DNA Mutation and Repair

A **mutation** is defined as an inherited change in genetic information.

Importance of mutations:

1. Mutation is the source of all genetic variation, the raw material of evolution. Without mutations and the variation that they generate, organisms could not adapt to changing environments and would risk extinction.

2. Most mutations have detrimental effects, and mutation is the source of many human diseases and disorders.

Causes of Mutations:

Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a mutagen. Mutations result from both internal and external factors.

1. Spontaneous Replication Errors

The primary cause of spontaneous replication errors was formerly thought to be tautomeric shifts, in which the positions of protons in the DNA bases change. Purine and pyrimidine bases exist in different chemical forms called tautomers. The standard base pairings—adenine with thymine, and cytosine with guanine—are between the common forms of the bases, but, if the bases are in their rare tautomeric forms, other base pairings are possible.

2. Spontaneous Chemical Changes in DNA

A- **Depurination:** the loss of a purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1- carbon atom of the deoxyribose sugar breaks producing an apurinic site-a nucleotide that lacks its purine base. An apurinic site cannot act as a template for a complementary base in replication. In the absence of base-pairing constraints, an incorrect nucleotide (most often adenine) is incorporated into the newly synthesized DNA strand opposite to the apurinic site, frequently leading to an incorporated error.

B- **Deamination:** the loss of an amino group (NH) from a base. Deamination may occur spontaneously or be induced by mutagenic chemicals. Deamination may alter the pairing properties of a base.

For example, the deamination of cytosine produces uracil, which pairs with adenine during replication. After another round of replication, the adenine will pair with thymine, creating a TA pair in place of the original CG pair (CG: UA: TA); this chemical change is a transition mutation. This type of mutation is usually repaired by enzymes that remove uracil whenever it is found in DNA.

3. Chemically Induced Mutations

A- **Base analogs:** chemicals with structures similar to that of any of the four standard bases of DNA. DNA polymerases cannot distinguish these analogs from the standard bases; so, if base analogs are present during replication, they may be incorporated into newly synthesized DNA molecules.

For example, 5-bromouracil (5**bU**) is an analog of thymine; it has the same structure as that of thymine except that it has a bromine (Br) atom on the 5-carbon atom instead of a

methyl group. Normally, 5- bromouracil pairs with adenine just as thymine does, but it occasionally mispairs with guanine, leading to a transition (TA: 5BUA: 5BUG: CG). Through mispairing, 5-bromouracil may also be incorporated into a newly synthesized DNA strand opposite guanine. In the next round of replication, 5-bromouracil may pair with adenine, leading to another transition (GC: G5BU: A5BU: AT).

B- **Alkylating agents:** chemicals that donate alkyl groups. These agents include methyl (CH3) and ethyl (CH3— CH2) groups, which are added to nucleotide bases by some chemicals.

For example, ethylmethanesulfonate (EMS) adds an ethyl group to guanine, producing 6-ethylguanine which pairs with thymine. Thus, EMS produces CG: TA transitions.

C- **Deamination:** in addition to its spontaneous occurrence, deamination can be induced by some chemicals. For instance:

1. Nitrous acid deaminates cytosine, creating uracil, which in the next round of replication pairs with adenine, producing a CG: TA transition mutation.

2. Nitrous acid changes adenine into hypoxanthine, which pairs with cytosine, leading to a TA: CG transition.

3. Nitrous acid also deaminates guanine, producing xanthine, which pairs with cytosine just as guanine does; however xanthine may also pair with thymine, leading to a CG: TA transition.

D- Hydroxylamine: is a very specific base modifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine. This conversion increases the frequency of a rare tautomer that pairs with adenine instead of guanine and leads to CG: TA transitions.

E- Oxidative reactions: reactive forms of oxygen (including superoxide radicals, hydrogen peroxide, and hydroxyl radicals) are produced in the course of normal aerobic metabolism, as well as by radiation, ozone, peroxides, and certain drugs. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes to DNA. For example, oxidation converts guanine into 8-oxy-7.8 dihydrodeoxyguanine, which frequently mispairs with adenine instead of cytosine, causing a GC: TA transversion mutation.

F- Intercalating agents: such as proflavin, acridine orange, ethidium bromide, and dioxin are about the same size as a nucleotide. They produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single-nucleotide insertions and deletions in replication. These insertions and deletions frequently produce frameshift mutations (which change all amino acids downstream of the mutation), and so the mutagenic effects of intercalating agents are often severe.

4. Radiation

A. Ionizing radiation:

The high energies of X-rays, gamma rays, and cosmic rays are all capable of' penetrating tissues and damaging DNA so greatly increase mutation rates in all organisms. They dislodge electrons from the atoms that they encounter, changing stable molecules into free radicals and reactive ions, which then alter the structures of bases and break phosphodiester bonds in DNA.

B. **Non-ionizing radiation:**

Non-ionizing radiation also frequently results in double-strand breaks in DNA. Attempts to repair these breaks can produce chromosome mutations.

UV radiation: is less energetic and therefore non-ionizing, dislodges electrons but is still highly mutagenic. Purine and pyrimidine bases readily absorb UV light, resulting in the formation of chemical bonds between adjacent pyrimidine molecules on the same strand of DNA and in the creation of structures called pyrimidine dimers. Pyrimidine dimers consisting of two thymine bases (called thymine dimers) are most frequent, but cytosine dimers and thymine– cytosine dimers also can be formed. Most pyrimidine dimers are immediately repaired by DNA repair mechanisms, but some escape repair and inhibit replication and transcription. When pyrimidine dimers block replication, cell division is inhibited and the cell usually dies; for this reason, UV light kills bacteria and is an effective sterilizing agent.

Heat: it clearly causes deamination of cytosine to form uracil, as does nitrous acid, thereby bringing about transitions from C-G to T-A. Heat also causes transversions from G-C to C-G by some unknown mechanism.

Phenotypic Effects of Mutations:

Geneticists use special terms to describe the phenotypic effects of mutations.

1. missense mutation referred to a base substitution that alters a codon in the mRNA, resulting in a different amino acid in the protein.

2. nonsense mutation changes a sense codon (one that specifies an amino acid) into a nonsense codon (one that terminates translation). If a nonsense mutation occurs early in the mRNA sequence, the protein will be greatly shortened and will usually be nonfunctional.

3. silent mutation changes the nucleotide but not the amino acid sequence because they affect the third position of the codon, which is usually less important in coding.

4. neutral mutation is a missense mutation that alters the amino acid sequence of the protein but does not change its function. Neutral mutations occur when one amino acid is replaced by another that is chemically similar or when the affected amino acid has little influence on protein function.

Major types of gene mutations and their distinguishing features (classification)

1-Gene mutations (point mutation), those that affect a single gene.

2- Chromosome mutations, those that affect the number or structure of chromosomes.

Basis of Classification	**Major Types of Mutations**	**Major features**
Origin	Spontaneous	Those that are a result of natural changes in DNA structure.
	Induced	Those that result from changes caused by environmental chemicals or radiation
Cell type	Somatic	Arise in somatic tissues (non-reproductive cells), which do not

		produce gametes
	Germ-line	Arise in cells that ultimately produce gametes (reproductive cells). These mutations can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germ-line cells.
Expression	Conditional	Expressed only under restrictive conditions (such as high or low temperature)
	Unconditional	Expressed under any conditions
Effect on Function	Loss-of -function	Eliminates normal function
	Hypomorphic	Reduces normal function
	Hypermorphic	Increases normal function
Type of Molecular	1–Substitution	One base pair in duplex DNA replaced with a different base pair
	2-Frameshift	Shifts triplet reading of codons out of the correct phase.

Here, we will categorize mutations primarily on the basis of the type of molecular change in DNA sequence:

1. **Base substitutions**

The simplest type of gene mutation is a base substitution, the alternation of a single nucleotide in the DNA. Because of the complementary nature of the two DNA strands, when the base of one nucleotide is altered, the base of the corresponding nucleotide on the opposite strand also will be altered in the next round of replication. A base substitution therefore usually leads to a base-pair substitution.

Base substitutions are of two types:

In a transition, a purine is replaced by a different purine or, alternatively, a pyrimidine is replaced by a different pyrimidine.

In a transversion, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine.

2. **Frameshift mutation**

Arise from the insertion or deletion of individual nucleotides and cause the rest of the message downstream of the mutation to be read out of phase, producing an incorrect protein from then on.

DNA Repair Systems

We can divide "repair" mechanisms into 3 categories:

1. **Damage reversal**--simplest; enzymatic action restores normal structure without

breaking backbone.

2. Damage removal--involves cutting out and replacing a damaged or inappropriate base or section of replacing nucleotides.

