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T.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[503]: Molecular biology and genetic engineering

Unit 4

MUTATION AND REPAIR

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CONTENT

- 4.1 Types of mutation- Spontaneous mutations and Induced mutations
- 4.2 Biochemical basis of mutation and mutation Reversion
- 4.3 Physical, Chemical and Biological Mutagenesis; Ames test
- 4.4 Experimental evidence of mutation: fluctuation analysis, mutation rate, Phenotypic and Phenomic lag
- 4.5 DNA repair mechanisms - Mismatch repair, Excision repair, Photo reactivation, Recombinational repair and SOS repair

TITLE EXPLANATION

- In this unit we are going to discuss about various types of mutation and its repair systeam.
- We can study about mutation and its types in detail.
- Study about induced ans spontaneous mutation.
- Study about mutation at chromosomne, gene and DNA level.
- Study about phenotypic effect and biochemical Basis of mutation.
- Learnirng about physical, chemical and biological mutagenesis.
- Study about DNA repair mechanism.

LEARNING OUTCOME

- The result of a mutation, **a change in the DNA sequence**. The effects of mutations can vary widely, from being beneficial, to having no effect, to having lethal consequences, and every possibility in between.
- A mutation is a change in the nucleotide sequence of a short region of a genome. Many mutations are point mutations that replace one nucleotide with another; others involve **insertion** or **deletion** of one or a few nucleotides.
- Mutations result either from errors in DNA replication or from the damaging effects of mutagens, such as chemicals and radiation, which react with DNA and change the structures of individual nucleotides.
- All cells possess **DNA-repair** enzymes that attempt to minimize the number of mutations that occur. These enzymes work in two ways.
- Some are pre-replicative and search the DNA for nucleotides with unusual structures, these being replaced before replication occurs; others are post-replicative and check newly synthesized DNA for errors, correcting any errors that they find. A possible definition of mutation is therefore *a deficiency in DNA repair*.

(For exam purpose)

MUTATION AND MUTATION RATE

- A mutation is a change in a genetic sequence. Mutations include changes as small as the substitution of a single DNA building block, or nucleotide base, with another nucleotide base.
- Meanwhile, larger mutations can affect many genes on a chromosome. Along with substitutions, mutations can also be caused by insertions, deletions, or duplications of DNA sequences.
- Some mutations are hereditary because they are passed down to an offspring from a parent carrying a mutation through the germ line, meaning through an egg or sperm cell carrying the mutation.
- There are also nonhereditary mutations that occur in cells outside of the germ line, which are called somatic mutations.
- Mutations can be introduced due to mistakes made during DNA replication or due to exposure to mutagens, which are chemical and environmental agents that can introduce mutations in the DNA sequence, such as ultraviolet light.
- Some mutations do not result in changes in the amino acid sequence of the encoded protein and can be described as silent mutations. Other mutations result in abnormal protein products.
- Mutations can introduce new alleles into a population of organisms and increase the population's genetic variation.

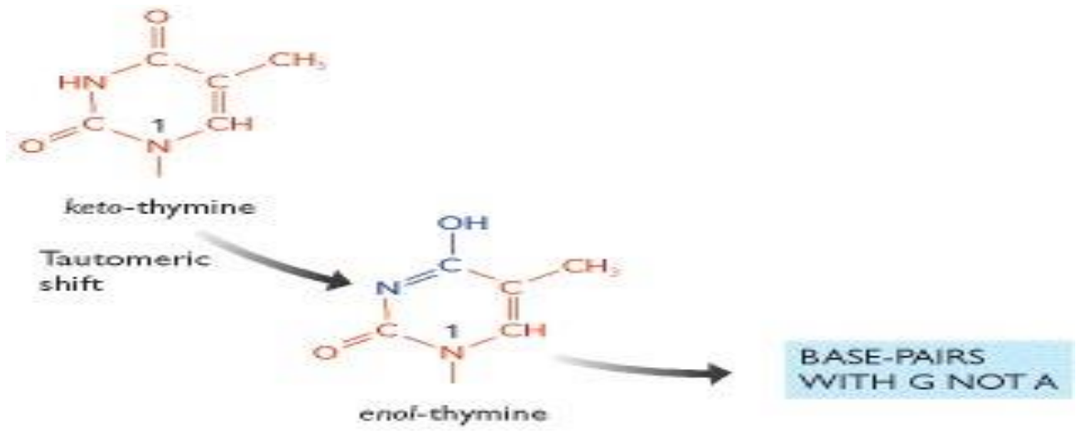
- **MUTATION RATE :** Mutation Rates The frequency with which a wild-type allele at a locus changes into a mutant allele is referred to as the mutation rate and is generally expressed as the number of mutations per biological unit, which may be mutations per cell division, per gamete, or per round of replication.

