E-CONTENT PREPARED BY

Dr. Sandip Majumder

Assistant Professor of Department of Conservation Biology

Durgapur Government College, Durgapur, West Bengal

(*Affiliated to Kazi Nazrul University, Asansol, West Bengal*)

NAAC Accredited "A" Grade College

(Recognized under Section 2(f) and 12(B) of UGC Act 1956)

E-Content prepared for students of

M.Sc.(Semester-II) in Conservation Biology

Name of Course:
Chemistry in Natural Management

Topic of the E-Content:
Biotic Health (Mutagens)

Mutagenesis and Mutagens

Mutagenesis is the process of inducing mutation by a number of physical, chemical or biological agents.

Spontaneous Mutations Arise from Replication Errors and Base Modifications

Since the process of DNA replication is imperfect. Occasionally, DNA polymerases insert incorrect nucleotides during replication of a strand of DNA. Although DNA polymerases can correct most of these replication errors using their inherent 3′ to 5′ exonuclease proofreading capacity, misincorporated nucleotides may persist after replication. If these errors are not detected and corrected by DNA repair mechanisms, they may lead to mutations. Replication errors due to mispairing predominantly lead to point mutations. The fact that bases can take several forms, known as tautomers, increases the chance of mispairing during DNA replication.

Tautomeric Shifts:

Purines and pyrimidines can exist in tautomeric forms—that is, in alternate chemical forms that differ by the shift of a single proton in the molecule. The biologically important tautomers are the **keto–enol forms of thymine and guanine** and the **amino–imino forms of cytosine and adenine**. **Tautomeric shifts** changes the covalent structure of the molecule, allowing hydrogen bonding with non-complementary bases, and hence, may lead to permanent base-pair changes and mutations.

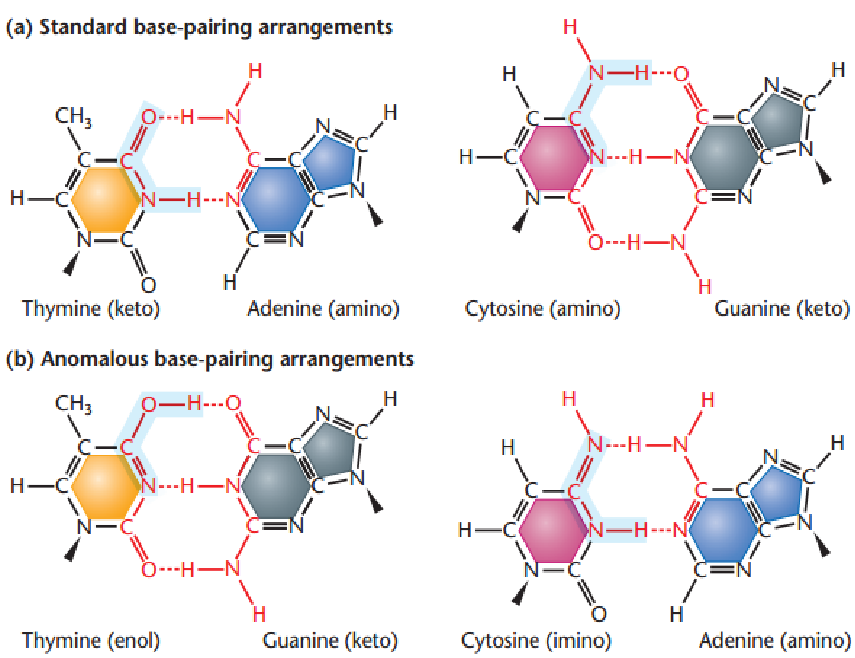


FIGURE Examples of standard base-pairing relationships (a) compared with examples of the anomalous base pairing that occurs as a result of tautomeric shifts (b). The long triangles indicate the point at which each base bonds to a backbone sugar.

