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Mutations: A View at Molecular Level

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ABSTRACT: Mutations play an important role in genetic variation and evolutionary process. Mutations are classified in number of ways, depending upon their origin, cells involved, effect on function, etc. The role of mutations in practical biology is evident when mutations are induced in bacteria for environmental, industrial and medical purposes. Therefore, understanding the basics of mutations, its classification and its importance in the fields such as evolutionary biology, microbial biotechnology, genetics, synthetic biology, etc. is important.

Keywords: Mutations, bacterial genetics, mutant, microbial mutation.

INTRODUCTION

A mutation is a change or alteration in the genetic composition (nucleotide sequences) brought on by transposition, replication mistakes, or exposure to mutagens. Evolution is driven by mutation, which is a powerful force. Species populations undergo sequence changes that allow them to adapt to changes in their surroundings.

The implications of mutations vary greatly depending on their size and level of occurrence. They have the ability to change anything from a single base pair to a large chromosomal section that contains numerous genes. While certain mutations are passed on to the following generation, others are restricted to the organism that contains them. Mutations must impact the genetic material and arise in germline cells in order to be passed on to the following generation. DNA mutations in germline cells can either have positive or negative impacts on phenotypic, or they can have no effect at all, making them neutral mutations. Positive selection fixes beneficial mutations in populations fast, while purifying or negative selection quickly removes other mutations with a bad phenotype from the population.

The top six applications of microbial mutations along with the examples are highlighted in the points that follow:

1. Determining Gene Function : Researchers can determine the function of a gene by causing mutations in particular genes and tracking the phenotypic changes that ensue. Disabling a gene (e.g., deleting recA) can show its role in DNA repair.

2. Understanding of Metabolic Pathways in Microorganisms : Three genes—galK, galT, and galE—are responsible for the breakdown of galactose in cells. By examining three different kinds of mutant cells (galK$^-$, galT$^-$, and galE$^-$), each lacking one of these genes, researchers were able to learn more about the functions of galactose. They traced the metabolic route using radioactive galactose (~14C-Gal).

Three radioactive substances are present in the medium at the beginning of the experiment:

14C-Galactose-1-phosphate (Gal-1-P), 14C-Uridine diphosphate galactose (UDP-Gal) and 14C-Uridine diphosphate glucose (UDP-Glc). Because galK$^-$ mutants are unable to convert galactose into Gal-1-P, galactose builds up. Mutants of galT$^-$ can produce Gal-1-P but are unable to further convert it, which causes Gal-1-P to accumulate. The accumulation of UDP-Gal in galE$^-$ mutants indicates that they are unable to convert it into product X.

This indicates the metabolic pathway follows these steps:

Galactose → (via **galK**) → **Galactose-1-phosphate** → (via **galT**) → **UDP-Galactose** → (via **galE**) → **Product X**

3. Understanding The Metabolic Regulation : Numerous bacterial mutants that exhibit variations in the quantity of a specific protein and their reactions to outside stimuli have been identified. Normally, the enzymes made by the **galK**, **galT**, and **galE** genes are not present in the bacteria unless **galactose** is added to the growth medium. However, some mutants make these enzymes all the time, even when galactose is not present. This suggests that a **regulatory gene** controls whether the enzymes are turned on or off. Thus,

understanding how metabolic pathways are impacted by mutations might help one better understand how the pathways are regulated and controlled.

4. For Matching a Biochemical Entity with a Biological Function : DNA polymerase is an enzyme that *E. coli* generates and aids in the synthesis of DNA. At first, researchers thought that DNA synthesis in *E. coli* was carried out by DNA polymerase I. But in a Pol A mutant (a strain with 50 times less DNA polymerase I activity) scientists found two more enzymes: DNA polymerase II and DNA polymerase III. These enzymes were also capable of synthesizing DNA. Thus, by studying how mutations impact cellular processes, it is possible to connect particular biochemical activity with their associated biological roles.

5. For Assessing the Targets of External Agents: Rifampicin is an antibiotic known to inhibit RNA synthesis. In the beginning it was unknown **exactly how rifampicin stops RNA production**. Two main possibilities were considered: **Does it block the DNA directly? Or does it interfere with RNA polymerase**, the enzyme that makes RNA from DNA? Researchers looked at bacterial mutants that had developed rifampicin resistance, meaning the antibiotic no longer had any effect on them. Two kinds of resistant mutants were found:

Cell Wall Mutants- Rifampicin was unable to penetrate the cell of these bacteria due to modifications in their cell wall. Resistance resulted from decreased drug entrance rather than a modification in the drug's mechanism of action.

RNA Polymerase Mutants- These had mutations in the RNA polymerase gene (rpoB). Rifampicin was no longer able to attach to RNA polymerase due to a small alteration in its structure. But the RNA polymerase continued to function, although without being inhibited by rifampicin.

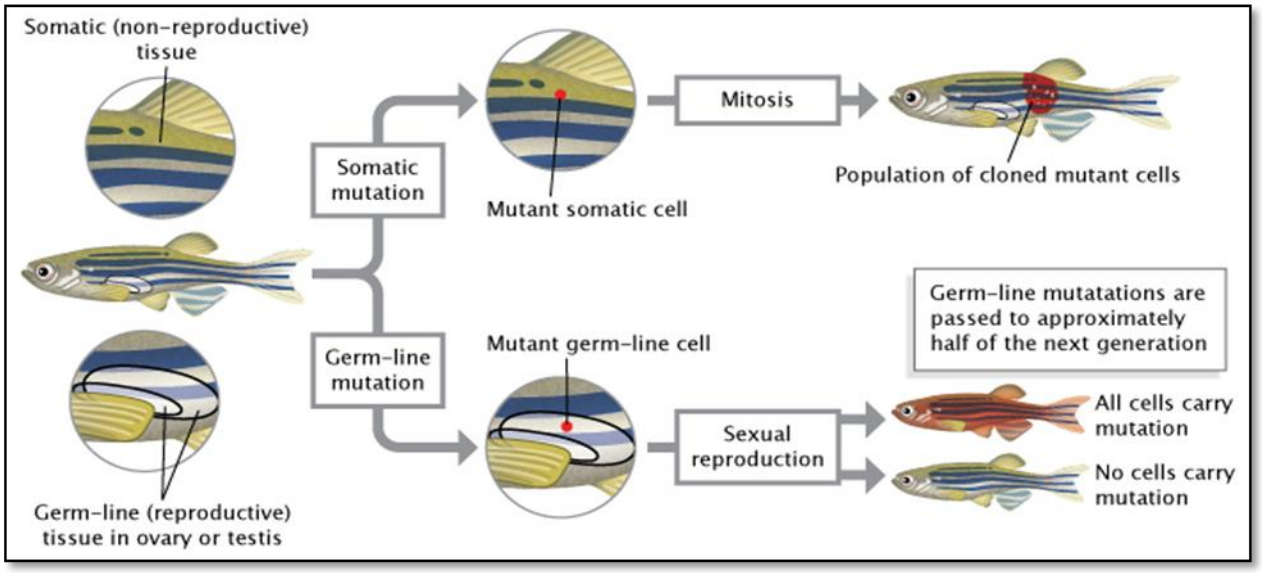
So, mutations helped scientists pinpoint RNA polymerase as the exact target of rifampicin.