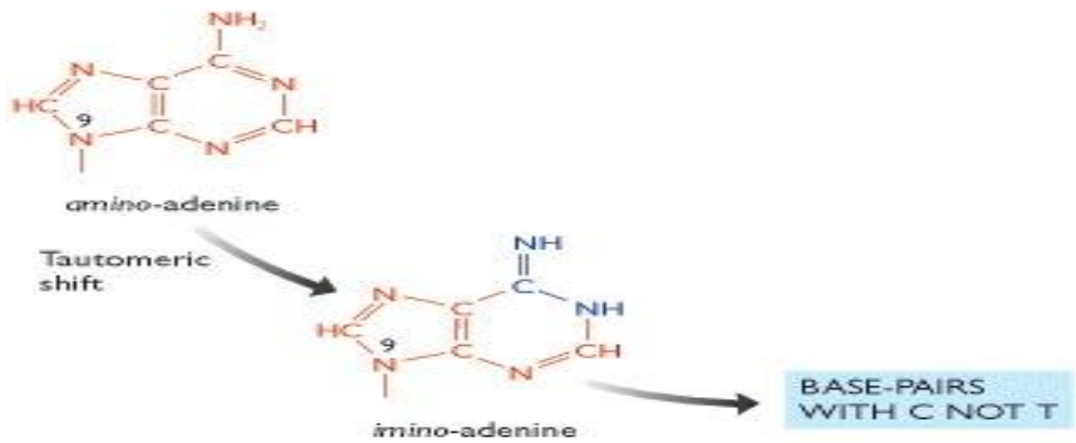
- For example, achondroplasia is a type of hereditary dwarfism in humans that results from a dominant mutation. On average, about four achondroplasia mutations arise in every 100,000 gametes, and so the mutation rate is 4/100,000, or 0.00004 mutations per gamete. The mutation rate provides information about how often a mutation arises.

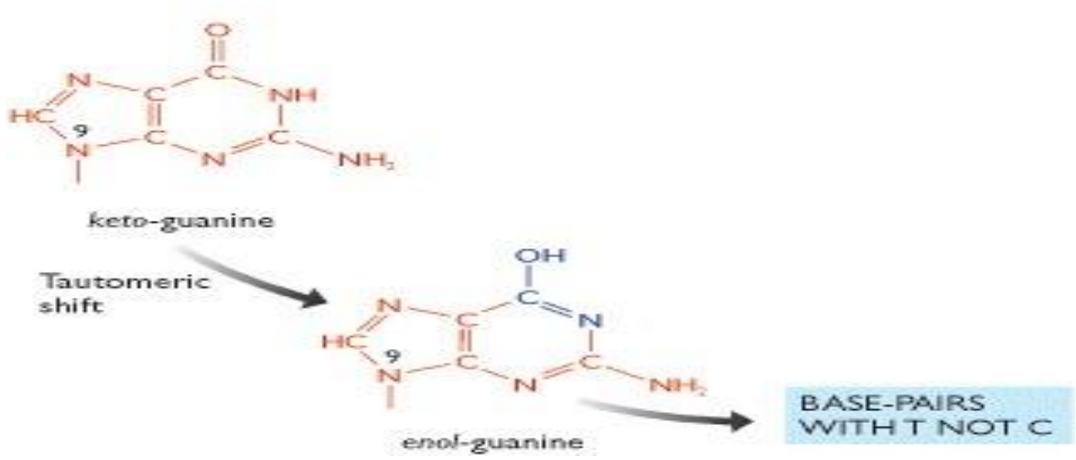
TYPES OF MUTATION

- Some mutations arise from spontaneous alterations to DNA structure, such as depurination and deamination, which may alter the pairing properties of the bases and cause errors in subsequent rounds of replication.
- Induced mutation are the result of exposure to a mutagen, which can be either a physical or a chemical agent. Mutation are characterized according to either the kind of genotypic change that has occurred or their phenotypic consequences.
- **Spontaneous mutation:** Replication is amazingly accurate less than one error in a billion nucleotides arises in the course of DNA synthesis. However, spontaneous replication errors do occasionally occur.
- Tautomeric shifts 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 formscalled tautomers.
- The two tautomeric forms of each base are in dynamic equilibrium, although one form is more common than the other. The standard Watson-and- Crick 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.

