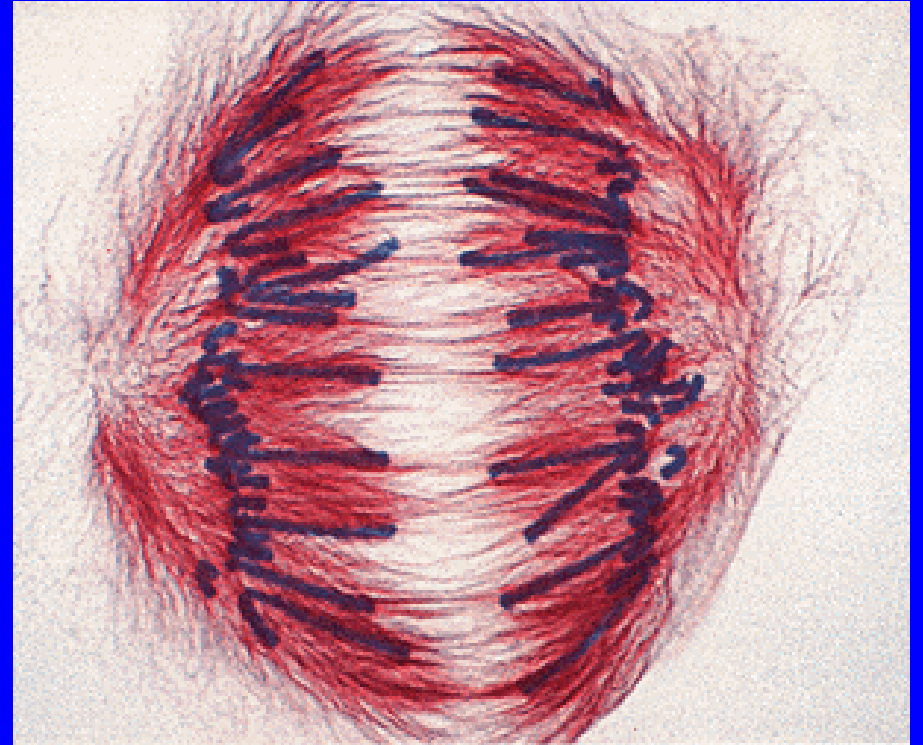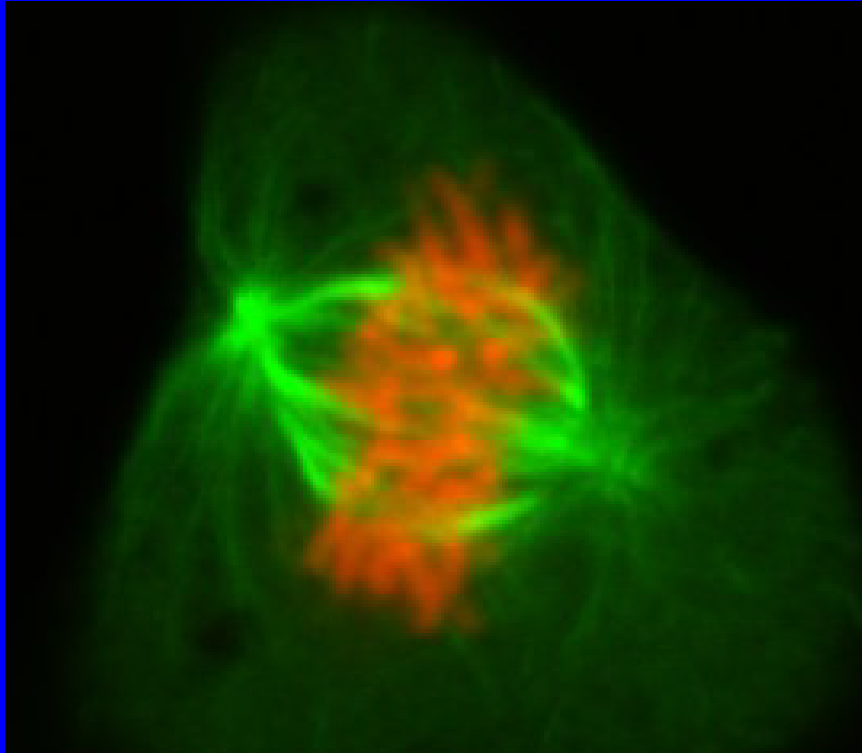
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Cell Division (mitosis)

Cell Division (mitosis)

40
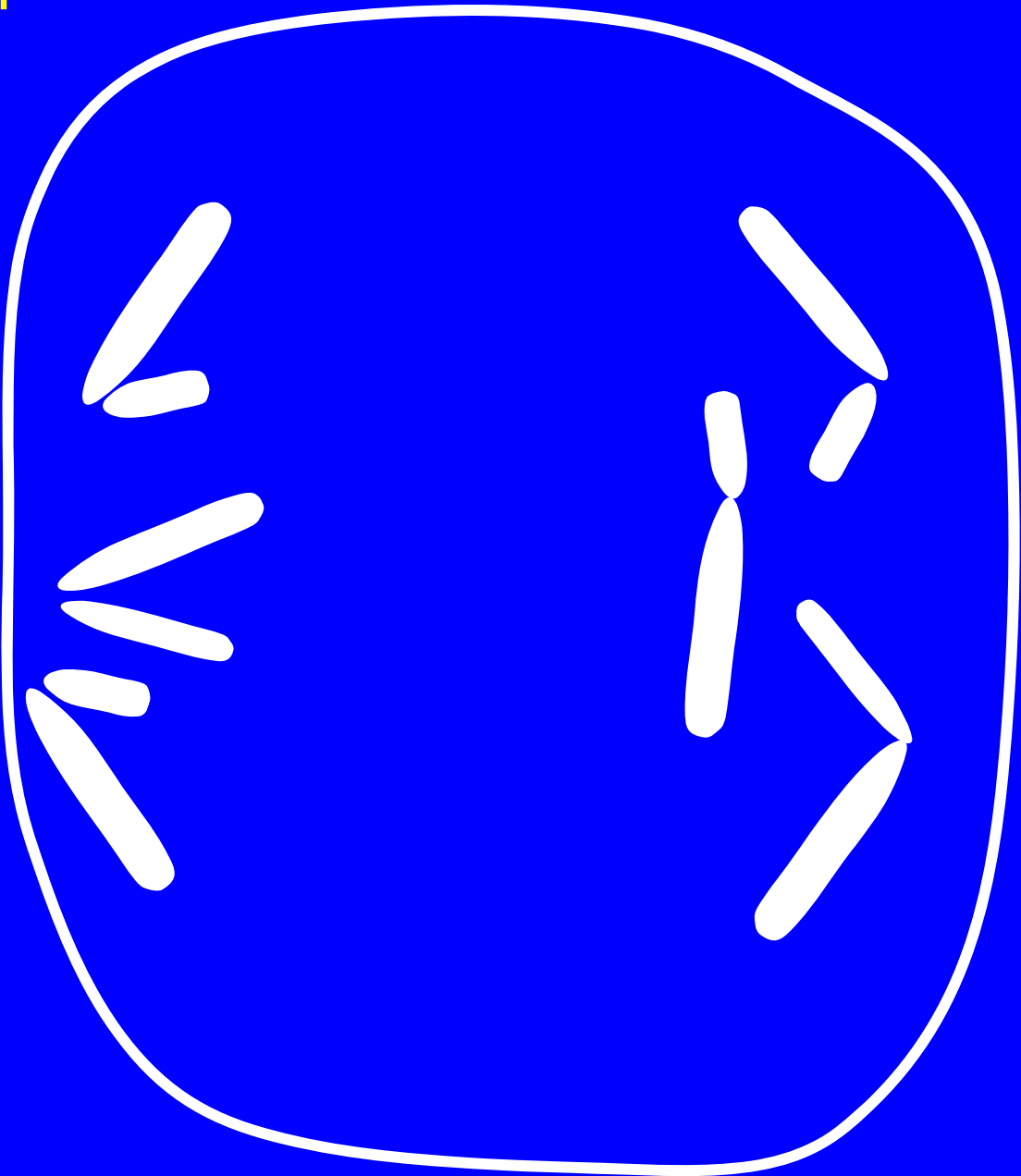
Cell Division (mitosis)
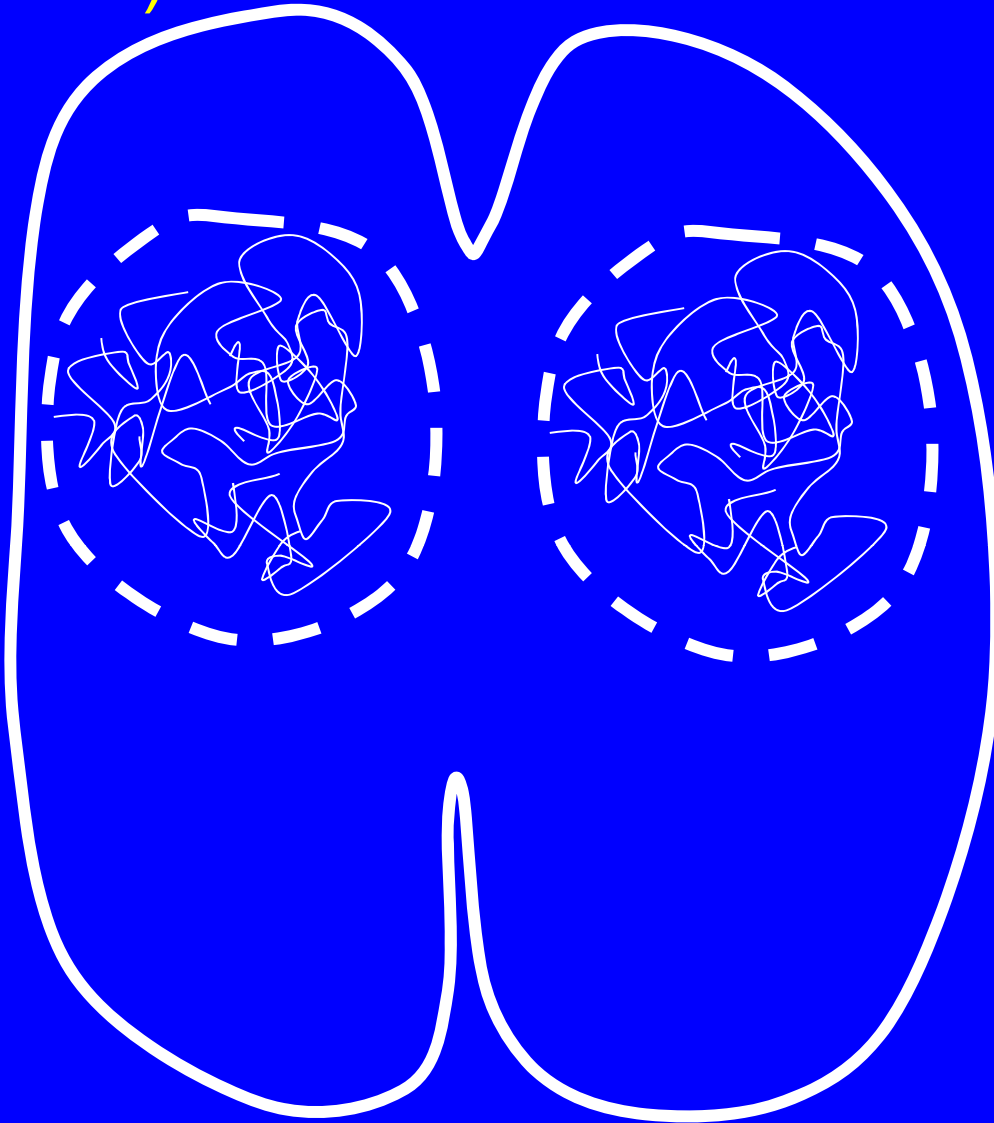
When the chromosomes have separated to the two sides of the cell, the nucleus reforms, the chromosomes lose their defined structure, and the cell pinches in two.

