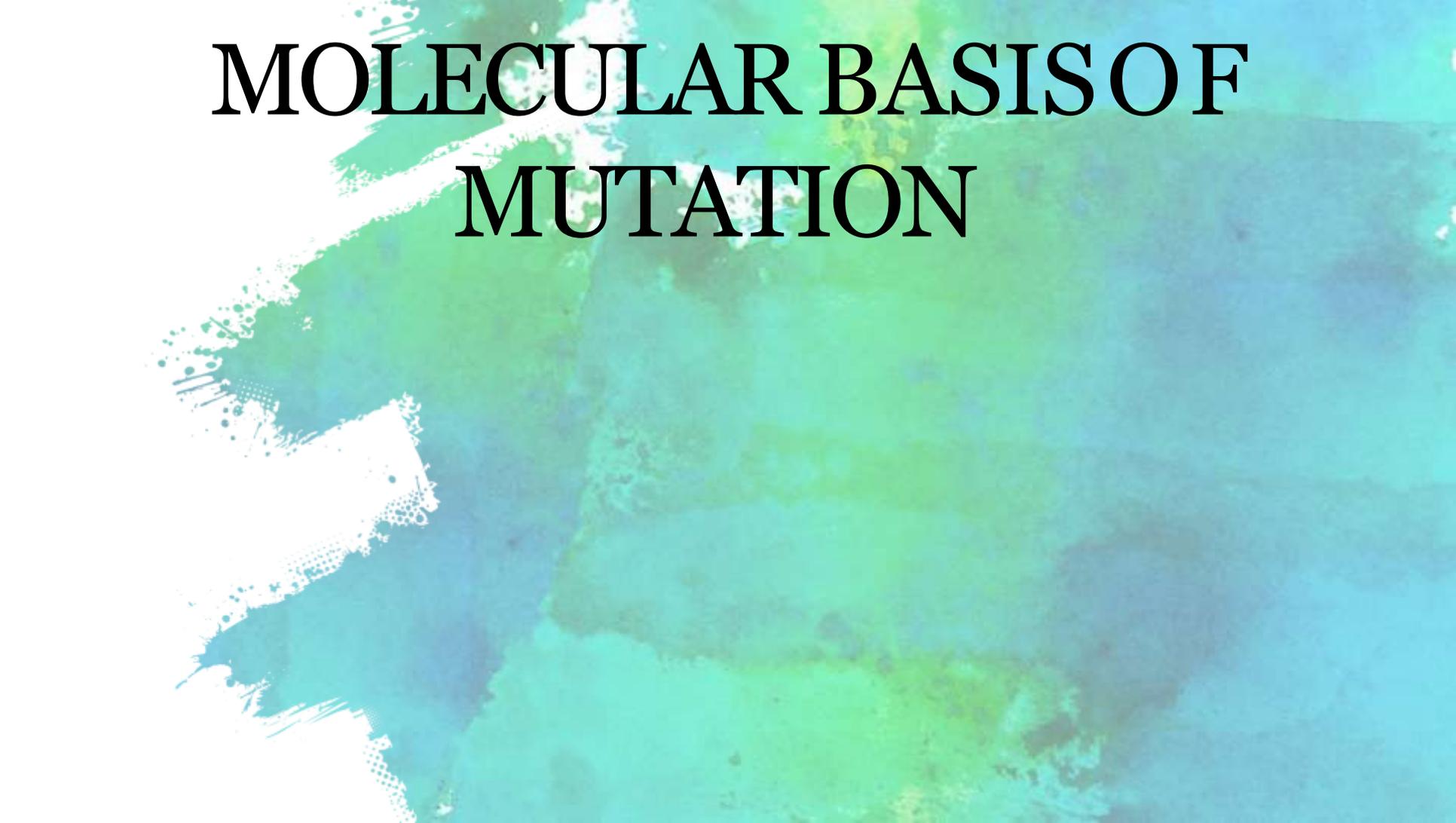


MOLECULAR BASIS OF MUTATION

The background of the slide is an abstract, artistic composition. It features a mix of vibrant green and teal colors, with some areas appearing as soft, watercolor-like washes. On the left side, there are white, textured shapes that resemble splatters or brushstrokes, some with a halftone dot pattern. The overall effect is a dynamic and modern aesthetic.

CONTENTS

- **BASIC TERMINOLOGY**
- **FEATURES OF MUTATION**
- **TYPES OF MUTATIONS**
- **MOLECULAR BASIS OF MUTATION**
- **MECHANISM OF MUTATION AT DNA LEVEL**
- **CASE STUDY.**

INTRODUCTION

“In gene mutation ,one allele of a gene changes into a different allele. Because such change takes place within single gene and maps to one chromosomal locus (point), a gene mutation is sometimes called as a point mutation”.

~Griffiths AJF, Gelbart WM, Miller JH,et al ,Modern Genetics Analysis.

Basic Terminologies

MUTATION

MUTAGEN

MUTANT

**SPONTANEOUS
MUTATION**

INDUCED MUTATION

FRAMESHIFT MUTATION

POINT MUTATION

MISSENSE MUTATION

NON-SENSE MUTATION

SILENT MUTATION

Features of Mutations :

- ❑ Mutations are sudden and heritable change in the nucleotide sequences of the gene.
- ❑ It is a permanent structural change in hereditary material (DNA).
- ❑ Mutations are generally recessive in nature.
- ❑ Mutations are recurrent in nature.
- ❑ Mutations may show Pleiotropic effects.
- ❑ Mostly mutations are deleterious and very rare are beneficial.
- ❑ Mutations may be caused by mistakes during the cell division or they may be caused by the exposure to DNA damaging agents in the environment such as radiation and mutagenic chemicals.
- ❑ Mutation occurs in both direction i.e. Reverse and Forward.
- ❑ It may be spontaneous or induced and it changes the DNA of a cell.
- ❑ Mutations are sometimes attributed to random chance events.