6. Production of Useful Products: Mutations can enhance the **production capacity** of microbes for valuable substances like **Antibiotics** (e.g., penicillin from *Penicillium*), **Amino acids** (e.g., glutamic acid from *Corynebacterium glutamicum*) etc. Mutations can change the structure of microbial enzymes, making them m**ore stable** (e.g., work at high temperature or extreme pH) and faster acting.

Classification of mutation

1. Based on type of cell involved:

- Germline mutations: These mutations occur in the body's germ tissues (one that produce gametes). These mutations are inherited and passed on to offspring.
- **Somatic mutations: These** mutations occur in body (somatic) cells, not in reproductive cells. These mutations pass to daughter cells during mitosis, but they are not inherited.



2. Based on the mode of origin

- **Spontaneous mutations: These are natural errors occuring during normal cellular processes such as DNA replication or repair, without any external cause (like radiation or chemicals).**
- **Induced mutations: These mutations are are deliberate changes in DNA caused by external agents i.e. mutagenic agents which may be physical (radiations) or chemical (base analogues) in nature.**

3. Based on the Direction of Mutation

- **Forward mutations: These types of mutations** convert a wild-type allele to a *mutant* allele (abnormal phenotype).
- **Reverse or back mutations: These mutations undo the forward mutations bringing mutant allele back to its wild form. These are normally rarer than forward mutations.**

4. Based on how mutations are detected

- **Morphological mutations: These mutations** involve changes in the physical appearance of an organism including colour, shape, size, etc.
- **Lethal mutations: These mutations affect the viability and kill the individual that carries it.**
- **Biochemical mutations: These mutations may not be visible but have a general affect on the ability to grow and proliferate.** Biochemical mutants need a

specific nutrient supplementation for its growth on a media.

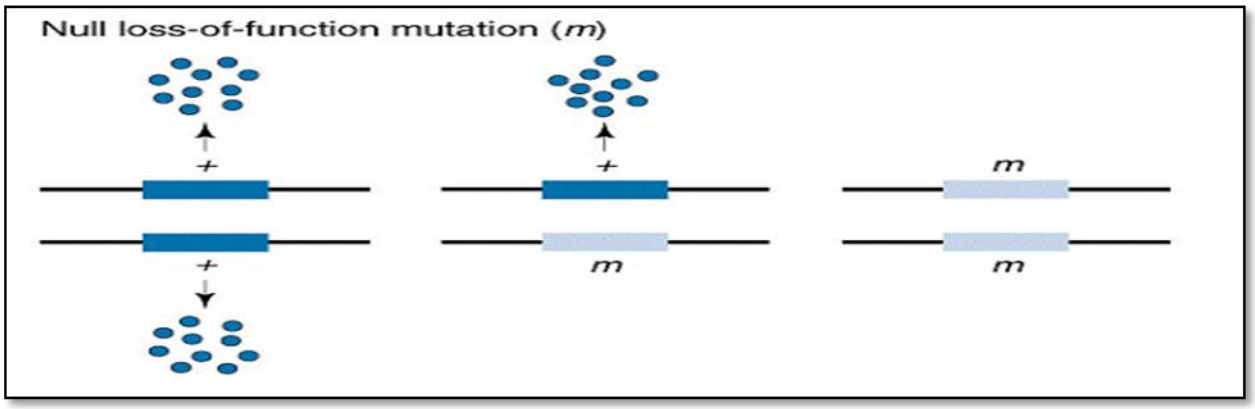
• **Resistant mutations: These types of mutations allow an organism (usually a microbe) to survive or grow in the presence of harmful substances to which the wild type is susceptible. These mutations make the organism resistant to something that would normally kill or inhibit it.**

• **Conditional mutations: Here, the mutant phenotype is** expressed only under certain conditions known as restrictive conditions. Once the permissive or natural conditions are restored the mutant expresses normal or wild phenotype.

5. Based on effect on function

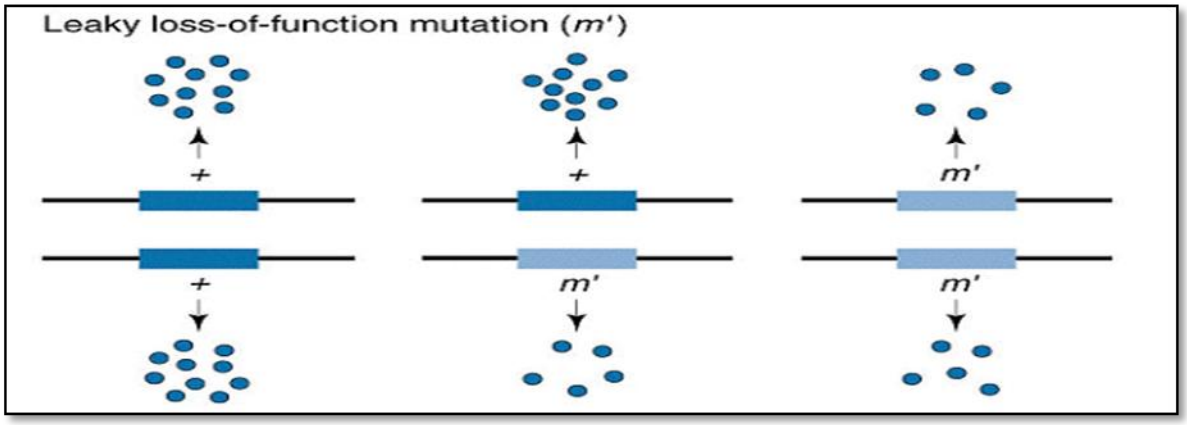
• Loss of function mutations: These are also known as inactivating mutations which destroy the function of gene product.

