1. (a) Mention the given statements are True or False :

- (i) Replication in E. coli takes place bidirectionally.
    - **True**
- (ii) Template in replication is in the direction of 5' to 3'.
    - **False** (The template strand is read in the 3' to 5' direction for DNA synthesis to occur in the 5' to 3' direction).
- (iii) Free 3' end of the primer is called the primer terminus.
    - **True**
- (iv) Tautomeric forms are required for the correct hydrogen bonding between the base pairs in DNA.
    - **False** (Tautomeric shifts can lead to *incorrect* base pairing and mutations, not correct hydrogen bonding).
- (v) Cells maintain DNA in an underwound state to facilitate its compaction by coiling.
    - **False** (Cells maintain DNA in an underwound state, which leads to negative supercoiling, to *facilitate unwinding* for processes like replication and transcription, not primarily for compaction by coiling in the same way as compaction by histones).
- (vi) Underwinding leads to the positive supercoiling.
    - **False** (Underwinding leads to **negative** supercoiling).
- (vii) Solenoidal supercoiling involves tight left-handed turns.
    - **True** (Solenoidal supercoiling in DNA is typically left-handed, leading to negative supercoiling).
- (viii) In interphase of cell cycle, the chromosomal material, chromatin, is condensed and segregated.

  - **False** (In interphase, chromatin is **decondensed** to allow for gene expression and replication. Condensation and segregation occur during mitosis/meiosis, not interphase).

- (ix) Beads-on-a-string arrangements are complexes of histones and DNA.
  - **True**
- (x) Histone H3 binds to the linker DNA.
  - **False** (Histone H3 is part of the core histone octamer. **Histone H1** binds to the linker DNA).

(b) Define the following (any two) :

- (i) Intercalating agents
  - Intercalating agents are planar, aromatic, and heterocyclic compounds that can insert themselves (intercalate) between adjacent base pairs in the DNA double helix.
  - This insertion causes distortions in the DNA structure, leading to an unwinding of the helix and an increase in the distance between base pairs.
  - They often interfere with DNA replication and transcription and can cause frameshift mutations during replication due to errors in template reading.
  - Examples include ethidium bromide and acridines.
- (ii) Base excision repair
  - Base excision repair (BER) is a DNA repair pathway that primarily removes damaged or modified individual bases from the DNA molecule.

- It typically deals with small, non-helix-distorting lesions caused by deamination, oxidation, or alkylation of bases.
- The process involves:
    i. A DNA glycosylase recognizes and removes the damaged base by hydrolyzing the N-glycosidic bond, leaving an apurinic/apyrimidinic (AP) site.
    ii. An AP endonuclease cleaves the phosphodiester backbone 5' to the AP site.
    iii. A phosphodiesterase or DNA glycosylase/lyase removes the deoxyribose phosphate.
    iv. DNA polymerase fills the gap with the correct nucleotide.
    v. DNA ligase seals the nick.

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2. (a) Differentiate the following :

- (i) A-DNA and Z-DNA
    - **A-DNA:**
        - **Handedness:** Right-handed helix.
        - **Conformation:** Broader and shorter than B-DNA.
        - **Grooves:** Major groove is narrow and deep, minor groove is wide and shallow.
        - **Bases per turn:** Approximately 11 base pairs per turn.
        - **Pitch:** ~2.3 nm per turn.
        - **Hydration:** Forms in conditions of lower humidity or in solutions containing specific salts.

        - **Biological Relevance:** Less common in biological systems than B-DNA, but may be adopted by RNA-DNA hybrids or double-stranded RNA.
    - **Z-DNA:**
        - **Handedness:** Left-handed helix.
        - **Conformation:** Longer and narrower than B-DNA, with a zigzag backbone appearance.
        - **Grooves:** Has only a single, deep, and narrow groove.
        - **Bases per turn:** Approximately 12 base pairs per turn.
        - **Pitch:** ~4.5 nm per turn.
        - **Hydration:** Forms under high salt concentrations or in the presence of specific sequences (e.g., alternating purine-pyrimidine repeats like GCGCGC).
        - **Biological Relevance:** Transiently forms in cells and is thought to play roles in gene regulation, recombination, and protection against viral infections.

