Mutation caused by chemical and physical mutagens:

Base analogs;
Deaminating agents;
Alkylating agents;
Intercalating agents;
Ionizing radiation;
Heat

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Mutagen

- In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.

- As many mutations can cause cancer, mutagens are therefore also likely to be carcinogens, although not always necessarily so.

- Not all mutations are caused by mutagens: so-called "spontaneous mutations" occur due to spontaneous hydrolysis, errors in DNA replication, repair and recombination.

- Powerful mutagens result in chromosomal instability, causing chromosome breakages and rearrangement of chromosomes such as translocation, deletion and inversion. Such mutagens are known as clastogens. Examples: Acridine Yellow, Benzene, Ethylene Oxide, Arsenic etc.
The international pictogram for chemicals that are sensitising, mutagenic, carcinogenic or toxic to reproduction.
History

• In 1567, Swiss physician Paracelsus suggested that an unidentified substance in mined ore (identified as radon gas in modern times) caused a wasting disease in miners.

• In England, in 1761, John Hill made the first direct link of cancer to chemical substances by noting that excessive use of snuff may cause nasal cancer.

• In 1775, Sir Percivall Pott wrote a paper on the high incidence of scrotal cancer in chimney sweeps, and suggested chimney soot as the cause of scrotal cancer.

• In 1822, John Ayrton Paris noticed that arsenic fumes ("arsenical vapour") might contribute to the occurrence of scrotal skin cancer in the copper-smelting works of Cornwall and Wales.

• In 1915, Yamagawa and Ichikawa showed that repeated application of coal tar to rabbit's ears produced malignant cancer.

• In the 1930s the carcinogen component in coal tar was identified as a polyaromatic hydrocarbon (PAH), benzo[a]pyrene. Polyaromatic hydrocarbons are also present in soot, which was suggested to be a causative agent of cancer over 150 years earlier.
History (Contd.)

• The mutagenic property of mutagens was first demonstrated in 1927, when Hermann Muller discovered that x-rays can cause genetic mutations in fruit flies, producing phenotypic mutants as well as observable changes to the chromosomes, visible due to presence of enlarged 'polytene' chromosomes in fruit fly salivary glands. His collaborator Edgar Altenburg also demonstrated the mutational effect of UV radiation in 1928.

• Chemical mutagens were not demonstrated to cause mutation until the 1940s, when Charlotte Auerbach and J. M. Robson found that mustard gas can cause mutations in fruit flies.
Timeline for Carcinogen Research

- Cancer was experimentally produced the first time by application of coal tar to the ear of rabbits in 1915.
- Induction of metastasizing skin cancer in mice by application of tar in 1918.
- First proof that single polycyclic aromatic hydrocarbons (PAHs) are capable of inducing malignant skin tumours in mice in 1921.
- The tricyclic aromatic hydrocarbon anthracene is hydroxylated in vivo in 1930.
- Production of bladder papillomas and carcinomas in dogs by 2-naphthylamine in 1933.
- Two-stage mouse skin chemical carcinogenesis model established in 1935.
- Microsomal-catalysed biotransformation of N,N-dimethyl-4-aminooazobenzene in a cell-free system in 1936.
- Initiation and promoting effects of chemical carcinogens are distinguished in 1938.
- Covalent binding of N,N-dimethyl-4-aminooazobenzene to proteins in rat liver in vivo in 1944.
- N-Hydroxylation of aromatic amine is discovered in 1947.
- Two-stage mouse skin chemical carcinogenesis model established in 1948.
- PAHs can induce metabolizing enzymes in rat liver in vivo in 1956.
Timeline for Carcinogen Research

- **1960**: N-Hydroxylation of aromatic amines/arylamides discovered
- **1962**: Discovery of cytochrome P450 in liver microsomes
- **1964**: Binding of 2-acetylaminofluorene to rat liver DNA discovered
- **1966**: BP binds to DNA through its 7,8-diol-9,10-epoxide
- **1970**: Stereoselective enzymatic conversion of BP leads to the 7R,8S-diol-9S,10R-epoxide, (+)-anti-BP diol-epoxide (BPDE)
- **1974**: Induction of activating Hras mutations in mouse skin following exposure to PAHs
- **1976**: Arylhydrocarbon-receptor-deficient mice are protected against TCDD-mediated carcinogenicity
- **1978**: Arylhydrocarbon-receptor-deficient mice are protected against PAH-induced skin tumorigenesis