- Watson and Crick proposed that tautomeric shifts might produce mutations, and, for many years, their proposal was the accepted model for spontaneous replication errors.
- However, there has never been convincing evidence that the rare tautomers are the cause of spontaneous mutations. Furthermore, research now shows little evidence of tautomers in DNA.







- **Mispairing due to other structures** Mispairing can also occur through wobble, in which normal, protonated,and other forms of the bases are able to pair because of flexibility in the DNA helical structure . These structures have been detected in DNA molecules and are now thought to be responsible for many of the mispairings in replication.
- **Incorporation errors and replication errors** When mismatched base has been incorporated into a newly synthesize nucleotide chain, an **incorporated error** is said to have occurred. Suppose that, in replication, thymine (which normally pairs with adenine) mispairs with guanine through wobble .
- In the next round of replication, the two mismatched bases separate, and each serves as template for the synthesis of a new nucleotide strand.
- This time, thymine pairs with adenine, producing another copy of the original DNA sequence. On the other strand, however, the incorrectly incorporated guanine serves as the template and pairs with cytosine, producing a new DNA molecule that has an error—a C · G pair in place of the original T :A pair (a T · A→C · G base substitution).
- The original incorporated error leads to a **replication error**, which creates a permanent mutation, because all the base pairings are correct and there is no mechanism for repair systems to detect the error.
- **Causes of deletions and insertions** Mutations due to small insertions and deletions also arise spontaneously inreplication and crossing over.
- **Strand slippage** can occur when one nucleotide strand forms a small loop. If the looped-out nucleotides are on the newly synthesized strand, an insertion results. At the next round of replication, the insertion will be replicated and both strands will

contain the insertion. If the looped-out nucleotides are on the template strand, then the newly replicated strand has a deletion, and this deletion will be perpetuated in subsequent rounds of replication.

2) Chemically Induced Mutations

- Although many mutations arise spontaneously, a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a **mutagen**.
- The first discovery of a chemical mutagen was made by Charlotte Auerbach, who started her career in Berlin researching the development of mutants in *Drosophila*.
- Faced with increasing anti-Semitism in Nazi Germany, Auerbach emigrated to Britain. There she continued her research on *Drosophila* and collaborated with pharmacologist John Robson on the mutagenic effects of mustard gas, which had been used as a chemical weapon in World War I.
- The experimental conditions were crude. They heated liquid mustard gas over a Bunsen burner on the roof of the pharmacology building, and the flies were exposed to the gas in a large chamber.
- After developing serious burns on her hands from the gas, Auerbach let others carry out the exposures, and she analyzed the flies.
- Auerbach and Robson showed that mustard gas is indeed a powerful mutagen, reducing the viability of gametes and increasing the numbers of mutations seen in the offspring of exposed flies.

- Because the research was part of the secret war effort, publication of their findings was delayed until 1947.
- **Base analogs** One class of chemical mutagens consists of **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 (5BU) 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 (T · A→5BU · A→ 5BU · G→C · G), Through mispairing, 5-bromouracil can also be incorporated into a newly synthesized DNA strand opposite guanine. In the next round of replication 5-bromouracil pairs with adenine, leading to another transition (G · C→G · 5BU→A · 5BU→A · T).
- Another mutagenic chemical is 2-aminopurine (2AP), which is a base analog of adenine. Normally, 2-aminopurine base pairs with thymine, but it may mispair with cytosine, causing a transition mutation (T · A→T · 2AP→C · 2AP→ C · G). Alternatively, 2-aminopurine may be incorporated through mispairing into the newly synthesized DNA opposite cytosine and then later pair with thymine, leading to a C · G→C · 2AP→T · 2AP→T · A transition. produce transition mutations.
- In the laboratory, mutations caused by base analogs can be reversed by treatment with the same analog or by treatment with a different analog.