TYPES OF MUTATIONS

SPONTANEOUS MUTATION

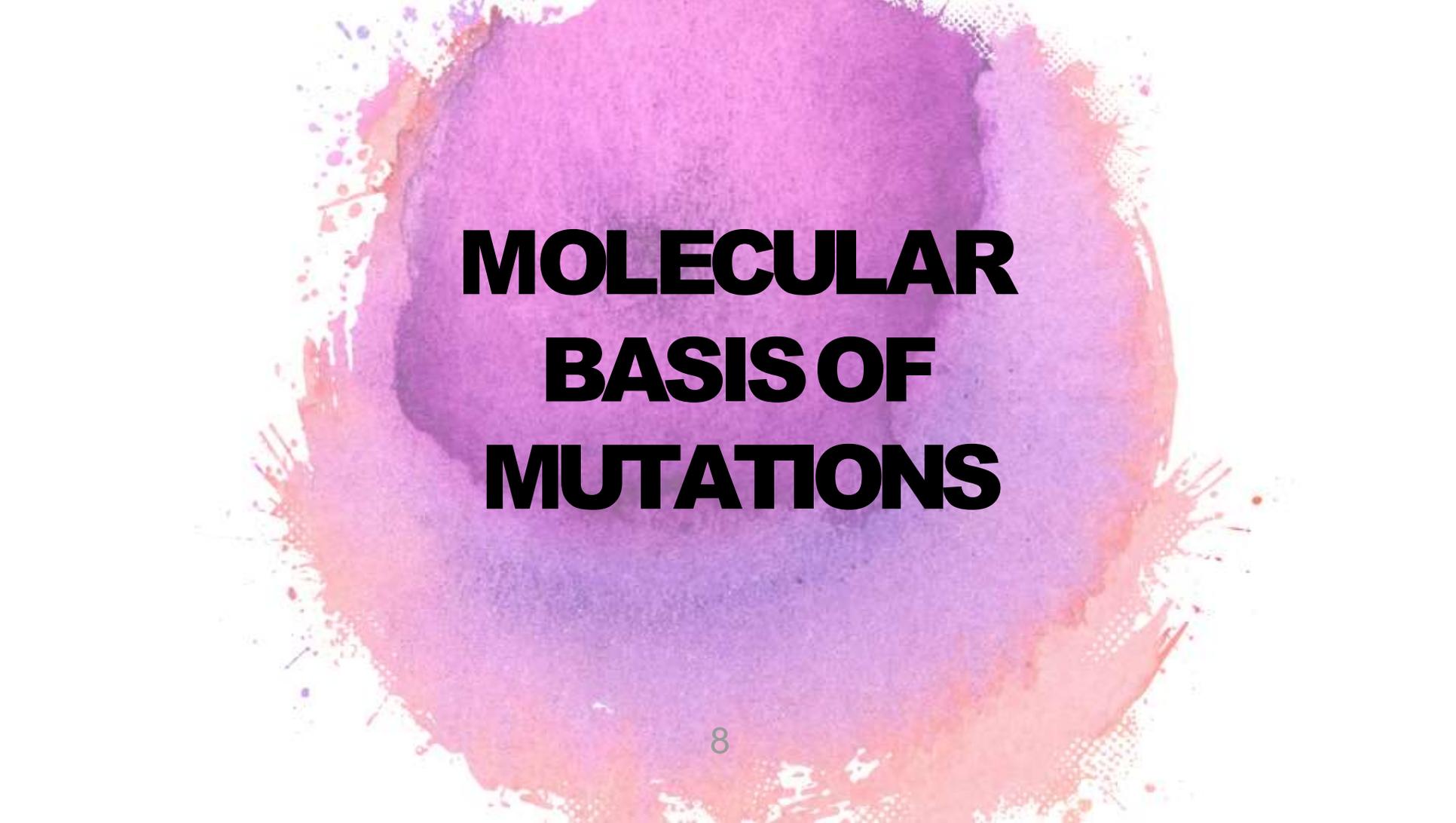
- Spontaneous mutations are naturally occurring mutations and can arise in any cells.
- Occurs in nature without any known cause.
- Spontaneous mutations occur infrequently and their frequencies vary from gene to gene and from organism to organism
- The rate of spontaneous mutation for various genes of prokaryotes range from about 10^{-5} to 10^{-7} detectable mutations/ nucleotide pair/ generation.
- The rate of spontaneous mutation in eukaryotes, ranges from about 10^{-4} to 10^{-7} detectable mutations /nucleotide pair /generation.

INDUCED MUTATION

- Induced mutations are those resulting from exposure of organisms to physical and chemical agents (mutagens) that cause changes in DNA.
- They are Physical Mutagen and Chemical Mutagens.
- Hugo de Vries (1900) Coined term ,and gave Mutation theory.
- Muller (1927)working with *Drosophila* provides proof of mutation induction by X-rays.
- Stadler (1929) described the mutagenic effect of x-rays in Barley.
- The rate of induced mutation is 10^{-3} detectable mutations/ nucleotide pair /generation.

Why we need to Know the Molecular basis of Mutation ?

- To understand the cause of mutation**
- To understand the actual change in DNA sequences**
- To understand the effects of nucleotide changes**
- To understand the one cause of evolution**
- To understand the one cause of variation**



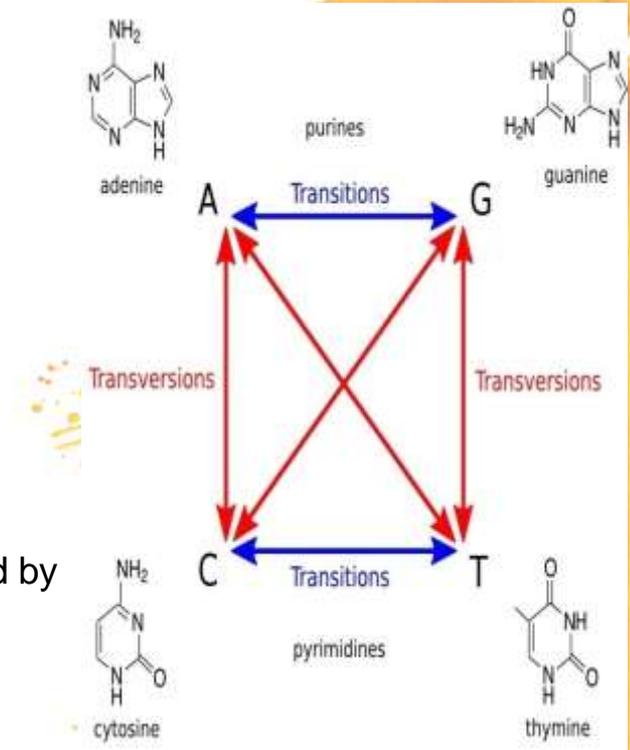
MOLECULAR BASIS OF MUTATIONS

THERE ARE FOUR MOLECULAR BASIS OF MUTATION

- ❑ BASE SUBSTITUTION
- ❑ BASE ADDITION/DELETION
- ❑ TRANSPOSITION
- ❑ TRINUCLEOTIDE REPEAT EXPANSION

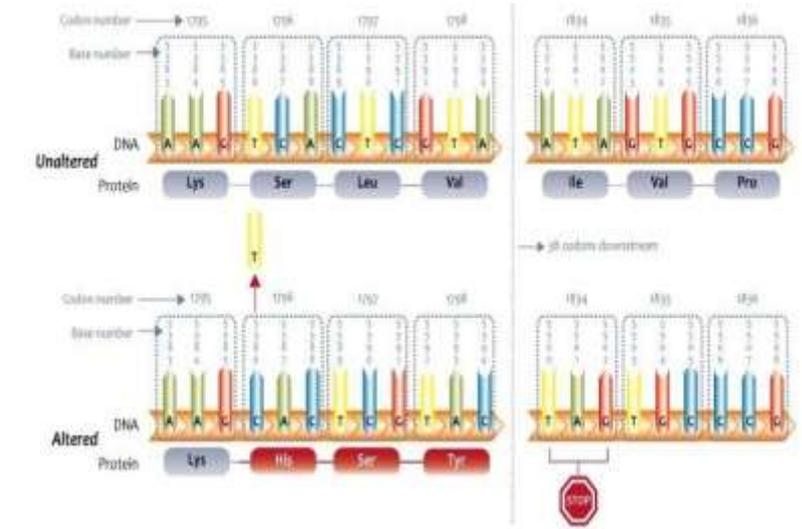
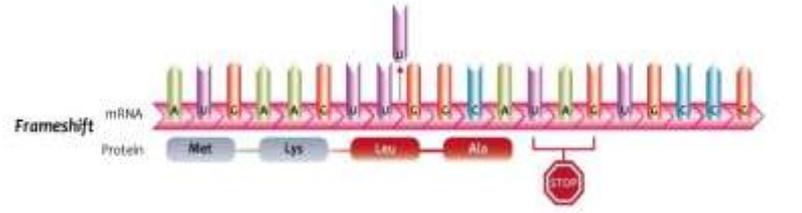
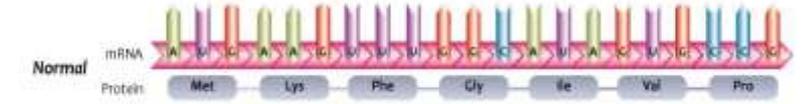
BASE SUBSTITUTION