— Null mutation: This loss of function mutation involves complete inactivation of gene product.



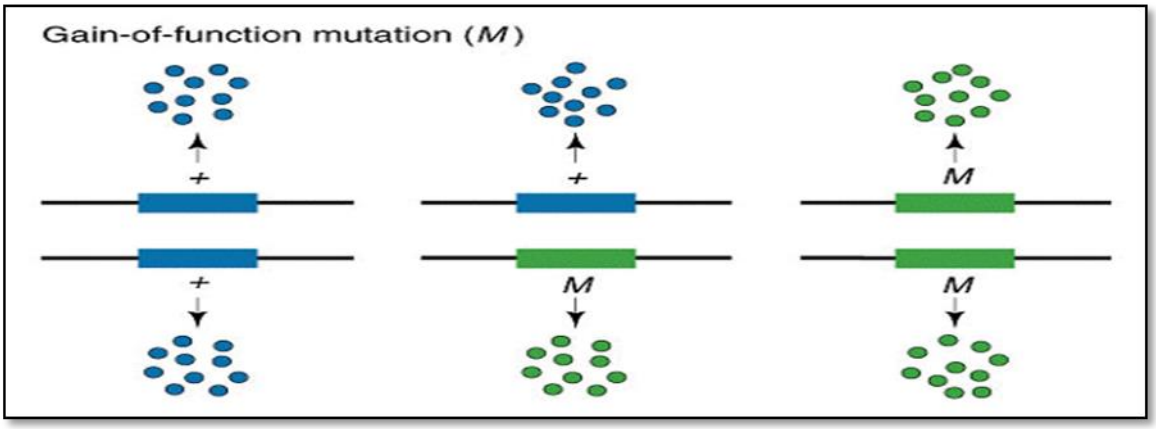
(Griffiths *et al*., 1996. An Introduction to Genetic Analysis, 6th edition)

— Leaky mutation: This loss of function mutation involves incomplete inactivation of gene product.



(Griffiths *et al*., 1996. An Introduction to Genetic Analysis, 6th edition)

• Gain of function mutations: These are the dominant mutations producing a new function for the gene product.



(Griffiths *et al*., 1996. An Introduction to Genetic Analysis, 6th edition)

6. Based on the Magnitude of Phenotypic Effect

• **Dominant mutations:** These mutations produce dominant phenotypic expressions.

• **Recessive mutations: These** mutations are recessive in nature and are not expressed phenotypically immediately.

• **Isoalleles: These** mutations slightly alter the phenotype of an organism and can be detected only by special techniques.

7. Based on structure involved in mutation

• **Gene mutation: These mutations involve** altering of sequence of the nucleotides within a part of the DNA molecule.

• **Chromosomal mutation: These mutations involve changes in** segments of chromosomes, whole chromosomes, or entire sets of chromosomes.

Chromosomal vs gene mutation

Chromosomal mutations are alterations that affect large segments of chromosomes or whole chromosomes rather than just single genes. These mutations can change the structure or number of chromosomes.

—Alteration or abnormality in structure of chromosome may be due to any of the following:

• Translocation

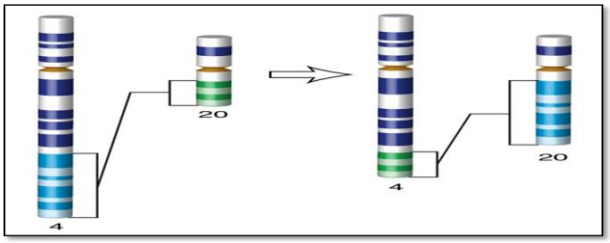
— Deletion

— Duplication

— Inversion

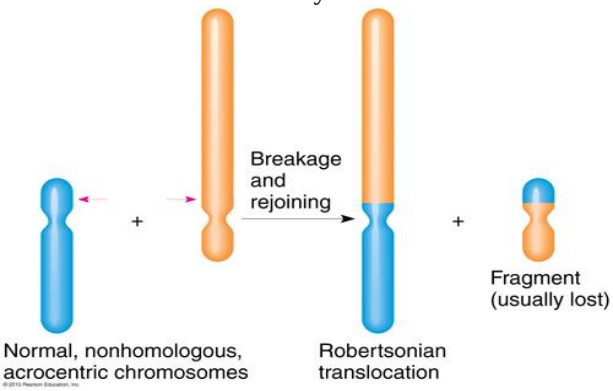
Translocation: This is a type of chromosomal mutation where a segment of one chromosome breaks off and attaches to a different, non-homologous chromosome (a chromosome that is not its matching pair). Translocation is of two main types: Reciprocal and Robertsonian translocation.

In Reciprocal translocation two different chromosomes exchange segments with each other. There is no loss of genetic material, only gene positions are changed.



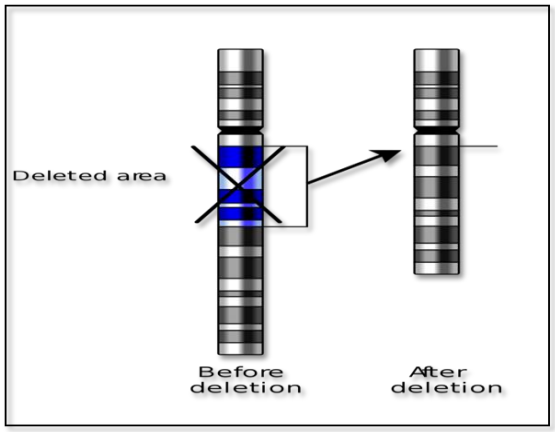
(National Institute of Health, 2011)

• In Robertsonian translocation there is break at or near two acrocentric chromosomes (with very short arms) and fusion occurs near their centromeres, forming one large chromosome and one extremely small chromosome that is eventually lost.



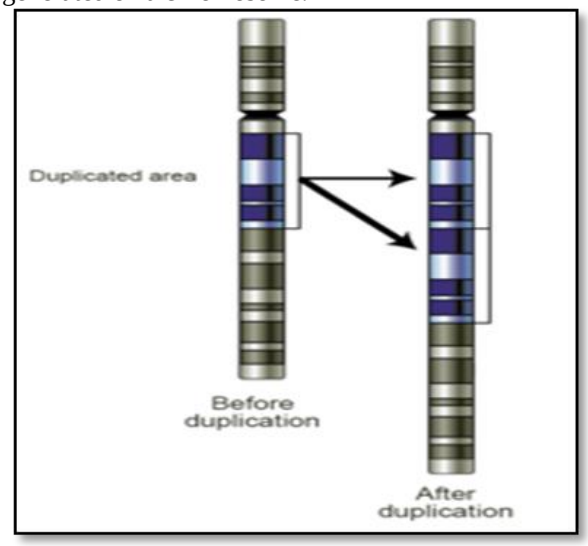
(Petter, 2010. iGenetics a Molecular Approach)

Deletion: In deletion, a part of a chromosome or a segment of DNA is lost or removed resulting in loss of genetic material during divison of cell.