3. Damage tolerance--not truly repair but a way of coping with damage so that life can go on.

1. Damage reversal

a. Photoreactivation:

This is one of the simplest and perhaps oldest repair systems: it consists of a single enzyme (photolyase enzyme) which can split pyrimidine dieters (break the covalent bond) in presence of light. It is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans).

b. Ligation of single-strand breaks:

X-rays and some chemicals like peroxides can cause breaks in the backbone of DNA. Simple breaks in one strand are rapidly repaired by DNA ligase.

2. Damage removal

a. Base excision repair

In base-excision repair, modified bases are first excised and then the entire nucleotide is replaced. The excision of modified bases is catalyzed by a set of enzymes called DNA. glycosylases, each of which recognizes and removes a specific type of modified base by cleaving the bond that links that base to the 1-carbon atom of deoxyribose. After the base has been removed, an enzyme called endonuclease cuts the phosphodiester bond, and other enzymes remove the deoxyribose sugar. DNA polymerase then adds new nucleotides to the exposed 3-OH group, replacing a section of nucleotides on the damaged strand. The nick in the phosphodiester backbone is sealed by DNA ligase, and the original intact sequence is restored.

b. Mismatch repair

Replication is extremely accurate: each new copy of DNA has only one error per billion nucleotides. Many incorrectly inserted nucleotides that escape detection by proofreading are corrected by mismatch repair. It is carried out by a group of proteins which can scan DNA and look for incorrectly paired bases (or unpaired bases) which will have aberrant dimensions in the double helix. After the incorporation error has been recognized, mismatch-repair enzymes cut out the distorted section of the newly synthesized strand and fill the gap with new nucleotides, by using the original DNA strand as a template.

c. Nucleotide excision repair

The nucleotide excision repair removes bulky DNA lesions that distort the double helix, such as pyrimidine dimmers. Nucleotide-excision repair can repair many different types of DNA damage. It is found in cells of all organisms from bacteria to humans and is one of the most important of all repair mechanisms.

The process of nucleotide excision is complex; in humans, a large number of genes take part. First, a complex of enzymes scans DNA, looking for distortions of its three-dimensional configuration. When a distortion is detected, additional enzymes separate the two nucleotide strands at the damaged region, and single-strand-binding proteins stabilize the separated strands. Part of the damaged strand is peeled away and the gap is

filled in by DNA polynierase and sealed by DNA ligase.

Sunlight includes a strong UV component, so exposure to sunlight produces pyrimidine dimers in the DNA of skin cells. Although human cells lack photolyase (the enzyme that repairs pyrimidine dimers in bacteria), most pyrimidine dimers in humans can be corrected by nucleotide excision repair. However, the cells of most people with xeroderma pigmentosum are defective in nucleotide excision repair, and many of their pyrimidine dimers go uncorrected and may lead to cancer.

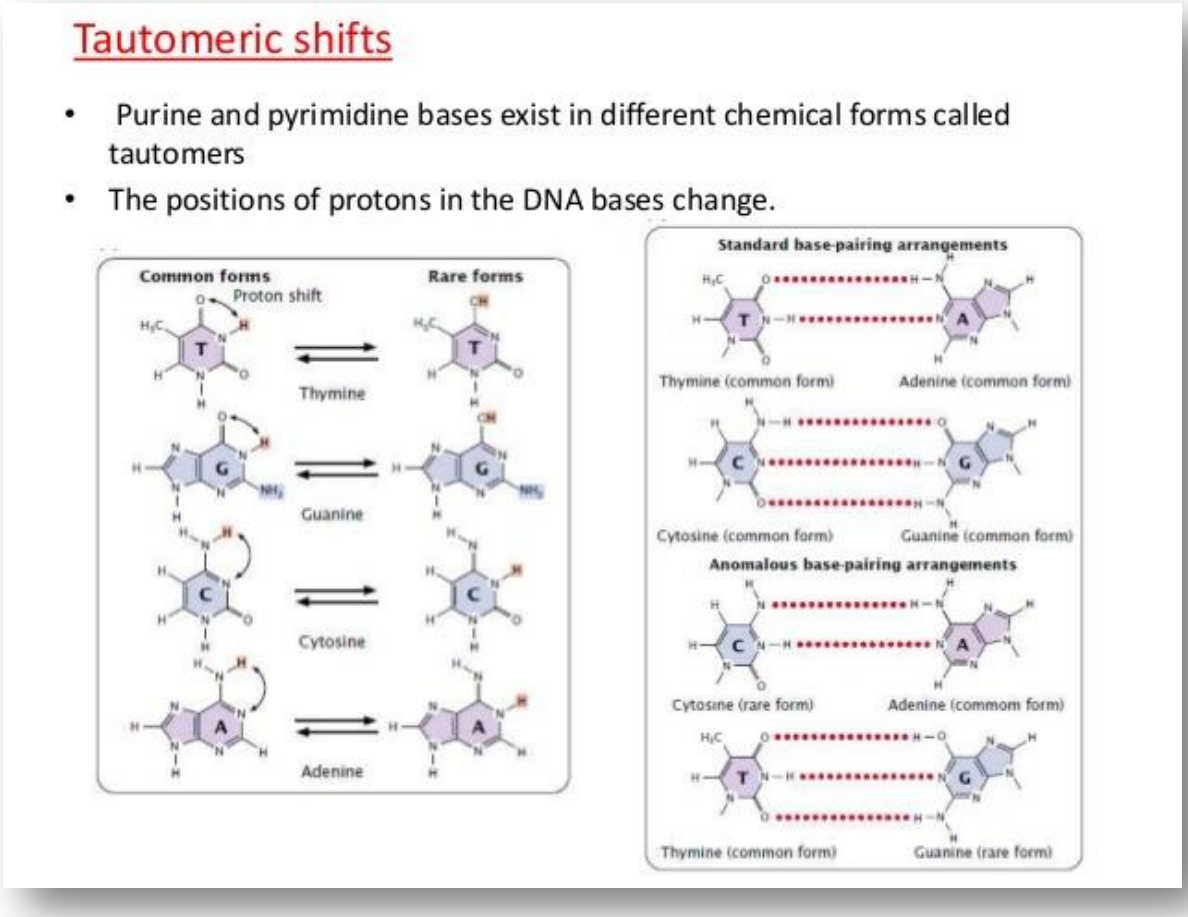
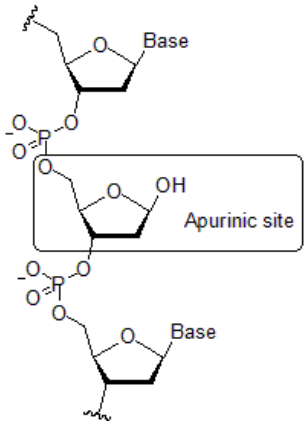
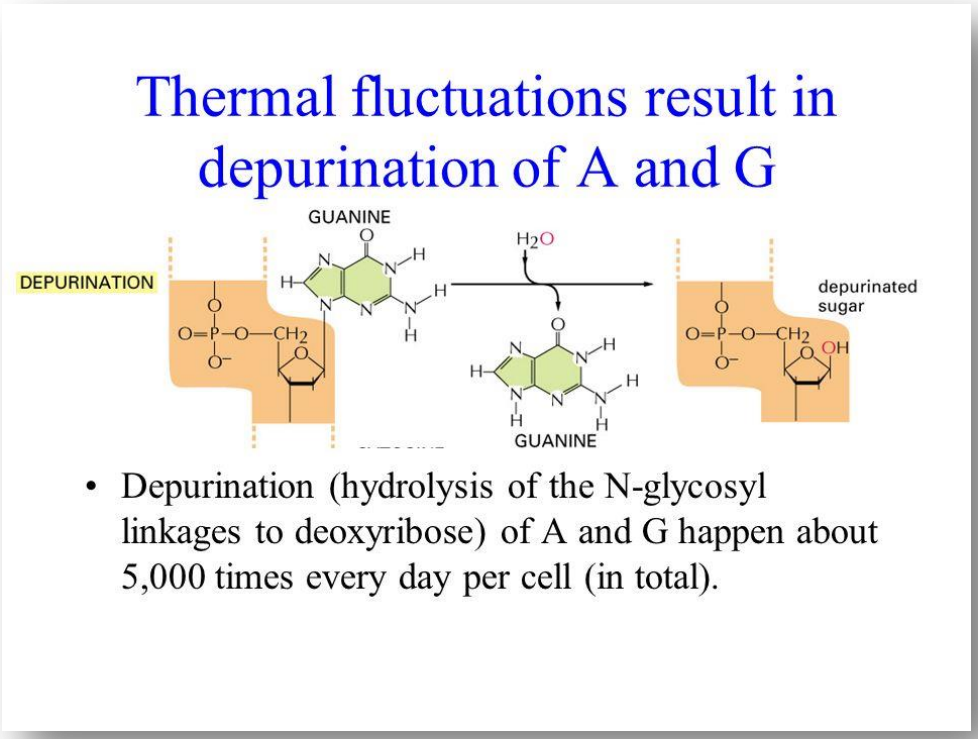