- One base in the DNA molecule is replaced by another base.
- Base Substitutions are of two types :
 - Transitions (Common Occurrence)
 - Transversions (Rare Occurrence)
- **Transition:** Purine replaces purine and Pyrimidine replaces pyrimidine.
- **Transversion:** Purine replaced by Pyrimidine and Pyrimidine replaced by Purine.
- Single base substitution affects the base sequence of only one codon and it may alter one amino acid in the concerned protein.
- Base substitutions may generate nonsense codon and terminate polypeptide chain.



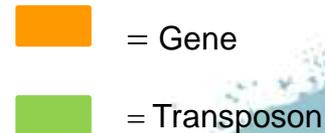
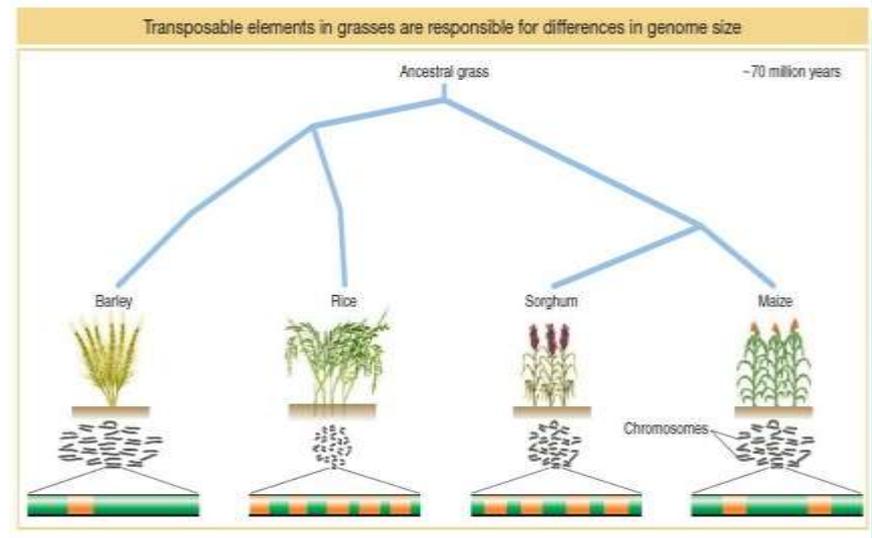
BASE ADDITION / DELETION

- Insertion of one or more bases in a DNA molecule is Base Addition.
- A loss of one or more base is known as Base Deletion.
- Both addition or deletion leads to mutations.
- If the number of bases added or lost is a multiple of three, then one to several amino acid would be either added or deleted from the concerned protein, such change may or may not have profound effect on the activity of polypeptide.
- If the number of bases added or deleted are not in multiple of three, base sequence of all the codons beyond this point is altered, and all codons will code different amino acid.
- Reading frame of subsequent codon is shifted in such mutations, called **FRAMESHIFT MUTATIONS**
- Proteins from frameshift mutations are more deleterious than the mutations caused by Base substitution (except for non sense mutations or silent mutations).



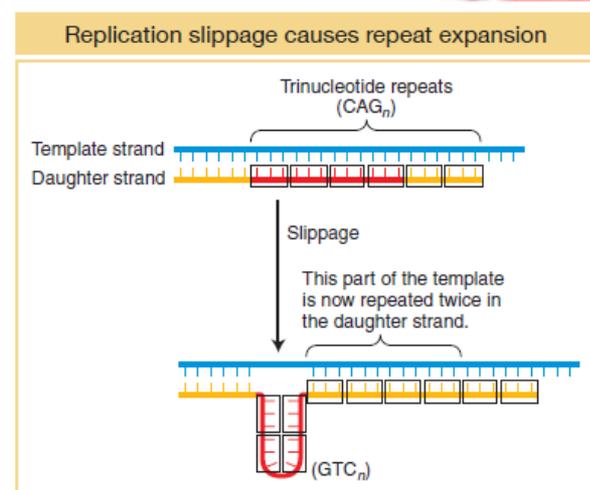
TRANSPOSITION:

- Eukaryotic and Prokaryotic genomes contain a variety of DNA sequences that can move in the genome and known as Transposable element.
- The movement of Transposable element in the genome is known as TRANSPOSITION.
- Integration of transposable elements into a gene often inactivates the gene, and if the gene encodes important protein, its inactivation leads to mutant phenotype.
- Examples : Mendel's *wrinkled* allele in the pea. The first mutation (*w1*) causing white eyes in *Drosophila* (resulted from the insertion of transposable elements).
- The grasses, including barley, rice, sorghum, and maize, diverged from a common ancestor about 70 million years ago.

