Part I
Brief summary on Radiobiology

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In this paper, we focus on the links between physical quantities (such as deposited energy, ionization and dose) and the DNA and cellular damage.

1 Physical and chemical effect of radiation

The passage of ionizing radiation through tissue produces both excited and ionized molecules; since the tissue is composed mostly of water ($70-85\%$), the most probable processes are those of *radiolysis*:

$$H_2O \overset{radiation}{\rightsquigarrow} H_2O^+ + e^- \tag{1}$$

and *excitation*:

$$H_2O \overset{radiation}{\rightsquigarrow} H_2O^* \tag{2}$$

of water molecule. These processes take place in the region close to the track at a time 10^{-15} s from the passage of the radiation.

The three chemical species, $\mathrm{H_2O^+}$, $\mathrm{H_2O^*}$ and $\mathrm{e^-}$ begin to diffuse in the space undergoing the following reactions:

a) in $\sim 10^{-14}$ s an ionized water molecule reacts with a neighboring molecule forming a hydronium and a hydroxyl radical:

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^\bullet \tag{3}$$

b) an excited water molecule releases its energy either by losing an electron, or by molecular dissociation in a time characterized by the vibrational period of the water molecule ($\sim 10^{-14}$ s):

$$H_2O^* \rightarrow \begin{cases} H_2O^+ + e^- \\ H^\bullet + OH^\bullet \\ H_2 + O^\bullet \end{cases} \tag{4}$$

c) a subexcited electron migrates losing energy by vibrational and rotational excitation of water molecules and is thermalized in $\sim 10^{-11}$ s; following this the electron orients the permanent dipole moments of the neighboring water molecules to form a cluster called *hydrated electron* (e^-_{aq}) (see fig. 1a). This reacts easily:

$$H_2O + e^-_{aq} \rightarrow H_2O^- \tag{5}$$

The two molecules $H_2O^+$and H_2O^- created in 4 and 5 are instable and dissociate yielding an ion and a free radical:

$$H_2O^+ \rightarrow H^+ + OH^\bullet \tag{6}$$

$$H_2O^- \rightarrow H^\bullet + OH^- \tag{7}$$

About 10^{-11} s after the passage of the radiation H_2 does not react further, O quickly forms stable hydrogen peroxide and only the four chemical species $H_3O^+, OH^\bullet, H^\bullet$ and e^-_{aq} remain active.

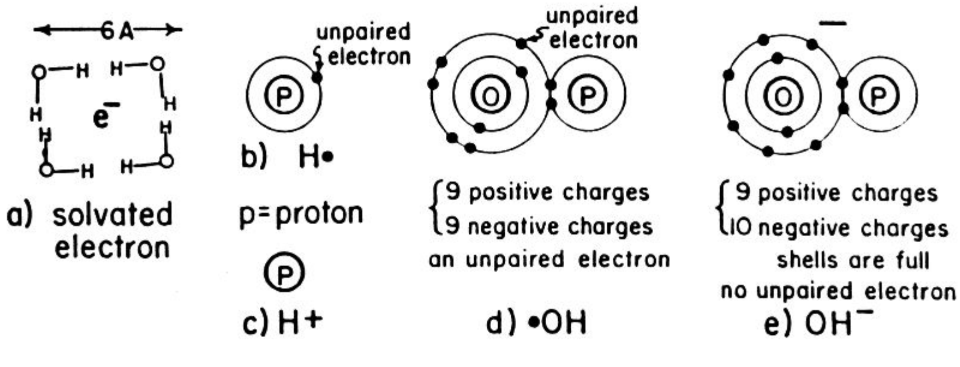


Figure 1: Nature of reactive species produced in water by ionizing radiation: a) solvated electron e^-_{aq}, b) hydrogen radical $H^\bullet$, c) hydrogen ion H^+, d) hydroxyl radical $OH^\bullet$, e) hydroxyl ion OH^-.

$OH^\bullet, H^\bullet$ and e^-_{aq} are *free radicals* [23], atoms or molecules electrically neutral with an unpaired electron in the last shell. These radicals are highly reactive because they tend to pair the unpaired electron or lose it through a transfer reaction.

Reactants diffuse in water in thermal motion for a distance λ:

$$\lambda = \sqrt{6\tau D} \tag{8}$$

in a time τ, where the diffusion constant D for the different reactants is shown in tab.1.