Cell Division (mitosis)
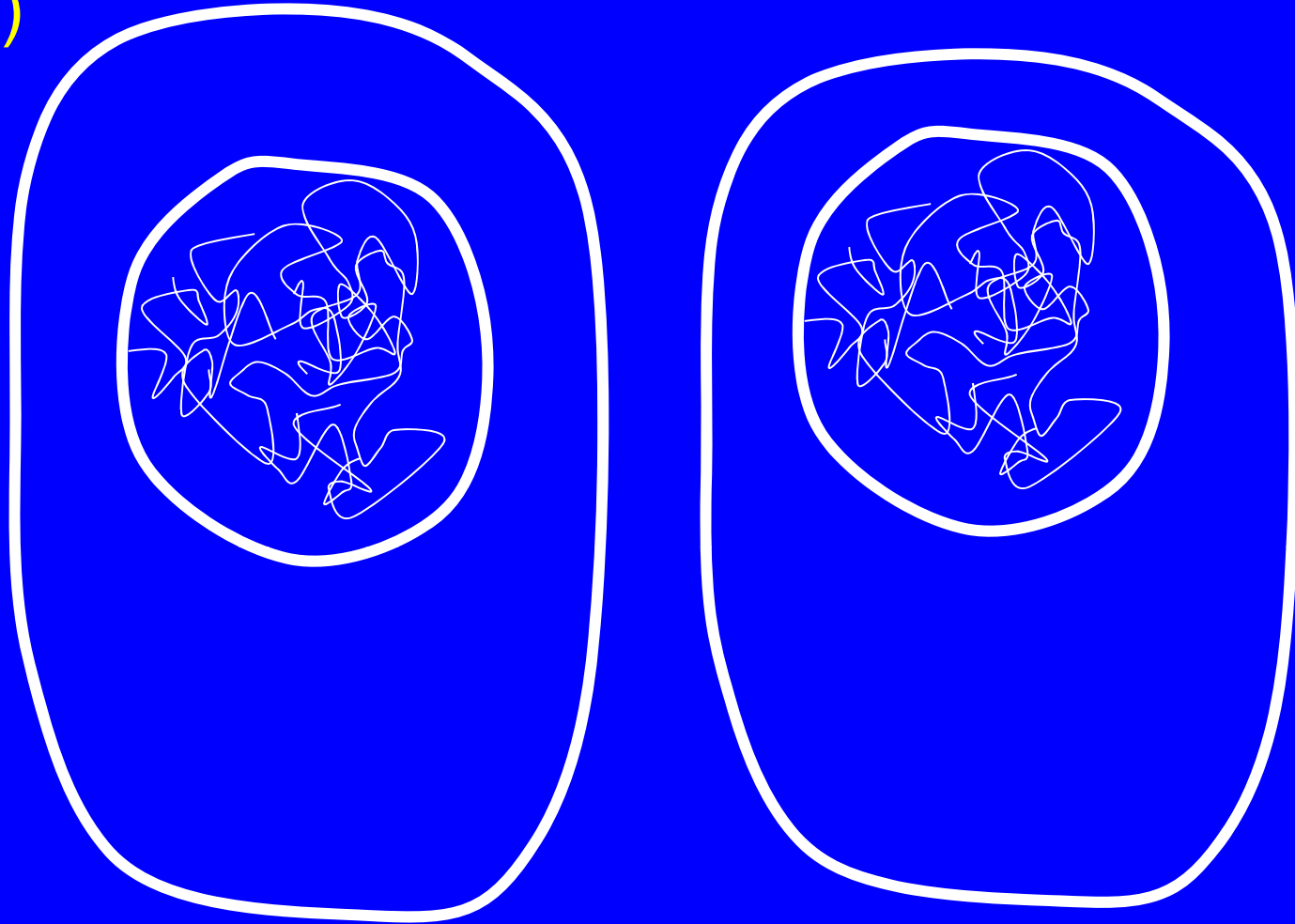
Two "daughter" cells, each with a complete complement of genetic material.

Interaction of Radiation with Cellular Constituents

Free Radical Production

Interactions of Radiation with Cellular Constituents

Gamma rays or x-rays interact with atoms in the cell by the photoelectric effect, Compton scattering, or pair production.

In human tissue, Compton scattering is the most likely interaction.

In these interactions the gamma-ray or x-ray transfers energy to an electron which then travels through the cell. This electron then interacts in the same way that other charged particles interact.

Interactions of Radiation with Cellular Constituents

Charged particles (such as alpha or beta particles) transfer energy to the molecules they pass near.

The result is that these molecules are excited, ionized, or broken.

In some cases free radicals are produced.

Radiolysis of Water

The splitting apart of water by radiation

Water is the major constituent of the cell. Something like 80% of the radiation energy absorbed by the body is absorbed by water.

The following reactions are brought about by the transfer of energy from betas (electrons) or alphas to the water molecule. Excitation of the water molecule is of little importance and will not be considered.

Radiolysis of Water

Ionization

$$Radiation + H_2O \rightarrow H_2O^+ + e^-$$

$$Radiation + H_2O \rightarrow H^+ + OH^-$$

Formation of Free Radicals

$$Radiation + H_2O \rightarrow \overset{\bullet}{H} + \overset{\bullet}{O}H$$
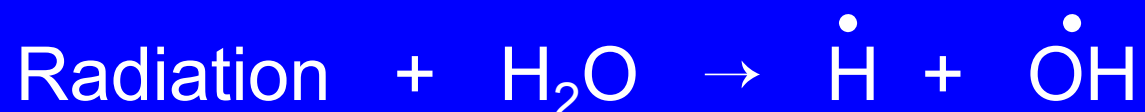
Free radicals are uncharged and have an unpaired electron. Sometimes symbolized as H^0 or OH^0

Free Radicals

The unpaired electron makes the free radical extremely reactive.

Within a nanosecond of its formation, a free radical will reacts with some nearby molecule (the target) in the cell and damages it.

The free radical might react with this molecule (RH) and produce a new radical species that proceeds to damage other molecules.

$$RH \ + \ \overset{\bullet}{O}H \ \rightarrow \ \overset{\bullet}{R} \ + \ H_2O$$

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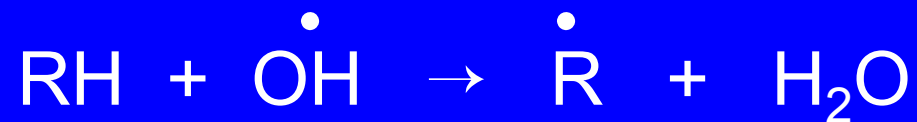
Free Radicals

Two hydroxyl free radicals can combine to produce hydrogen peroxide. Since the latter, a powerful oxidizer, is relatively long lived, it has a substantial range of damage.

$$\overset{\bullet}{O}H + \overset{\bullet}{O}H \rightarrow H_2O_2$$

Two hydrogen free radicals can combine together to produce hydrogen.

$$\overset{\bullet}{H} + \overset{\bullet}{H} \rightarrow H_2$$

Free Radicals

A hydrogen free radical might combine with an oxygen molecule to produce a hydroperoxyl radical. The latter might then combine with another hydroxyl free radical to produce hydrogen peroxide.

$$\dot{H} + O_2 \rightarrow \dot{H}O_2$$

$$\dot{H}O_2 + \dot{H} \rightarrow H_2O_2$$

Other types of free radicals can combine with oxygen to produce organic peroxy free radicals:

$$\dot{R} + O_2 \rightarrow \dot{R}O_2$$

Free Radicals

Much of the damage brought about by radiation is believed to be due to oxygen containing radicals, ions and peroxides collectively known as reactive oxygen species (ROS).