- **Alkylating agents** Alkylating agents are chemicals that donate alkyl groups, such as methyl (CH3) and ethyl (CH3–CH2) groups, to nucleotide bases.

- For example, ethylmethylsulfonate (EMS) adds an ethyl group to guanine, producing *O*6-ethylguanine, which pairs with thymine . Thus, EMS produces C · G→T · A transitions. EMS is also capable of adding an ethyl group to thymine, producing 4-ethylthymine, which then pairs with guanine, leading to a T · A→C · G transition. Because EMS produces both C · G→ T · A and T · A→C · G transitions, mutations produced by EMS can be reversed by additional treatment with EMS.

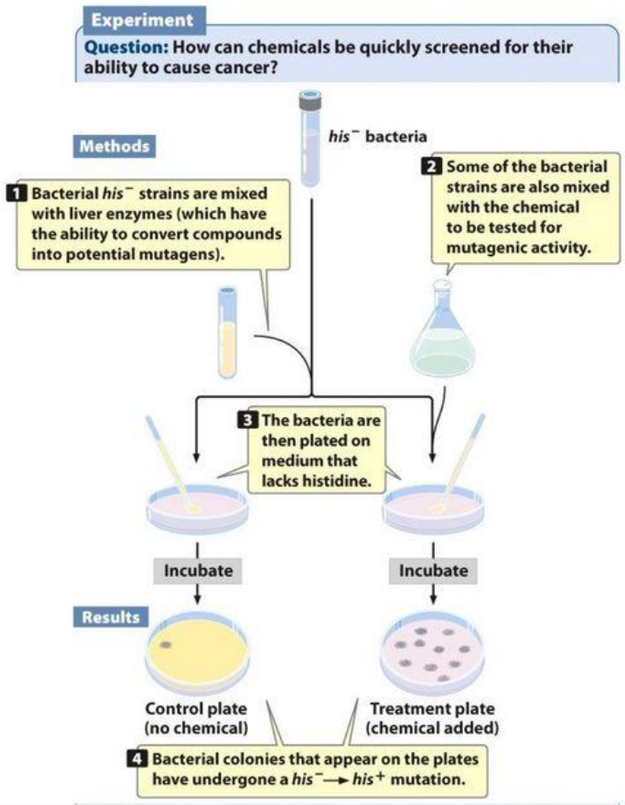
- Mustard gas is another alkylating agent. **Deamination** In addition to its spontaneous occurrence , deamination can be induced by some chemicals. For instance, nitrous acid deaminates cytosine, creating uracil, which in the next round of replication pairs with adenine, producing a C · G→T A transition mutation.
- Nitrous acid changes adenine into hypoxanthine, which pairs with cytosine, leading to a T · A→C · G transition.
- Nitrous acid also deaminates guanine, producing xanthine, which pairs with cytosine just as guanine does; however, xanthine can also pair with thymine, leading to a C · G→T · A transition. Nitrous acid produces exclusively transition mutations and, because both C · G→T· A and T · A→C · G transitions are produced, these mutations can be reversed with nitrous acid.

- **Hydroxylamine** Hydroxylamine is a very specific basemodifying 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 C · G→T · A transitions. Because hydroxylamine acts only on cytosine, it will not generate T · A→C · G transitions; thus, hydroxylamine will not reverse the mutations that it produces.
- **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 in DNA.
- For example, oxidation converts guanine into 8-oxy-7,8-dihydrodeoxyguanine which frequently mispairs with adenine instead of cytosine, causing a G · C→T · A transversion mutation.
- **Intercalating agents** Proflavin, acridine orange, ethidium bromide, and dioxin are **intercalating agents** , which 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, and so the mutagenic effects of intercalating agents are often severe.
- Because intercalating agents generate both additions and deletions, they can reverse the effects of their own mutations

