Mutation and DNA Repair

A **mutation:** is any heritable change in the genetic material, resulting in an alteration of the DNA sequence**.**

* **Mutations are usually considered as a change that alters gene function and thus the phenotype of the organism**.
* Some results in genetic diseases, but others have no physical effects.
* Some mutations consist of an alteration of the **number or structure of chromosomes** in a cell (abnormalities involve loss or gain of chromosomes or breakage and rejoining of chromatids).
* Other mutations can take place in **coding DNA** or in regulatory sequences **(single-gene mutation).**

❑ Types of mutation

Mutations, which can alter the coding properties of a DNA segment are of several types:

1- Base- pair substitutions

Convert or replaced one type of base pair into another.

* **G-C ⇨ A-T** and **A-T ⇨ G-C** changes are referred to as **transition mutations (replacement of a purine to pyrimidine base pair by a purine to pyrimidine base pair).**
* **T-A ⇨ G-C, G-C ⇨ C-G, A-T ⇨ T-A** are called **transversions (replacement of a pyrimidine - purine base pair by a purine - pyrimidine base pair).**

CATTCACCTGTACCA
GTAAGTGGACATGGT
Normal Sequence

Transition (T-A to C-G)	Transversion (T-A to G-C)
CATCCACCTGTACCA GTAGGTGGACATGGT	CATGCACCTGTACCA GTACGTGGACATGGT
➢ **base pair substitutions** Transition: pyrimidine- purine bas pair to pyrimidine- purine base pair	➢ **base pair substitutions** Transversion: pyrimidine- purine base pair to purine- pyrimidine base pair

- This type of mutation (**Base- pair substitutions**) can **change the codon** to that of another amino acid, thus altering the protein.
- In addition, such changes can also **create a stop codon** in which one base pair is replaced by another. This can result in a change in amino acid sequence.
- Although transitions are more common than transversions, both kinds of mutations occur as a **consequence of replication errors**, both can result from chemical damage to DNA, and both have been implicated as causative factors in inherited genetic disease and cancer.

There are two types of base- pair substitutions:

A. Missense mutations: which produce change in a single amino acid

B. Nonsense mutations: which produce one of the three stop codons (**UAA, UAG, or UGA**) in the mRNA.

* Because the stop codons terminate the translation of mRNA, nonsense mutation results in a premature termination of the polypeptide chain.
* Conversely, if a stop codon is altered so that it encodes an amino acid, an abnormally elongated polypeptide is produced.

2- Small insertions / deletions

comprise a second common class of mutation.

* Genetic changes involve insertion or loss of a small number of base pairs **(one to several hundred).**
* These mutations can results in **extra** or **missing** amino acids in a protein.

- **An example** of such mutation is the 3 bp deletion that causes **cystic fibrosis.**

CATTCACCTGTACCA
GTAAGTGGACATGGT
Normal Sequence

Insertion
- CATGTCACCTGTACCA
- GTACAGTGGACATGGT

Deletion
- CATCACCTGTACCA
- GTAGTGGACATGGT

➢ deletions and insertions can involve one or more base pairs

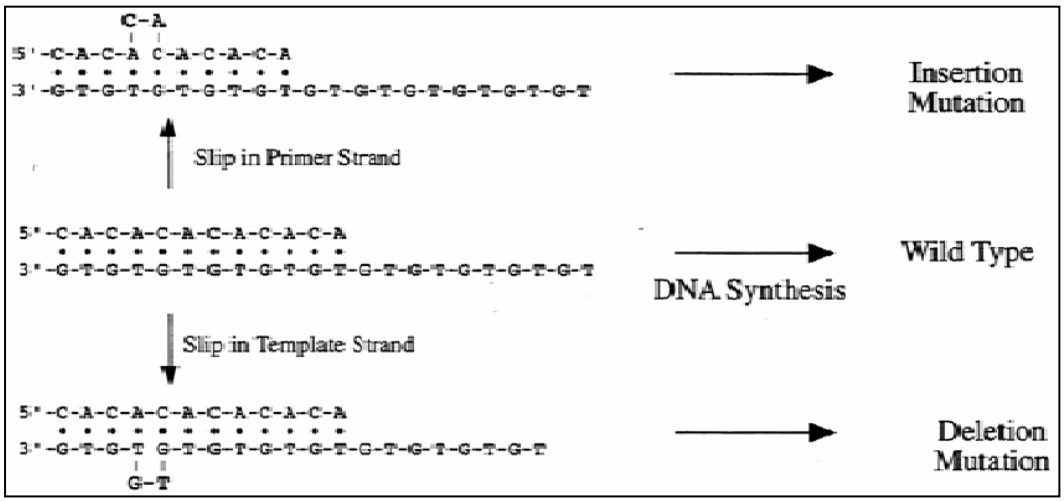
2.1: Frameshift mutation:

Another type of deletion and insertion tend to be especially harmful when the number of **missing or extra base pairs is not a multiple of three. Because codons consist of groups of 3bp,** such insertions or deletions can alter all the downstream codons, and produced truncated polypeptide.

2.2: Promoter mutation:

The affinity of RNA polymerase to bind a promoter site is decrease, resulting in reduced production of mRNA, and final result is decreased production of a protein. Also mutations of **transcription factor genes or enhancer sequences** have the similar effects.

* Repetitive runs of a mono, di-, or trinucleotide sequence are extremely prone to insertion /deletion mutation, an effect that has been attributed to **slippage of template and primer strands during DNA replication**



2.3: Splice site mutation:

Mutation interfere with the splicing of introns as a mature mRNA is formed from the primary mRNA transcript, and alter the splicing signal that is necessary for proper excision of an intron.

* **Mutations can be also caused by expanded tandem repeats DNA sequences (satellite)** which are prone to insertion /deletion as a result of unequal crossover or unequal sister chromatid exchanges

So the principle types of mutations are:

Missens, Nonsense, Frameshift, Promoter, Splice site

❑ Causes of Mutation

* **Mutations** can be induced in our DNA by exposure to a variety of mutagens occurring in our external environment or to mutagens generated in the intracellular environment.
* A large number of agents known to cause **induced mutations.** These mutations due to known environmental causes are called **spontaneous mutations,** which are naturally arising during the process of DNA replication and repair (*endogenous)*.
* **Mutagens:** Agents that cause induced mutations

Types of Mutagens

1. Radiation:

* **Ionizing radiation,** produced by X- rays and nuclear fall-out, can eject electrons from atoms, forming electrically charged ions.
  When these ions are situated within or near the DNA molecule, they can promote chemical reactions that **change DNA bases, and can also break the bonds of the double-stranded DNA.**
* **Nonionizing radiation:** produced by UV radiation which occurs naturally in sunlight.
  It does not form charged ions but **can move electrons from inner to outer orbits within an atom, and the atom becomes chemically unstable**.
* UV radiation causes **the formation of covalent bonds between adjacent pyrimidine base,** and are unable to pair properly with purines during DNA replication, this results in a base pair substitution
* **Sunlight is particularly damaging to DNA**. This figure shows the formation of a thymine dimer, catalyzed by UV light. The thymine dimer bridges two adjacent thymine residues on the same DNA strand. Dimer is (a molecule having two subunits).
* Because UV radiation is absorbed by the epidermis, it does not reach the germ line but can cause skin cancer.

photolyase

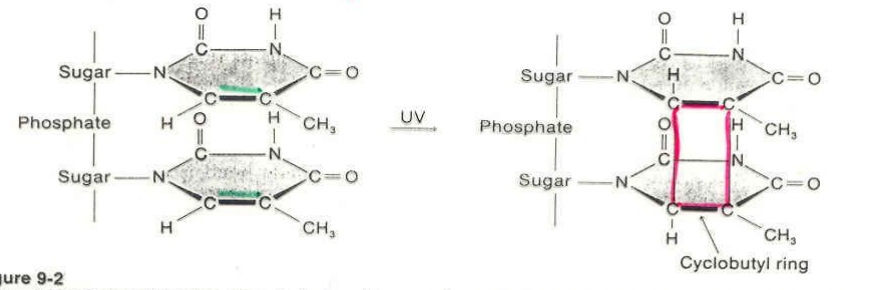


Figure 9-2
Structure of a cyclobutylthymine dimer. Following ultraviolet (UV) irradiation, adjacent thymine residues in a DNA strand are joined by formation of the bond shown in red. Although not drawn to scale, these bonds are considerably shorter than the spacing between the planes of adjacent thymines, so that the double-stranded structure becomes distorted. The shape of the thymine ring also changes as the C=C double bond of each thymine is converted to a C—C single bond in each cyclobutyl ring.