Fig. 82: Tautomeric shifts



(a)



(b)

Fig. 83 (a,b): Depurination

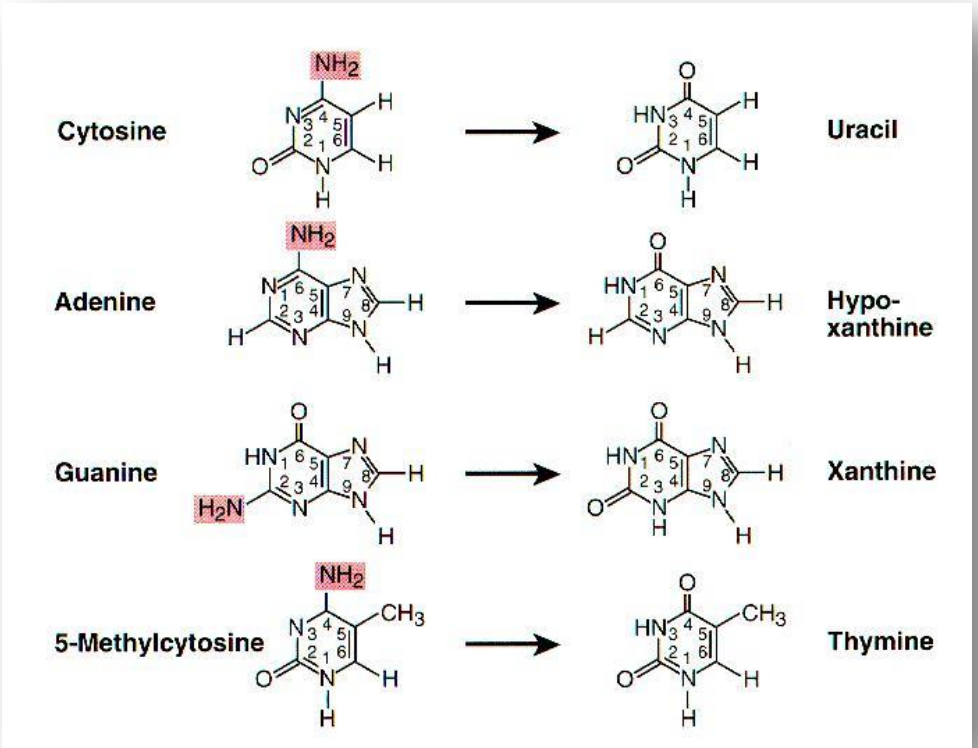


Fig. 84: Deamination of nitrogenous bases

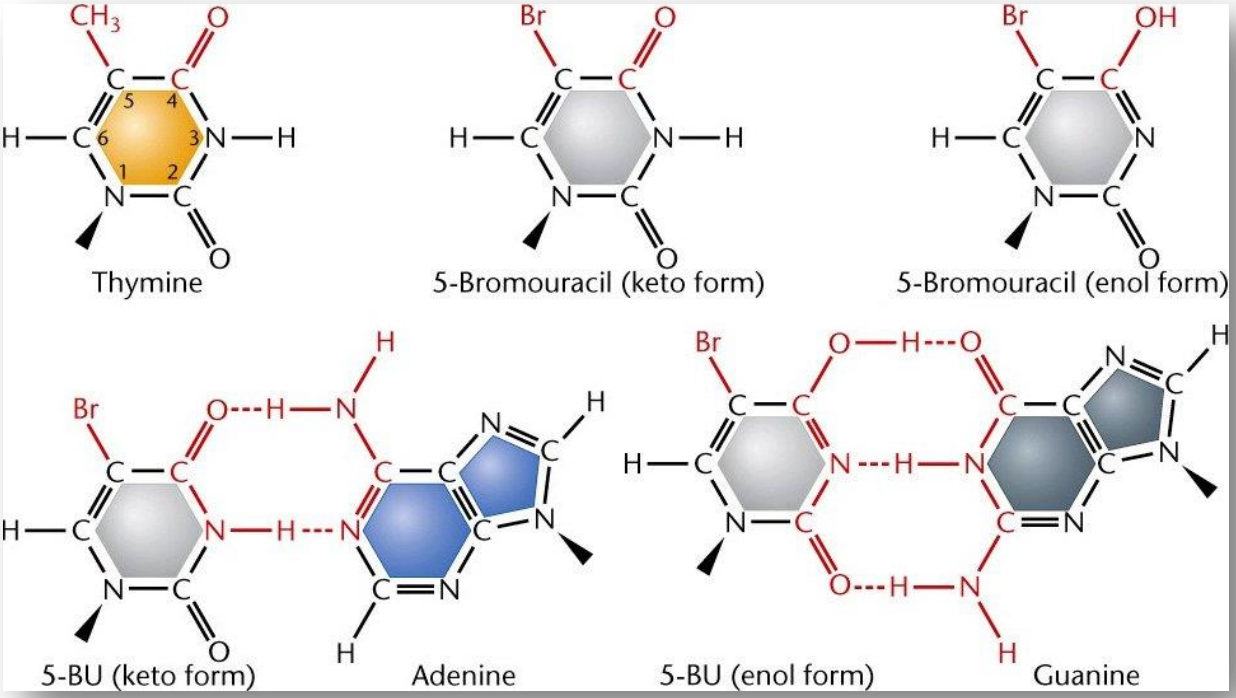


Fig. 85: Base analogs

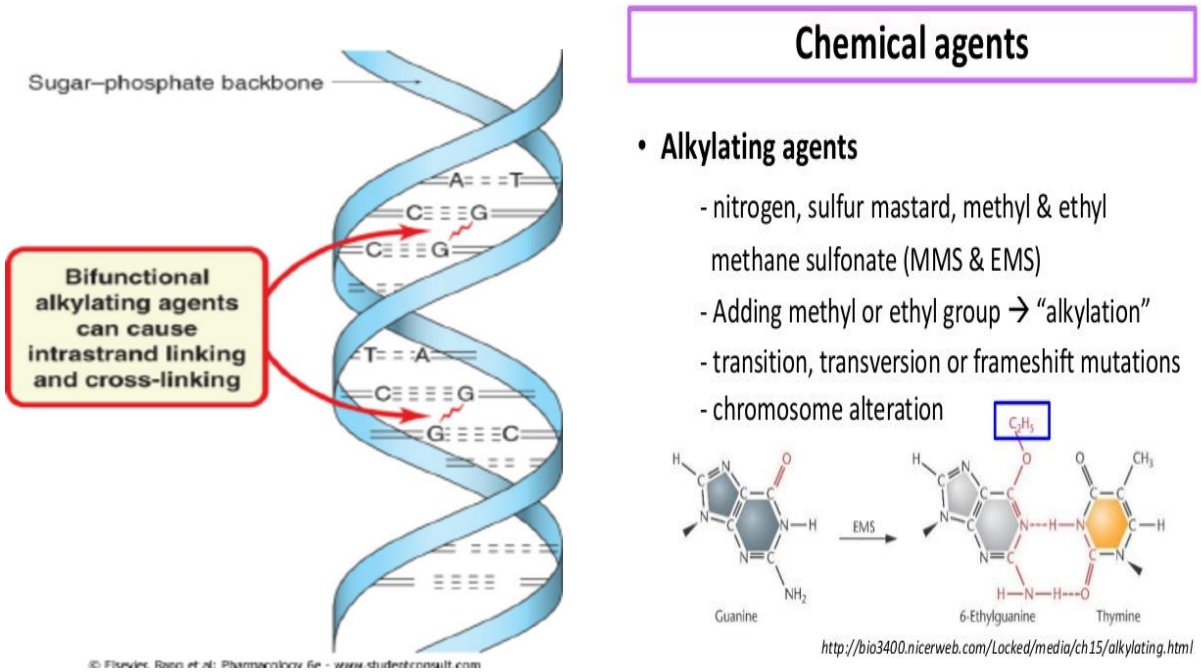