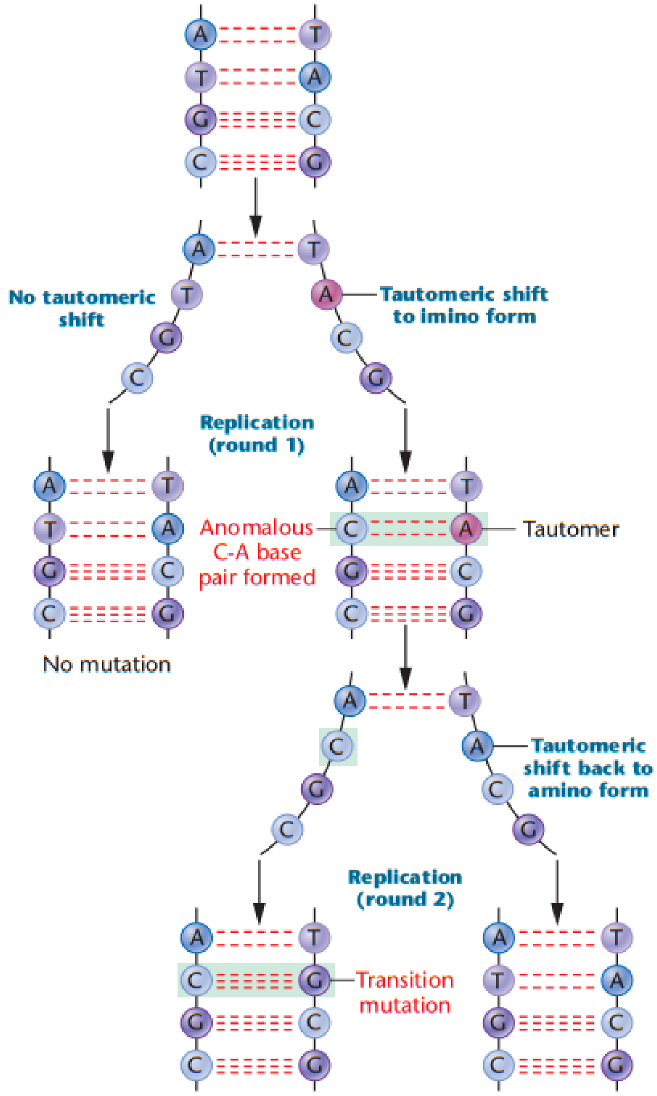


FIGURE Formation of an A-T to G-C transition mutation as a result of a transient tautomeric shift in adenine.

Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

What is mutagen?

"Mutagens are the known agents either physical, chemical or biological causes mutations by altering the genotype or gene expression which results in genetic abnormality."

or

"A natural or human-made agent which can alter the structure or sequence of genetic material and induce mutation."

Type of mutagens:

There are three main types of mutagens classifying by their sources:

- **Chemical Mutagens**
  - Base analogs
  - Chemical modification agents
  - Intercalating agents
  - Metal ions
- **Physical Mutagens**
  - Ionizing Radiation
  - UV Radiation
- **Biological Mutagens**
  - Transposable element
  - Virus
  - Bacteria

Chemical mutagens:

- Chemicals structurally resemble normal bases, purines and pyrimidines
- Incorporate into DNA during replication
- Lead to incorrect insertion of nucleotides opposite them in replication

Base analogs:

One category of mutagenic chemicals is base analogs, compounds that can substitute for purines or pyrimidines during nucleic acid biosynthesis. For example, **5-Bromouracil (5-BU)**, a derivative of uracil, behaves as a thymine analog but with a Bromine atom substituted at the number 5 position of the pyrimidine ring. The following figure compares the structure of 5-BU with that of thymine. The presence of the bromine atom in place of the methyl group increases the probability that a tautomeric shift will occur. If BrdU is incorporated into DNA in place of thymidine and a tautomeric shift to the enol form of 5-BU occurs, 5-BU base-pairs with guanine. After one round of replication, an A-T to G-C transition results. Furthermore, the presence of 5-BU within DNA increases the sensitivity of the molecule to UV light, which itself is mutagenic.

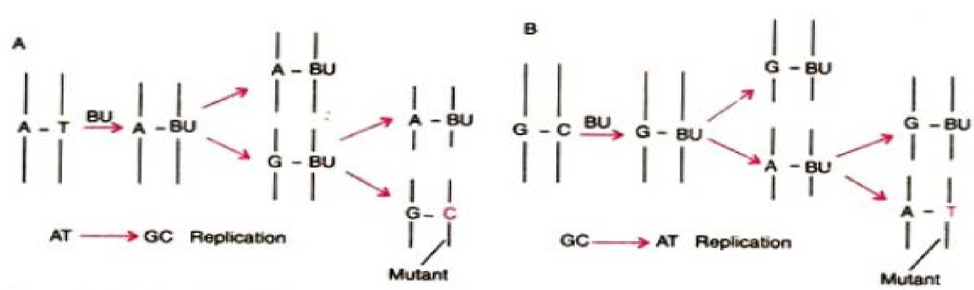


Fig. : Mechanism of 5-bromouracil (BU)-induced mutagenesis. A, AT→GC replication; B, GC→AT replication.