Species	$D\ 10^{-5} cm^2/s$	$R(\AA)$
$OH^\bullet$	2	2.4
e^-_{aq}	5	2.1
H_3O^+	8	0.3
$H^\bullet$	8	0.42

Table 1: Diffusion constants D and reaction radii R for reactive species. For comparison, water molecule diameter is 2..9 Å.

The most frequent reactions the free radicals have are:

$$\begin{aligned}
OH^\bullet + OH^\bullet &\rightarrow H_2O_2 & (9)\\
OH^\bullet + H^\bullet &\rightarrow H_2O & (10)\\
OH^\bullet + e^-_{aq} &\rightarrow OH^- & (11)\\
H_3O^+ + e^-_{aq} &\rightarrow H^\bullet + H_2O & (12)\\
e^-_{aq} + e^-_{aq} + 2H_2O &\rightarrow H_2 + 2OH^- & (13)\\
e^-_{aq} + H^\bullet + H_2O &\rightarrow H_2 + OH^- & (14)\\
H^\bullet + H^\bullet &\rightarrow H_2 & (15)
\end{aligned}$$

These radicals can interact in $\sim 10^{-11}\ s$ with organic molecules RH (in which H is the hydrogen atom and R the other components of the molecule) producing secondary radicals:

$$RH + OH^\bullet \rightarrow R^\bullet + H_2O \tag{16}$$

$$RH + H^\bullet \rightarrow R^\bullet + H_2 \tag{17}$$

Primary and secondary free radicals interact with biologically relevant molecules producing indirect radiobiological damage.

A direct action of the radiation can also take place:

$$RH \overset{radiation}{\rightsquigarrow} RH^+ + e^- \rightarrow R^\bullet + H^+ + e^- \tag{18}$$

¿From a radiobiological point of view it is obviously irrelevant if the action on the molecule is direct or indirect.

Free radicals can interact with oxygen molecules, generating dangerous radicals. This is the reason why biological effects increase in oxygenated tissue (*oxygen effect*):

$$O_2 + H^\bullet \rightarrow HO_2^\bullet \tag{19}$$

$$O_2 + e^- \rightarrow O_2^- \tag{20}$$

$$O_2^- + H^+ \rightarrow HO_2^\bullet \tag{21}$$

Oxygen can react with free radicals of the organic molecule RH, starting a chain reaction:

$$R^\bullet + O_2 \rightarrow RO_2^\bullet \tag{22}$$

$$RO_2^\bullet + RH \rightarrow RO_2H + R^\bullet \tag{23}$$

2 Molecular effect

Several studies have demonstrated that the sensitive region to the radiation is localized in the cell nucleus. The damage of the DNA molecule can produce cellular degeneration or death, although there is some evidence that even membrane damage can cause cellular damage. Several experiments have shown that a dose in the nucleus of 1/100 of that delivered in the cytoplasm is enough to kill one cell [23].

To have a better understanding of the changes induced in the DNA, we need to consider the structure of this molecule.

2.1 DNA structure

The fundamental chemical building block of DNA (**deoxyribonucleic acid**) is the nucleotide; this consists of three different parts:

- a nitrogen-containing pyrimidine (cytosine and thymine) or purine (adenine and guanine) base;

- a deoxyribose sugar;
- a phosphate group that acts as a bridge between adjacent deoxyribose sugars.

A DNA molecule is composed of two unbranched polynucleotide chains (strands) that wind about each other into a structure called a **double helix** (see fig. 2). The chain is stabilized by Van der Waals bounding (hydrogen bridge between complementary bases) and by the hydrophobic interaction between the molecule and the surrounding water molecules.

The medium diameter of DNA is 2.3 nm with 0.32-0.33 nm between two subsequent bases on the same chain. In tab.2 we can see the number of **base pairs** of the haploid genome of some eucaryotes.

Cell type	Genome size (Mbp)
Homo Sapiens	3000-3300
Chinese Hamster	2341
All mammals	1400-5700
Drosophila melanogaster	176
Saccharomyces cerevisiae	12.069404

Table 2: Size of the haploid genome of some eukaryotes.

2.2 Chromatin

The fundamental structural unit of chromatin is the nucleosome, an assembly composed of five different types of histones and the DNA.