Fig. 86: Alkylating agents

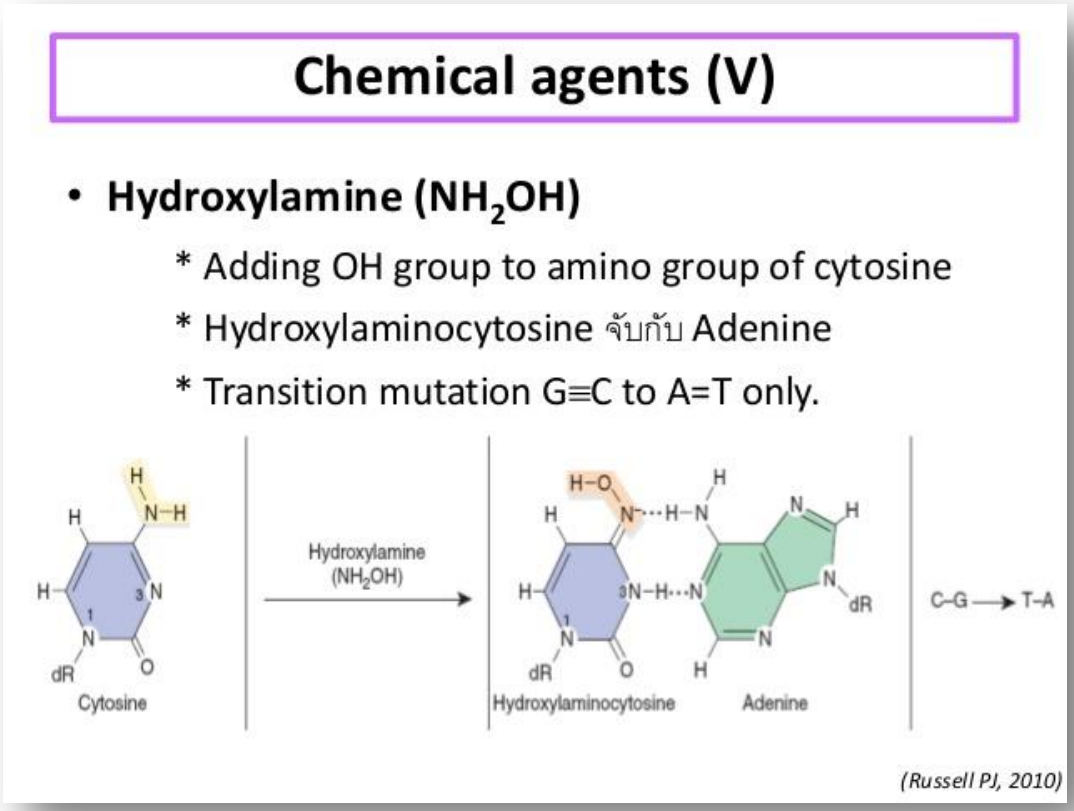


Fig. 87: Hydroxyamine

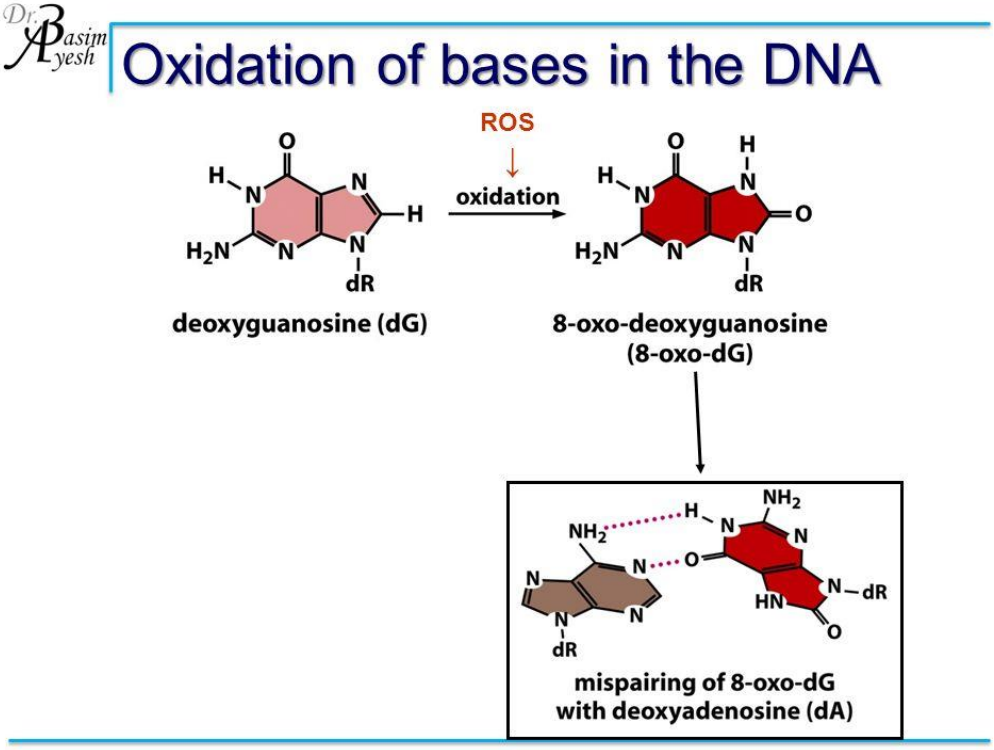


Fig. 88: Oxidation of bases

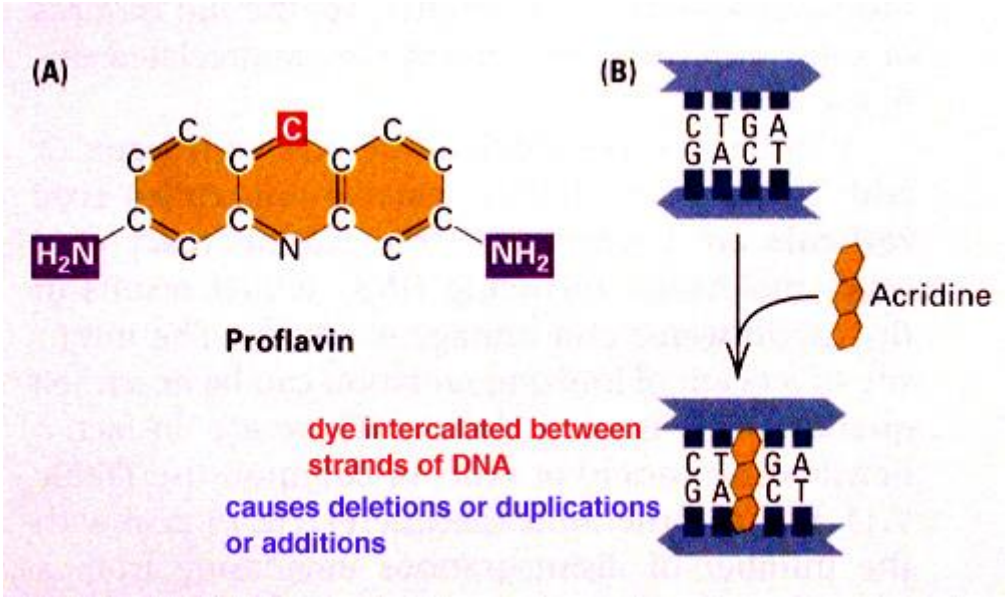


Fig. 89: Intercalating agents

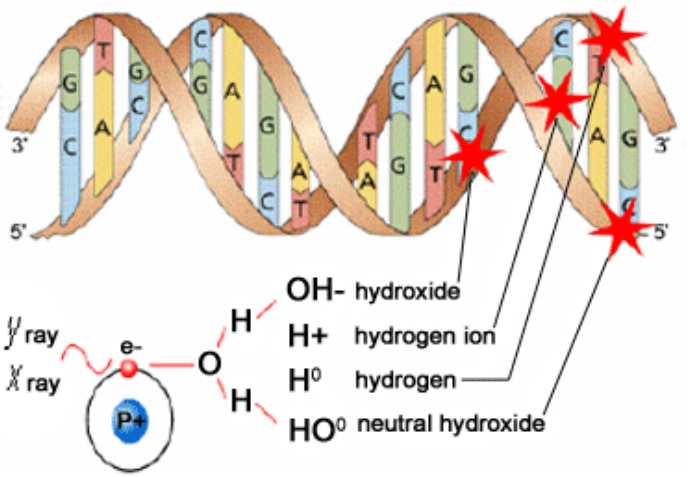