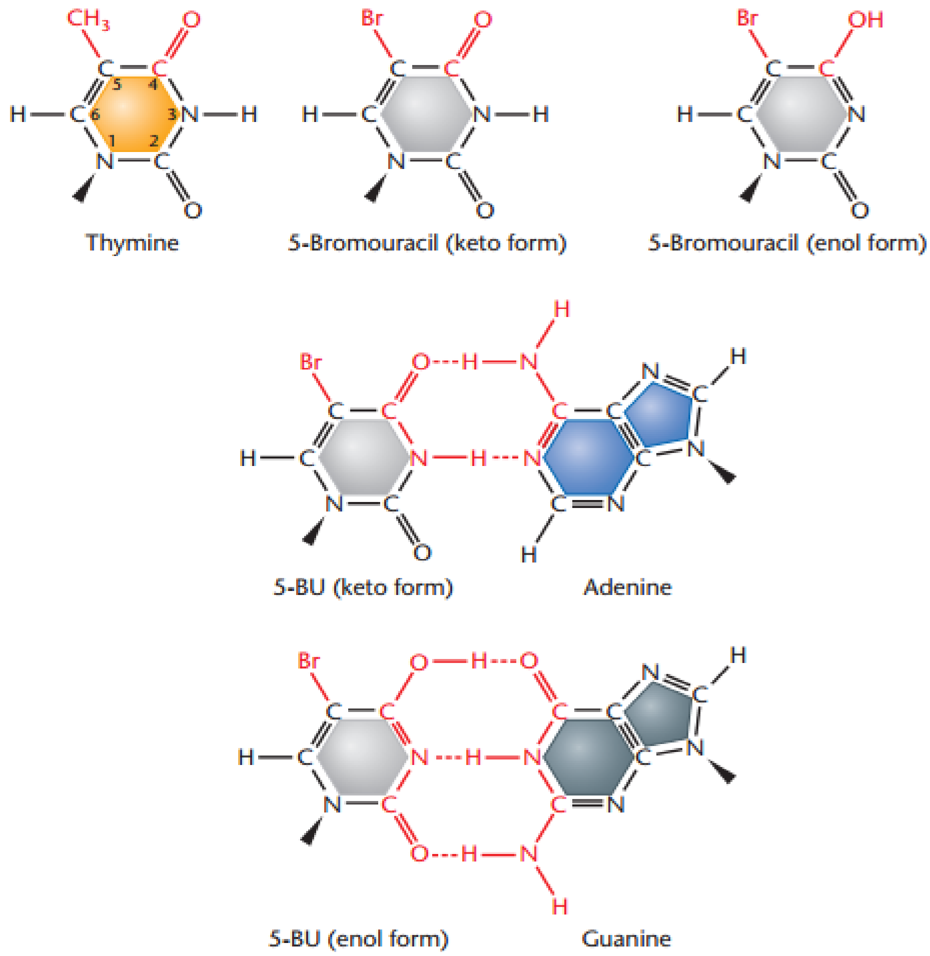


FIGURE Similarity of the chemical structure of 5-bromouracil (5-BU) and thymine. In the common keto form, 5-BU base-pairs normally with adenine, behaving as a thymine analog. In the rare enol form, it pairs anomalously with guanine.

Another most common base analog is **2-Aminopurine (2-AP)**, which is similar to the adenine and can pair with either T or C, although pairing with C is less frequent. It can also cause AT to GC or GC to AT transition during the replication.