- (ii) DNA polymerase I and DNA polymerase III
    - **DNA Polymerase I (Pol I):**
        - **Primary Role:** Primarily involved in **DNA repair** and **primer removal/gap filling**.
        - **Polymerization Rate:** Slower polymerization rate.
        - **Processivity:** Lower processivity (falls off the template more frequently).
        - **Exonuclease Activity:** Possesses three enzymatic activities: 5' → 3' polymerase, 3' → 5' exonuclease (proofreading), and crucial **5' → 3' exonuclease** (for primer removal).

- **Abundance:** More abundant in the cell than Pol III.
- **Function in Replication:** Removes RNA primers (synthesized by primase) from Okazaki fragments and fills the resulting gaps.

- **DNA Polymerase III (Pol III):**
  - **Primary Role:** The **main replicative enzyme** responsible for bulk DNA synthesis during replication.
  - **Polymerization Rate:** Very fast polymerization rate.
  - **Processivity:** Very high processivity (remains associated with the template for long stretches).
  - **Exonuclease Activity:** Possesses 5' → 3' polymerase and 3' → 5' exonuclease (proofreading) activities. **Lacks 5' → 3' exonuclease activity.**
  - **Abundance:** Less abundant than Pol I.
  - **Function in Replication:** Synthesizes the leading strand continuously and the lagging strand discontinuously (Okazaki fragments).

- (iii) Origin of replication in prokaryotes and eukaryotes
  - **Origin of Replication in Prokaryotes (e.g., E. coli - oriC):**
    - **Number:** Typically **single** origin of replication per chromosome.
    - **Size:** Relatively small (e.g., ~245 bp in E. coli).
    - **Complexity:** Less complex, usually consists of specific DNA sequences recognized by initiator proteins.
    - **Replication Forks:** Creates two replication forks that move bidirectionally.

- **Regulation:** Primarily regulated by factors like DnaA protein binding and ATP hydrolysis.
- **Chromosomes:** Circular chromosomes.

- **Origin of Replication in Eukaryotes:**
  - **Number: Multiple** origins of replication per chromosome.
  - **Size:** Varies greatly, can be hundreds to thousands of base pairs.
  - **Complexity:** More complex, often involving specific sequence motifs (e.g., ARS in yeast) and chromatin structure.
  - **Replication Forks:** Each origin forms two replication forks, allowing for simultaneous replication at multiple sites along the linear chromosome.
  - **Regulation:** Highly regulated to ensure each origin fires only once per S phase, involving origin recognition complexes (ORC), licensing factors (MCM helicase), and cyclin-dependent kinases (CDKs).
  - **Chromosomes:** Linear chromosomes.

(b) Define linking number and find out the linking number of a closed circular DNA molecule of 4200 bp. Suppose four turns are removed from it, what will be the ΔLk and Specific linking difference ?

- **Linking Number (Lk):**
  - The linking number is a topological property of a closed circular DNA molecule that represents the **total number of times one strand of the DNA helix crosses over the other strand** when the molecule is constrained to lie in a plane.

- It is a fixed integer for a covalently closed circular DNA molecule and can only be changed by breaking and rejoining the phosphodiester backbone of one or both strands (catalyzed by topoisomerases).
- It is the sum of **Twist (Tw)** and **Writhe (Wr)**: $Lk = Tw + Wr$.
  - **Twist (Tw):** The number of helical turns of one DNA strand around the duplex axis.
  - **Writhe (Wr):** The number of superhelical turns or supercoils.

- **Calculation for a closed circular DNA molecule of 4200 bp:**
  - Assuming B-form DNA, which has approximately 10.5 base pairs per helical turn.
  - **Initial Linking Number (Lk_0):**
    - $Lk_0 = \frac{\text{Number of base pairs}}{\text{Base pairs per turn}}$
    - $Lk_0 = \frac{4200 \text{ bp}}{10.5 \text{ bp/turn}}$
    - $Lk_0 = 400$ turns

- **If four turns are removed from it:**
  - Removing turns means the DNA is being underwound, leading to a decrease in the linking number (negative supercoiling).
  - **Change in Linking Number (ΔLk):**
    - $\Delta Lk =$ Number of turns removed $= -4$ (negative because turns are removed/underwound)
  - **Specific Linking Difference (σ):**
    - The specific linking difference (also known as superhelical density) is a normalized measure of supercoiling.