Source: *Wikipedia* (2020) *(*National Human Genome Research) https://en.wikipedia.org/w/index.php?title=Deletion_(genetics)&oldid=964037078

Duplications occur when genes are duplicated and are generated on a chromosome.

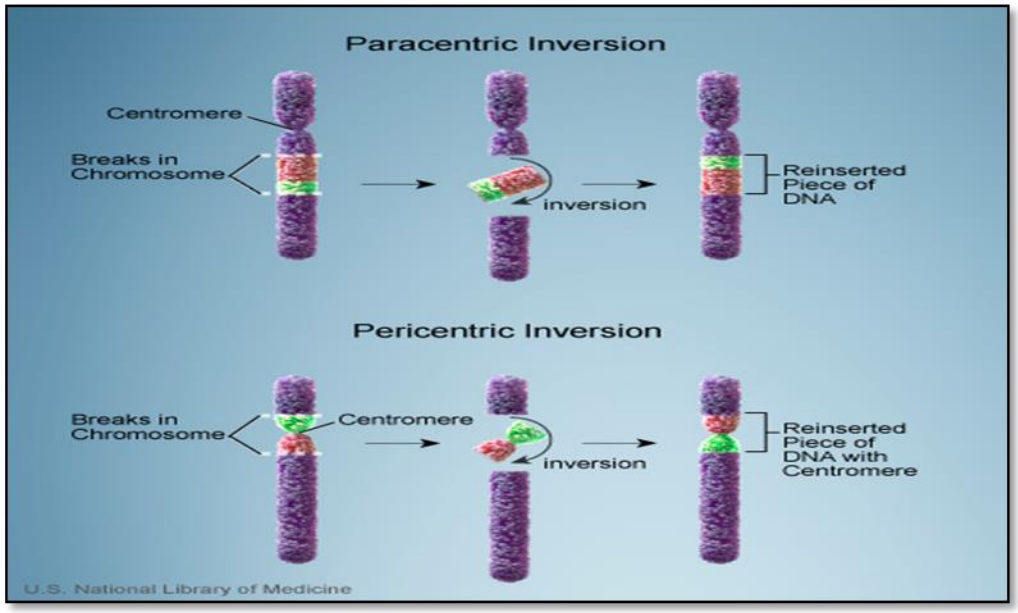


Source: *Wikipedia*. Gene duplication. 2020. https://en.wikipedia.org/w/index.php?title=Gene_duplication&oldid=962671162

An Inversion mutation occurs when a segment of a chromosome breaks off, flips around, and reattaches in the reverse direction.

Pericentric Inversion: Inversion involving the centromere of the chromosome

Paracentric Inversion: If inversion does not include the centromere and both breaks occur on one arm of the chromosome



Alteration or abnormality in number of chromosome: It is mainly due to non-disjunction.

Aneuploidy is a type of mutation in the chromosome number arising as a result of the **nondisjunction of chromosomes.**

Polyploidy occurs when an individual bears more than one haploid set of chromosomes.

Gene mutation

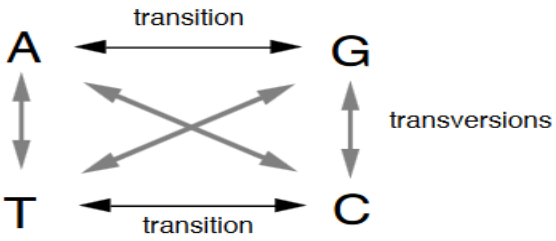
A gene mutation is the alteration in the DNA sequence of a gene. These mutations may impact a single nucleotide of DNA or more extensive gene sequences. They can change the protein that the gene codes for, change its structure or function, or have no effect at all.

Types of gene mutation:

- Point mutation
- Substitution
- Insertion
- Deletion
- Frameshift mutation

A point mutation involves alteration in a single nucleotide base. This is the most common type of gene mutation. These may of any following types:

- Silent substitutions: Here for the same amino acid, one codon changes into another codon. No change in the amino acid → no visible effect.
- Missense mutations: Here the amino acid changes with the swapping of codons
- Nonsense mutations: Here a codon is changed into a stop codon resulting in shorter protein.
- Base substitutions: A base substitution is a type of point mutation where one nitrogen base (A, T, G, or C) is replaced by another in the DNA sequence. These may be:
- Transitions: (purine replaces a purine; pyrimidine replaces a pyrimidine). These are more common than transversions and often less disruptive.
- Transversions: (pyrimidine replaces a purine; purine replaces a pyrimidine). these are more likely to affect protein function



Genes can also get *mutated* by the *deletion* or *insertion* of a number of nucleotide bases.

Frame shift Mutations: Insertion or deletion of one or more basepairs (not a multiple of three) within the translated portion of a gene results in change in the reading frame causing frame shift mutations. Since the genetic code is read in triplets (codons), this shifts the entire "reading frame" of the gene from that point onward.

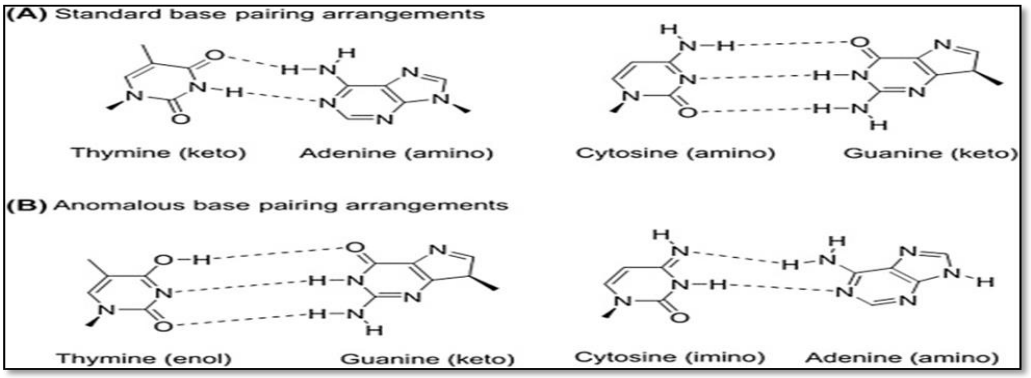
Molecular basis of mutations

Depending upon the type of mutation, there are several molecular factors or mechanisms giving rise to such mutations.