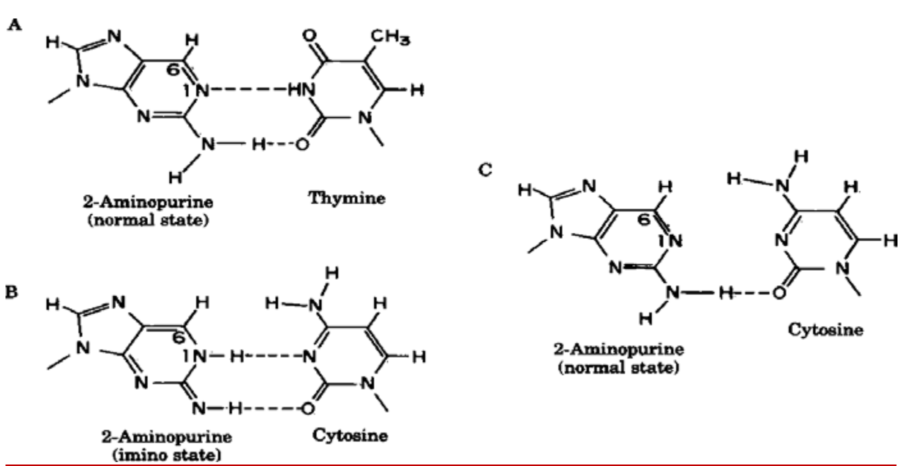


Fig. Hydrogen bonding properties of APur. (A) "Normal" pairing with thymine by means of two hydrogen bonds. (B) Pairing of the rare imino form with cytosine, resulting from a tautomeric shift of a hydrogen to the N' position. (C) Pairing of the normal form with cytosine by means of a single hydrogen bond

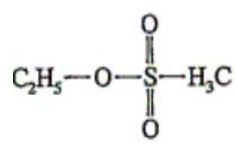
Chemical modification agents:

A number of naturally occurring and human-made chemicals alter the structure of DNA and cause mutations.

- Chemicals which alter structure and pairing properties of normal bases
- Active on both replicating and non-replicating DNA
- Result in mutation upon DNA replication by forming baseless sites or mispair
- Two common chemical modification agents are
  - Alkylating agents
  - Deaminating agents

Alkylating agents:

The sulfur-containing mustard gases, discovered during World War I, were some of the first chemical mutagens identified in chemical warfare studies. Mustard gases are alkylating agents—that is, they donate an alkyl group, such as CH_3 or CH_2CH_3, to amino or keto groups in nucleotides. **Ethylmethane sulfonate (EMS)**, for example, alkylates the keto groups in the number 6 position of guanine and in the number 4 position of thymine. As with base analogs, base-pairing affinities are altered, and transition mutations result. For example, 6-ethylguanine acts as an analog of adenine and pairs with thymine.



EMS

Fig. Structure of Ethylmethane sulfonate (EMS)

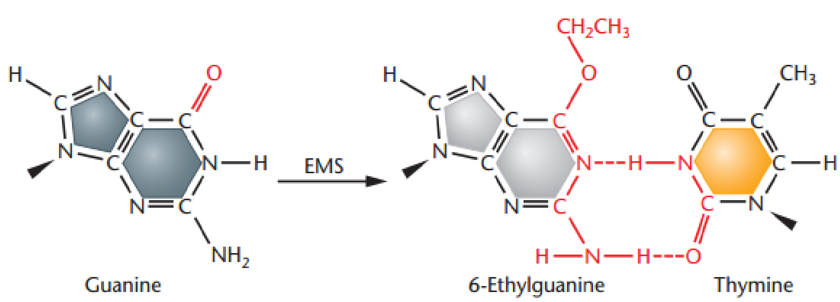


FIGURE Conversion of guanine to 6-ethylguanine by the alkylating agent ethylmethane sulfonate (EMS). The 6-ethylguanine base-pairs with thymine.

Deaminating agents:

Nitrous Oxide (HNO_2) is one of common deaminating agents which convert the amino group ($-NH_2$) of bases into keto group (=O) through oxidative deamination and changes H-bonding potential of the modified bases.

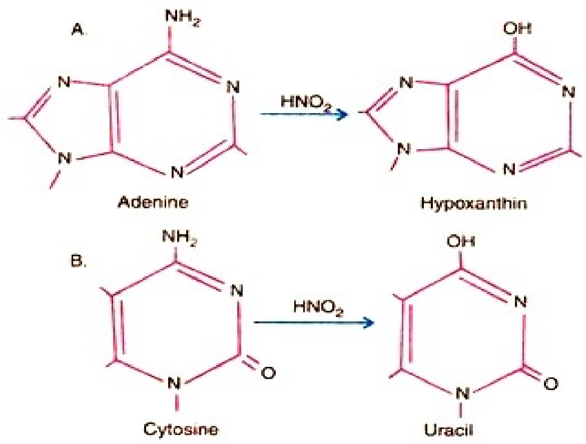


Fig. : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs. In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation. Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion or insertion after replication of DNA molecule. Deletion or insertion of intercalating agents leads to frameshift mutations.