In fig. 3 we can see the nucleosome's structure in which DNA turns 1.8 times around an histone's octamer interacting with this via electrostatic forces. It is generally assumed that the chromatin in a cellular environment winds into a regular coil called the solenoid (see fig. 3), which contains 6-8 nucleosomes per turn and has a total outside diameter of about 34 nm, although this has not yet been clearly verified.

The chromatin in a cell (see fig. 4) can be visually divided into condensed chromatin *heterochromatin* and unfolded chromatin *euchromatin*.

Folded DNA is relatively inaccessible to the polymerases and proteins involved in DNA transcription; thus before a polymerase can transcribe

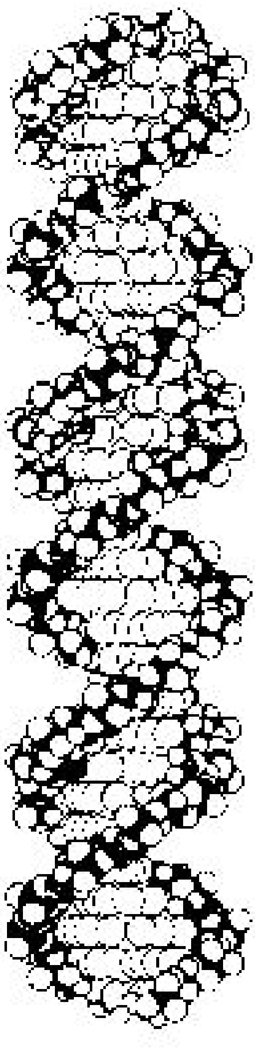


Figure 2: DNA's structure

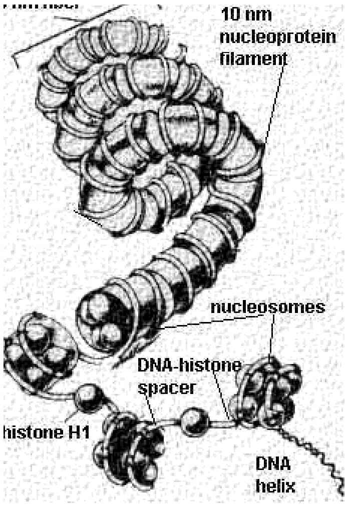


Figure 3: The solenoid model of a chromatin fiber.

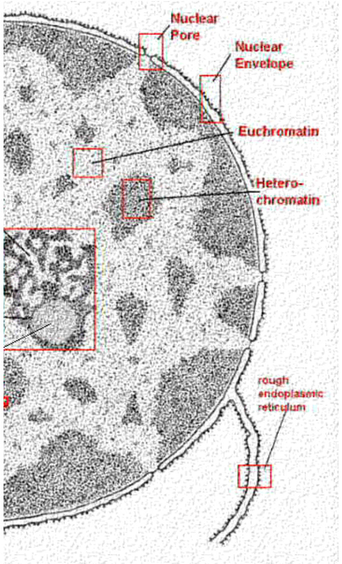


Figure 4: An idealized schematic of euchromatin and heterochromatin in a typical eukaryotic cell nucleus.

a section of DNA into RNA (ribonucleic acid) the heterochromatin must unfold into a more open euchromatin structure; nonetheless not all of the euchromatin is trancriptionally active.

The folded and condensed DNA is organized in structures called *chromosomes* (5).

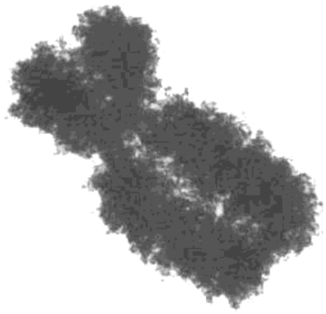


Figure 5: Image of a chromosome taken with electronic microscopy.

The nucleus of a human cell contains about 1 meter of DNA in 46 different chromosomes. A chromosome has a typical bastoncello form and is divided in two parts joined together by the centromere. The dimension of a typical chromosome is of the order of a tenth of micrometer.