- $\sigma = \frac{\Delta Lk}{Lk_0}$
- $\sigma = \frac{-4}{400}$
- $\sigma = -0.01$

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3. (a) Explain the double-strand break repair model of homologous recombination.

- The **double-strand break (DSB) repair model** is a major pathway for homologous recombination in eukaryotes and is also important in bacteria (RecBCD pathway). It proposes a mechanism by which a broken DNA molecule with two free ends can be repaired using an intact homologous DNA molecule (e.g., sister chromatid or homologous chromosome) as a template.

- **Steps of the DSB Repair Model:**

    b. **Double-Strand Break Formation:** The process begins with a clean double-strand break in one of the DNA molecules. This can be caused by radiation, chemical agents, or programmed events (e.g., meiotic recombination).

    c. **Resection (5' → 3' Exonucleolysis):** Enzymes (like the MRN complex and subsequently nucleases like Exo1 or Sgs1/Dna2 in eukaryotes, or RecBCD in E. coli) process the broken ends. This involves a **5' → 3' exonuclease activity** that degrades the 5' ends of the broken DNA, leaving behind **3' single-stranded overhangs**. These 3' overhangs are crucial for invading the homologous DNA.

    d. **Strand Invasion (D-Loop Formation):**

        - The 3' single-stranded overhang from one of the broken ends **invades** the intact homologous DNA duplex (the donor molecule). This invasion is mediated by

recombinase proteins (e.g., **RecA in bacteria, Rad51 in eukaryotes**), which bind to the single-stranded DNA and facilitate its search for homology.

- Upon finding a homologous sequence, the invading strand displaces one of the strands of the donor duplex, forming a **displacement loop (D-loop)**. The invading strand then base-pairs with the complementary strand of the donor.

e. **DNA Synthesis and D-loop Extension:**

- The invading 3' end serves as a **primer** for DNA polymerase. DNA synthesis extends this invading strand, using the intact homologous strand in the D-loop as a template.
- As DNA synthesis proceeds, the D-loop expands, leading to further displacement of the original strand from the donor DNA.

f. **Second End Capture and Ligation (Formation of Double Holliday Junctions):**

- The displaced strand from the D-loop can then anneal with the **other 3' single-stranded overhang** from the *original broken molecule* (the second end of the break).
- DNA synthesis fills any remaining gaps, and DNA ligase seals the nicks, resulting in the formation of **two Holliday junctions** (double Holliday junction, dHJ).

g. **Holliday Junction Migration:**

- The two Holliday junctions can migrate along the DNA, driven by helicases and branch migration proteins (e.g., RuvAB in E. coli, Rad54/Mph1 in eukaryotes). This expands the region of heteroduplex DNA.

h. **Resolution of Holliday Junctions:**

- The two Holliday junctions are then resolved by specific nucleases called **resolvases** (e.g., RuvC in E. coli, various nucleases like MUS81-EME1 or SLX1-SLX4 in eukaryotes).
- Resolution can occur in two ways, depending on how the cuts are made at each junction:
  - **Non-crossover products:** If the cuts are made in the same orientation at both junctions (e.g., both vertical or both horizontal), the original flanking markers remain linked.
  - **Crossover products:** If the cuts are made in opposite orientations at the two junctions (e.g., one vertical and one horizontal), the DNA segments flanking the recombined region are exchanged, leading to genetic crossover.

- **Significance:** This model explains how genetic information can be accurately restored at a DSB using an undamaged homologous chromosome as a template, ensuring genome stability and serving as a key mechanism for genetic diversity through meiotic recombination.

(b) Write a note on the enzymes and proteins required for homologous recombination and their roles.

Homologous recombination (HR) is a complex process involving numerous enzymes and proteins that work in a coordinated manner. Key players include:

- **1. RecBCD Complex (E. coli) / MRN Complex (MRE11-RAD50-NBS1) (Eukaryotes):**

- **Role:** These complexes are typically the first responders to a double-strand break (DSB).
- **RecBCD (E. coli):** Possesses helicase (unwinds DNA) and nuclease (exonuclease) activities. It unwinds the DNA from the break point and degrades it in a 5' → 3' direction until it encounters a Chi site (χ), at which point it shifts to primarily 3' → 5' degradation, creating the crucial 3' single-stranded overhang required for RecA binding.
- **MRN Complex (Eukaryotes):** Recognizes DSBs, tethers the broken DNA ends, and initiates the resection of the 5' strands, creating 3' overhangs. It also recruits other repair proteins.