Detecting Mutations with the Ames Test

- People in industrial societies are surrounded by a multitude of artificially produced chemicals: more than 50,000 different chemicals are in commercial and industrial use today, and from 500 to 1000 new chemicals are introduced each year.
- Some of these chemicals are potential carcinogens and may cause harm to humans.
- One method for testing the cancer-causing potential of chemicals is to administer them to laboratory animals (rats or mice) and compare the incidence of cancer in the treated animals with that of control animals.
- These tests are unfortunately time consuming and expensive.
- Furthermore, the ability of a substance to cause cancer in rodents is not always indicative of its effect on humans.
- After all, we aren't rats! In 1974, Bruce Ames developed a simple test for evaluating the potential of chemicals to cause cancer.
- The **Ames test** is based on the principle that both cancer and mutations result from damage to DNA, and the results of experiments have demonstrated that 90% of known carcinogens are also mutagens.
- Ames proposed that mutagenesis in bacteria could serve as an indicator of carcinogenesis in humans.
- The Ames test uses different auxotrophic strains of the bacterium *Salmonella typhimurium* that have defects in the lipopolysaccharide coat, which normally protects the bacteria from chemicals in the environment.
- Furthermore, the DNA-repair system in these strains has been inactivated, enhancing their susceptibility to mutagens.



- The most recent version of the test (called Ames II) uses several auxotrophic strains that detect different types of base-pair substitutions.
- Other strains detect different types of frameshift mutations. Each strain carries a *his*– mutation, which renders it unable to synthesize the amino acid histidine, and the bacteria are plated onto medium that lacks histidine (**Figure)**.
- Only bacteria that have undergone a reverse mutation of the histidine gene (*his*—→*his*+) are able to synthesize histidine and grow on the medium.
- Different dilutions of a chemical to be tested are added to plates inoculated with the bacteria, and the number of mutant bacterial colonies that appear on each plate is compared with the number that appear on control plates with no chemical (i.e., that arose through spontaneous mutation).

- Any chemical that significantly increases the number of colonies appearing on a treated plate is mutagenic and is probably also carcinogenic.
- Some compounds are not active carcinogens but can be converted into cancer-causing compounds in the body.
- To make the Ames test sensitive for such *potential* carcinogens, a compound to be tested is first incubated in mammalian liver extract that contains metabolic enzymes.
- The Ames test has been applied to thousands of chemicals and commercial products. An early demonstration of its usefulness was the discovery, in 1975, that many hair dyes sold in the United States contained compounds that were mutagenic to bacteria.
- These compounds were then removed from most hair dyes.

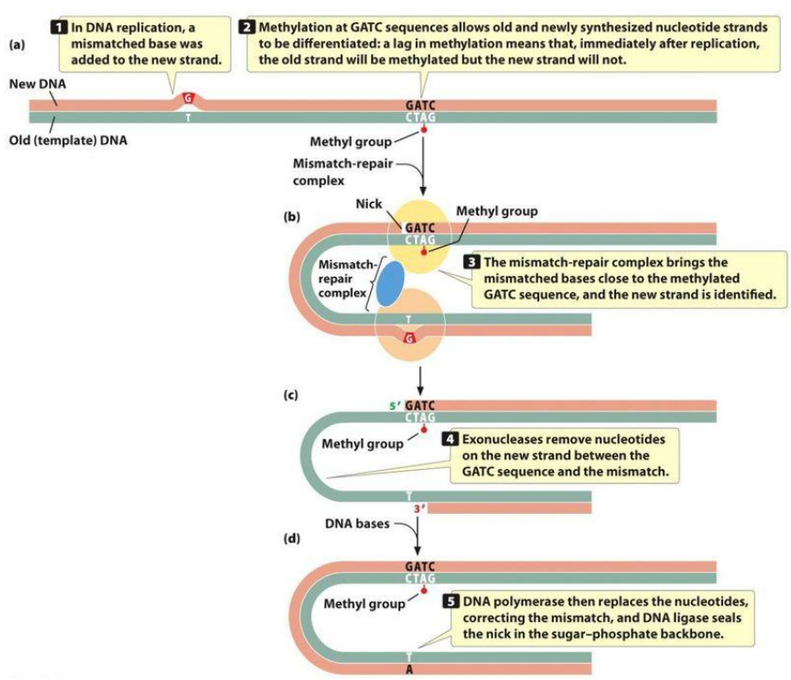
Repair mechanism

- The integrity of DNA is under constant assault from radiation, chemical mutagens, and spontaneously arising changes.
- In spite of this onslaught of damaging agents, the rate of mutation remains remarkably low, thanks to the efficiency with which DNA is repaired.
- There are a number of complex pathways for repairing DNA, but several general statements can be made about DNA repair.
- First, most DNA-repair mechanisms require two nucleotide strands of DNA because most replace whole nucleotides, and a template strand is needed to specify the base sequence A second general feature of DNA repair is redundancy, meaning that many types of DNA damage can be corrected by more than one pathway of repair.
- This redundancy testifies to the extreme importance of DNA repair to the survival of the cell: it ensures that almost all mistakes are corrected.