2.3 DNA damage

Several experiments have demonstrated that pyrimidine bases are easily degenerated by free radicals compared with purines. Even if it is difficult to classify the damage to DNA because every break is different for position, dimension, genes involved, etc., an intuitive classification of DNA damage can be outlined as follows:

Base alteration (Ba): the chemical properties of an organic base (A,T,G or C) are abnormally modified from the form found in undamaged DNA;

Base deletion (Bd): an organic base is removed from a nucleotide;

Sugar alteration (Sa): the chemical properties of deoxyribose sugar are abnormally modified from the form found in undamaged DNA;

Strand break (Sb): the covalent bond between the deoxyribose sugar unit and the phosphate group is broken;

Mismatched base: the natural coupling between complementary bases A-T and G-C is altered.

2.4 Cellular and molecular answer to damage

Not all of the previously described damage mechanisms are lethal to cells. These mechanisms must be analysed taking into account the transcription phases and the genome integrity control apparatus.

Some damage are repaired immediately in loco:

- sulphydryl group in the glutathione form double bound between Sulphur to give Hydrogen (see fig.6) ;

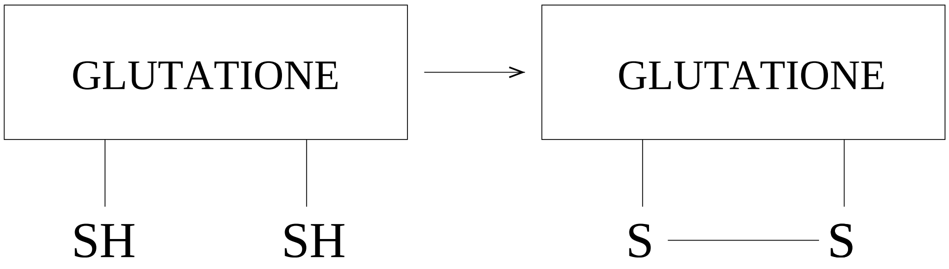


Figure 6: The gluthatione is an oxydereducent and can yield Hydrogen.

- the highly reactive superoxide radical O_2^- becomes inactive

  after the action of two enzymes, the superoxide dismutase

$$2O_2^- + 2H^+ \xrightarrow{S.D.} H_2O_2 + O_2$$

  and the catalase

$$2H_2O_2 \xrightarrow{C.} 2H_2O + O_2$$

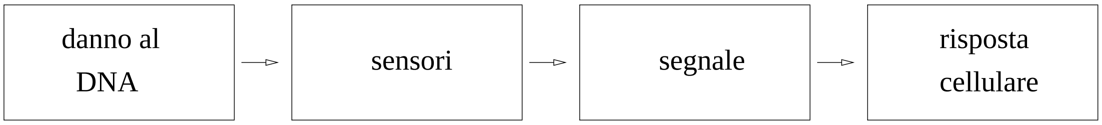


Figure 7: Chain reaction to a DNA damage following a repair mechanism.

The cell repair of DNA damage is a long and delicate process that follows certain phases (see fig. 7). Actually certain parts of this chain of events are unknown, but the following general developments can be assumed.

1. A frequent damage induced by the radiation (as well as by UV-rays and Platinum) is the substitution of the Hydrogen bond between complementary pyrimidine bases with a covalent bond leading to a physical DNA distortion (see fig. 8). When the DNA segment is transcripted the protein will reach the damage site and will send the signal for DNA repair.

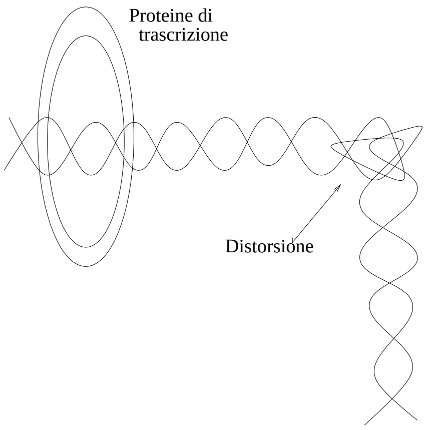


Figure 8: Physical distortion of DNA caused by base damage.

2. The molecules involved in the detection of DNA damage are the proteins. Only in some cases we actually know the way in which the

radiation damage can be identified: it is presumed, for example that in the CHO xrs-6 cell line the Ku80 protein can recognize the DSB (double strand break).