- **2. Single-Stranded Binding Protein (SSB) (E. coli) / Replication Protein A (RPA) (Eukaryotes):**
  - **Role:** Binds to the single-stranded DNA (ssDNA) generated during resection.
  - **Function:** Prevents the ssDNA from forming secondary structures (like hairpins), protects it from degradation by nucleases, and facilitates the loading of recombinase proteins (RecA/Rad51).
- **3. RecA (E. coli) / Rad51 (Eukaryotes):**
  - **Role:** These are the central recombinase proteins, critical for strand invasion and homologous pairing.
  - **Function:** Binds cooperatively to the 3' ssDNA overhangs, forming a nucleoprotein filament. This filament then actively searches for homologous sequences in an intact duplex DNA molecule. Once homology is found, RecA/Rad51 catalyzes the displacement of one strand of the homologous duplex and the invasion of the ssDNA, forming the D-loop and facilitating homologous pairing and strand exchange.

- **4. DNA Polymerase (e.g., Pol III in E. coli, various polymerases in eukaryotes):**
    - **Role:** Synthesizes new DNA.
    - **Function:** Uses the invading 3' end as a primer and the intact homologous strand as a template to extend the invading strand, filling gaps and synthesizing DNA across the recombination junction.
- **5. DNA Ligase:**
    - **Role:** Seals nicks in the phosphodiester backbone.
    - **Function:** Joins the newly synthesized DNA fragments and the original strands after strand exchange and gap filling, completing the phosphodiester backbone.
- **6. RuvAB Complex and RuvC (E. coli) / Various Resolvases (e.g., MUS81-EME1, SLX1-SLX4, Gen1) (Eukaryotes):**
    - **Role:** Proteins involved in processing and resolving Holliday junctions (HJs).
    - **RuvA:** Recognizes the Holliday junction.
    - **RuvB:** Is a helicase that powers the branch migration of the Holliday junction, moving the crossover point along the DNA.
    - **RuvC:** Is an endonuclease (resolvase) that specifically cleaves the Holliday junction to resolve it into separate DNA molecules, either resulting in crossover or non-crossover products.
    - **Eukaryotic Resolvases:** Multiple enzymes with similar roles, often acting as heterodimers, to cleave Holliday junctions.
- **7. Topoisomerases (e.g., Topo I, Topo II):**
    - **Role:** Manage DNA supercoiling.

- **Function:** Important for relieving torsional stress that builds up during DNA unwinding (e.g., during D-loop formation and branch migration) and for disentangling DNA molecules after recombination.

- **Accessory Factors (examples):**
  - **RecFOR pathway (E. coli):** Involved in loading RecA onto ssDNA at gaps.
  - **Rad52 (Eukaryotes):** Helps load Rad51 onto ssDNA and mediates strand annealing.
  - **Rad54 (Eukaryotes):** A motor protein (helicase) that remodels chromatin and facilitates Rad51 filament activity, including D-loop formation and branch migration.

These proteins ensure the fidelity and efficiency of homologous recombination, which is crucial for DNA repair and genetic exchange.

(c) How site-specific recombination is different from Homologous recombination ?

Site-specific recombination (SSR) and homologous recombination (HR) are both mechanisms of genetic recombination, but they differ significantly in their requirements, mechanisms, and biological roles.

- **Homologous Recombination (HR):**
  - **Sequence Requirement:** Requires **extensive sequence homology** (typically hundreds to thousands of base pairs) between the recombining DNA molecules. It searches for and aligns identical or nearly identical sequences.
  - **Enzymatic Machinery:** Involves a complex set of proteins including recombinases (RecA/Rad51), helicases, nucleases, polymerases, and ligases.