**Later Events**

- **1983**: Arylhydrocarbon hydroxylase is induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and carcinogenic PAHs through a cytoxic receptor protein
- **1986**: Binding of BP to DNA in vivo occurs predominantly through (+)-anti-BPDE
- **1988**: The exo-8,9-epoxide is the DNA-binding metabolite of aflatoxin B1 (REF: 106)
- **1996**: DNA-binding signature of BPDE in TP53 of lung epithelial cells correspond to human lung cancer mutational hotspots

*Source: Nat Rev Cancer © 2005 Nature Publishing Group*
Mutagenesis

• Mutagenesis is described as the exposure or treatment of biological material to a mutagen, i.e., a physical, chemical or biological agent that raises the frequency of mutation above the spontaneous rate.

• Based upon the type, the mutagen could be:
  – Physical
  – Chemical
    • Reactive Oxygen Species
    • DNA Reactive Chemicals
    • Intercalating Agents
    • Base Analogs
    • Metals
  – Biological
Physical Mutagens

- Physical mutagen is a mutation agent which is in the form of physical substances, such as short wave (ultraviolet and radiation ray such as alpha, beta, and gamma). Some physical mutagens can cause ionization while some others cannot.

- When we watch black and white television in an hour, we will be hit by 1 mrem radiation every hour. If we watch colored television, the effect becomes doubled. If we are diagnosed by X-ray, we will be hit by 150 mrem radiation, and every time we have our teeth portrayed, we get hit by 20 mrem radiation. Note that 1 to 2 dosage of mrem is already able to induce mutation.

- Radiation

- Heat
Radiation

• There are many kinds of radiations that can increase mutations.

• Most adverse health effects of radiation exposure may be grouped in two general categories:
  – **Deterministic effects** (harmful tissue reactions) due in large part to the killing/malfunction of cells following high doses; and
  – **Stochastic effects**, i.e., cancer and heritable effects involving either cancer development in exposed individuals owing to mutation of somatic cells or heritable disease in their offspring owing to mutation of reproductive (germ) cells.

• Radiation is often classified as ionizing or non-ionizing depending on whether ions are emitted in the penetrated tissues or not.

• The ability of radiation to cause human cancer, especially leukemia, was dramatically shown by the increased rates of leukemia among survivors of the atomic bombs dropped in World War II, and more recently by the increase in skin cancer in individuals exposed to too much sunlight (UV radiation).
Radiation

• Accumulated evidence in radiobiological studies has suggested DNA as the principle target for the biologic effects of radiation. It is now well established that radiation produces a wide spectrum of DNA lesions, which include damages to nucleotide bases (base damages), DNA single-strand breaks (SSBs) and double-strand breaks (DSBs).
Radiation

The chart illustrates the spectrum of radiation, distinguishing between non-ionizing and ionizing types. Non-ionizing radiation includes extremely low frequency, radio, infrared, microwave, and visible light. Ionizing radiation includes ultraviolet, x-ray, and gamma rays.

Non-thermal radiation, which includes power line, radio-TV, and microwave oven, induces low currents. Thermal radiation, such as heating, microwave oven, and heat lamp, induces high currents. Optical radiation, including heat lamp and tanning booth, excites electrons and produces photochemical effects. Broken bonds and damages DNA, as seen in medical x-ray.
Ionizing radiation as physical mutagen

- Ionizing radiation is a high-energy kind of radiation that causes ions and free radicals to form.
- Ionizing radiation is made up of energetic subatomic particles, ions or atoms moving at high speeds (usually greater than 1% of the speed of light), and electromagnetic waves on the high-energy end of the electromagnetic spectrum.
- **X rays, gamma rays (γ), beta particle radiation**, and **alpha particle radiation** (also known as alpha rays) are ionizing form of radiation.
- Ionization usually occurs because the radiation source has very large energy. For example, radiation from radioactive substances (uranium, radium, cobalt), X-ray, and cosmic ray. If DNA molecules are hit by the radiation, the DNA chain will loose. In consequence, the DNA chain cannot function in protein synthesis.
Ionizing radiation as physical mutagen

• Aside from hydrogen bonds, covalent bonds also hold DNA together. The backbones of the two DNA strands are made of nucleotides linked together by covalent bonds. So, if ionizing radiation comes along and breaks these bonds, the DNA will be chopped up into tiny little pieces! The cell will try to repair these DNA breaks, but it is very difficult for the cell to correctly put all those DNA pieces back together again.