— Spontaneous mutations: These are caused mainly by spontaneous replication errors or inherent instability of nucleotides, in the absence of known mutagen treatment. These involve:

- Tautomeric shifts- Temporary changes in base pairing
- Wobble base pairing-non-standard hydrogen-bonded pairing between two RNA nucleotides
- Strand slippage-also called slipped-strand mispairing or replication slippage
- Unequal crossing over-misalignment leading to asymmetric genetic exchange
- Spontaneous chemical changes- e.g. Depurination/Deamination

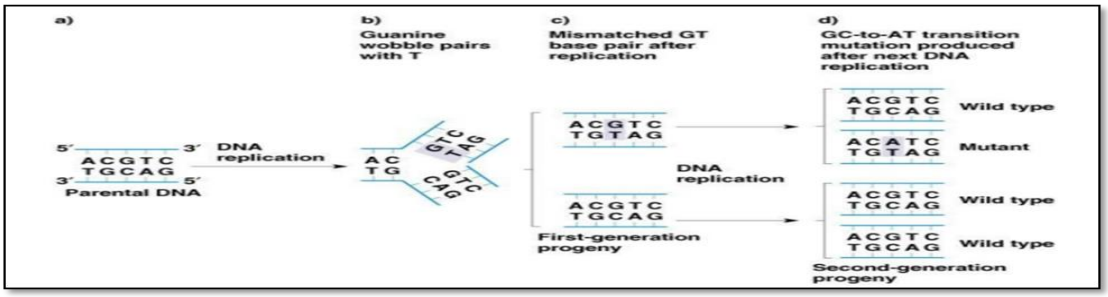
Tautomeric shifts: Each of the bases in DNA can appear in tautomeric forms. A tautomer is a structural isomer that is interconvertible and exists at equilibrium with the standard, "normal" molecule. DNA normally has keto form for each base, but they can rarely shift to an "enol" or "imino" (rare) form.



(Ackerman and Horton 2018)

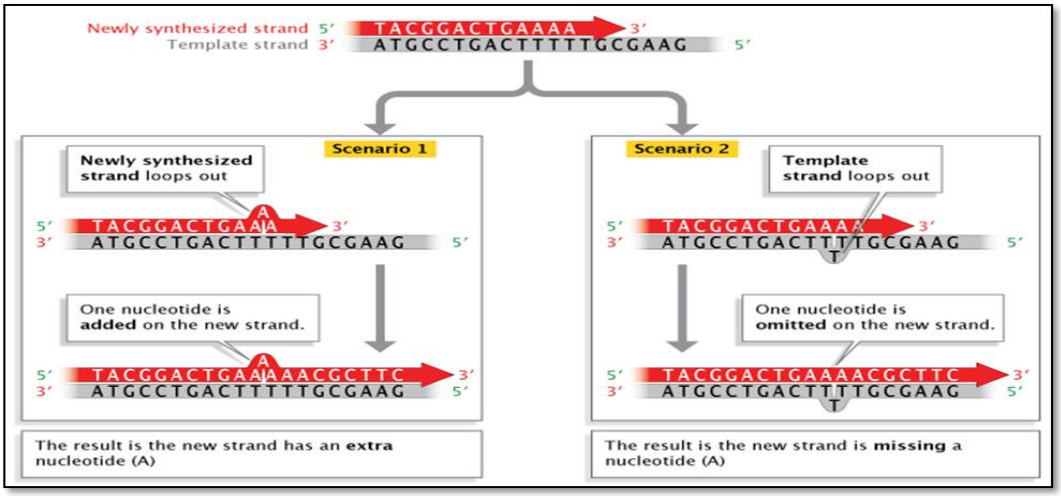
Wobble base pairing: The wobble position is the 3rd nucleotide base in a codon of m-RNA. The first two bases of the codon pair **strictly** with the tRNA anticodon (following Watson-Crick base pairing rules).

The **third base** can **"wobble"**, meaning it is much "looser" in the third position, due to which several types of non-Watson–Crick base pairing occurs allowing **a single tRNA to recognize multiple codons**.



(Russell, 2010. iGenetics a Molecular Approach)

Slipped strand mispairing (SSM) is also known as strand slippage or replication slippage. This type of mutation occurs during DNA replication the newly synthesized strand or the template strand "slips", causing small loops or misalignment leading to insertions and deletions.

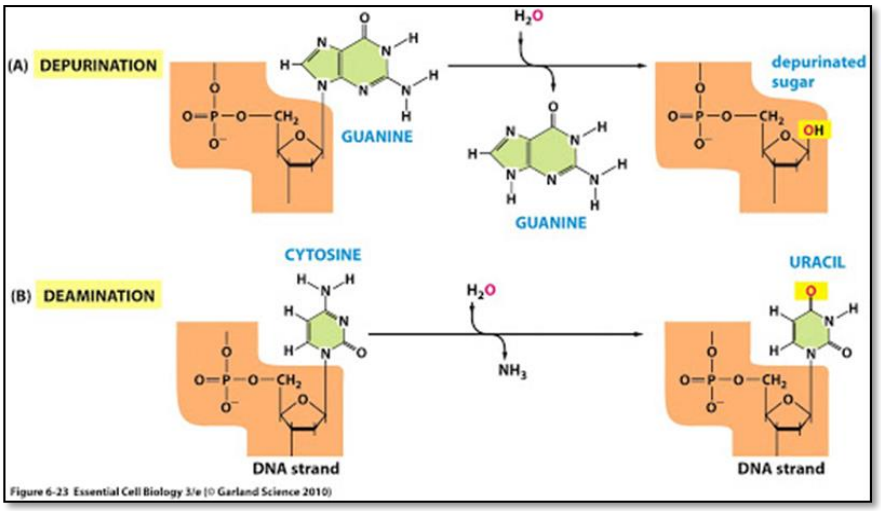


(Pray, 2008)

In unequal crossing over homologous chromosomes misalign and exchange unequal segments of DNA.This is a type of gene duplication or deletion event.

Spontaneous chemical changes: Depurination and deamination are two of the most common spontaneous lesions, with the former occurring more frequently.