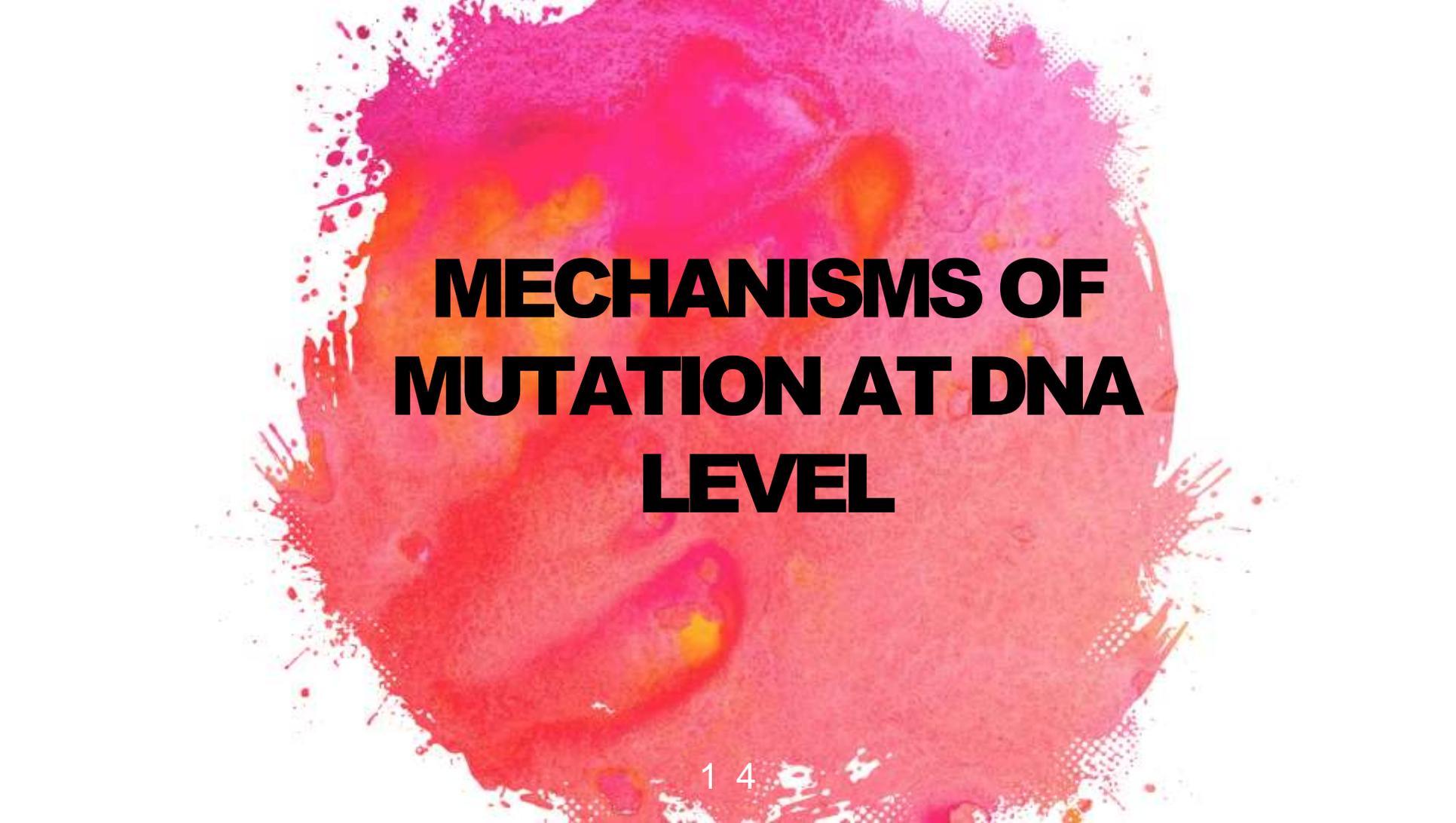


TRINUCLEOTIDE REPEAT EXPANSION

- Complex form of mutation in which duplications of short repeated sequences occurs which lead to expansion of three base repeats.
- The expected mechanism for this repeat expansion is due to the Replication slippage. When slippage takes place, the presence of nearby duplicate bases stabilizes the slippage so that replication can proceed.
- Several human genetic disorders are result of these tri-nucleotide repeat expansion.
- Ex. Fragile X syndrome, common form of mental retardness, results from change in the number of (CGG)_n repeats in region of FMR-1 gene that is transcribed but not translated.



(b)	FMR-1 gene	Phenotype	Transmission	Methylation	Transcription
Normal	(CGG) ₆₋₅₅ ATG 	Normal	Stable	No	Yes
Premutation	(CGG) ₇₀₋₂₀₀ ATG 	Largely normal	Unstable, prone to expansion	No	Yes
Full mutation	(CGG) _{>200} ATG 	Affected	Unstable	Yes	No



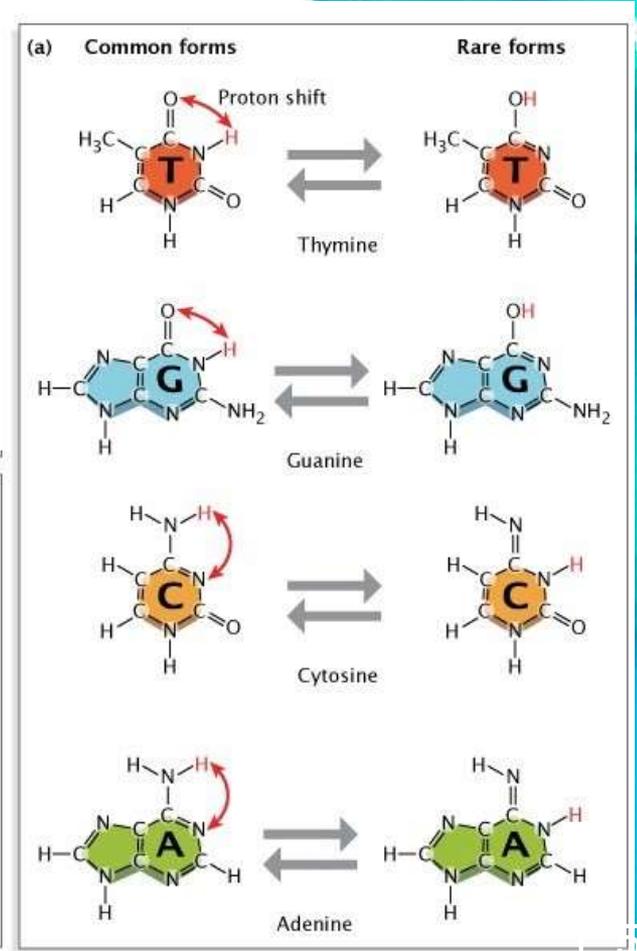
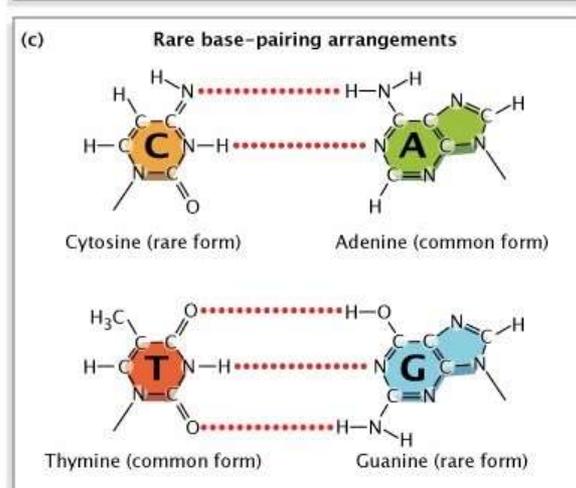
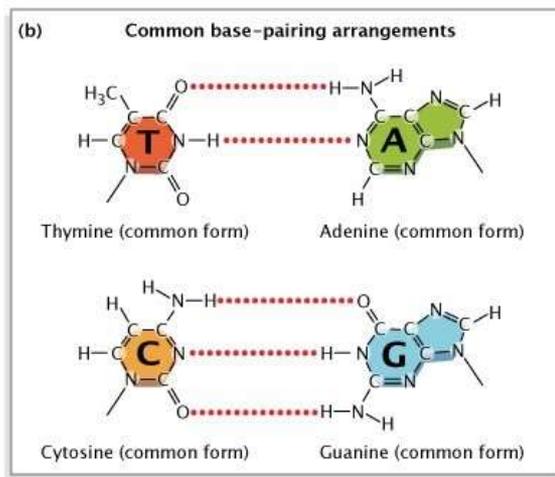
MECHANISMS OF MUTATION AT DNA LEVEL

(A) TAUTOMERIC SHIFTS

• Watson and Crick pointed out that the specific base pairing and structures of the bases in DNA are not static, Hydrogen atoms and Amino nitrogen shifts their position known as **TAUTOMERIC SHIFTS**.