• Inevitably, some mistakes may be made, and these mistakes are mutations because they change the DNA sequence. This means that ionizing radiation can cause mutations in cells that are deep inside, not just the cells on the surface of body. These mutations can eventually lead to cancer; ionizing radiation is known to cause leukemia and thyroid cancer.

• DSBs caused by ionizing radiation or other carcinogenic chemicals are considered the most relevant lesion for mutations and carcinogenesis. Unrepaired and misrepaired DSBs are serious threats to the genomic integrity. DSBs lead to chromosomal aberrations, which simultaneously affect many genes to cause malfunction and death in cells.
 Ionizing radiation as physical mutagen

- When cells are exposed to ionizing radiation, radiochemical damage can occur either by direct action or indirect action.
- Direct action occurs when alpha particles, beta particles or x-rays create ions which physically break one or both of the sugar phosphate backbones or break the base pairs of the DNA.
- Ionizing radiation can also impair or damage cells indirectly by creating free radicals. Free radicals are molecules that are highly reactive due to the presence of unpaired electrons on the molecule. Free radicals may form compounds, such as hydrogen peroxide, which could initiate harmful chemical reactions within the cells. As a result of these chemical changes, cells may undergo a variety of structural changes which lead to altered function or cell death.
Ionizing radiation as physical mutagen

Direct action of ionizing radiation on DNA.

Single strand and double strand breaks
Ionizing radiation as physical mutagen

Indirect action of ionizing radiation on DNA.
Ionizing radiation as physical mutagen

Ionising radiation: uranium atoms break into smaller atoms and particles, which enter a human cell, strike the nucleus, and damage the DNA, causing it to divide in an uncontrolled way - cancer
Non – Ionizing radiation as physical mutagen

• Non-ionizing radiation refers to any type of electromagnetic radiation that does not carry enough energy per quantum (photon energy) to ionize atoms or molecules—that is, to completely remove an electron from an atom or molecule.

• Causes molecular vibration, electron raised to higher level and new bonds can be formed like thymine dimer.
Non – Ionizing radiation as physical mutagen

• UV is normally classified in terms of its wavelength:
  – **UV-C** (180-290 nm)--"germicidal"--most energetic and lethal, it is not found in sunlight because it is absorbed by the ozone layer;
  – **UV-B** (290-320 nm)--major lethal/mutagenic fraction of sunlight;
  – **UV-A** (320 nm--visible)--"near UV"--also has deleterious effects (primarily because it creates oxygen radicals) but it produces very few pyrimidine dimers. Tanning beds will have UV-A and UV-B.

• Ultraviolet ray generally do not cause ionization, However, the energy from ultraviolet ray will be absorbed by purine and pyrimidine so that the atom becomes more reactive (the electron undergoes excitation). Consequently, DNA double-helix becomes in disorder and inhibits replication, One of the effects caused by ultraviolet ray is skin cancer.

• The major lethal lesions are pyrimidine dimers in DNA (produced by UV-B and UV-C)--these are the result of a covalent attachment between adjacent pyrimidines in one strand
Non – Ionizing radiation as physical mutagen

When cells are exposed to UV light in the 240- to 300-nm range, nucleic acid bases acquire excited energy states, producing photochemical reactions between DNA bases. The principal products in DNA at biologically relevant doses of UV light are cyclobutane dimers formed between two adjacent pyrimidine bases in the DNA chain. Both thymine-thymine and thymine-cytosine dimers are formed.
Non – Ionizing radiation as physical mutagen

Two thymine residues

Deoxyribose

O

C

H

C = O

Deoxyribose

O

C

H

C = O

Deoxyribose

O

C

H

C = O

Deoxyribose

O

C

H

C = O

Cyclobutane dimer

C

H

C = O

C

H

C = O

Thymine-thymine dimer residue

T

O

N

C

O

N

H

CH₃

O

N

C

O

N

H

CH₃

T

O

N

C

O

N

H

CH₃

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O

N

H

CH₃

T

O

N

C

O

N

H

CH₃

O

N

C

O

N

H

CH₃

UV

irradiation

UV

PR

UV

PR
RADIATION DAMAGE TO DNA

- H2-Bond Breakage
- Pyrimidine Dimer
- DNA Cross-Linkage
- Protein Cross-Linkage
- Double-Strand Break
- Base Loss
- Base Change
- Cross-Linkage
- Single-Strand Break
Heat as mutagen

• GC - A*T transitions are induced by heat, and arise from the deamination of cytosine (5- hydroxy methyl cytosine in the case of bacteriophage T4) generating uracil.