The hydroxyl free radicals are believed to be particularly important.

Direct and Indirect Effects

Two general categories of radiation effects:

direct effects

indirect effects

Direct Effects

Direct effects of radiation involve a transfer of energy from the radiation (e.g., alpha or beta) directly to the target molecule (typically DNA).

Direct effects are believed to be the most important type of effect for high LET radiation (e.g., neutrons and alpha particles).

Indirect Effects

Indirect effects occur when radiation produces free radicals which react with the target molecule (typically DNA).

The free radicals must be produced very close to the target (e.g., within a few nm) since they have such short lifetimes.

Indirect effects seem to be most important for low LET radiation (e.g., gammas, x-rays and betas). About 65% of low LET damage has an indirect component whereas 35% is exclusively direct (UNSCEAR 2000).

Subcellular Targets

Everything in the cell is subject to direct and indirect damage, but some targets are considered to be more important than others:

DNA

membranes (especially the nuclear membrane)

The DNA molecule is considered to be the most important site of damage because it serves as the master blue print for the cell.

DNA Damage and Repair

DNA Damage

With low LET radiation, the damage tends to be spatially isolated along the DNA molecule.

With high LET radiation, clusters of damage occur on a given chromosome.

Such clustered damage is more complex and difficult to repair than the isolated damage caused by low LET radiation. Repair may require enzymes from multiple repair pathways.

Clustered damage rarely occurs in the absence of radiation, i.e., it is somewhat "unique" to radiation.

DNA Damage

The following table (UNSCEAR 2000) indicates the estimated DNA damage per gray (100 rads).

Base damage	500 per cell
Single strand breaks	1,000 per cell
Double strand breaks	40 per cell
DNA-protein crosslinks	150 per cell

1. DNA Base Damage

This involves alterations to individual nitrogenous bases.

These alterations are primarily due to the formation of free radicals in the water that is closely bound to the chromosomes.

As an example of such damage, the free radicals might induce the deamination of the nitrogenous base cytosine and convert it to uracil. It is even possible that the base might be completely removed.

1. DNA Base Damage and Repair

DNA base damage is often (although not always) what is meant by the term mutation. It is sometimes referred to as a genetic (vs. chromosomal) mutation.

Large stretches of the chromosome consist of "nonsense" repetitive DNA. Base damage to these regions is inconsequential.

1. DNA Base Damage and Repair

Base damage is usually repaired. The classical base-excision repair mechanism operates as follows:

- First, the damaged section is excised by a DNA glycosylase enzyme. Various glycosylases recognize different types of base damage.

- Then a DNA endonuclease cuts the DNA "backbone"

- Next, a phosphodiesterase removes the sugar and phosphate remnants.

1. DNA Base Damage and Repair

- After this, the excised segment is resynthesized by a polymerase using the undamaged strand as a template.

- Finally, ligases attach the newly synthesized segment in place.

Sometimes the damage necessitates the removal of a large segment of one side of the DNA molecule, not just one base and the associated sugar and phosphate groups.

1. DNA Base Damage and Repair

Damaged base

A T
C G
G*C
A T
C G

Damaged base removed

A T
C G
C
A T
C G

Sugar and phosphate removed

A T
C G
C
A T
C G

Resynthesis

A T
C G
G C
A T
C G

Repair complete

A T
C G
G C
A T
C G
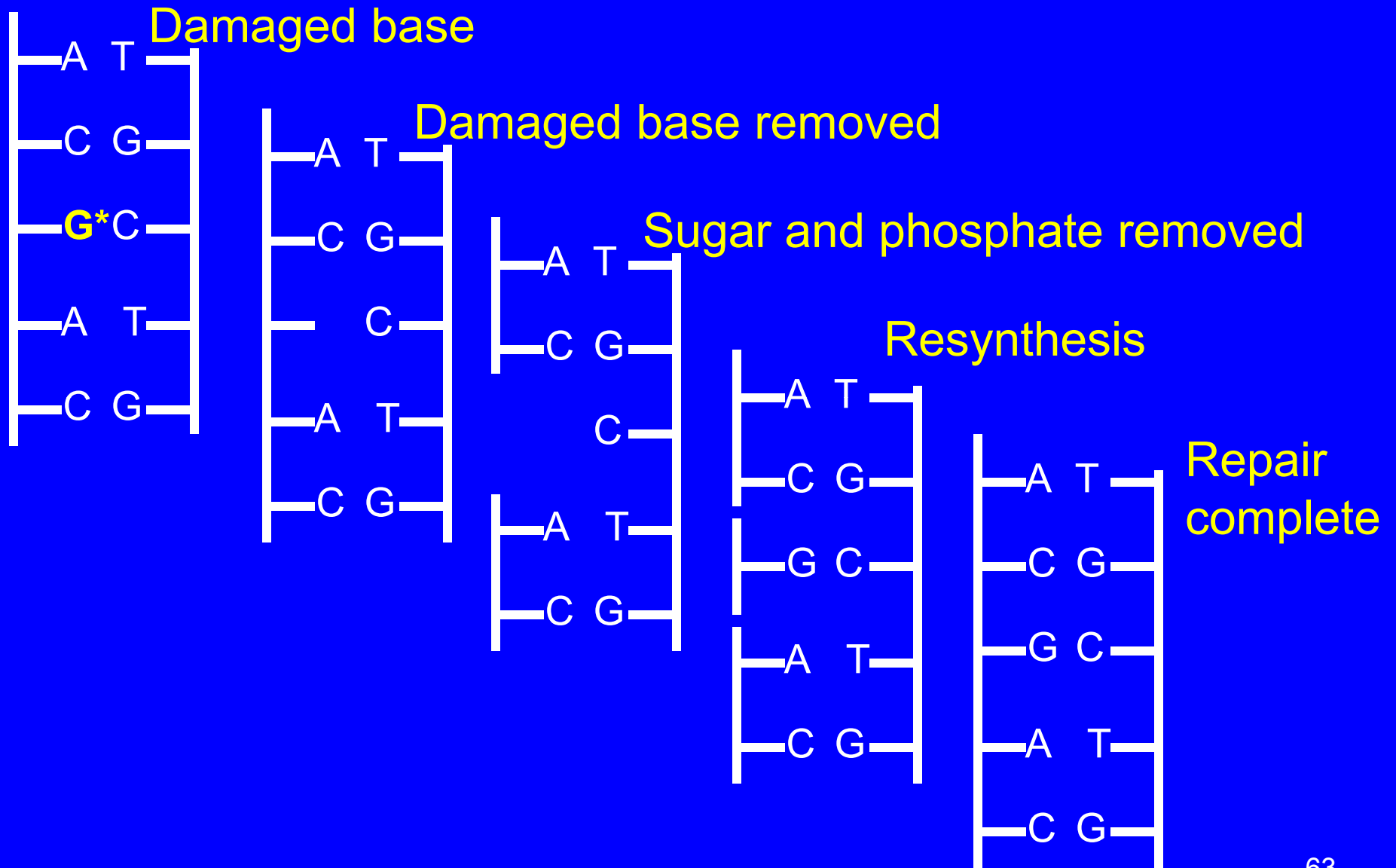
2. DNA Single Strand Breaks (SSBs)

One side of the DNA molecule is broken. This is the most common type of DNA damage, but perhaps the least important. As a rule, an SSB also involves damage to the sugar group.
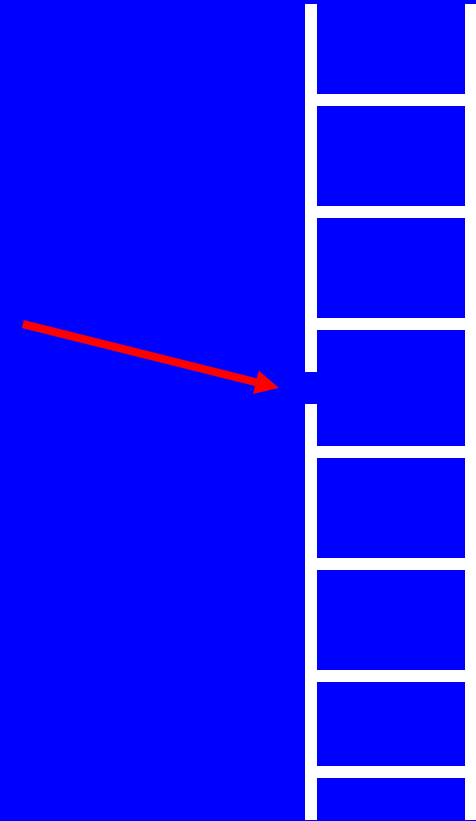
Repair of Single Strand Breaks (SSBs)

Single strand breaks are the easiest to repair. The repair mechanism is similar to that for base damage.

3. DNA Double Strand Breaks (DSBs) and Repair

Both sides of the DNA molecule are broken.

The least common type of DNA damage, but perhaps the most important.

One concern is that the broken two pieces of DNA (and hence the chromosome) might not rejoin. If this happens, the chromosome has broken into two separate pieces.

Another concern is the possibility of mis-repair.