- Depurination is a spontaneous DNA damage where a purine base — adenine (A) or guanine (G) — is removed from the DNA molecule. This leaves behind a "blank spot" in the DNA, called an abasic site or AP (apurinic) site.
- Deamination occurs because an amino group ($–NH_2$) is removed from a nitrogenous base in DNA.

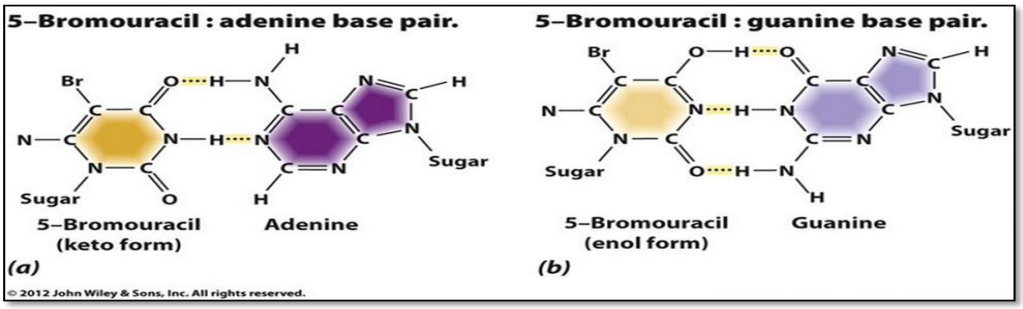


(Alberts *et al*., 2010 *Essential cell biology*. Garland Science.)

• Induced mutations are alterations in the gene due to contact with mutagens and environmental causes. These may be due to physical, chemical or biological mutagen.

A chemical mutagen can alter the structure of DNA bases, interfere with base pairing, or damage the DNA backbone, leading to point mutations, insertions, deletions, or strand breaks.

Base analog mutagens: Chemical substances known as base analogues share structural similarities with typical DNA bases. Mispairing and mutations may result from their inadvertent incorporation into DNA during replication. For example: 5-bromouracil (5-BU) is a derivative of uracil and behaves as a thymine analog. **5-BU in keto form** pairs with **A** (like normal T) and **in enol form** pairs with **G.**



• Alkylating agents transfer methyl or ethyl groups to bases in DNA. As a consequence, the altered bases pairing is evident. Some examples of alkylating agents are mustard gas, vinyl chloride, nitroso amines and ethyl nitrosourea etc.

• Deaminating agents remove amino group from a base. Nitrous acid reacts with DNA by removing an amino group ($–NH_2$) from certain nitrogenous bases and converts them. For e.g. cytosine is converted into uracil, adenine into hypoxanthine, and guanine into xanthine.

• Hydroxylamine adds a hydroxyl group to cytosine residues.

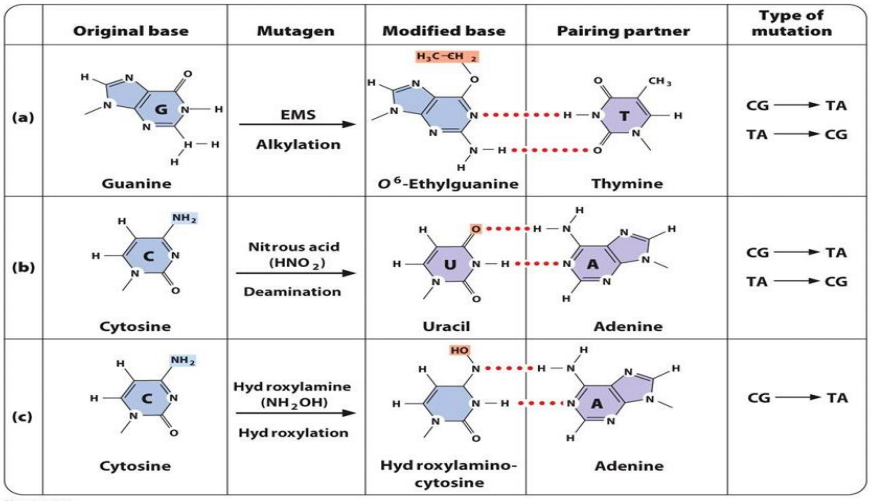


Figure 18.18
Genetics: A Conceptual Approach, Fifth Edition
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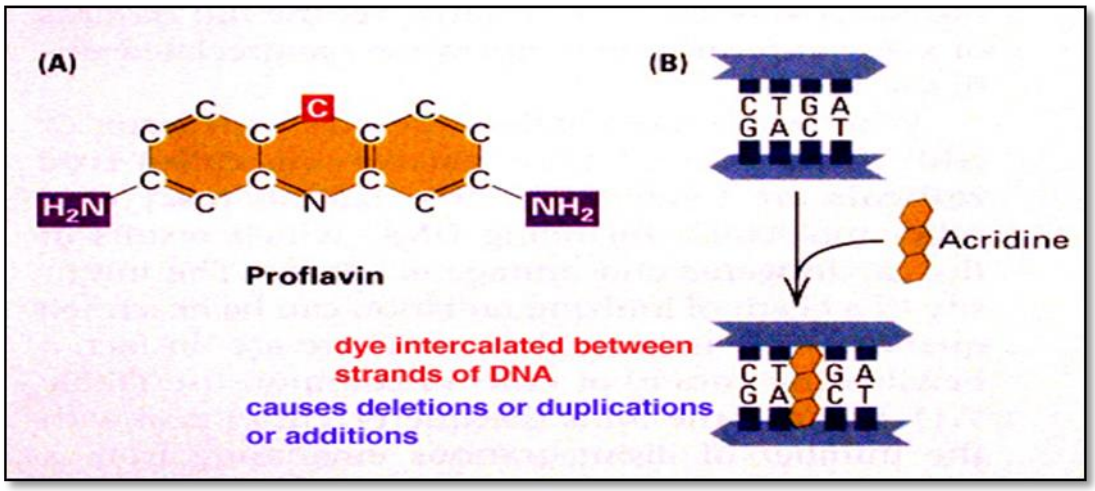
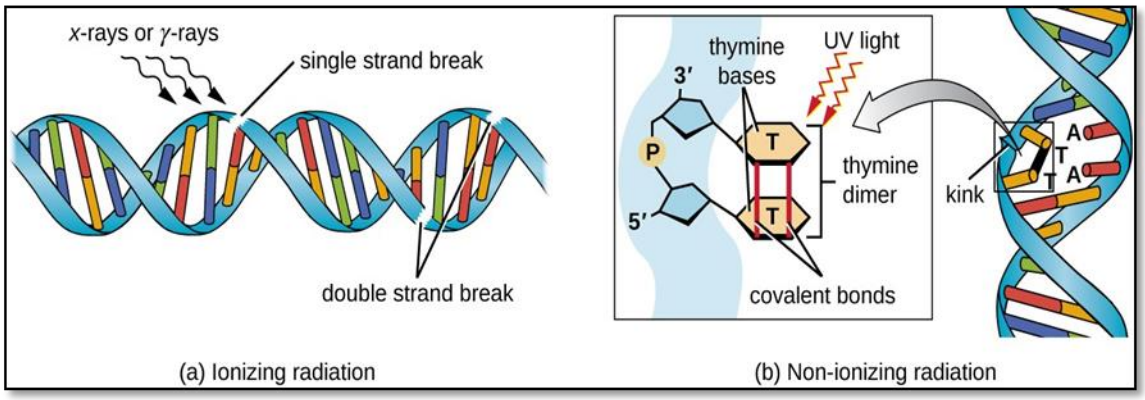
The intercalating agents are usually flat planar molecules that slide between stacked base pairs in double-stranded DNA and pushes the bases apart, causing DNA polymerase to either add an extra base (insertion) or skip a base (deletion). This group of compounds includes proflavin, acridine orange etc.