Fig. 90: Ionizing radiation

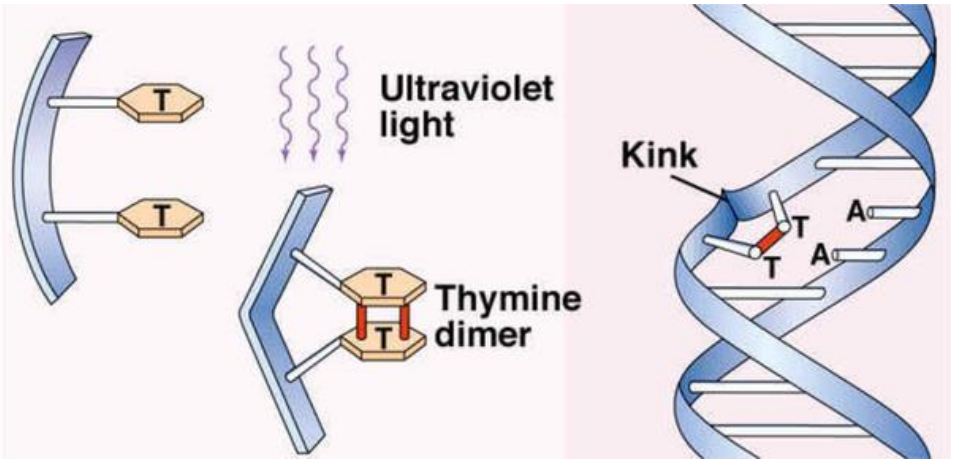


Fig. 91: Non-ionizing radiation

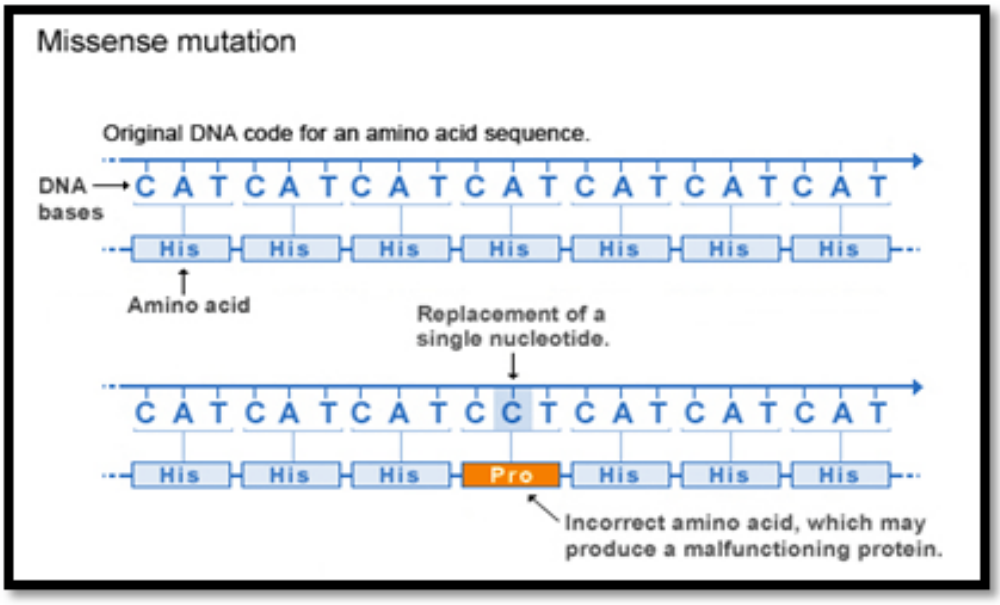


Fig. 92: Missense mutation

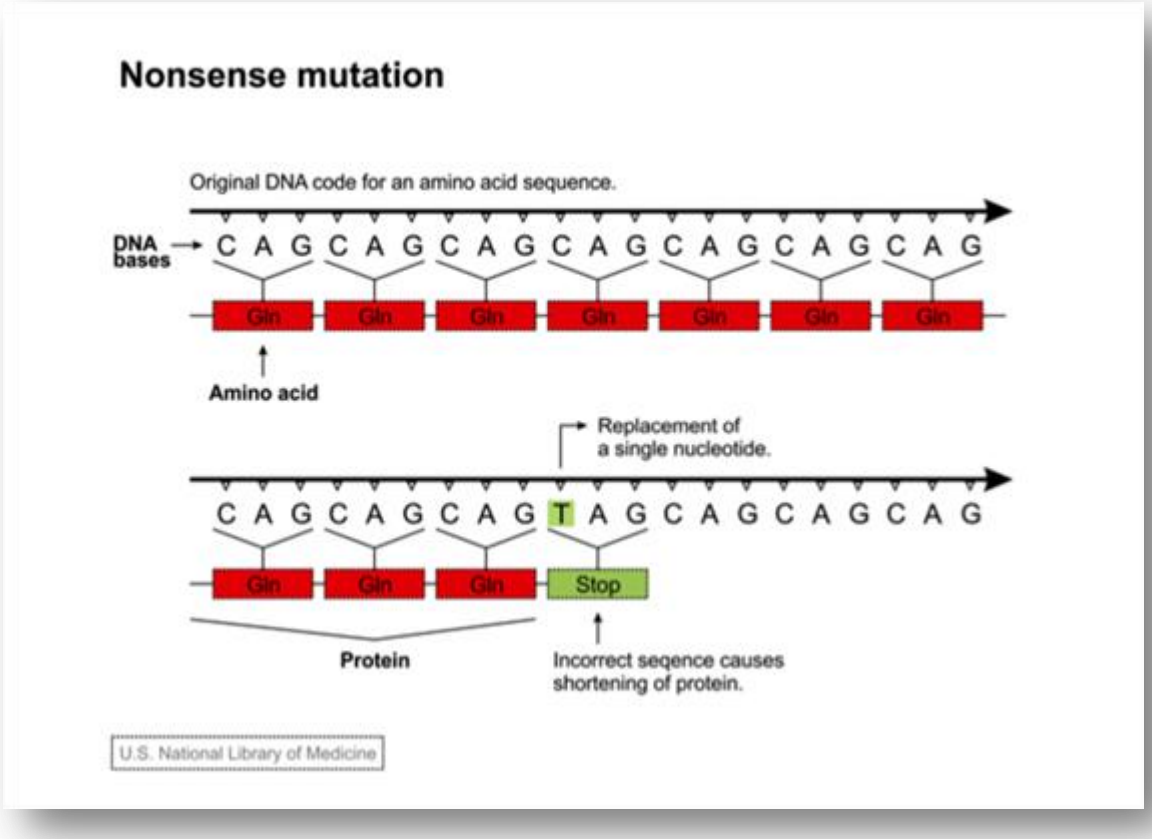


Fig. 93: Nonsense mutation