• Tautomeric shifts are rare and alters pairing potential.

• The bases would be expected to exist in their less stable tautomeric forms for only short periods of time. If a base existed in the rare form at the moment that it was being replicated or being incorporated into a nascent DNA chain, a **mutation** would result.



(B) **ERRORS IN THE DNA REPLICATIONS:**

- DNA polymerase I enzymes are very accurate about the addition of the correct bases and their complementary base pairing with the template strand.
- Polymerase enzymes do make mistakes at a rate of about 1 per every 100,000 nucleotide.
- To fix the mistakes immediately during replication DNA polymerase I do *proofreading* (3' to 5'), by recognizing and replacing the incorrectly inserted nucleotide so that replication can continue.
- Proofreading fixes about 99% of these type of errors.
- After replication, Mismatch repair reduces the final error rate even further
- Error rates : Before proof reading : 1 in 1 lakh bases
After proof reading : 1 in 10 lakh bases
After Mismatch Repair : 1 in 10 crore bases.
- Incorrectly paired nucleotides cause deformities in the secondary structure of the final DNA molecule.
- During mismatch repair, enzymes recognize and fix these deformities by removing the incorrectly paired nucleotide and replacing it with the correct nucleotide.

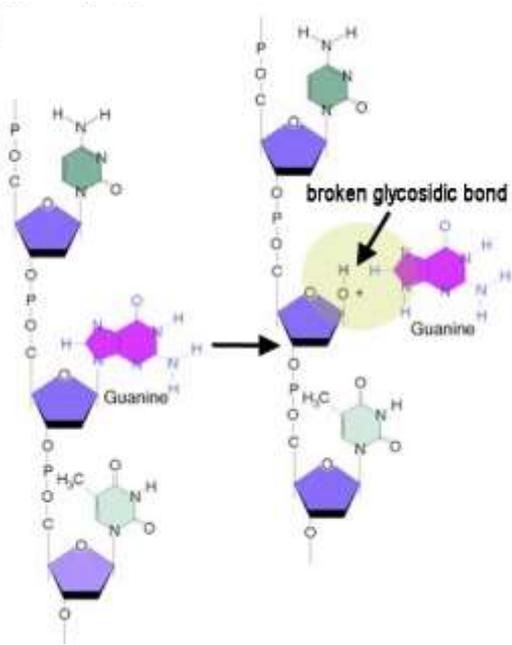
(C) SPONTANEOUS LESIONS :

Naturally occurring damage to the DNA, generate mutations known as spontaneous lesions.
Common spontaneous lesions are

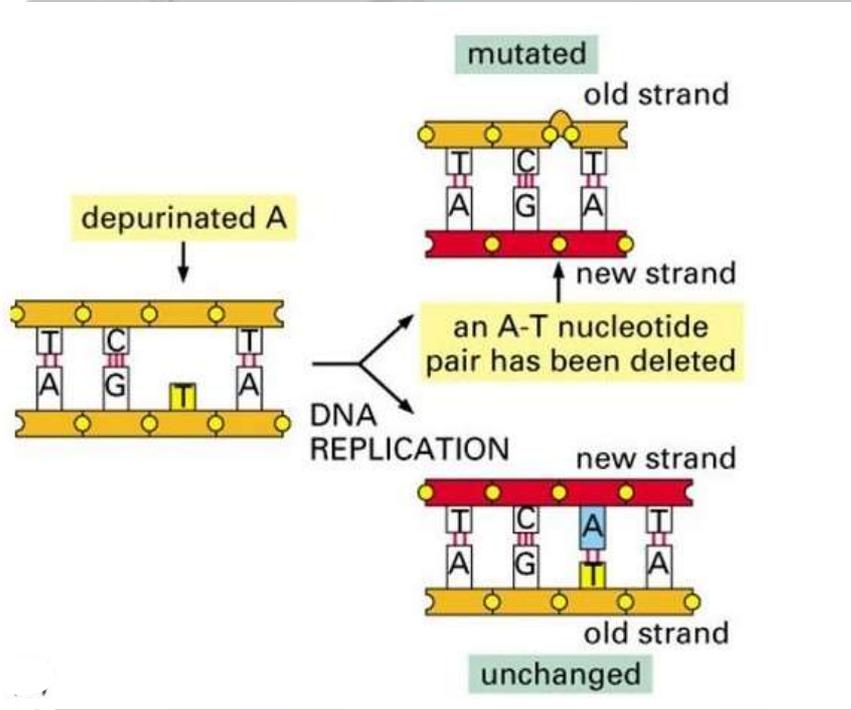
- I . D E P U R I N A T I O N
- II. DEAMINATION
- III. OXIDATIVELY DAMAGED BASES

I. DEPURINATION

- Loss of a purine (A/G) after the breakage of the glycosidic bond between base and the deoxyribose.
- Apurinic sites are removed by the DNA repair mechanism but if a base inserted across from apurinic site, results to mutation.
- Lesions are known as APURINIC SITES, these sites cannot specify a base complementarity.
- Transversion occurs .