Physical mutagens may include ionizing or non-ionizing radiations.

• Ultraviolet radiation causes formation of pyrimidine dimers- mainly thymine dimers. A thymine dimer forms when two adjacent thymine bases on the same DNA strand become covalently bonded due to UV exposure. This distorts the DNA helix and prevents normal replication and transcription.

• The gamma and X rays have enough energy to ionize atoms and break chemical bonds. These can either can interact directly with the DNA molecule, or else generate free radicals causing single strand breaks- or double-strand breaks.



Biological mutagens: In bacterial population, biological mutagens are the biological products that can cause mutations in the DNA like transposons and viruses.

• Viral or transposon DNA inserts into a gene, disrupting its function.

• Bacterial toxins cause oxidative stress, leading to base changes or strand breaks. E.g. Aflatoxin B_1 (AFB_1) is a powerful carcinogen.

Repair mechanisms. A range of enzyme repair mechanisms have been developed by living cells to fix DNA damage in different ways. A higher mutation rate is apparent when these mechanisms malfunction.

DNA repair mechanisms:

• Prevention of errors,
• Proof reading of DNA polymerase
• Reversal of damage,
• Excision repair, and
• Post replication repair.

Prevention of errors: Before they even interact with DNA, certain enzymatic systems neutralise potentially harmful substances. The superoxide radicals are converted to hydrogen peroxide by the enzyme superoxide dismutase, which is then converted to water by the enzyme catalase.

Proof reading of DNA polymerase: The error rate for DNA polymerase enzymes is approximately 1 per 100,000 nucleotides. Through a procedure called proofreading, some of the errors are fixed right away. The DNA helix is somewhat distorted if the wrong base is introduced. This causes the DNA strand to move from the synthesis active site to the 3′→5′ exonuclease site, slowing down polymerisation. The mismatched nucleotide is removed by the exonuclease, which also moves backward (or "proofreads") one or more nucleotides to guarantee a repaired 3′ end. The strand returns to the polymerase active site, and correct nucleotide incorporation resumes.

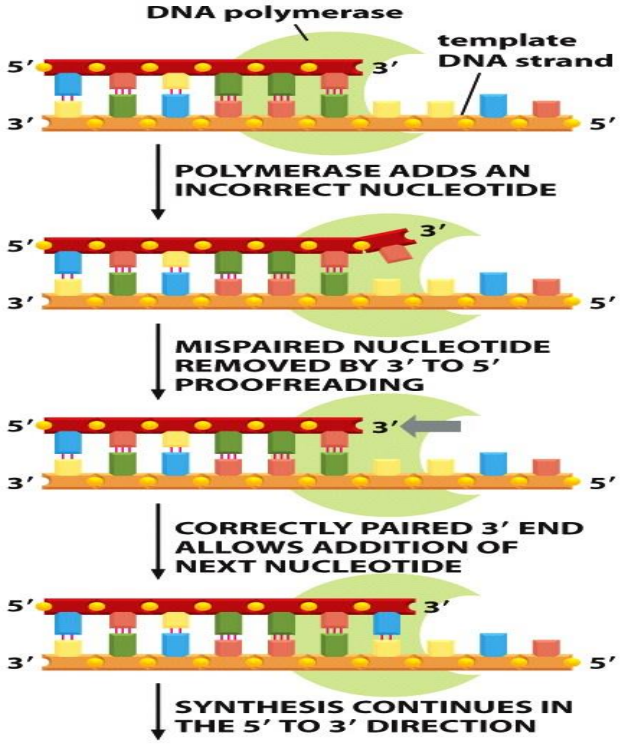
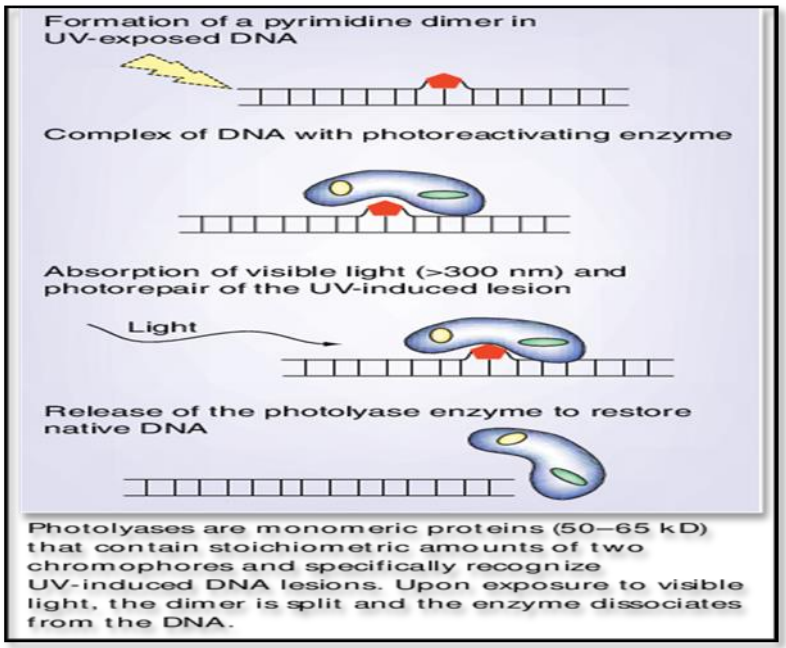


Figure 6-13 Essential Cell Biology 3/e (© Garland Science 2010)

Reversal of damage-Photoreactivation: Directly reversing a lesion to regenerate the normal base is the most fundamental method of repair. UV-induced mutagenic photodimer, for instance. The photolyase enzyme uses this energy to break down the dimer into its constituent monomers when light is present.