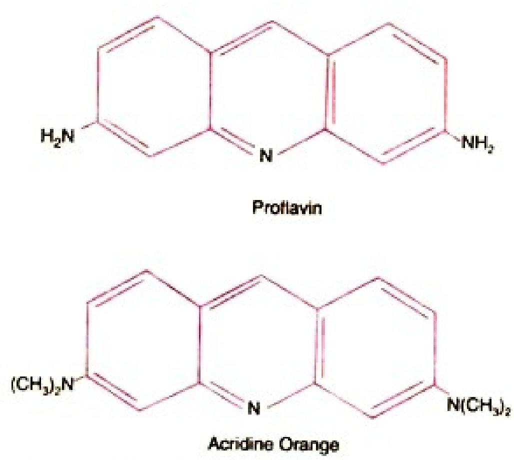


Fig. : Chemical structure of two mutagenic acridine derivatives.

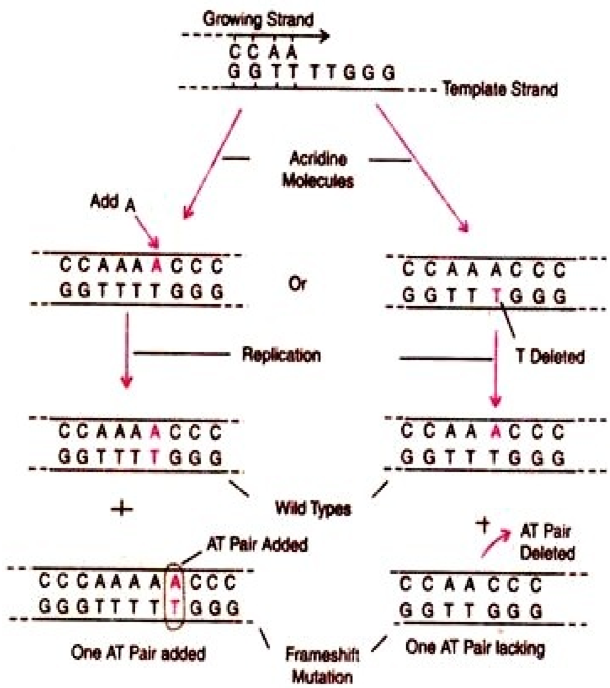


Fig. : Mechanism of intercalation of an acridine molecule in the replication fork.

Another intercalating agent which is used for DNA stains is **ethidium bromide**, a fluorescent compound that is commonly used in molecular biology laboratories to visualize DNA during purifications and gel electrophoresis.

Adduct-Forming Agents:

Another group of chemicals that cause mutations are known as **adduct-forming agents**. A DNA adduct is a substance that covalently binds to DNA, altering its conformation and interfering with replication and repair. Two examples of adduct-forming substances are acetaldehyde (a component of cigarette smoke) and **heterocyclic amines (HCAs)**. HCAs are cancer-causing chemicals that are created during the cooking of meats such as beef, chicken, and fish. HCAs are formed at high temperatures from amino acids and creatine. Many HCAs covalently bind to guanine bases. At least 17 different HCAs have been linked to the development of cancers, such as those of the stomach, colon, and breast.

Metal ions:

Metal ions are also dangerous to our DNA as it acts in varieties of different ways. Nickel, chromium, cobalt, cadmium, arsenic, chromium and iron are some of the common metal ions that cause mutations. The metal ions work by producing ROS (reactive oxygen species), hindering the DNA repair pathway, causing DNA hypermethylation or may directly damages the DNA.

Physical mutagens:

Radiations are the first mutagenic agent reported in 1920. UV rays, X-rays, alpha rays, neutrons, and other ionizing and non-ionizing radiations are mutagenic. Usually, radiation directly damages the DNA or nucleotide structure which might be either lethal or sub-lethal.

Ionizing Radiation:

X-rays, gamma rays, cosmic rays are ionizing radiation which ionizes water of the cell to release hydroxyl free radical (OH). The hydroxyl radical is a powerful oxidizing agent. Hydroxyl radical oxidises the phosphodiester bond of DNA. Higher dose of X-rays can even causes death of an organism. At the molecular level, the lethal dose of X-ray (350-500 rems) breaks the phosphodiester bonds between the DNA and thus results in strand breakages. It creates multiple strand breakage and results in the deletion of the portion of DNA. If the strand breakage occurs in both strands, it will become lethal to the cell. Electromagnetic radiation is also one of the known mutagens that cause lethal or sub-lethal mutations.

UV Radiation:

UV light is a non-ionizing radiation. It causes the formation of thymine dimer (Pyrimedine dimer). If two thymine occur together in one strand of DNA, UV light causes fusion to form thymine dimer. Nitrogenous bases absorb UV lights and the absorption is maximum at 260 nm. At the site of thymine dimer confirmation of DNA is changed, so rate of error during DNA replication is high.