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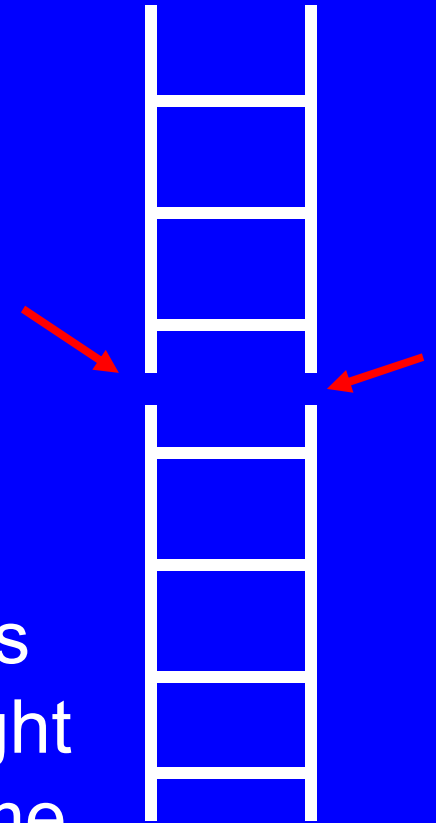
3. DNA Double Strand Breaks (DSBs) and Repair
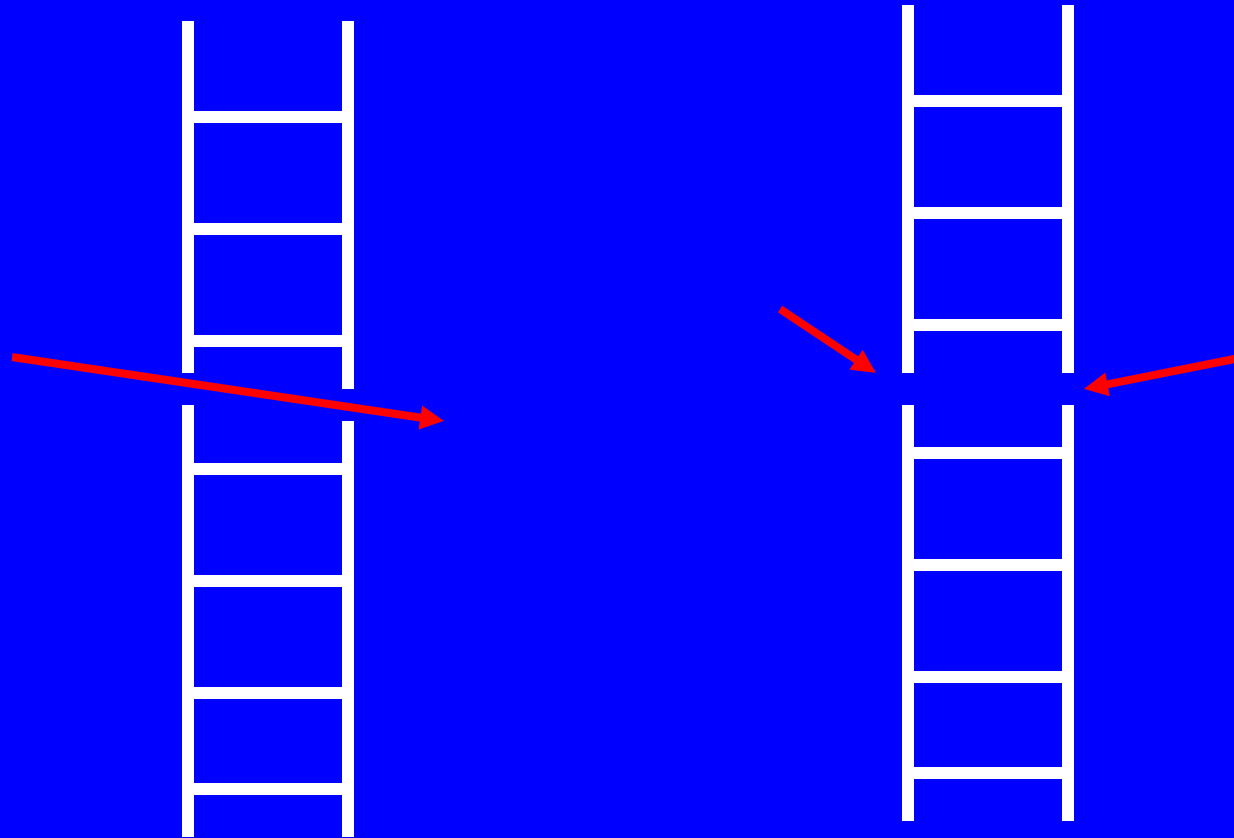
Single event DSB   Double event DSB

With a double event DSB, opposite strands of the DNA molecule are broken separately, but close together in space and time.

3. DNA Double Strand Breaks (DSBs) and Repair

Since the hydrogen bonds joining the nitrogenous base pairs are weak, the breaks in the two strands don't have to be immediately opposite each other. They just need to within 10 to 20 base pairs of each other.

Fragmented DNA molecule (chromosome)
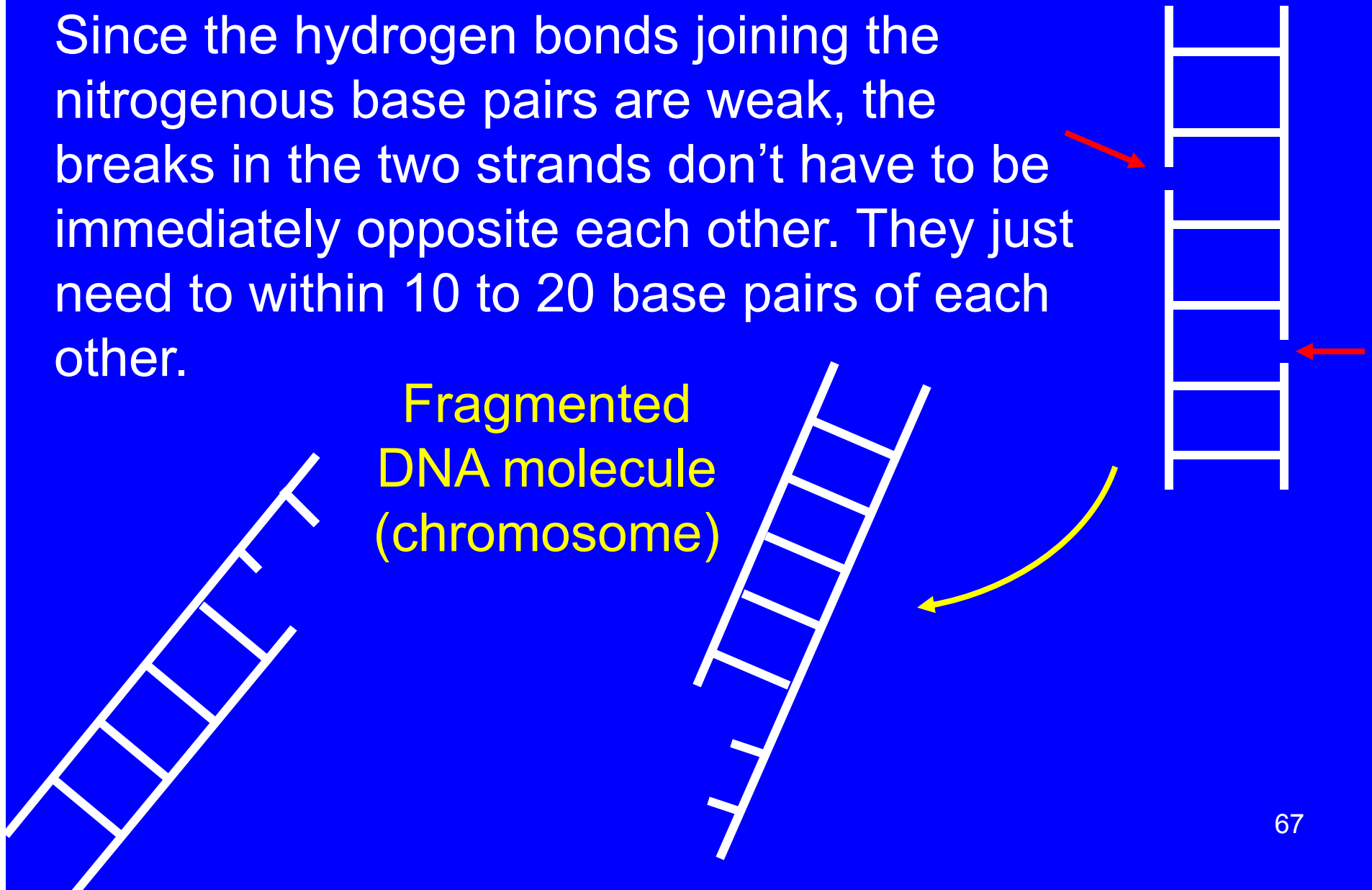
3. DNA Double Strand Breaks (DSBs) and Repair

Two types of repair mechanisms for DSBs:

    non-homologous end joining (NHEJ)
    homologous recombination (HRR)

NHEJ predominate in mammals. "Quick and dirty."

Simply involves attaching the broken ends (which might be missing material).

More error prone than HRR, but this is not so much of a concern since mammalian chromosomes contain a great deal of repetitive DNA.

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3. DNA Double Strand Breaks (DSBs) and Repair

Homologous recombination is more complex, but less prone to error.

HRR is only possible in S or G_2.

In HRR, the broken end of a single strand of the DNA "invades" the appropriate unbroken section of an homologous DNA molecule (e.g., that of its sister chromatid).

The homologous DNA is used as a template to produce any missing DNA on the broken section.

3. Double Strand Breaks (DSBs) and Repair

DSBs are more likely to result in the death of the cell than base damage. Even so, only a small percentage of DSBs are lethal.

Most DSBs are repaired and do not result in visible chromosomal aberrations.

The number of DSBs is 40 to 100 times the number of lethal events. Presumably, these lethal events are due to the failure of the repair mechanisms to repair 1 to 2.5% of the DSBs.

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Chromosomal Aberrations

Chromosomal Aberrations

Unrepaired DSBs can lead to chromosomal aberrations (gross structural abnormalities visible under a microscope during mitosis).

Some of these aberrations are due to the fact that the broken ends of the chromosome at the site of the DSB are "sticky."   That is, they tend to attach to the ends of other broken, or unbroken, chromosomes.