- If a mistake escapes one repair system, it's likely to be repaired by another system.

Mismatch Repair

- Replication is extremely accurate: each new copy of DNA has less than one error per billion nucleotides.
- However, in the process of replication, mismatched bases are incorporated into the new DNA with a frequency of about 10−4 to 10−5; so most of the errors that initially arise are corrected and never become permanent mutations.
- Some of these corrections are made in proofreading by the DNA polymerases.
- Many incorrectly inserted nucleotides that escape detection by proof-reading are corrected by *mismatch repair*.
- Incorrectly paired bases are detected and corrected by mismatch-repair enzymes.
- In addition, the mismatch-repair system corrects small unpaired loops in the DNA, such as those caused by strand slippage in replication.

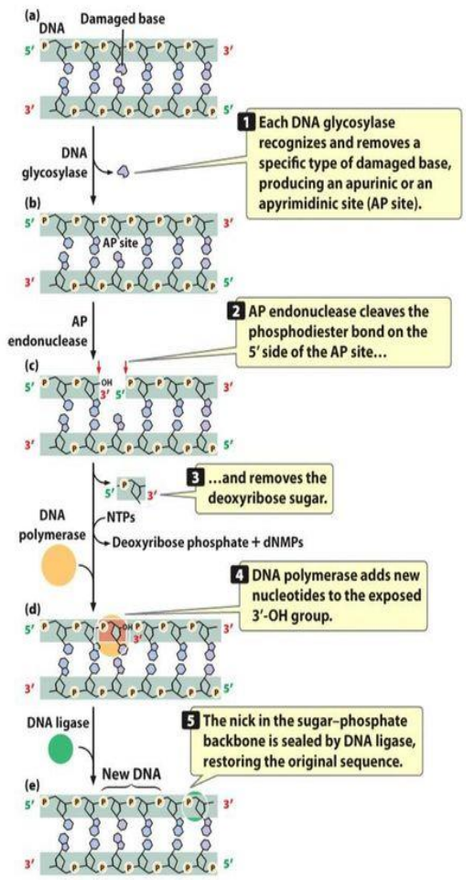


- Some nucleotide repeats may form secondary structures on the unpaired strand (see Figure), allowing them to escape detection by the mismatch-repair system.
- After the incorporation error has been recognized, mismatch-repair enzymes cut out a section of the newly synthesized
- strand and fill the gap with new nucleotides by using the original DNA strand as a template.
- For this strategy to work, mismatch repair must have some way of distinguishing between the old and the new strands of the DNA so that the incorporation error, but not part of the original strand, is removed.
- The proteins that carry out mismatch repair in *E. coli* differentiate between old and new strands by the presence of methyl groups on special sequences of the old strand.
- After replication, adenine nucleotides in the sequence GATC are methylated.
- The process of methylation is delayed and so, immediately after replication, the old strand is methylated and the new strand is not (**Figure a**).
- The mismatchrepair complex brings an unmethylated GATC sequence in close proximity to the mismatched bases. It nicks the unmethylated strand at the GATC site (**Figure b**), and degrades the strand between the nick and the mismatched bases (**Figure c**).
- DNA polymerase and DNA ligase fill in the gap on the unmethylated strand with correctly paired nucleotides (**Figure d**).
- Mismatch repair in eukaryotic cells is similar to that in *E. coli*, but how the old and new strands are recognized in eukaryotic cells is not known.

- In some eukaryotes, such as yeast and fruit flies, there is no detectable methylation of DNA, and yet mismatch repair still takes place.
- Humans who possess mutations in mismatch-repair genes often exhibit elevated somatic mutations and are frequently susceptible to colon cancer.

Base-Excision Repair

- In **base-excision repair**, a modified base is 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 sugar(**Figure a**).