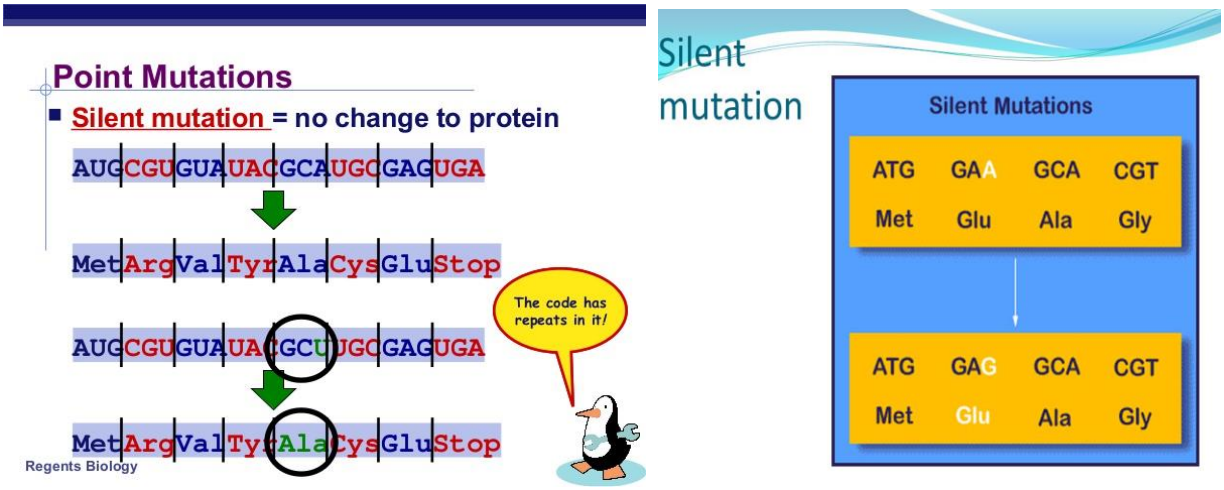


Fig. 94: Silent mutation

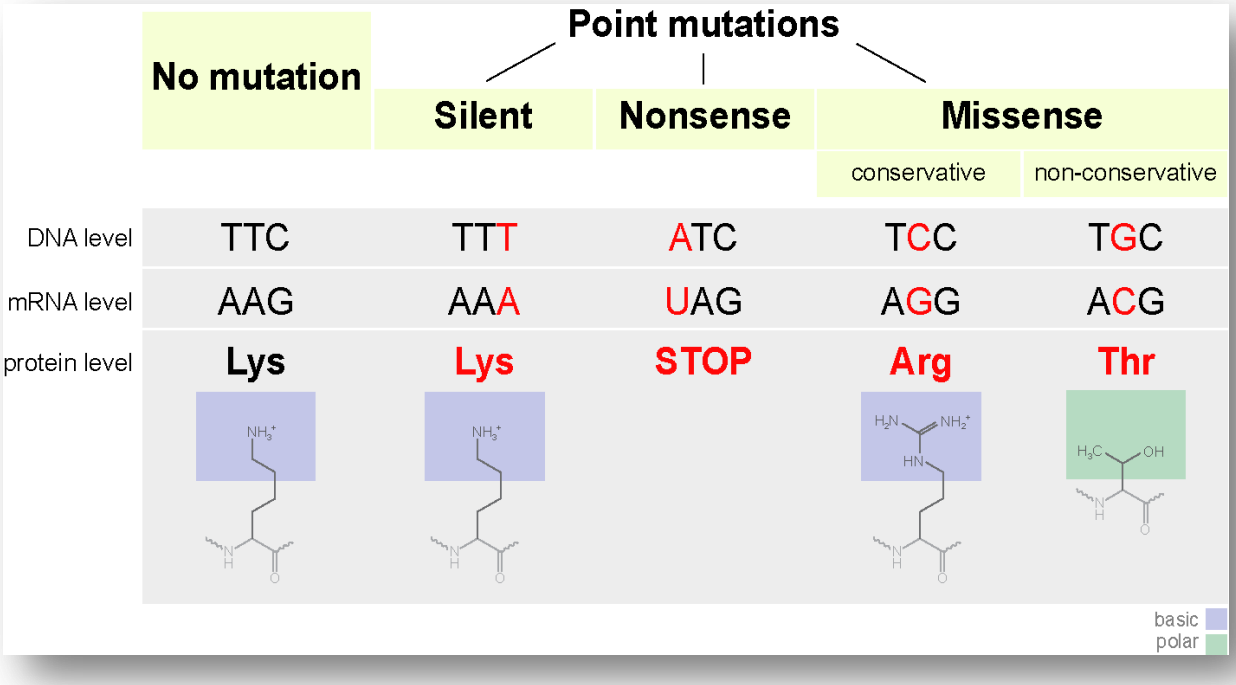


Fig. 95: Phenotypic effect of mutation

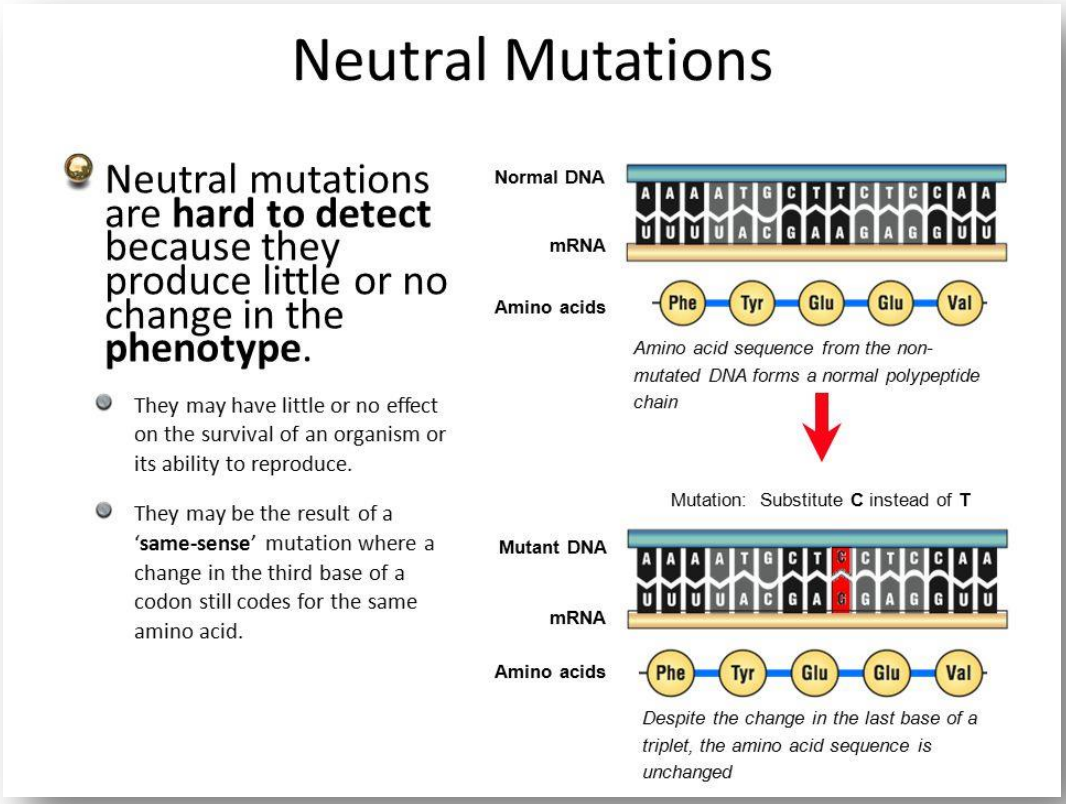
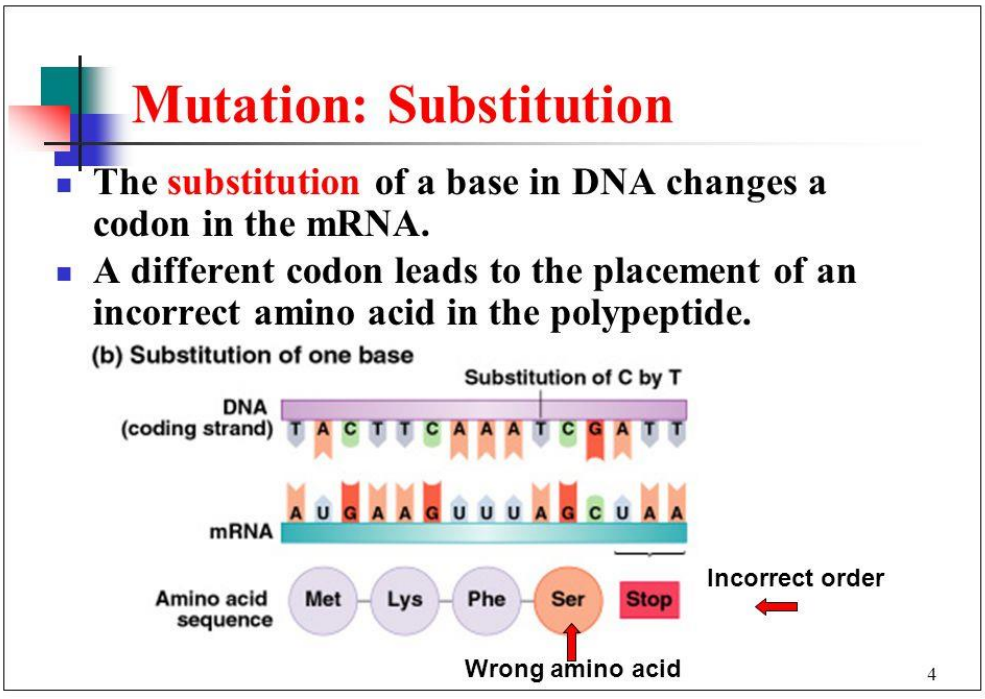
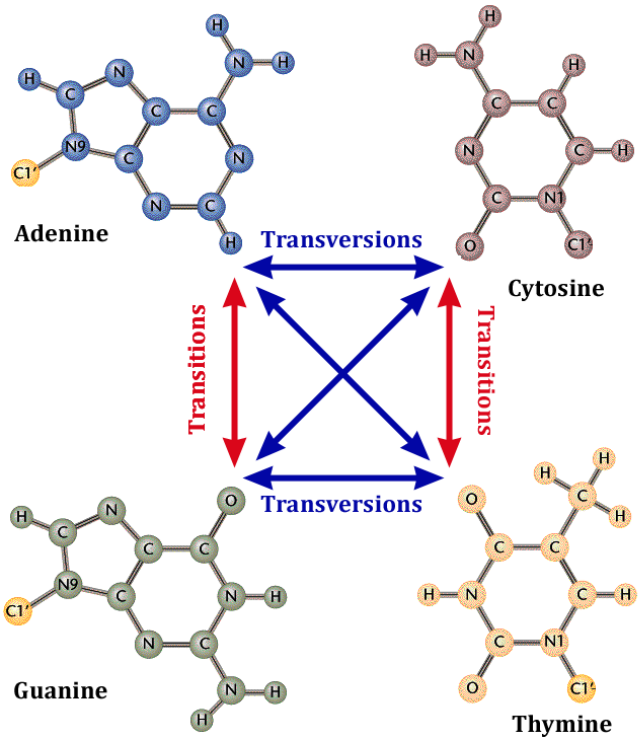