Chromosomal Aberrations

The nature of the aberration depends on the stage of the cell cycle at which the exposure occurred. In G_1 each chromosome consists of one DNA molecule. In G_2 each chromosome consists of two DNA molecules (one per chromatids).

The aberrations that result from DSBs in G_1 are called chromosomal aberrations.

Those that result from DSBs in G_2 are sometimes called chromatid aberrations.

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Chromosomal Aberrations

These figures show common chromosomal and chromatid aberrations, e.g., rings, fragments, dicentrics
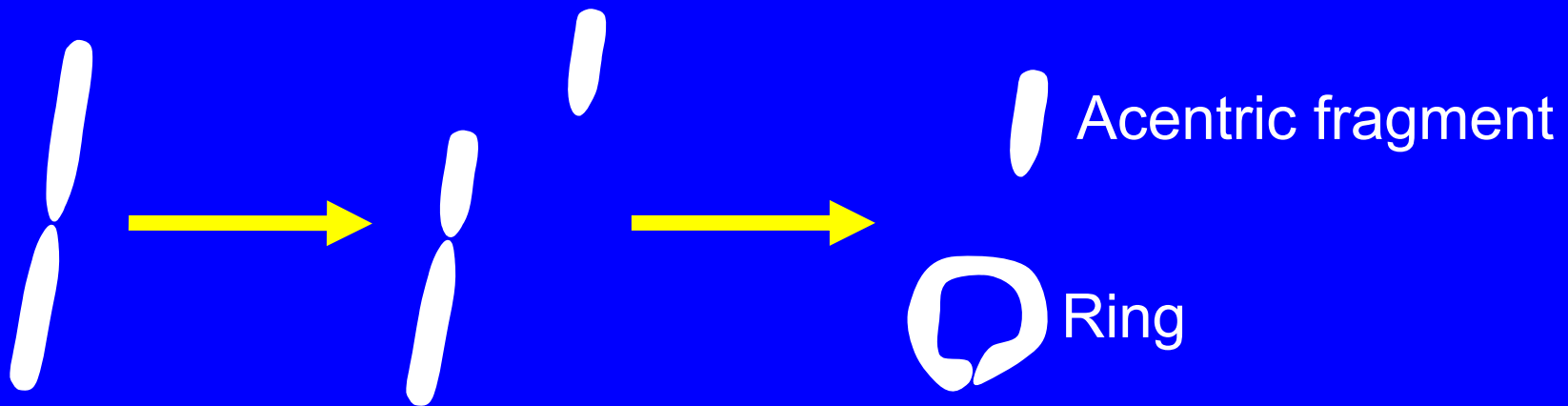
74

Chromosomal Aberrations

Acentric fragment

Centric fragment

Acentric fragment

Ring

Chromosomal Aberrations

Acentric fragment

Centric fragment

Dicentric
Chromosome

Unbroken chromosome

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Likely Results of Chromosomal Aberrations

If the cell attempts to divide, it is likely that some of the chromosomal material will not be properly distributed between the two daughter cells.

For example, the spindle fibers cannot attach to and separate the acentric fragments. In late mitosis when the two daughter cell nuclei are being formed, this type of remainder genetic material might end up being incorporated into a "micronucleus."

Dicentric chromosomes might even be pulled in both directions!

Chromosomal Aberrations

Photo of a cell at a later stage of mitosis.

Dicentric Bridge
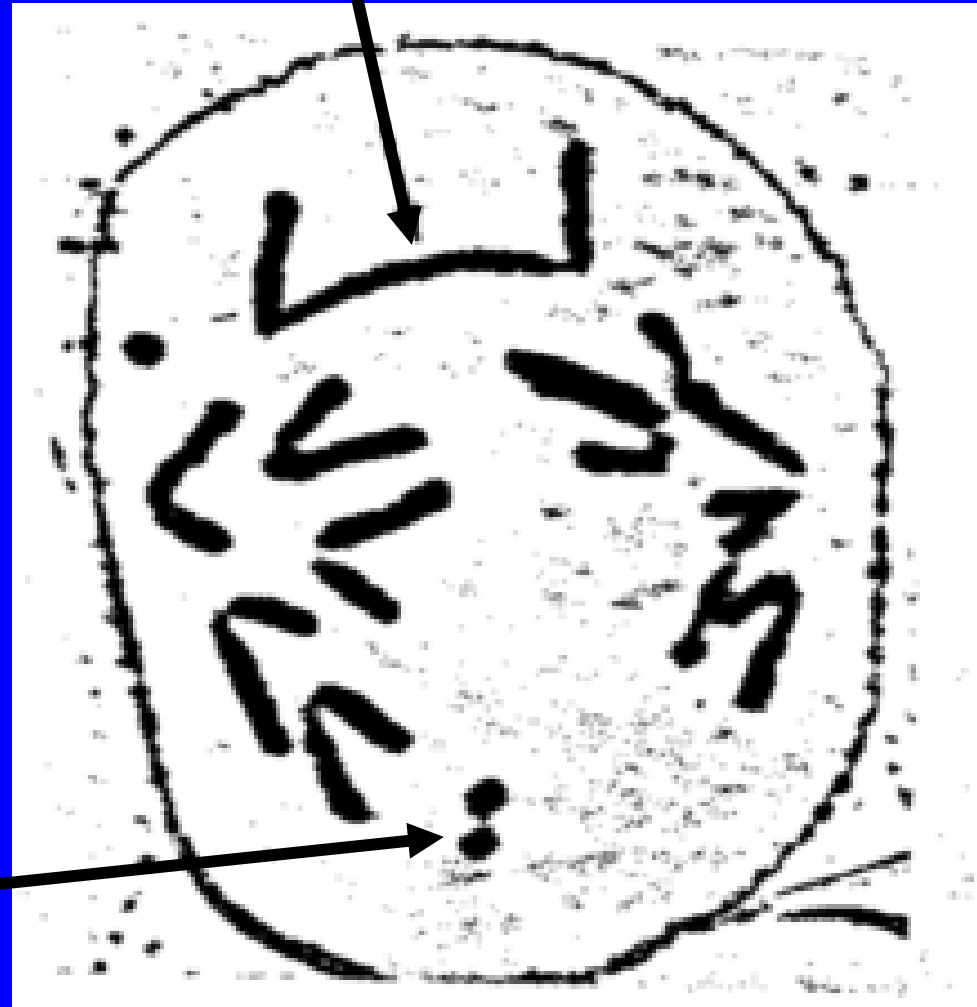
Acentric fragments

Likely Results of Chromosomal Aberrations

Due to the chromosomal aberrations, the cell might:

- Survive but never attempt to divide.

- Die at mitosis without dividing.

- Divide successfully, but the daughter cells die because they lack a complete complement of DNA.

This is referred to as "mitotically-related death," or simply "mitotic death."

Radiation can also induce cell death via apoptosis.

Cytogenetic Dosimetry

Cytogenetic Dosimetry

For low LET radiation, the relationship between dose and the number of DSBs (and therefore dicentric chromosomes) seems to be linear-quadratic.

At low doses, the number of DSBs increases linearly due to the increased number of cases where a DSB results from a single event.

At high doses, the number of DSBs increases quadratically due to the increased frequency of DSBs caused by two discrete events.

Cytogenetic Dosimetry

Following an acute radiation exposure, a blood sample can be taken and the peripheral blood lymphocytes cultured. Since these cells are relatively long-lived, this can be done up to several weeks after the exposure without the cells entering mitosis and the aberrations being "lost".

Using colchicine, the lymphocytes are "arrested" in mitosis and then, the average number of dicentrics per cell is determined. The probable dose is obtained from a calibration curve similar to the following:

Cytogenetic Dosimetry

High LET Radiation

Low LET Radiation

Dicentrics/cell

Dose (Gy)

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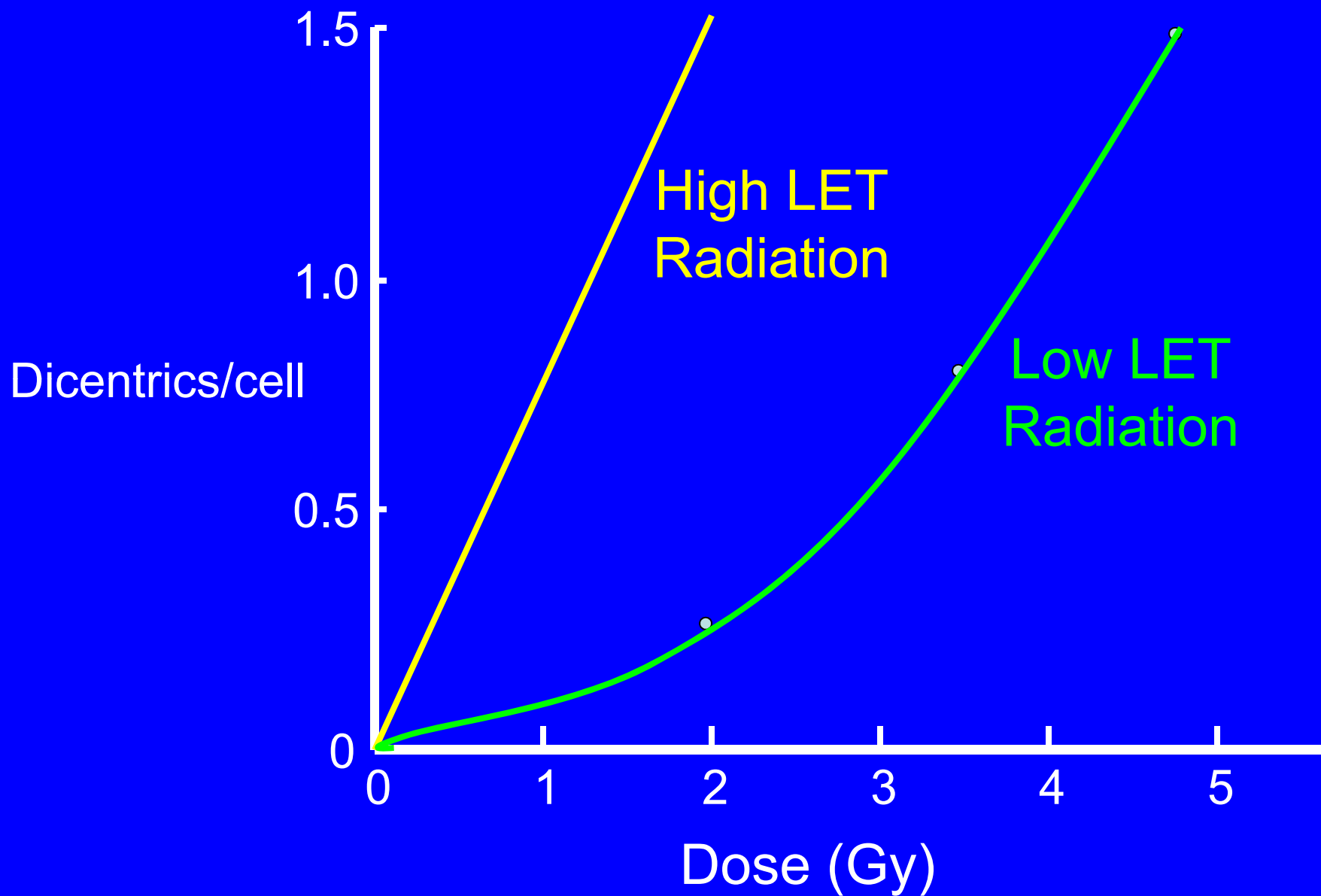
Cytogenetic Dosimetry