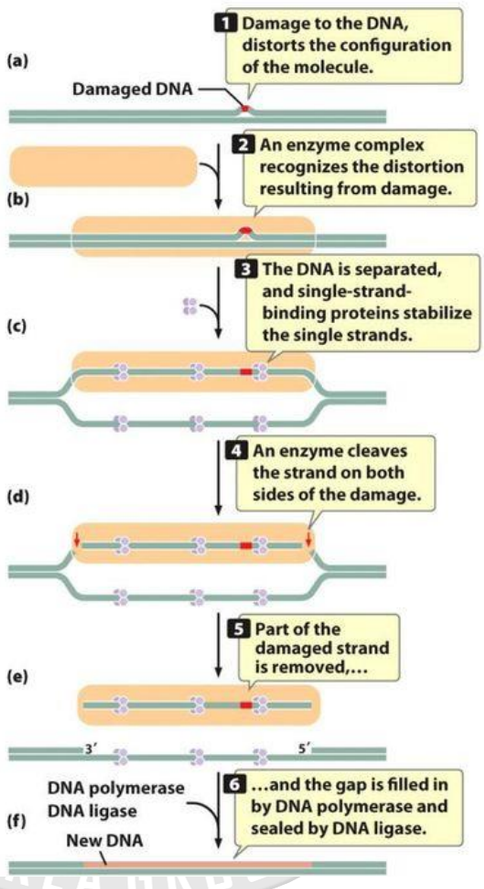


- Uracil glycosylase, for example, recognizes and removes uracil produced by the deamination of cytosine.

- Other glycosylases recognize hypoxanthine, 3-methyladenine, 7-methylguanine, and other modified bases. apurinic or apyrimidinic endonuclease cuts the phosphodiester bond, and other enzymes remove the deoxyribose sugar (**Figure b**).
- DNA polymerase then adds one or more new nucleotides to the exposed 3′-OH group (**Figure c**), replacing a section of nucleotides on the damaged strand.
- The nick in the phosphodiester backbone is sealed by DNA ligase (**Figure d**), and the original intact sequence is restored (**Figure e**). Bacteria use DNA polymerase I to replace excised nucleotides, but eukaryotes use DNA polymerase ß, which has no proofreading ability and tends to make mistakes.
- On average, DNA polymerase ß makes one mistake per 4000 nucleotides inserted.
- About 20,000 to 40,000 base modifications per day are repaired by base excision, and so DNA polymerase ß may introduce as many as 10 mutations per day into the human genome.
- How are these errors corrected? Recent research results show that some AP endonucleases have the ability to proofread. When DNA polymerase ß inserts a nucleotide with the wrong base into the DNA, DNA ligase cannot seal the nick in the sugar–phosphate backbone, because the 3′-OH and 5′-P groups of adjacent nucleotides are not in the correct orientation for ligase to connect them.
- In this case, AP endonuclease 1 detects the mispairing and uses its 3′→5′ exonuclease activity to excise the incorrectly paired base. DNA polymerase ß then uses its polymerase activity to fill in the missing nucleotide.
- In this way, the fidelity of base-excision repair is maintained.

Nucleotide-Excision Repair

- Another repair pathway is **nucleotide-excision repair**, which removes bulky DNA lesions (such as pyrimidine dimers)
- that distort the double helix. Nucleotide-excision repair is quite versatile and can repair many different types of DNA
- damage. It is found in cells of all organisms from bacteria to humans and is among 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 (**Figure a** and **b**). 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 (**Figure c**).
- Next, the sugar–phosphate backbone of the damaged strand is cleaved on both sides of the damage (**Figure d**).

- Part of the damaged strand is peeled away by helicase enzymes (**Figure e**), and the gap is filled in by DNA polymerase and sealed by DNA ligase (**Figure f**).