The UV light can be classified into three different categories:

- **UV-A:** nearly visible range (320nm) causes pyrimidine dimers.
- **UV-B:** (290-320nm) emitted by the sunlight. These UV rays are highly lethal to our DNA.
- **UV-C:** (180-290nm) is one of the most energy-consuming forms of UV which is extremely lethal.

Biological mutagens:

Transposable Elements:

Transposable elements are DNA sequences that can move within genomes. These mobile elements are present in the genomes of all organisms, from bacteria to humans, and often constitute large portions of these genomes. Transposable elements can act as naturally occurring mutagens. If in moving to a new location they insert themselves into the coding region of a gene, they can alter the reading frame or introduce stop codons. If they insert into the regulatory region of a gene, they can disrupt proper expression of the gene. Transposable elements can also create chromosomal damage, including double-stranded breaks, inversions, and translocations.

Virus:

We all know about HIV, a causative agent of AIDS. Viruses are common mutagens that are well known to us and create lethal health issues.

Viruses insert their DNA into our genome and disrupt the normal function of DNA or genes. Once it inserts DNA, the DNA is replicated, transcribed and translated into viral protein instead of our own protein. Mature viral particle forms in a cell.

Bacteria:

Some bacteria are also dangerous for our DNA- cause inflammation. It provokes DNA damage and DNA breakage.

Effect of mutagens:

- The mutagens are genotoxic- harmful to our DNA in many ways, some directly affect the DNA some indirectly. And therefore, the exact effect of each mutagen is still unknown to us.
- At the chromosomal level, the mutagens can alter the structure or number of chromosomes. Deletion, duplication, insertion, translocation, monosomy and nondisjunction are some of the chromosomal abnormalities produced by mutagens.
- The mutagens also affect or dysregulate the molecular central dogma process- replication, transcription and translation. At the molecular level, the mutagens create different gene mutations results in loss of function, altered function or non-functional protein.
- It also alters the codon, deletes bases, alters bases, breaks hydrogen bonds or phosphodiester bonds and changes gene expression. Some mutagens dysregulate cell proliferation and cell death process and thus cause cancer, those are called carcinogens.
- Biological mutagens slower down the DNA repair or DNA synthesis process. Some of the common types of mutagens based on their effect are enlisted below:

Teratogens: teratogens are the class of mutagens that causes congenital malformations. X-rays, valproate and toxoplasma are common physicals, chemical and biological teratogens, respectively.

Carcinogens: The carcinogens are the class of mutagens that induces tumor formation and thus cause cancer. A wide variety of agents are categorized as carcinogens. X-rays/ UV-rays, Aflatoxins and retroviruses are common physicals, chemical and biological carcinogens, respectively.

Clastogens: Clastogens are the class of mutagens responsible for chromosomal- breakage, deletion, duplication and rearrangements. UV-rays, Bleomycins and HIV viruses are common types of physical, chemical, and biological clastogens, respectively.

Use of mutagens:

- Physical, chemical or biological mutagens are used for various purposes. For example, an EtBr (ethidium bromide) is used as an intercalating dye during agarose gel electrophoresis. It emits fluorescent and the DNA bands can be visualized on a gel.
- The heat method is used during the polymerase chain reaction for the denaturation of DNA. This facilitates single-stranded DNA for various applications. The UV-rays are utilized for decontamination or sterilization processes in genetics as well as microbiology. The UV light destroys all the bacteria or viruses present in a culture room or laminar hood.
- Carcinogens and teratogens are used in cancer research.
- Transposons are used as a vehicle for transferring a gene of interest at a particular location in a genome. Thus it is used in gene therapy experiments.

Conclusion:

With the help of our DNA repair system, almost all abnormal DNA mutations are repaired by it. By adopting a healthy lifestyle we can save our DNA from the harmful effect of mutagens. For example, use sun scream while coming out in sunlight. Eat healthy food and stay away from unnecessary radiation. Mutagens are harmful, but not always. Some mutations are also beneficial to us, however, those are rare. In fact, different phenotypes for our survival are originated due to different mutations but those are not likely originated from the mutagens.

Sources:

T. J. Schrader. Mutagens, Encyclopedia of Food Science and Nutrition. 2003; 2:4059-67.

W. S. Klug, M. R. Cummings. Concepts of Genetics. 12th Ed, Pearson Education, Inc., 2018.