Cytogenetic dosimetry can be effective for evaluating doses above 0.1 Gy (10 rads) or so.

In the US, there are two groups with expertise in cytogenetic dosimetry:

REACTs (part of Oak Ridge Associated Universities)

AFFRI (Armed Forces Radiobiology Research Institute).

Consequences of DNA Damage

DNA damage has no effect if:

- The damage is completely repaired

- The damage is unrepaired (or misrepaired) but it is limited to a non-functional (meaningless) segment of DNA

- The damage is unrepaired (or misrepaired) and the damaged cells die or become non-functional. There is no significant effect if only a small number of cells die or become non-functional

Deterministic effects (e.g., cataracts) occur if:

- The damage is unrepaired (or misrepaired) and many cells die or became non-functional

  This can involve mitotically-related death and/or apoptosis

Stochastic effects (cancer) might occur if:

- The damage goes unrepaired (or misrepaired). The cells survive and ultimately produce many malfunctioning cells.

Even unexposed cells might be affected

Cells "interact" with each other via the transfer of various chemicals

In the bystander effect, a radiation exposure to one cell affects adjacent cells.

e.g., DNA repair mechanisms in adjacent cells might be stimulated thus providing a protective effect

Alternatively, the metabolism of the adjacent cells might be damaged, possibly leading to cell death.

The effects of radiation are not unique – the same effects can result from other causes, e.g., exposure to chemicals.

Cell Survival Curves

Cell Survival Curves

While it is difficult to determine the number of non-lethal mutations induced by radiation, it is relatively easy to quantify cell death. In radiation biology, cell death is defined as the inability of individual cells to reproduce.

The relationship between dose and cell death may be a simple exponential as seen in the next slide.

The mean lethal dose is the dose that kills 63% of the original population. An optimist would say that it is the dose at which 37% survive.

Cell Survival Curves

$$N = N_0 \, e^{-\frac{D}{D_0}}$$

N   number of surviving cells
N_0 original number of cells
D   dose
D_0 mean lethal dose

N
(number of surviving cells)

N_0

$0.37 \, N_0$

D_0

0

Dose (Gy)
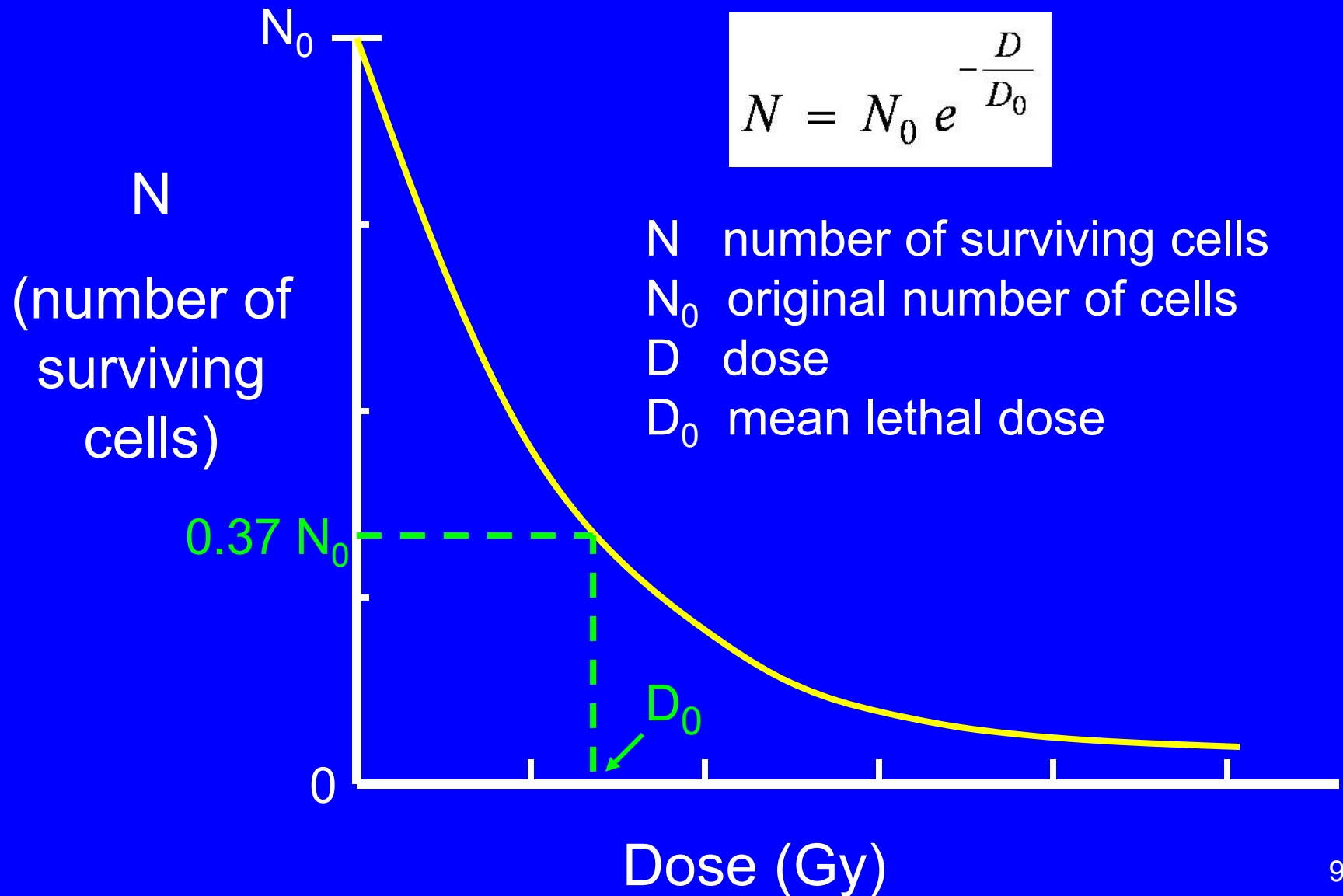
Cell Survival Curves

The surviving fraction (S) plotted against dose on semi log paper is referred to as a **survival curve**.

$$S = \frac{N}{N_0} = e^{-\frac{D}{D_0}}$$

S

Surviving Fraction

n

D_0

D_q

Dose (Gy)