3. The signal sent to the cell can be:

   - cell cycle arrest
   - apoptosi
   - repair

   (a) The cell cycle is primarily regulated in the **biochemical checkpoints** G1/S and G/M [1]. Cells irradiated in the G1 phase stop in the checkpoint G1/S, regulated by the protein p53[2]; it is supposed that the stop in the G1 phase permit the cell to repair the DNA damage . Cytotoxic effects (perhaps even SSB) are not stopped in G1/S and, if they are, probably repaired in S or G2. Cells irradiated in G2 are stopped in an unknown way in G2/M.

2.4.1 Cell cycle arrest

Sometimes damaged cells are stopped in their evolution throughout the cell cycle, inhibiting their ability to duplicate.

2.4.2 Apoptosi

Apoptosi is a genetically induced mechanism which leads to the death of damaged or old cells without the inflammatory processes typical of necrosis.

Lymphocytes, germinal cells and cells of the intestinal crypt react to ionization with apoptosi instead of repair or cell cycle arrest.

Apoptosi is a complicated processes that can be induced by a range of factors at different times.

Most cells react to DNA damage with a p53-dependent apoptosi and it has been discovered that some radioresistant cells have lost their ability to induce apoptosis.

[1] For abbreviations, see paragraph 2.6.

[2] Usually this protein is addressed as "genome's guardian" beacause it does not allow the duplication of mutated DNA.

2.4.3 Repair

The cells can repair DNA damage in three steps:

1. incision,
2. digestion,
3. synthesis.

An endonuclease finds the damaged site and cuts the chain at this point. Following this, an exonuclease digests the fragment and a DNA-polymerase synthetises the lost part using the complementary section as copy.

2.5 The fate of fatal lesion

In the repair mechanism the undamaged part of the DNA is used by the DNA-polymerases as stamp to rebuild the lost part of the chain, so it is clear why the *double strand breaks (DSb, DSB or Sb_2)* are relatively important to determine the fate of the cell. A DSB is created when a lesion composed of two or more Sb breaks the molecule in several small fragments. In this case is *single strand breaks (SSB, SSb o Sb)* are very close to one another and the polymerases cannot discriminate between them.

Considering that the number of the DSBs created by the passage of ionizing radiation is 40 to 100 times the number of lethal events ([10], [11] and fig. **??**) it is clear that not all the DSBs are lethal .

Another proof that the break of the double helix is not always fatal is that the cell has the ability of rejoining the truncated segment by a mechanism called *recombination*; for example the elicasi proteins are able to transform a DSB into a SSB that can be further repaired by ligase-proteins.

Overall we can assume that from 1 to 2.5% of DSBs induced in DNA by ionizing radiation can lead to cell death.

2.5.1 Chromosomic aberration

The cell trying to repair a DNA lesion (*restitutio ad integrum*) can create one or more *pointlike mutations*[3], a movement (*translocation*) or a duplication (*amplification*) of a part of DNA between different chromosomes or between different arms of the same chromosomes. This kind of modifications are

[3] A triplet of three nucleotides that codify for genetic information is called *codone.* A pointlike mutation occurs when the sequence of codons is altered (eg. AGC in AGA).

usually called *aberrations*. In figure 9 the most frequent mechanisms that can lead to chromosomic alteration are represented.

The aberration alters the expressed characteristic of the cell (*phenotype*) and this can alter the normal evolution of the cell leading both to carcinogenesis or to apoptosi.

2.6 Cell cycle

The non germinal cells evolve throughout periods of growing and division called *cell cycle* which has four phases represented in fig.10; the whole cycle lasts from few hours to some days, depending on the cell type and on the influence of external factors such as temperature or presence of food.

G1 (gap 1): in this phase the cell has an intensive biochemical activity, the cell doubles its dimension, enzymes, ribosomes, mitochondria and other molecules and cytoplasmic structures; other structures such as microtubule, microfilaments, Golgi's bodies, lysosomes, vacuoles and vesicula are constructed ex novo. When the cell has completed its growth, it stops in the G1 phase; it is assumed that internal factors can also influence the cell in its evolution throughout the cell cycle to the S phase.

S (syntesi): here the cell chromosomes are doubled.