• Heat-induced mutagen formation from creatine and fat-soluble constituents of food (Vithayathil et al., 1983)
Chemical Mutagens

- Chemicals which alter structure and pairing properties of normal bases
- Active on both replicating and nonreplicating DNA
- Result in mutation upon DNA replication by forming baseless sites or mispair

Types:
- Intercalating Agents
- DNA Reactive Agents
  - Alkylating Agents
  - Deaminating Agents
  - Hydroxylating Agents
- Base Analogs
- Metals
Intercalating Agents

- A group of aromatic organic molecules
- Roughly the same dimensions as nitrogenous base pair
- Intercalate or wedge between the base pair
- Insertion stretches the DNA duplex and the DNA polymerase is fooled into inserting an extra base opposite an intercalating molecule.
- Examples:
  - 2,8-Diamino acridine (proflavin)
  - Acridine orange
  - Ethidium Bromide
Intercalating Agents (Anthracycline antibiotics)

• Primary mechanism:
  – intercalation in major groove of DNA causing several cytotoxic actions:
  – inhibition of topoisomerase II
  – single- and double-strand breaks (mutagenic) or sister chromatid exchange (carcinogenic)

• Secondary mechanisms:
  – interact with cell membranes to alter fluidity and ion transport
  – in the presence of NADPH, they react with cytochrome P450 reductase to form superoxide anion radicals à may also cause unique cardiotoxicity
Intercalating Agents

a) Mutation by addition

Template DNA strand

5’ ATCAGTTTACT 3’

New DNA strand

3’ TAGTTCGAATGA 5’

Molecule of intercalating agent

A randomly chosen base is inserted opposite intercalating agent; here, the base is G.

Subsequent replication of new strand

5’ ATCAGTTTACT 3’

TAGTTCGAATGA 5’

Result: frameshift mutation due to insertion of one base pair (CG).

b) Mutation by deletion

Template DNA strand

5’ ATCAGTTTACT 3’

New DNA strand

3’ TAGTTCATGA 5’

Intercalating agent

Replication of new strand after intercalating agent lost

5’ ATCAGTGACT 3’

TAGTCATGA 5’
**Alkylating agents**

- Electrophilic chemicals add alkyl (methyl) group also in nitrogen bases and alter the base character of nucleotide residues.

\[
\begin{align*}
\text{Di-(2-chloroethyl)sulfide (Sulfur mustard)} & \quad \text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl} \\
\text{Di-(2-chloroethyl)methylamine (Nitrogen mustard)} & \quad \text{CH}_2\text{CH}_2\text{NCH}_2\text{CH}_2\text{Cl} \\
\text{Ethylmethane sulfonate (EMS)} & \quad \text{CH}_3\text{CH}_2\text{O}\text{SO}_2\text{CH}_3
\end{align*}
\]
Alkylating agents

(a)

CH$_3$SO$_2$O$\cdot$CH$_3$

H$_2$N$\cdot$C$\cdot$N$\cdot$CH$_2$CH$_3$

MethyImethane sulfonate

Ethynitrosourea

(b)

7-Methylguanine

3-Methyladenine

O$^6$-Methylguanine

Fig. 2. Examples of (a) alkylating agents; (b) alkylated bases.
Alkylating agents

- Alkylated DNA either does not coil or uncoil properly, or cannot be processed by information-decoding enzymes. This results in cytotoxicity with the effects of inhibition the growth of the cell, initiation of programmed cell death or apoptosis. However, mutations are also triggered, including carcinogenic mutations, explaining the higher incidence of cancer after exposure.
Alkylating agents

- alkylating agents

alkylation

rare tautomer

$O^6$-meG

Alkylating agents

dimethylNitrosamine
diethylNitrosamine

dimethylsulfate
methylsulfonate

ethylNitrosourea

nitrogen mustard
Deamination Agents

- Most frequent and important hydrolytic damage
- Loss of amino group so that \( C \) (2-oxy-4-amino pyrimidine) change to \( U \) (2,4-dioxy-pyrimidine), 5-methyl cytosine changes to \( T \).
- Possible spontaneously (1 of \( 10^7 \) cytosine/24 h). \( G.C \rightarrow A.T \) (transition). The change if remain unrepaired pair with A instead of G during replication. 100 events/day in mammalian cell
Deamination Agents

1) Guanine
   - Original base: \( \text{dR} \) \( \text{N} \) \( \text{H} \) \( \text{C} \) \( \text{N} = \text{C} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \)
   - Mutagen: Nitrous acid (\( \text{HNO}_2 \))
   - Modified base: Xanthine
   - Pairing partner: Cytosine
   - Predicted transition: None