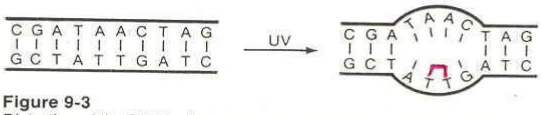
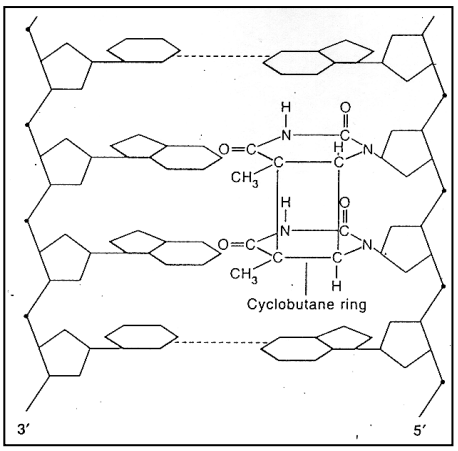


Figure 9-3
Distortion of the DNA helix caused by two thymines moving closer together when joined in a dimer. The dimer is shown as two joined lines.



2. Chemicals: Because of their chemical similarity to DNA bases (base analogs).

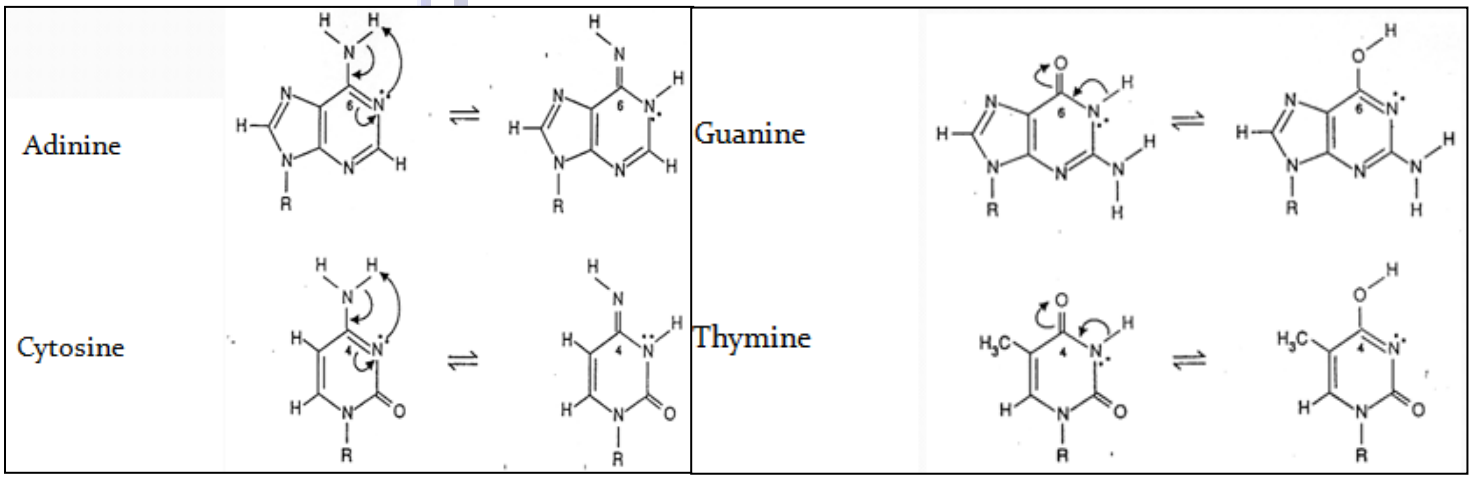
* **Bromouracil:** can be substituted for a true DNA base during replication.

* **Acridin dyes:** can physically insert themselves between existing bases causing frameshift mutation.

* **Nitrous acid:** removes an **amino group from cytosine, converting it to uracil,** and pairs with adenine instead of guanine, as the original cytosine would have done. The end result is a base pair substitution.