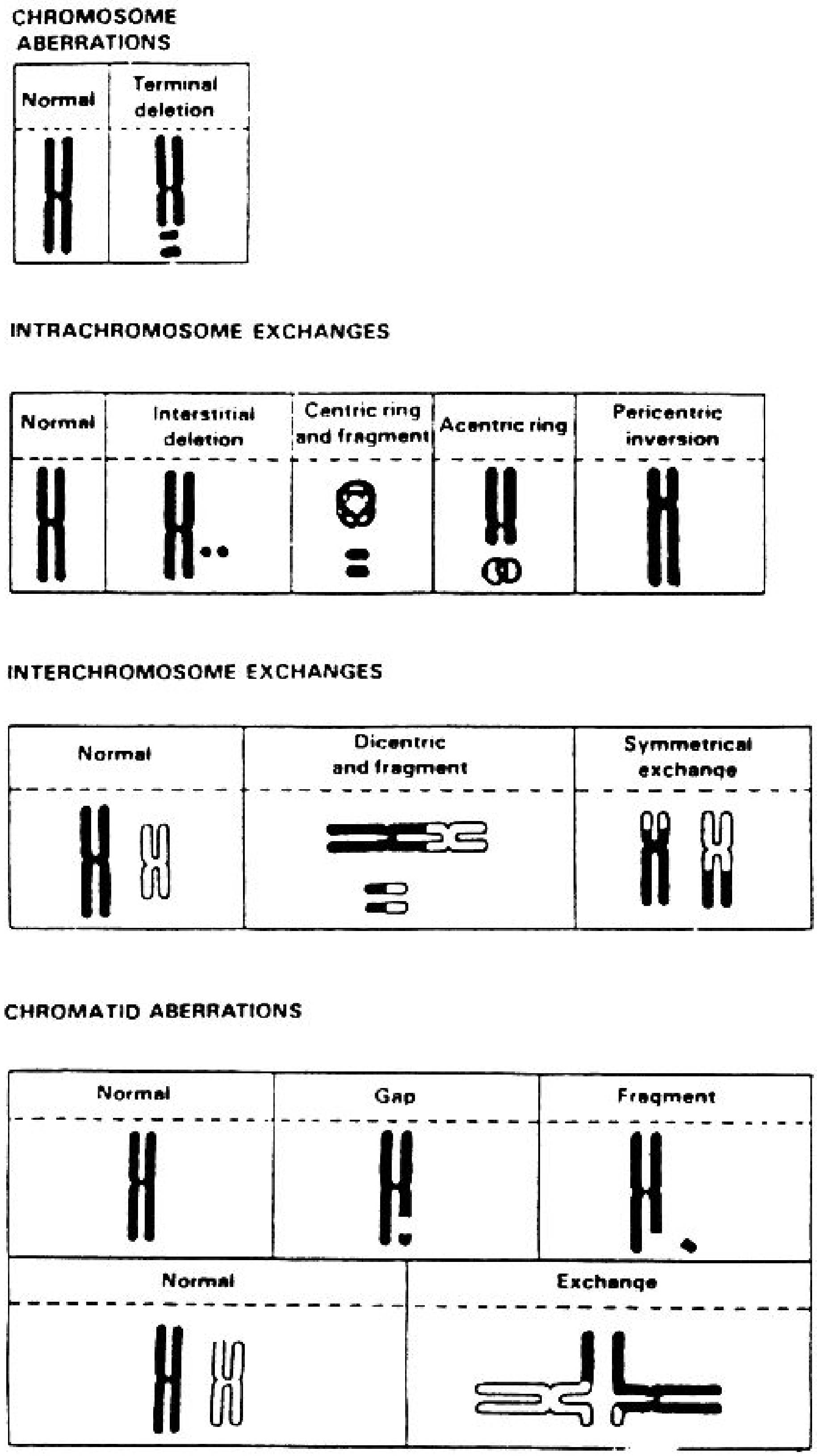
G2 (gap 2): in this phase the cell assembles the special structures that are needed to divide the chromosomes into two perfectly identical parts for the two daughter cells.

M (mytosi): here the DNA is divided up into two identical parts (every daughter will receive the same genetical information the parent had), the cytoplasm is separated and the membrana for the two daughters is formed.

The phase of non growth of the cell **G0 (gap 0)**, is controlled by external stimuli: communication between adjacent cells via receptors located in the membrana, by temperature, presence of food, etc...