Fig. 96: Neutral mutation



(a)



(b)

Fig. 97 (a,b): Base substitution mutation

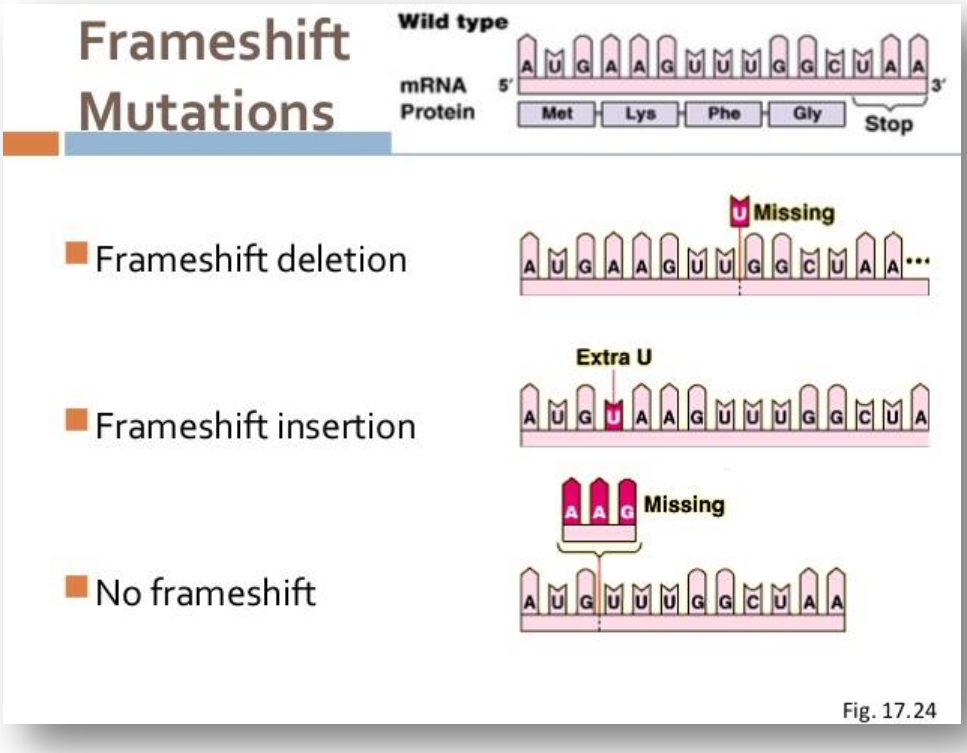


Fig. 98: Base substitution mutation

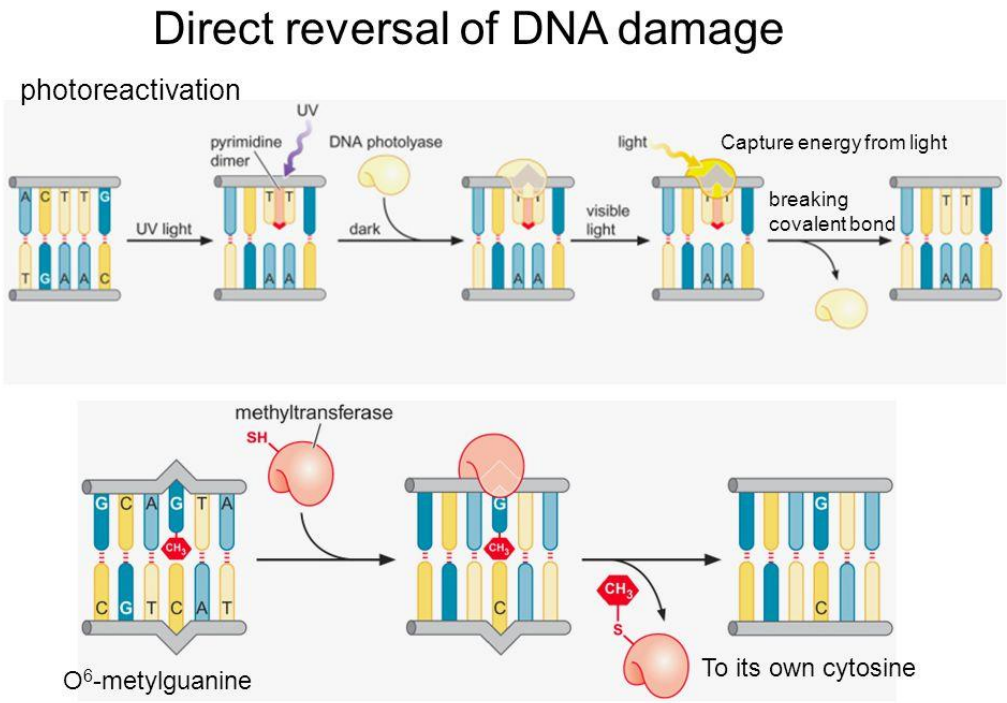


Fig. 99: Repair of DNA by damage reversal

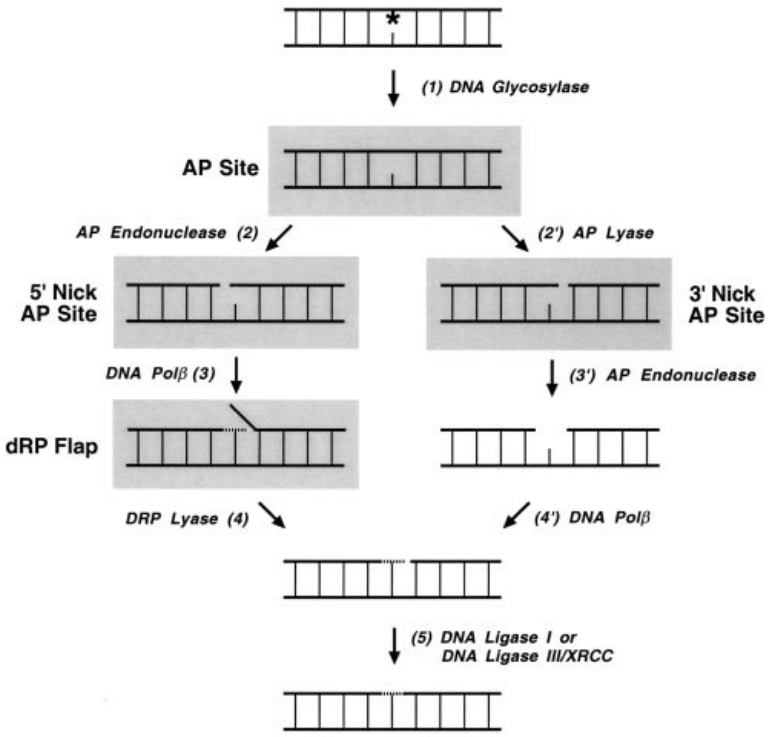


Fig. 100: Repair of DNA by base excision repair

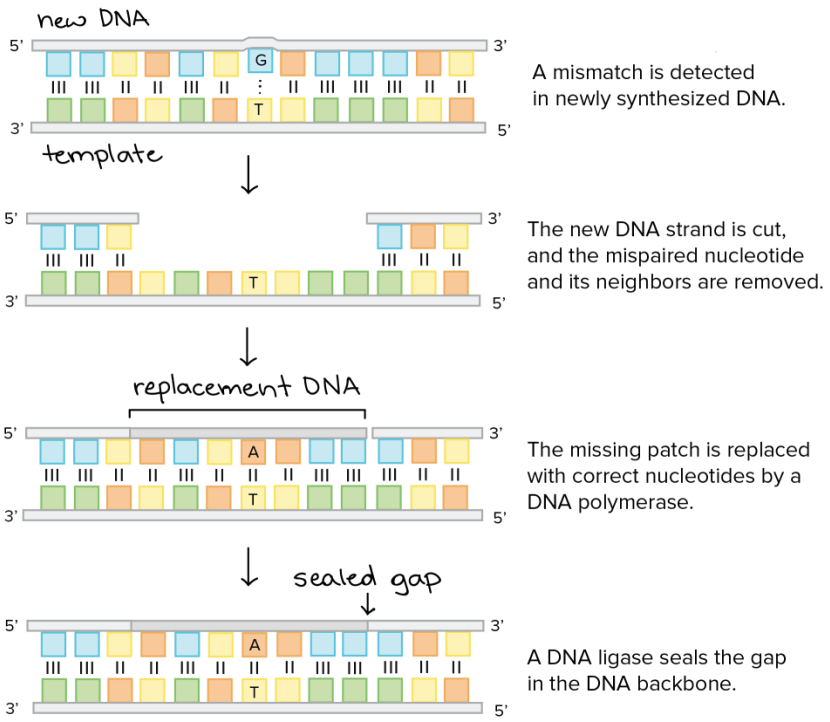


Fig. 101: Mismatch repair of DNA

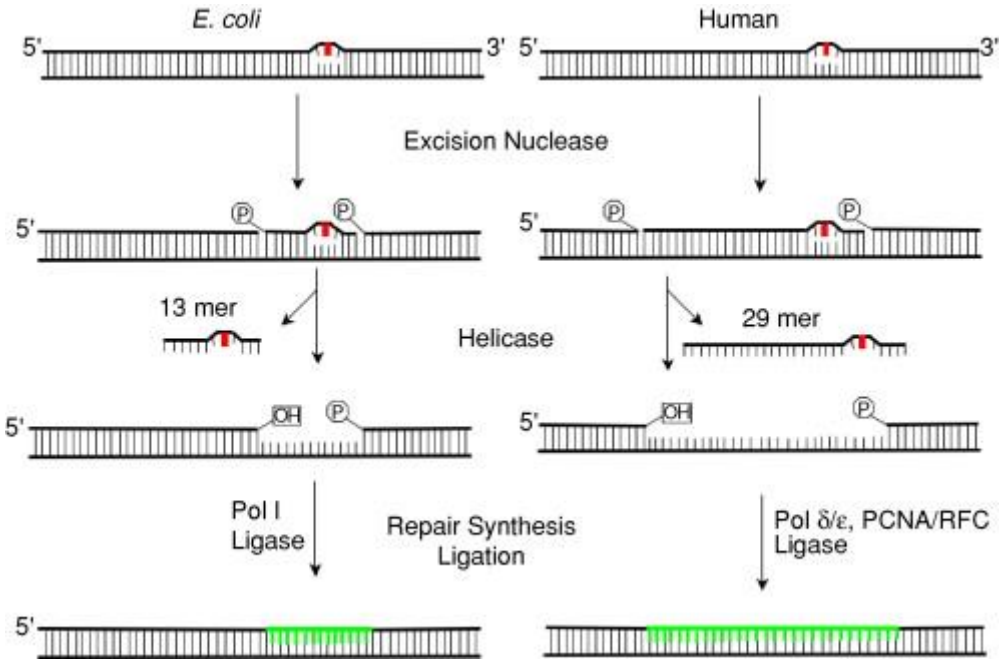


Fig. 102: Repair of DNA by nucleotide excision repair

Nucleic Acid Hybridization

I- Southern blotting:

- The Southern blot is used to detect the presence of a particular DNA fragment in a sample.
- The DNA detected can be a single gene, or it can be part of a larger piece of DNA such as a viral genome.

Principle

The key to this method is hybridization.

Hybridization here means: It is the process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target DNA.

There are 2 important features of hybridization-.

- The reactions are specific-the probes will only bind to targets with a complementary sequence.
- The probe can find one molecule of a target in a mixture of millions of related but non-complementary molecules.

Summary of procedure

1. Extract and purify DNA from cells
2. DNA is restricted with enzymes
3. Separated by electrophoresis
4. Denature DNA
5. Transfer to nitrocellulose paper
6. Add labeled probe for hybridization to take place
7. Wash off the unbound probe
8. Autoradiograph

Application

- To identify specific DNA in a DNA sample
- To Isolate desired DNA for construction of rDNA
- Identify mutations, deletions, and gene rearrangements
- Used in the prognosis of cancer and in prenatal diagnosis of genetic diseases
- In RFLP
- Used in phylogenetic analysis
- Diagnosis of HIV-1 and infectious disease
- In DNA fingerprinting:
- Paternity and Maternity Testing
- Criminal Identification and Forensics

- Personal Identification

2. Northern Blotting

The Northern blot is used to detect the presence of a particular mRNA in a sample.

Principle

The key to this method is hybridization.

Hybridization here means: It is the process of forming a double-stranded DNA-RNA hybrid molecule between a single-stranded DNA probe and a single-stranded target RNA. There are 2 important features of hybridization:

- The reactions are specific-the probes will only bind to targets with a complementary sequence.
- The probe can find one molecule of a target in a mixture of millions of related but non-complementary molecules.

Summary of procedure

1. Extract and purify mRNA from cells
2. Separated by gel electrophoresis
3. This gel is immersed in depurination buffer for 5-10 minutes and washed with water
4. Transfer to aminobenzyloxymethyl filter paper. The transfer of RNA from gel to membrane is called blotting
5. After transfer, the membrane is baked at 800C
6. Add labeled DNA probe for hybridization to take place
7. Wash off the unbound probe

S. Autoradiograph to detect mRNA –DNA hybrid

Applications

- Detecting a specific mRNA in a sample.
- Used in the screening of recombinants by detecting the mRNA produced by the transgene.
- In disease diagnosis.
- In gene expression studies.

Difference between Northern and Southern blotting (Southern vs Northern blotting)

Southern blotting	Northern blotting
Southern name of inventor	Northern a misnomer
Separation of DNA	Separation of RNA
Denaturation needed	Denaturation not needed