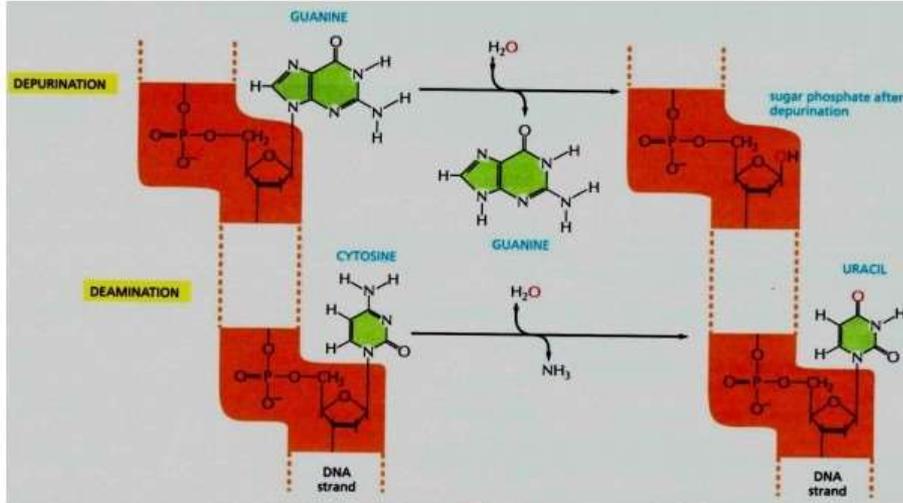
Depurination at DNA level



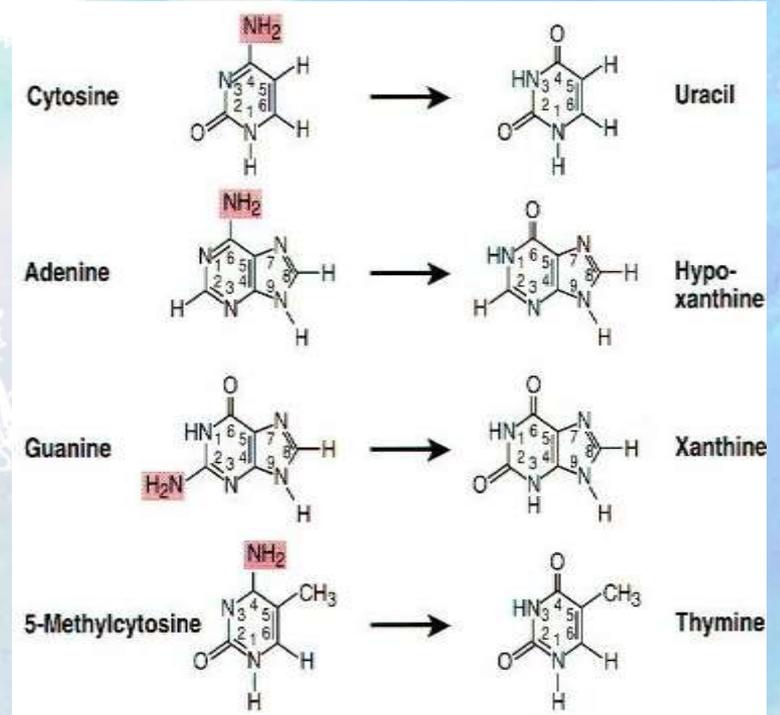
Depurination effects at DNA Replication

II. DEAMINATION :

- Loss of amino group in A / C / G converts the amino groups to keto groups and changes the hydrogen-bonding potential of the modified bases
- Deamination of guanine produces xanthine, it base-pairs with cytosine.
- Deamination of guanine is not mutagenic.
- Deamination of adenine results in A:T → G:C transitions.
- Deamination of cytosine produces G:C → A:T transitions.



Generation of a basic site by depurination & base alteration by deamination



Deamination of Bases

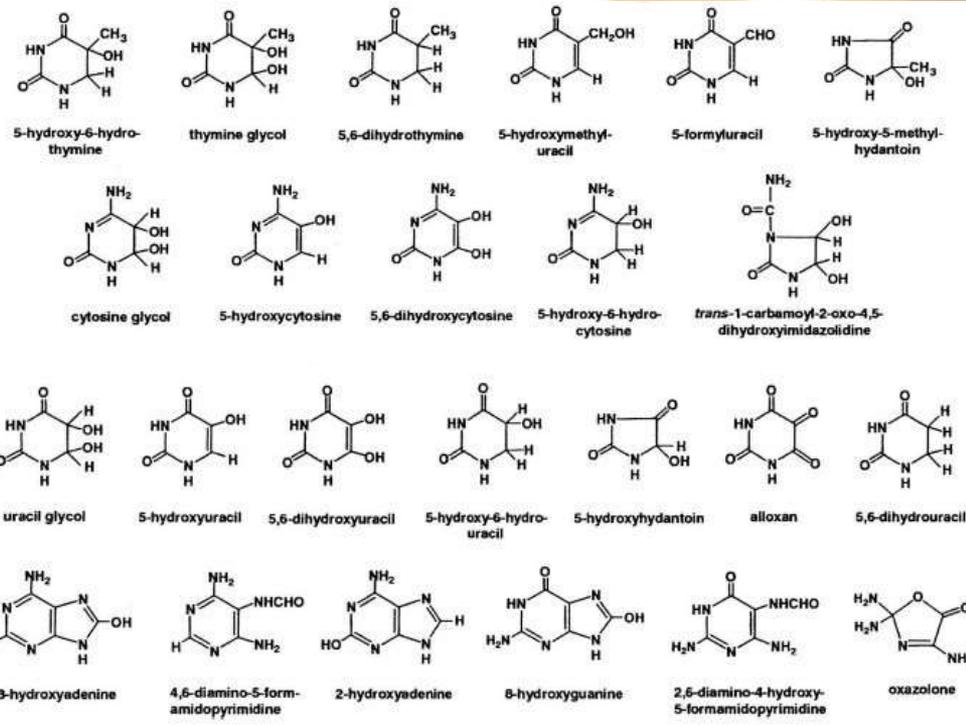
III. OXYDATIVELY DAMAGED BASES

- Active oxygen species produced by normal aerobic metabolism

- Superoxide radicals (O_2^-),
- Hydrogen peroxide (H_2O_2),
- Hydroxyl radicals ($-OH$)

- Cause oxidative damage to DNA, also to precursors of DNA (such as GTP), resulting in mutation.

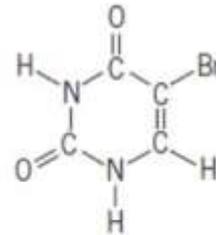
- Eg. The 8-oxo-7-hydroxydeoxyguanosine (8-oxo dG, or GO) mispairs with A, resulting in a high level of G T transversions.



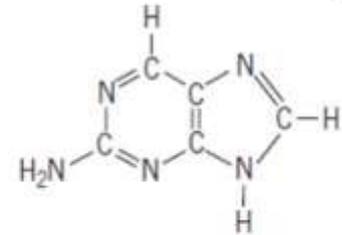
Products generated as a result of interaction of DNA bases with reactive oxygen and free radical species.

(D) **BASE ANALOGS**

- Chemical compounds **mimic the nitrogenous** bases, possess the pairing properties like that of the normal nitrogenous bases.
- Base analogs **incorporate incorrect** nucleotides during replication and causes mutation.
- Base analogs exist only in single strand, but substitutes the nucleotides in all replicating DNA copies descended from the original strand.
- Base analogue will result in altered base structures as affects DNA replication and transcription of genes.
- Eg. 5-Bromouracil (5 BU)& 2 Aminopurine (2AP).



5-Bromouracil
(5-BU)



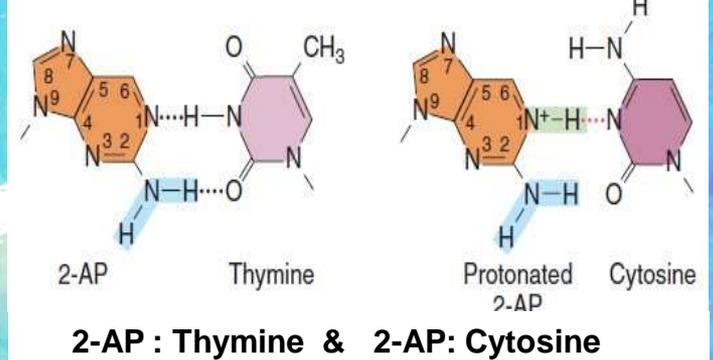
2-Aminopurine
(2-AP)

- 2-amino-purine (2-AP) is analog of adenine can pair with thymine and produce A-T → G-C transitions

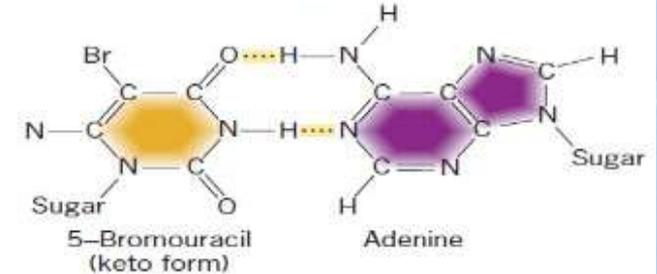
- Protonated 2-AP is incorporated by mis-pairing with cytosine, leads to G-C → A-T transitions

- 5-BU is a thymine analog, the bromine at this position changes the charge distribution and increases the frequency of tautomeric shifts.