3. Spontaneous mutations (those that result from no external cause)

* Spontaneous mutations can occur by rearrangement of bonds and by the repositioning of hydrogens in the purine and pyrimidine bases



Mechanisms of DNA Repair

1. Mutations that occur during DNA replication are repaired when possible by proofreading by the DNA polymerases

2. Mutations that are not repaired by proofreading are repaired by mismatch (post-replication) repair followed by excision repair

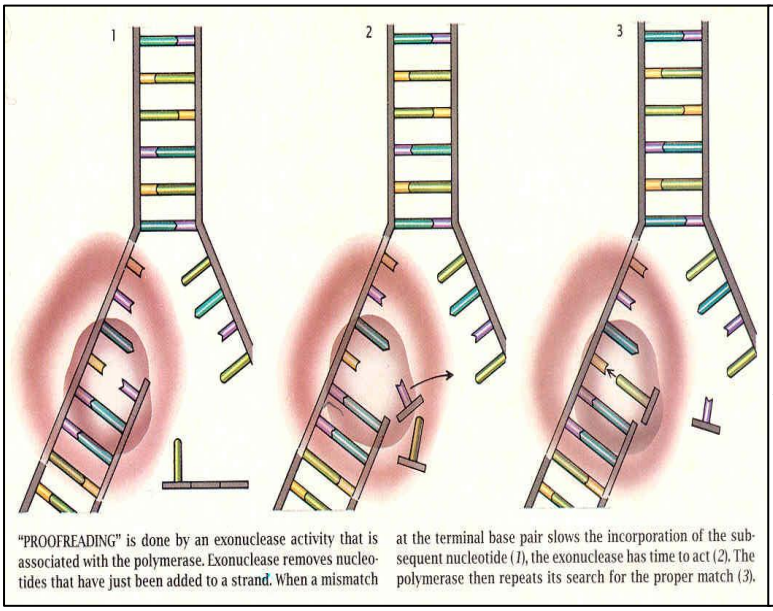
3. Mutations that occur spontaneously any time are repaired by excision repair (base excision or nucleotide excision)

* For DNA to be repaired properly following the **mismatch** incorporation of a nucleotide into the newly synthesized DNA strand, the replication machinery must have a means by which to distinguish between the "old" (template) strand and the "new" (daughter).
* Several dozen enzymes are involved in the repair of damaged DNA. They collectively recognize the altered base, excise it by cutting the DNA strand, replace it with the correct base and reseal the DNA.

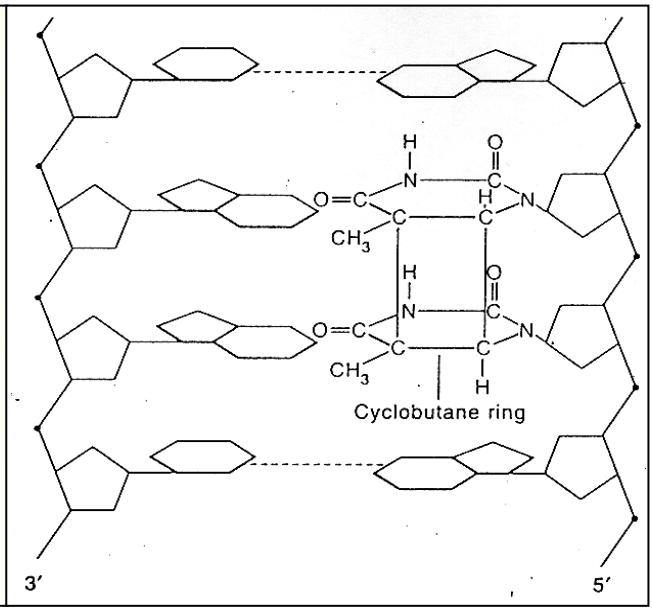
* **There are two types of excision repair:**
- base excision repair and
- nucleotide excision repair .

* Defects in DNA repair system can lead to many types of disease. For example, inherited mutation in genes responsible for **DNA mismatch repair** result in the persistence of cells with replication errors (mismatches) and lead to some kinds of cancers.
* Fail to repair double- stranded DNA breaks can lead specifically to ovarian and /or breast cancer.