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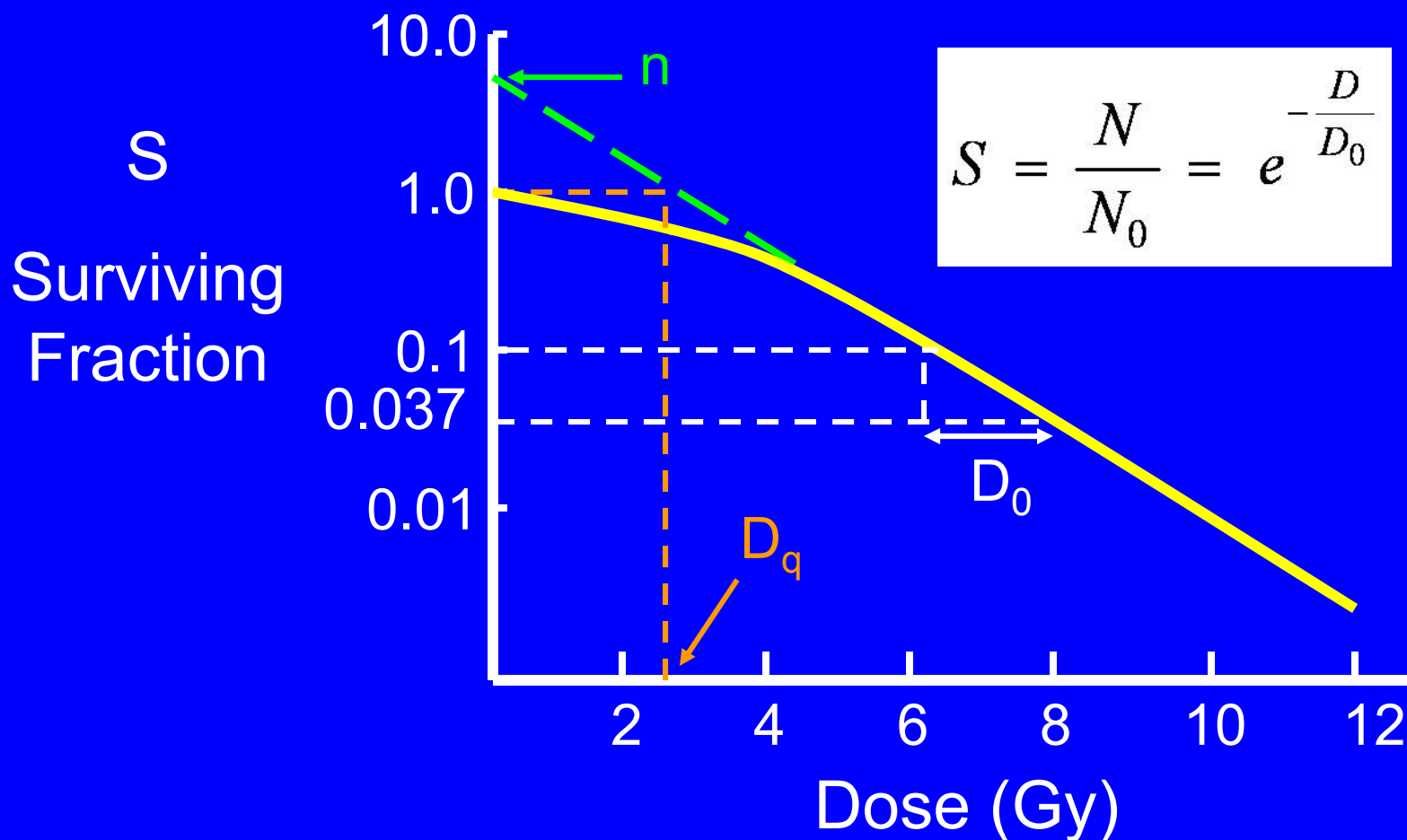
Cell Survival Curves

The most significant feature of this type of curve is the shoulder at low doses. At higher doses the relationship becomes exponential (the straight line portion of the curve).

A mathematical description of this curve not only requires D_o, but also n (the extrapolation number). The latter is the value for the survival fraction obtained when the exponential (straight line) portion of the curve is extrapolated back to D = 0.

Cell Survival Curves

In most mammalian cell lines, D_o is 1 to 2 Gy (100 to 200 rads) and n is between 1 and 5.

The quasi-threshold dose (D_q), is often specified. This is the dose at which the extrapolated exponential portion of the curve crosses the 100% survival point (S = 1).

The following slides discuss three ways in which the survival curves can be interpreted.

Cell Survival Curves

1. Q Repair.

One explanation for the shoulder on the survival curve is that it indicates the presence of a DNA repair mechanism, the so called Q repair process.

D_q then becomes a measure of the effectiveness of this repair process

D_o a measure of the cell's sensitivity to radiation induced death.

The extrapolation number (n) is given no "real" biological meaning.

Cell Survival Curves

2. Target Theory (multitarget/multihit processes).

   Survival curves are explained in terms of the number of targets in a cell that must be hit (or the number of hits that an individual target in the cell must receive) for cell death to occur.

   At the center of such theories are equations that attempt to describe the observed relationship between cell survival and dose. One goal is that these equations should be biologically justifiable.

Cell Survival Curves

2. Target Theory (multitarget/multihit processes).

   As an example of such an equation:

$$S = 1 - \left(1 - e^{-\frac{D}{D_0}} \right)^n$$

   This equation is interpreted to mean that two or more unspecified cellular targets must each receive a "hit" for death to occur. The extrapolation number (n), represents the number of targets that must be hit (a "hit" is taken to mean a direct ionizing event). However, this model predicts a slope of zero for the curve at low doses--something rarely observed.

Cell Survival Curves

3. Target Theory (multitarget/multihit processes)

Cell death is assumed to be due to DSBs. The latter can be produced by a single event when both strands are broken at once, or by two separate events when each strand is broken individually

$$S = e^{-\left(\alpha D + \beta D^2\right)}$$

α represents the probability of single-event DSBs

β reflects the probability of double-event DSBs

Factors Affecting Radiosensitivity

1. Radiation Quality

While different types of radiation produce the same effects, the magnitudes of the effects per unit dose can be quite different.

The amount of biological damage per unit dose increases with the linear energy transfer (LET) of the radiation.

The higher the LET, the greater the radiation energy lost per unit distance, and the greater the density of ions and free radicals in the charged particle tracks.

As LET increases, more energy is deposited in the individual cells, but fewer cells are affected per unit dose.

Increasing the LET of the radiation increases the complexity of the damage to the DNA. This makes repair more difficult.

1. Radiation Quality

In radiation protection the effectiveness of different types of radiation is indicated by a quality factor (Q).

In radiation biology these differences are indicated by the relative biological effectiveness (RBE):

$$RBE = \frac{dose\,of\,250\,kVp\,xrays\,required\,to\,produce\,a\,given\,magnitude\,of\,a\,specified\,effect}{dose\,of\,a\,different\,radiation\,needed\,to\,produce\,the\,same\,magnitude\,of\,the\,same\,effect}$$

1. Radiation Quality

	Low LET (gammas, x-rays, betas)	High LET (neutrons, alphas)
Dose Rate Effect	dependent	independent or inverse dependence
Dose Response	often linear quadratic	often linear
DNA Damage	primarily indirect	primarily direct
	DNA damage simpler	DNA damage more complex
DNA Repair	easier	harder
Effect of Oxygen	sensitizes tissue	none
Effect of Radical Scavengers	reduces damage	none

2. Dose Rate Effect

The dose delivery can be classified as a:

- Chronic Exposure. The dose is delivered at a low rate over a long time, e.g., 0.1 rad/hr for 10,000 hours (total 1000 rad).

- Fractionated Exposure. The dose is delivered in discrete quantities, e.g., 100 rad are delivered per week for 10 weeks (total dose: 1000 rad).

- Acute Exposure. The total dose is delivered at once, or in a very short time (total 1000 rad).

2. Dose Rate Effect
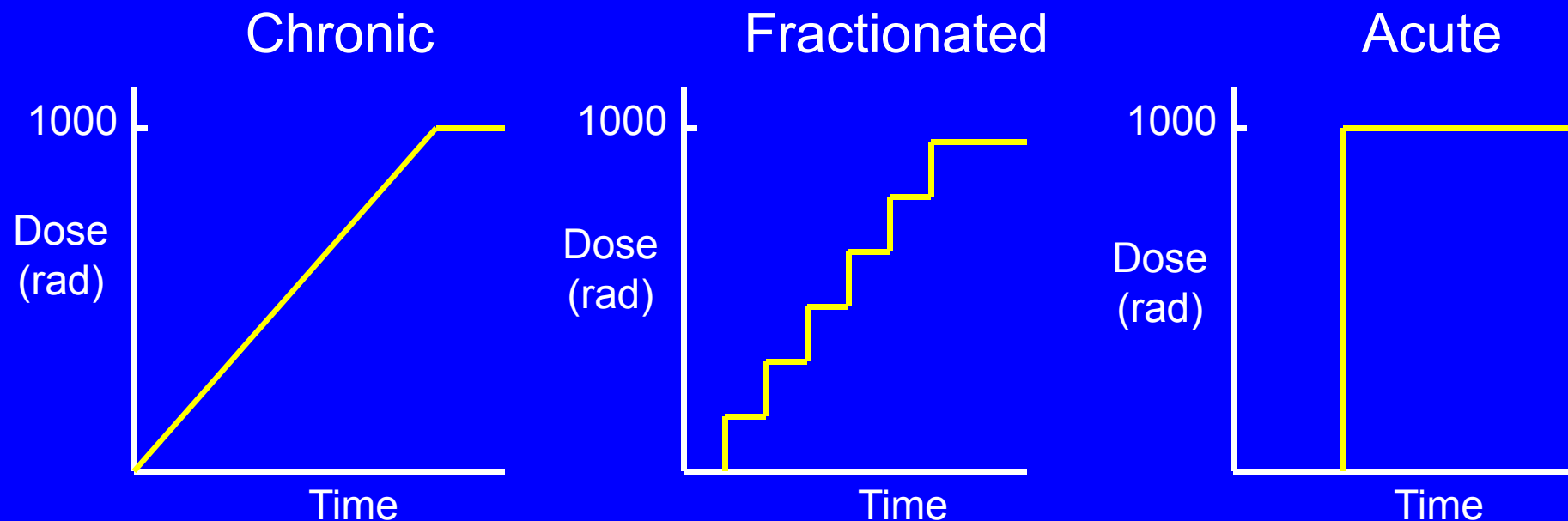
For low LET radiation, the magnitude of the effect per unit dose is greatest following acute exposures and least with chronic exposures. This applies to dose rates between 10 and 5,000 rad/hr. Lowering the dose rate below 10 rad/hr doesn't further reduce the magnitude of the effect. Increasing the dose rate above 5,000 rad/hr has no additional effect.

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2. Dose Rate Effect

For high LET radiation, this "dose rate effect" is less pronounced or absent.

There is some evidence that chronic exposures with high LET radiation may be more carcinogenic than acute exposures, i.e., an inverse dose rate effect.

2. Dose Rate Effect

One possible explanation involves the cell's repair mechanisms: below 10 rad/hr the repair mechanisms are maximally effective, from 10 to 5,000 rad/hr the repair rate falls behind the rate of damage, above 5,000 rad/hr any repair is superfluous.

Another possible explanation involves the fact that the number of two event DSBs per unit dose increases with dose rate. For two single strand breaks to produce a DSB, they must occur close together on the DNA molecule and close together in time; the higher the dose rate, the greater the probability that two SSBs will be sufficiently close in time.