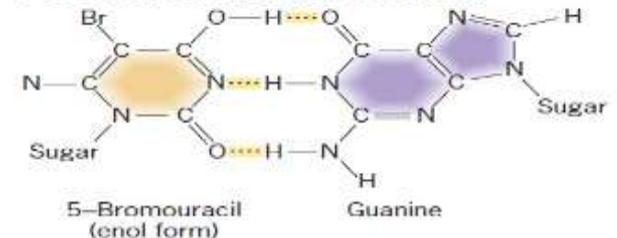
- 5-BU pairs with Adenine in keto form. After a tautomeric shift to its enol form, 5-bromouracil pairs with Guanine.



5-Bromouracil : adenine base pair.

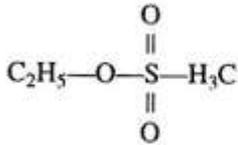


5-Bromouracil : guanine base pair.

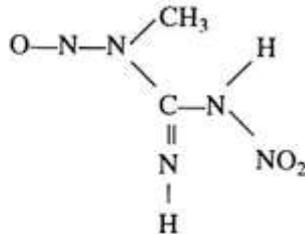


(E) SPECIFIC MISPAIRING:

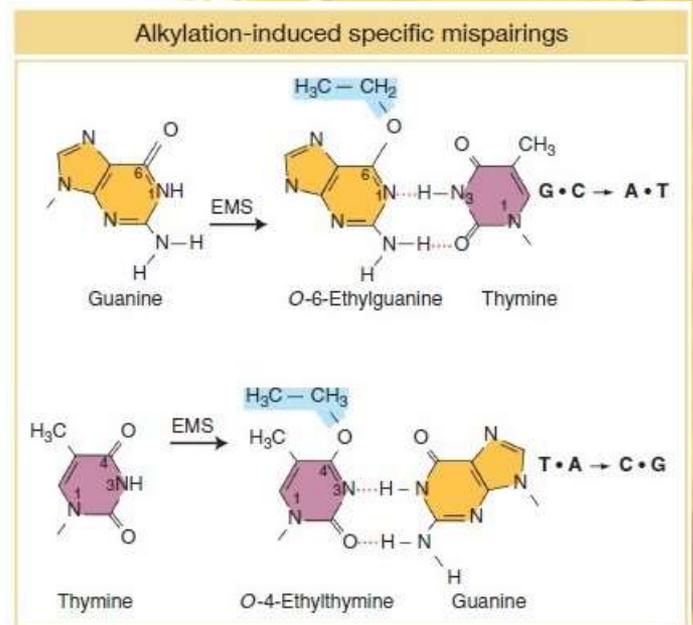
- Some mutagens are not incorporated into the DNA but alter a base to form a specific mis-pair.
- Alkylating agents such as ethylmethanesulfonate (EMS), nitrosoguanidine (NG), operate by this pathway.
- EMS and NG add alkyl groups (an ethyl group in EMS and a methyl group in NG) to many positions on all four bases.
- Addition of oxygen at position 6 of guanine to create an O-6-alkylguanine. This addition leads to direct mispairing with thymine, and would result in G•C → A•T transitions.
- Alkylating agents can also modify the bases in dNTPs (where N is any base), which are precursors in DNA synthesis.



ethyl methane sulphonate

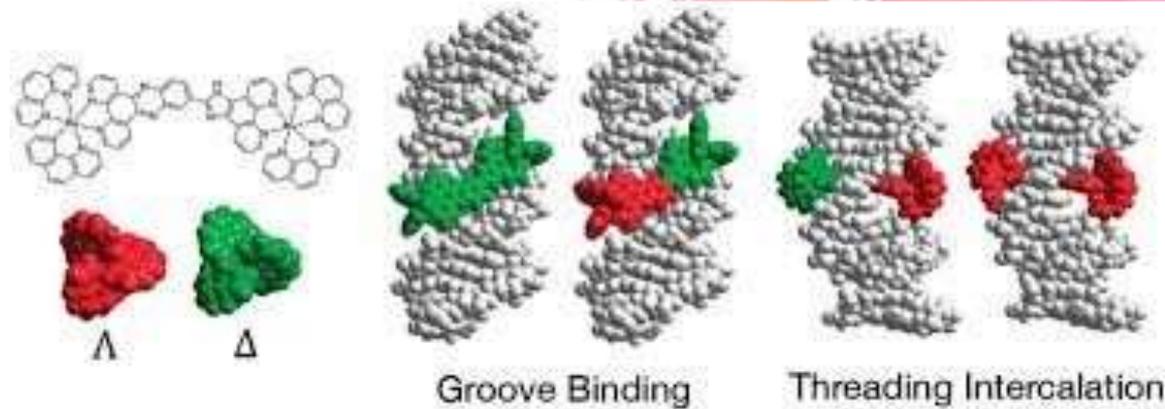
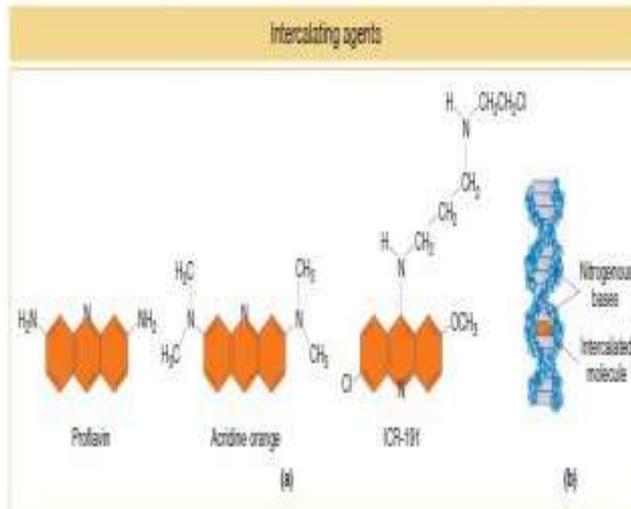


nitrosoguanidine



(F) INTERCALATING AGENTS

- The intercalating agents form another important class of DNA modifiers includes proflavin, acridine orange and Class of chemicals termed as ICR compounds.
- These agents are planar molecules that mimic base pairs and are able to slip themselves in (intercalate) between the stacked nitrogen bases at the core of the DNA double helix .
- In this intercalated position, such an agent can cause an insertion or deletion of a single nucleotide pair



(G) BASE DAMAGE

- Large number of bases damage, affects the specific base pairing and blocks the replication and causes mutation.