* **Nucleotide excision repair** occurs when the DNA lesion is larger, for example when there is **a thymine dimer**.
* In this case, a special repair exconuclease removes about 30 nucleotides, including the lesion. The DNA is then resynthesized and ligated together as with base excision repair.
* **Defect in excision repair** lead to a number of diseases like **xeroderma pigmentosum**



"PROOFREADING" is done by an exonuclease activity that is associated with the polymerase. Exonuclease removes nucleotides that have just been added to a strand. When a mismatch at the terminal base pair slows the incorporation of the subsequent nucleotide (*1*), the exonuclease has time to act (*2*). The polymerase then repeats its search for the proper match (*3*).



xeroderma pigmentosum

* **Xeroderma pigmentosum:** A genetic disease characterized by such extraordinary sensitivity to sunlight that it results in the development of skin cancer at a very early age. Children with xeroderma pigmentosum (XP) can only play outdoors safely after nightfall. They have been called midnight children.
* **Xeroderma pigmentosum (XP)** occurs due to failed to remove pyrimidine dimers in the DNA of the skin cells after exposure to UV radiation, and the resulting replication errors lead to **base pair substitution** in skin cells.
* **XP** usually begins within the first 10 years of life. The skin is dry and scaly (xeroderma), and extensive freckling and abnormal skin pigmentation (pigmentosum) are followed by numerous skin tumors. Patients with XP can develop sever malignancies that some times result in death before 20 years of age.

Mutation Rates

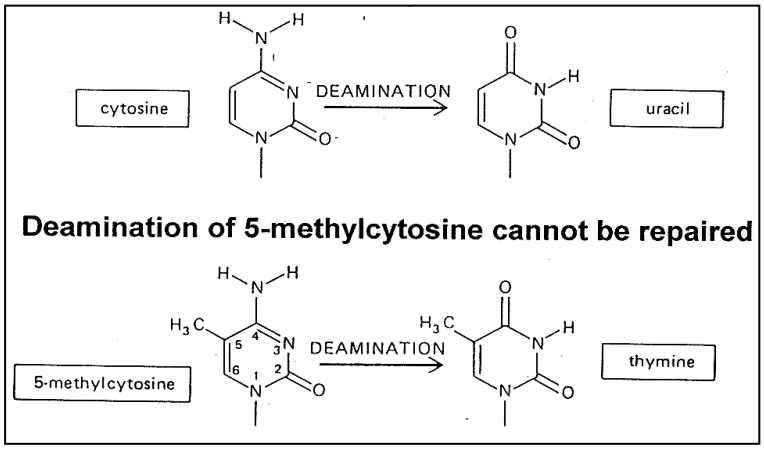
At the level of the gene, the mutation rate ranging **from 10^{-4} to 10^{-7} per locus per cell division.**

There are at least two reasons for the large range of variation:

1. Gene size: larger genes present large **"targets"** for mutation than do smaller genes.

2. Certain nucleotide sequences are susceptible to mutation**.** These are called **mutation hot spots**. The best known example is the two- base (dinucleotide) sequence **CG**. In mammals about 80% of **CG dinucleotides** are **methylated**: a methyl group is attached to the cytosine base. The methylated cytosine, 5-methylcytosine easily loses an amino group, **converting it to thymine**, resulting mutation from cytosine to thymine.
The problem that arises from these methylations is that deamination of **a 5 methyl cytosine** results in the production of **thymine**, which is not foreign to DNA. Thus, while a base pair mismatch is seen in the DNA by the repair machinery, it does not know which of the two strands to repair.

3. Mutation rates also affected with the age of the parent



Molecular consequences of Mutation:

Mutations can produce either:

***** a gain of function or ***** loss of function of the protein product

*** Gain- of- function** result in over expression or inappropriate expression of the product (in the wrong tissue or in the wrong stage of development).
Gain- of- function mutations produce **dominant disorders.**

*** Dominant negative mutations,** result in a protein product that is not only nonfunctional but also inhibits the function of the protein produced by the normal allele

*** Loss- of- function mutations are seen in recessive** diseases. In such diseases, the mutation results in the loss of 50% of the protein product (e.g. metabolic enzymes), but the 50% that remains is sufficient for normal function.

*** Diseases involving haplo insufficiency:** in which **50% of the gene product is insufficient** for normal function and **dominant disorder** can result.

E.g. **familial hypercholesterolemia**, in this disease, a single copy of mutation reduces the number of low-density lipoprotein (LDL) receptors by 50%, so cholesterol levels are double than normal resulting in increase the risk of heart disease.