The progression in the cell cycle is controlled in the checkpoints by proteins called **cyclines**. The studies on the cell clock are important both concerning the growth of cancer (there is evidence of alteration of this control) and on the fate of a cell damaged by radiation.

Figure 9: Most freqent chromosomic aberrations.



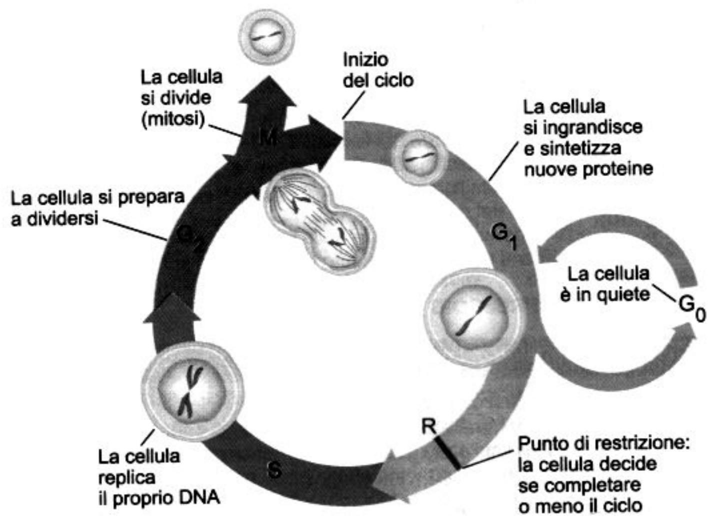


Figure 10: Cell cycle.

As previously noted the answer of the cell to radiation damage is different dependending on the phase of the cell cycle, and also during the phase, both for geometrical reasons (chromosome visibility and folding) and due to the different reactions in the checkpoints.

2.7 Dependence on radiation. Relative Biological Effectiveness (RBE).

As can be seen in fig. **??**, different types of ionizing radiation have different effects on cells. High LET radiation (ions, neutrons, low energy protons) have a higher efficiency for damaging cells than low LET radiation.

We define *Relative Biological Effectiveness (RBE)* as the ratio between the absorbed dose from a γ source of ^{60}Co and that of the radiation we consider to have the same biological effect:

$$RBE = \frac{D_\gamma}{D} \tag{24}$$

where D_γ is the photon dose.

The RBE depends on the phenomena we are considering (cell death, double strand break, chromosomic aberration, etc...) and on the type of damage inflicted.

In tab. 25 we can see the analysis done by Rydberg et al [59] on the percentage of timydine glycole (a base damaged by the passage of radiation) remaining unrepaired two hours after a 10 Gy irradiation. It can be clearly seen that the damage increases if produced by high LET radiation.

ionizing radiation	LET (keV/μm)	% of tymidine glycole
x	$\sim$0.2	17
N	97	27
Fe	150	32
He	70	36
He	120	43

(25)

The same authors have measured (see tab. 26) the number of incorrectly repaired and unrepaired DSB (for DNA fragment between 1 and 200 kbp) in the same experiment demonstrating that the repair efficiency decreases with increasing LET.

ionizing radiation	LET (keV/μm)	DSB non rejoined	DSB non correctly rejoined
x	$\sim$0.2	2	30
He	7	2	37
He	71	27	40
N	97	10	40
He	125	18	47
Fe	150	10	38

(26)

In fig. 11 it can be seen that high LET radiation is up to three times more efficient than photons in damaging the cell. This can be easily explained if we compare the different ionization events (see fig. 12) of radiation with different LET compared with a biological target such as chromatin, nucleosomes and DNA. For high LET, the ionization density increases and so does the probability of having unrepaired damage, such as, for example DSB. For very high LET ($LET > 200\ keV/\mu m$) this is no longer true because the energy released per unit path length is much more than that required to kill the cell (*overkill*).