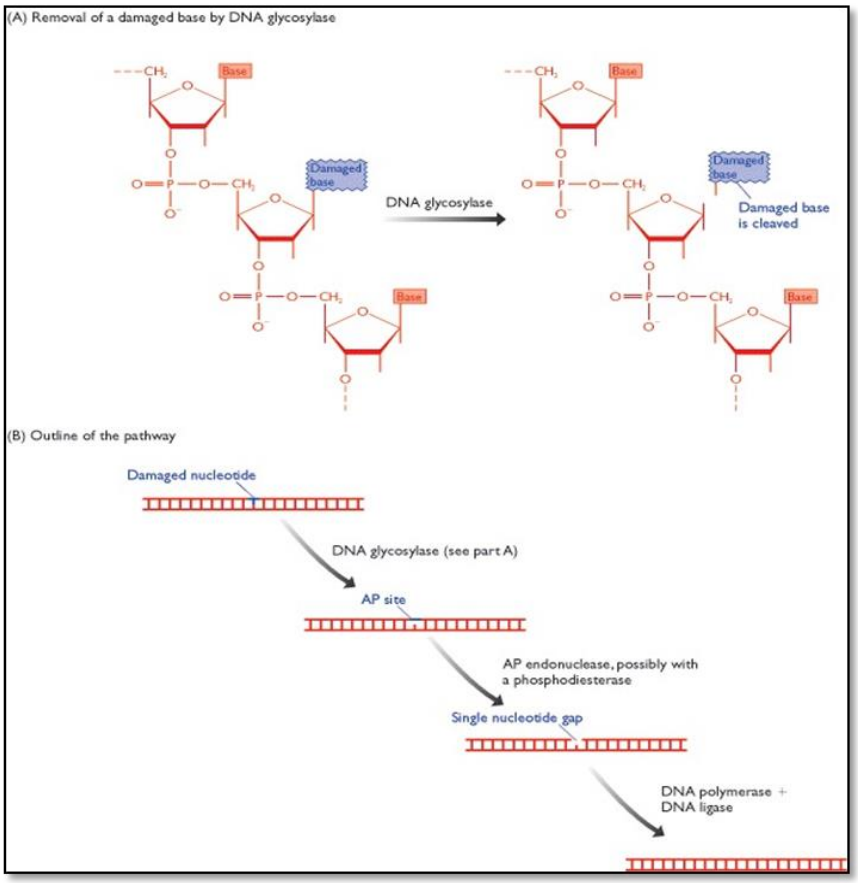


(Garinis *et al*., 2006)

The general excision-repair system causes an oligonucleotide to be expelled by breaking a phosphodiester link on the same strand on either side of the lesion. A ligase closes the cracks, and repair synthesis fills the void left by this.

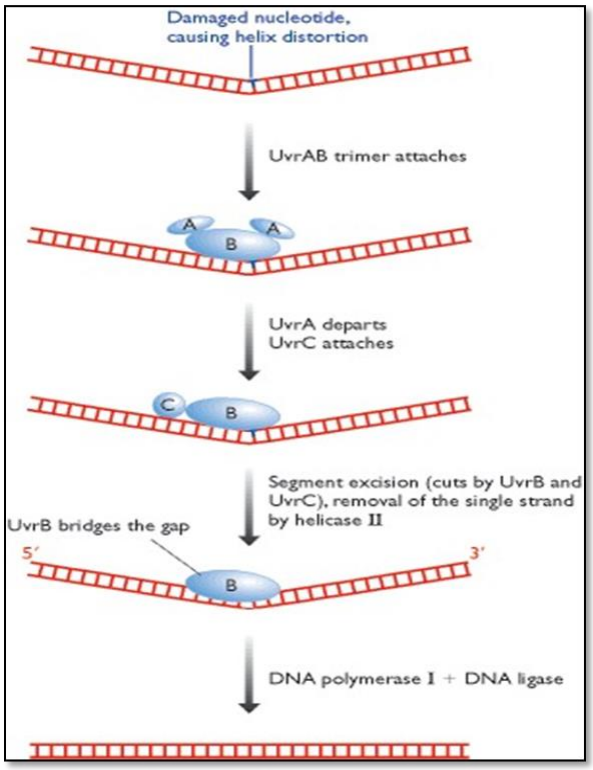
Some lesions are too small for the general excision-repair mechanism to detect. Therefore, more targeted excision mechanisms are required.

Base excision repair: DNA glycosylases perform base-excision repair by breaking N-glycosidic (base-sugar) linkages, which releases the modified bases and creates apurinic or apyrimidinic sites (AP sites). An endonuclease repair mechanism unique to AP sites then fixes these sites. These enzymes cleave the phosphodiester bonds at AP sites, causing chain breakage. Three more enzymes—an exonuclease, DNA polymerase I, and DNA ligase—mediate the excision-repair process that is triggered by this.



(Brown, 2002)

Nucleotide excision repair can handle more severe types of damage and has a higher specificity than the base excision method. Nucleotide excision repair involves removing the damaged nucleotide or nucleotides from a section of single-stranded DNA and replacing them with fresh DNA. A larger stretch of polynucleotide is excised and no selective base removal occurs beforehand unlike base excision.



(Brown, 2002)

Fig. Short patch nucleotide excision repair in *Escherichia coli*.

Post Replication Repair pathways: Even after DNA has undergone replication, post-replication repair pathways like the mismatch-repair system can identify mistakes. These are able to identify not just mismatched base pairs but also the erroneous base inside the mismatch. Ultimately, the wrong base is removed, and repair synthesis happens.

CONCLUSIONS

Mutations play a crucial role in bacterial genetics by being a major source of genetic variability. They represent a major force in microbial evolution and adaptation.

FUTURE SCOPE

Understanding mutation in bacteria is important for tackling major public health issues, particularly in tracking and combating antibiotic resistance. Moreover, it also prove to be an essential tool for molecular genetics research, biotechnology applications and synthetic biology.

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