3. Tissue Exposed

A tissue is a group of similar cells organized to perform a common function.

The Law of Bergonie and Tribondeau (1906)

The "law" characterizes those tissues in the body that are most radiosensitive.

Like all laws in biology, it is a generalization and has exceptions.

Although radiobiologists tend to dismiss the "law", it does have its uses.

3. Tissue Exposed

The law states that the most radiosensitive tissues possess cells are:

- dividing at the time of exposure (mitosis is the most sensitive stage of the cell cycle)

- of an undifferentiated type, i.e., unspecialized in structure and function

The law describes those tissues where early (acute) effects are observed.

These early effects are due to large doses in short periods of time and primarily involve cell death.

3. Tissue Exposed

Examples of Radiosensitive Tissues:

- germinal cells of the ovary and testis (spermatogonia)

- hematopoietic (blood forming) tissues:
  - red bone marrow
  - spleen
  - lymph nodes

- epithelium of skin

- epithelium of gastrointestinal tract

- lymphocytes (exception to the "law")

- oocytes (exception to the "law")

3. Tissue Exposed

Examples of Radioresistant Tissues:

- bone

- muscle

- cartilage

- nervous tissue

During embryonic and fetal development, the cells of these tissues are reproducing and much more sensitive to radiation.

4. Exposure Time During the Cell Cycle

For low LET radiation, the most sensitive stages of the cell cycle, with respect to cell death, are mitosis and late G1 (at the G1 -S border).

This might be because the chromosomes are condensed during mitosis and the repair mechanisms have poor access to the DNA molecule.

For high LET radiation, all phases of the cell cycle appear equally sensitive.

4. Exposure Time During the Cell Cycle

In radiation therapy, the cancer cells most likely to be killed are those in the sensitive stage of the cell cycle. Cells in other stages of the cell cycle survive. This is how radiation exposures can synchronize cells in the cell cycle. Subsequent radiation exposures to the synchronized cancer cells are particularly effective.

Radiation can slow down a cell's passage through G1 and G2. One possible effect of this is to give the cell more time to repair damage prior to division.

5. Adaptive Response

Low doses of radiation appear capable of initiating changes in cells that reduce the consequences of subsequent exposures.

For example, a conditioning dose of 5 to 200 mGy (500 to 20,000 mrad) to lymphocytes can result in an adaptive response some four to six hours later. This adaptive response results in a lower than expected number of chromosomal aberrations following a second "challenge" dose.

This adaptive response seems to involve the activation of certain genes that increase the production of enzymes involved in DNA repair.

6. Oxygen Tension

Cells with normal concentrations of oxygen (40+ mm Hg) tend to be 2-3 times as sensitive to low LET radiation as hypoxic (low in oxygen) cells. For a given effect, this difference is referred to as the oxygen enhancement ratio (OER).

Poorly vascularized tissue, i.e., tumors, tend to be hypoxic. Tissues well supplied with blood tend to have normal oxygen tensions.

The relationship between oxygen and radiosensitivity is most pronounced below 20 mm Hg. Above this, an increase in oxygen concentration does little to increase the radiosensitivity of a tissue.

6. Oxygen Tension

This effect of oxygen is probably due to several things, e.g.,:

- a resulting increase in the production of H_2O_2 and other reactive oxygen species.

- increasing the stability and toxicity of free radicals. Because oxygen is electronegative, it can combine with the free electrons produced during the radiolysis of water. This might slow down the recombination of certain radiolytic products and increase the capacity for damage by extending the latter's effective lifespan.

- oxygen might combine with DNA damage sites and interfere with repair.

7. Chemical Protective Agents

Certain chemicals, injected in substantial quantities 30 minutes or so prior to an acute exposure, can significantly reduce the effective dose of the radiation. Post-irradiation and oral administrations are less effective.

The dose reduction factor (DRF) is the ratio of the LD_{50}s for unprotected and protected animals. Typical DRFs for these radioprotective agents range from 1.5 – 2.0.

Examples of radioprotective drugs include cysteine, cystamine, and glutathione. A sulphydryl group is common to many of these agents.

7. Chemical Protective Agents

The interest in radioprotective drugs usually focuses on the protection afforded to large acute exposures. This is evidenced by the fact that the DRF is defined in terms of the LD_{50}.

Nevertheless, some of these chemicals (e.g., Amifostine) may provide protection from late effects such as cancer.

The protective mechanisms vary. Radioprotectants can work by scavenging (quenching) free radicals, enhancing cellular repair mechanisms, stabilizing membranes, enhancing hypoxia, etc.

7. Chemical Protective Agents

While many of these agents are toxic and must be used at near-lethal levels, some have been found to reduce the radiosensitivity of tissues when administered in relatively low doses.

Of these, WR-3689, is of particular interest since it appears to work better with healthy (well oxygenated) tissue than neoplastic (hypoxic) tissue. Furthermore, it is slightly less toxic than WR-2721 and may be effective when administered orally.

The aminothiol WR-2721 (better known as Amifostine) has been approved by the FDA and is used to protect the healthy tissue of patients undergoing radiotherapy.

Radioprotector[1]	Toxicity LD$_{50}$ (mg/kg)	Protective doses (mg/kg)	DRF
Cysteine	1700	1200	1.7
MEA	200	150	1.7
Cysteamine	220	150	1.7
AET	480	400	2.1
WR-638	1120	500	2.0
WR-2721	950	500	2.7
WR-3689	1120	450	2.2
WR-77913	3574	2200	2.0
WR-151327[2]	785	315	1.9
Mercapto-propionyl glycin[3]	2100	20	1.4
Glutathione	4000	4000	1.3

[1] Results from the research for LD$_{50/30}$ on mice radiated with X or gamma radiation
[2] Achieves high protectiveness from neutron radiation
[3] Achieves considerable protection applied after radiation

8. Temperature

In many experiments, a dramatic increase in radioresistance has been produced by lowering an animal's body temperature. For example, in mice the LD_{50} can be doubled by reducing the body temperature to 5 ^{o}C.

The increase in radioresistance is apparently due to a reduction in oxygen tension that accompanies a lower body temperature.

In some cases the lowered temperature merely serves to delay the effect, not reduce it. If the effect is simply delayed, it may be due to a reduced mitotic rate; once the animal warms up and mitosis resumes, mitotic cell death can occur.

9. Sex

In some species females tend to be slightly more radioresistant than males. That this is related to differences in hormonal levels is suggested by the fact that castrated males reverse this tendency.

Differences in sensitivity depend on the effect being looked for. For example, human males are more susceptible to radiation-induced leukemia, while females are more susceptible to radiation-induced thyroid cancer.

Lymphocytes of males and females differ in their radiosensitivity, something that can introduce uncertainty into cytogenetic dosimetry. Variability in female hormonal levels has been linked to variations in lymphocyte radiosensitivity.

10. Age

More Radiosensitive   Embryo

                                        Fetus

                                        Child

Less Radiosensitive   Adult

11. Species

For animals, the more primitive the species the more radioresistant the organism. The more advanced the organism, the more radiosensitive it is.

With plant species, the larger the interphase chromosome volume (ICV) the greater the radiosensitivity. The ICV is defined as the average volume of the nuclei divided by the number of chromosome characteristic of the species.

11. Species

More Radiosensitive   mammals

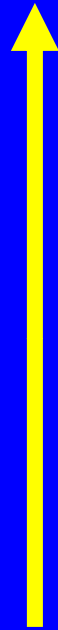   reptiles, fish, amphibians

                                       insects

                                       protozoa

Less Radiosensitive   bacteria

11. Species

A useful way to compare the radiosensitivities of different species is by comparing their LD_{50}s.

The LD_{50} is the dose from an acute exposure that will kill off 50% of the population (with the exposed organisms left untreated).

We usually specify that the deaths must occur within a certain time after the exposure. For example the $LD_{50/30}$ is the dose that will kill 50% of the population within 30 days. The $LD_{50/60}$ indicates that the deaths occur within 60 days.

11. Species

There is probably no single reason as to why various species show such a range of sensitivities.

Possible explanations include differences in the effectiveness of the DNA repair mechanisms, differences in mitotic rates, differences in oxygen tension, differences in the number and size of their chromosomes, etc.

The high radiosensitivity of mammals (i.e., the low $LD_{50/30}$ s) is possibly related to their complexity. The more complex the system and the wider the range of tissue types, the greater the likelihood certain essential tissues will be radiosensitive.

Species	LD-50 (rad)	Species	LD-50 (rad)
amoeba	ca. 100,000	guinea pig	200-400
Paramecium	ca. 300,000	pig	275-400
Ctenophore	ca. 1,200	goat	350
snail (Radix)	2,000	dog	325-365
snail (Thais)	17,000	laboratory mouse	400-600
"amphipod"	600	cattle	534
amphipod (Daphnia)	3,000	monkey	500-600
cockroach	10,000	burro	585-785
beetle (Tribolium)	ca. 100,000	hamster	610-725
fruit fly (Drosophila)	ca. 100,000	man	250-500
wasp (Bracon)	ca. 300,000	rabbit	750-825
grain weevil	ca. 5,000	laboratory rat	600-800
salmon	1,500	bobcat	ca. 500
swordtail	1,000	raccoon	ca. 600
goldfish	670-800	gray fox	ca. 700
"frog"	700	opossum	ca. 750
"newt"	1,500-3,000	wild mouse	1125
turtle	850-1,500	harvest mouse	1130
"snakes"	300-400	cotton rat	1200
lizard	1,200-2,000	pocket mouse	1200-1300
Eastern bluebird	2,500	pocket mouse	ca. 1520
pigeon	2,000-3,160	bat	ca. 15,000
parakeet	ca. 2,300		
chicken	600-1,630		
canary	1,015		

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