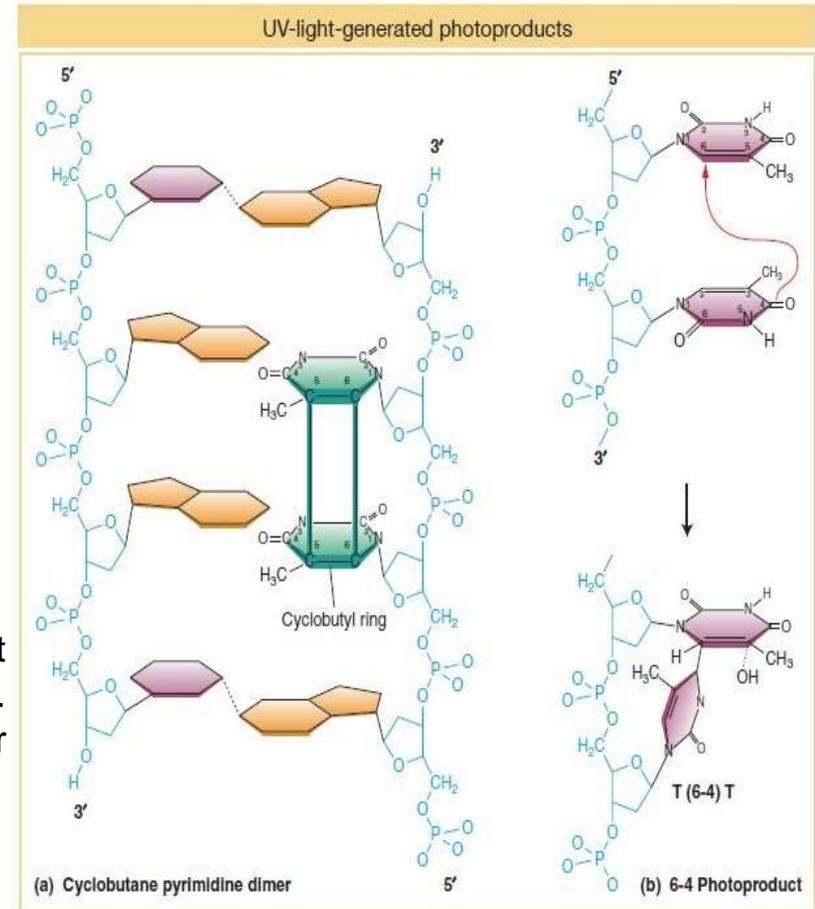
- Reasons :

1. *Ultraviolet rays*
2. *Ionization radiation*
3. *Aflatoxin*

1. Ultraviolet rays

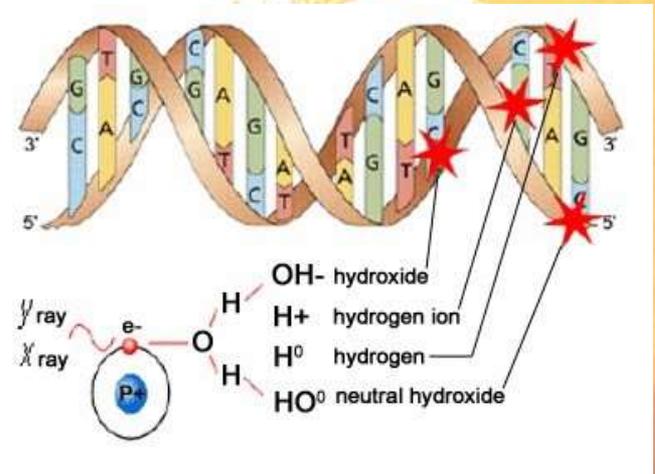
- UV light damages the bases by generating different type of alterations in DNA called as photoproducts .

- Photoproducts causes mutation by uniting two different lesions adjacent to pyrimidine residues in the same strand. These lesions are the cyclobutane pyrimidine, photodimer and the 6-4 photoproduct



2. IONIZING RADIATION

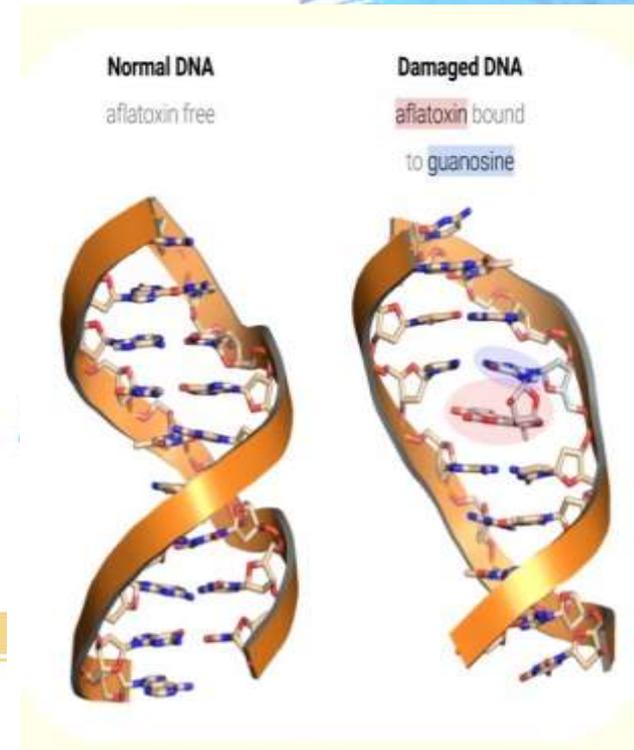
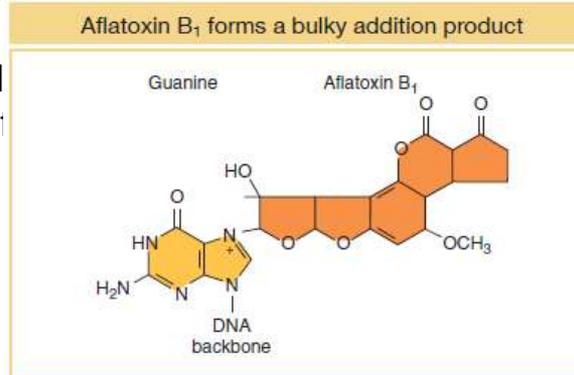
- Forms ionized and excited molecules that can damage DNA directly.
- Ionizing radiations ionizes the water of the cell, produce reactive oxygen species (\bullet OH, O_2^- , and H_2O_2) which damages the DNA by forming different adducts and degradation products.
- Thymidine glycol and 8-oxo dG, both can result in mutations.
- Radiations breaks the *N*-glycosydic bond and form apurinic or apyrimidinic sites, and it can cause strand breaks this is the most lethal effects of ionizing radiation.



3. AFLATOXIN

- Aflatoxin B1 is a powerful carcinogen that attaches to guanine at the N-7 position, and breaks the bond between the base and the sugar, liberate the base and generates an apurinic site.
- Aflatoxin B1 is a member of a class of chemical carcinogens known as bulky addition products and they bind covalently to DNA.
- Other example diol epoxides of benzo(a)pyrene, a compound

internal combustion engines, al
O'



CONCLUSION

1. Molecular basis helps to understand the phenomena of mutation in more précised way.
2. This can increase the efficiency of mutation breeding .
3. Helps to understand techniques like TILLING and ECO-TILLING.
3. Can help to develop new techniques of mutation detection in near future.



THANK YOU