Nitrocellulose filter membrane	Amino benzyloxymethyl filter paper Membrane
DNA-DNA Hybridization	RNA-DNA Hybridization

3. Western Blotting:

Definition

A technique for detecting specific proteins separated by electrophoresis by the use of labelled antibodies.

Principle

Antigen-antibody interaction, an immunodetection method.

Summary of Procedure

1. Extract and purify protein from cells.

2. Separated by SDS-PAGE (Sodium dodecyl sulphate-Poly Acrylamide Gel Electrophoresis) Function of SDS: SDS is an anionic detergent. Here it denatures protein and impart an overall negative charge. Therefore separation is based on size.

3. Transfer proteins from gel to nitrocellulose paper.

4. Blocking nonspecific antibody sites on the nitrocellulose paper with bovine serum albumin (BSA) or milk powder.

5. Probing electroblotted proteins with primary antibody.

6. Washing away nonspecifically bound primary antibody.

7. Detecting bound antibody by horseradish peroxidase-anti-Ig conjugate or formation of a diaminobenzidine (DAB) precipitate, radiolabelling or use of fluorescently labelled secondary antibody.

8. Autoradiography or photographing or fluorescence detection.

Different samples of equal quantity are loaded in each lane. From the blot it is clear that protein expression (40,000 MW) is more in lane 4 and 5.

Applications

- Highly sensitive method to detect a specific protein even in very low quantity.
- Used in clinical diagnosis
- Quantifying a gene product (gene expression studies)

4. Dot Blot Technique:

Definition:

Non fractionated or non-electrophoresed samples are directly blotted and immobilized on a nitrocellulose or nylon membrane as dots or spots for hybridization.

Advantage over Southern blotting:

Sample from different sources can be tested in a single run Principle:

The key to this method is hybridization.

Hybridization: It is the process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target DNA.

There are 2 important features of hybridization:

- The reactions are specific-the probes will only bind to targets with a complementary sequence.

- The probe can find one molecule of a target in a mixture of millions of related but non-complementary molecules.

Summary of Procedure:

1. Extract and purify DNA or RNA from different sources.
2. Apply directly as small dots on nitrocellulose or nylon membrane.
3. If DNA, denature it with mild alkali treatment to form single strands.
4. Immobilize by baking at 70-80 C for, 2-3 H.
5. Add a labeled probe for hybridization to take place.
6. Wash off the unbound probe.
7. Autoradiograph.

Application:

- Detection of specific DNA or RNA in a sample without the step of electrophoresis.
- Many samples can be screened in a single run.

5. Southwestern blot:

Southwestern blotting, involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA) by their ability to bind to specific oligonucleotide probes. The proteins are separated by gel electrophoresis and are subsequently transferred to nitrocellulose membranes similar to other types of blotting.

The name southwestern blotting is based on the fact that this technique detects DNA-binding proteins, since DNA detection is by Southern blotting and protein detection is by western blotting.

6. Northwestern blot

The Northwestern blot, also known as the Northwestern assay, is a hybrid analytical technique of the Western blot and the Northern blot, and is used in molecular biology to detect interactions between RNA and proteins.