2) Cytosine
   - Original base: \( \text{dR} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{O} \)
   - Mutagen: Nitrous acid (\( \text{HNO}_2 \))
   - Modified base: Uracil
   - Pairing partner: Adenine
   - Predicted transition: \( \text{C-G} \rightarrow \text{T-A} \)

3) Adenine
   - Original base: \( \text{dR} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \) \( \text{N} \) \( \text{H} \)
   - Mutagen: Nitrous acid (\( \text{HNO}_2 \))
   - Modified base: Hypoxanthine
   - Pairing partner: Cytosine
   - Predicted transition: \( \text{A-T} \rightarrow \text{G-C} \)
Base Analogs

- Base analogs are chemicals that are structurally very similar to the bases normally found in DNA. Base analogs can get incorporated into DNA during replication because of their structural similarity to normal
- Some cause mis-pairing (e.g., 5-bromouracil).
- Not all are mutagenic.
- Base analogues become incorporated into daughter strands during DNA replication
  - For example, 5-bromouracil is a thymine analogue
    - It can be incorporated into DNA instead of thymine

![Diagram showing base pairing and tautomeric shift](image)

**Figure 16.14**  (a) Base pairing of 5Bu with adenine or guanine

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Base Analogs

a) Base pairing of 5-bromouracil in its normal state

b) Base pairing of 5-bromouracil in its rare state

5-bromouracil
(behaves like thymine; normal state)

Adenine
(normal state)

5-bromouracil
(behaves like cytosine; rare state)

Guanine
(normal state)
In this way, 5-bromouracil can promote a change of an AT base pair into a GC base pair.

(b) How 5BU causes a mutation in a base pair during DNA replication.
c) Mutagenic action of 5BU

AT-to-GC transition mutation

Add 5BU

DNA replication

5BU incorporated in normal state

5BU shifts to rare states

DNA replication

5BU shifts back to normal state

GC-to-AT transition mutation

Add 5BU

DNA replication

5BU incorporated in rare state

5BU returns to normal state

DNA replication

Transition mutation (instead of T–A, it is C–G)

DNA replication

Transition mutation (instead of C–G, it is T–A)
<table>
<thead>
<tr>
<th>No.</th>
<th>Mutagen</th>
<th>Characteristics</th>
<th>Note</th>
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</table>
| 1.  | Nitric Acid (HNO₂) | - Deamination of adenine and guanine so that it disturbs the process of replication and transcription  
                             - Mutagen against bacteria, fungi, and virus                                                      | Mostly used as food preservative, e.g. meat, fish, and cheese                                             |
| 2.  | Bróm-Uracil (Bu)   | - Mimic of timin, so when attaching to DNA strand will couple to adenine       | During transcription, Bu will print guanine instead of adenine                                             |
| 3.  | Hidroxilamine (NH₂OH) | - Causing mutation and aberration on the cell culture of human, mouse, bacteria, and fungi  
                             - Disturb the process of replication and transcription because of the ability to couple with timine and guanine | Mostly used in industry of textile, paper, photo, glue, and paint                                          |
| 4.  | Peroxide (H₂O₂)    | - Causing mutation on oncom bacteria and fungi and aberration on mouse          | Mostly used in industry of wood, rubber, plastic, flour, and cosmetics                                     |
| 5.  | Acridine           | - Able to insert between nitrogen base pair and make the DNA strand stiff     |                                                                                                            |
|     |                    | - Causing addition and deletion in replication                                 |                                                                                                            |
 Metals

- Many metals, such as arsenic, cadmium, chromium, nickel and their compounds are mutagenic in action.

- **Arsenic, chromium, iron** and **nickel** may be associated with the production of ROS.

- Some metals alters the DNA replication mechanism.

- **Nickel** involved in DNA hypermethylation.

- **Cobalt, arsenic, nickel** and **cadmium** may also affect DNA repair processes such as DNA mismatch repair and base and nucleotide excision repair.
Biological Mutagen

- **Transposon** are also known as jumping gene these transposons causes DNA fragments and its Its insertion into chromosomal DNA disrupt functional elements of the genes.

- Virus – Virus causes insertion of their DNA into the genome of host organism and disrupts genetic function. Some viruses causes the cancer e.g., Rous sarcoma virus

- Bacteria– some bacteria such as *Helicobacter pylori* cause inflammation during which oxidative species are produced, causing DNA damage and reducing efficiency of DNA repair systems, thereby increasing mutation.