Several experimental data confirm that different particles with same LET have different RBE, for example protons are more efficient than α-particles

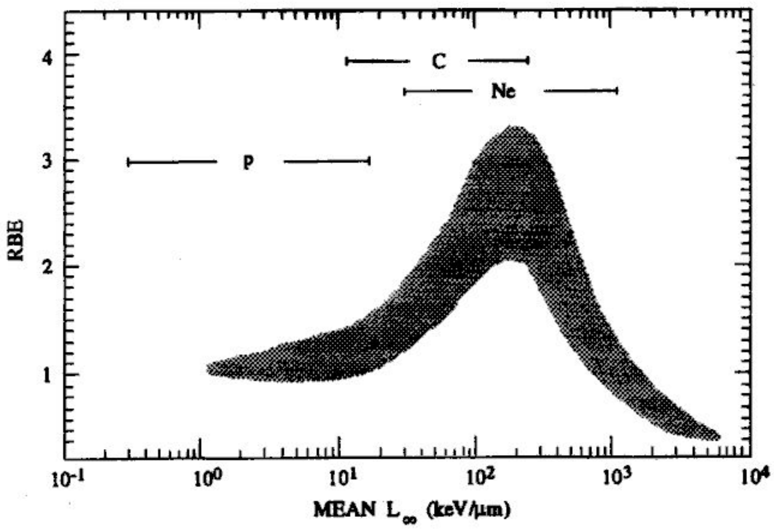


Figure 11: RBE-LET dependency for a survival level of 10% for protons, Carbon and Neon ions.

in the same LET range ($7 - 31\ keV/\mu m$) [32]. For this reason LET is not the only measure of the effects of radiation along the track.

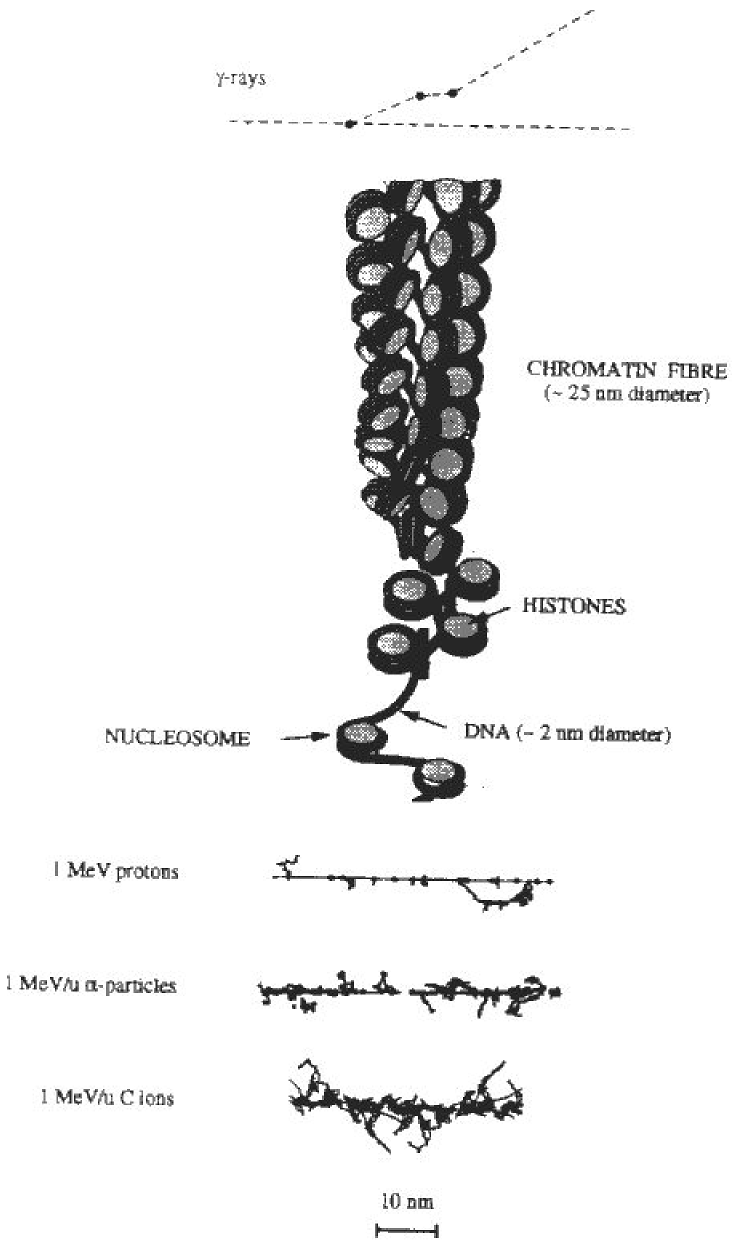


Figure 12: Radiation track compared to biological target.

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