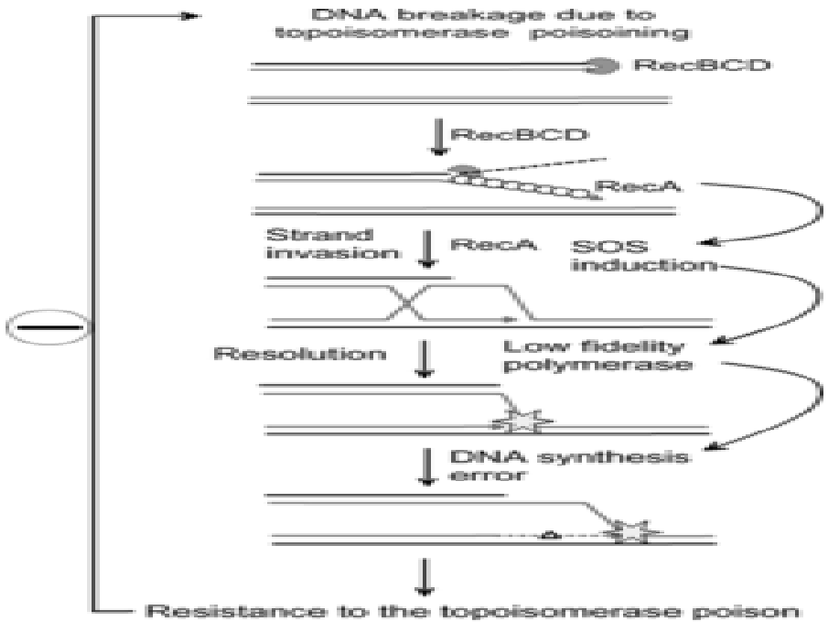
- **Repair of Double-Strand Breaks**
- A common type of DNA damage is a double-strand break, in which both strands of the DNA helix are broken.
- Double strand breaks are caused by ionizing radiation, oxidative free radicals, and other DNA-damaging agents.
- These types of breaks are particularly detrimental to the cell because they stall DNA replication and may lead to chromosome rearrangements, such as deletions, duplications, inversions, and translocations.
- There are two major pathways for repairing double-strand breaks: homologous recombination and nonhomologous end joining.
- **Homologous recombination** Homologous recombination repairs a broken DNA molecule by using the identical or nearly identical genetic information contained in another DNA molecule, usually a sister chromatid.
- DNA repair through homologous recombination uses the same mechanism employed in the process of homologous recombination that is responsible for crossing over.
- Homologous recombination begins with the removal of some nucleotides at the broken ends, followed by strand invasion, displacement, and replication.
- Many of the same enzymes that carry out crossing over are utilized in the repair of double-strand breaks by homologous recombination.
- Two enzymes that play a role in homologous recombination are BRCA1 and BRCA2.

- The genes for these proteins are frequently mutated in breast cancer cells.
- **Nonhomologous end joining** Nonhomologous end joining repairs double-strand breaks without using a homologous template.
- This pathway is often used when the cell is in G1 and a sister chromatid is not available for repair through homologous recombination.
- Nonhomologous end joining uses proteins that recognize the broken ends of DNA, bind to the ends, and then joins them together.
- Nonhomologous end joining is more error prone than homologous
- recombination and often leads to deletions, insertions, and translocations.

SOS repair

The SOS response was discovered and named by Miroslav Radman in 1975.

- During normal growth, the SOS genes are negatively regulated by LexA repressor protein dimers. Under normal conditions, LexA binds to a 20-bp consensus sequence (the SOS box) in the operator region for those genes.
- Some of these SOS genes are expressed at certain levels even in the repressed state, according to the affinity of LexA for their SOS box.
- Activation of the SOS genes occurs after DNA damage by the accumulation of single stranded (ssDNA) regions generated at replication forks, where DNA polymerase is blocked. RecA forms a filament around these ssDNA regions in an ATP-dependent fashion, and becomes activated.
- The activated form of RecA interacts with the LexA repressor to facilitate the LexA repressor's self-cleavage from the operator.



- Once the pool of LexA decreases, repression of the SOS genes goes down according to the level of LexA affinity for the SOS boxes.
- Operators that bind LexA weakly are the first to be fully expressed. In this way LexA can sequentially activate different mechanisms of repair. Genes having a weak SOSbox (such as *lexA*, *recA*, *uvrA*, *uvrB*, and *uvrD*) are fully induced in response to even weak SOS-inducing treatments.
- Thus the first SOS repair mechanism to be induced is nucleotide excision repair (NER), whose aim is to fix DNA damage without commitment to a full-fledged SOS response. If, however, NER does not suffice to fix the damage, the LexA concentration is further reduced, so the expression of genes with stronger LexA boxes (such as *sulA*, *umuD*, *umuC* - these are expressed late) is induced.
- SulA stops cell division by binding to FtsZ, the initiating protein in this process. This causes filamentation, and the induction of UmuDC-dependent mutagenic repair.
- As a result of these properties, some genes may be partially induced in response to even endogenous levels of DNA damage, while other genes appear to be induced only when high or persistent DNA damage is present in the cell.