- **Mechanism:** Initiated by DNA double-strand breaks or single-strand gaps. Involves strand invasion, D-loop formation, branch migration, and resolution of Holliday junctions.
- **Products:** Can result in either **crossover** (exchange of flanking DNA) or **non-crossover** products.
- **Biological Roles:**
  - **DNA Repair:** Primary mechanism for repairing double-strand breaks in a high-fidelity manner, especially important in actively dividing cells and during S/G2 phases of the cell cycle.
  - **Meiotic Recombination:** Essential for genetic diversity during meiosis, leading to the exchange of genetic material between homologous chromosomes.
  - **Maintenance of Genome Integrity:** Repairs collapsed replication forks.
- **Flexibility:** Can occur anywhere along homologous DNA segments.

- **Site-Specific Recombination (SSR):**
  - **Sequence Requirement:** Requires **very short, specific DNA sequences** (typically 10-30 base pairs) on both recombining DNA molecules. These sites are recognized directly by the recombinase enzymes. The sequences usually contain inverted or direct repeats.
  - **Enzymatic Machinery:** Catalyzed by a small number of specialized recombinase enzymes (often a single protein or a small complex), belonging to either the Integrase (Tyrosine recombinase) family or the Resolvase/Invertase (Serine recombinase) family. No extensive DNA synthesis or repair machinery is usually involved.

- **Mechanism:** Involves precise recognition of target sites, cleavage of DNA at these sites, rejoining of DNA ends in a new configuration, often without a Holliday junction intermediate (or with a transient, easily resolvable one). The reaction is typically highly regulated and oriented.
- **Products:** The outcome is highly specific and predictable, resulting in **integration, excision, inversion, or gene cassette exchange**, depending on the orientation of the recognition sites.
- **Biological Roles:**
  - **Phage Integration/Excision:** Crucial for bacteriophages (e.g., λ phage integration into bacterial genome) and transposons to integrate into or excise from host genomes.
  - **Genome Rearrangements:** Involved in bacterial inversion events (e.g., phase variation of flagellar antigens), gene regulation, and gene activation.
  - **Immune System Development:** In vertebrates, V(D)J recombination, a type of SSR, is vital for generating antibody and T-cell receptor diversity.
  - **Genetic Engineering:** Widely used in biotechnology (e.g., Gateway cloning, Cre-loxP, Flp-FRT systems) for precise manipulation of DNA.
- **Flexibility:** Occurs only at predefined recognition sites.

In summary, HR is a general repair and exchange mechanism requiring extensive homology, while SSR is a highly specialized and precise recombination event driven by specific DNA sequences and dedicated recombinase enzymes, serving distinct biological functions, often related to genome rearrangements and integration/excision events.

4. (a) Explain Ames test and its significance.

- **Ames Test:**
    - The Ames test is a widely used and relatively inexpensive biological assay developed by Bruce Ames in the 1970s to **assess the mutagenic potential of chemical compounds**. It is a rapid and sensitive screening tool to identify substances that can cause mutations in DNA, which are often correlated with carcinogenic potential.
    - **Principle:** The test utilizes special strains of the bacterium *Salmonella typhimurium* that are auxotrophic for histidine, meaning they cannot synthesize histidine and thus require it in their growth medium. These mutant strains have specific point mutations (e.g., in the *hisG* or *hisD* genes) that prevent them from growing without added histidine.
    - **Procedure:**
        i. **Preparation:** A small amount of the test chemical is added to a petri dish containing a histidine-deficient agar medium.
        ii. **Bacterial Strains:** *Salmonella typhimurium* strains (usually multiple strains, each with a different type of point mutation, e.g., frameshift or base substitution) are inoculated onto the plate. These strains also often have other mutations that increase their sensitivity to mutagens (e.g., defective DNA repair systems or increased cell wall permeability).
        iii. **Metabolic Activation:** To account for the fact that many compounds are not mutagenic themselves but are converted into mutagens by metabolism in the liver, the test often includes a **rat liver extract (S9 fraction)**. This extract contains metabolic enzymes (e.g., cytochrome

P450 oxidases) that can mimic mammalian metabolism, activating promutagens into their active mutagenic forms.

iv. **Incubation:** The plates are incubated for a period (e.g., 48 hours).

v. **Observation:**

- If the test chemical is a mutagen, it will cause **reverse mutations (reversions)** in the *Salmonella* strains, restoring their ability to synthesize histidine.
- These "revertant" bacteria will then be able to grow and form visible colonies on the histidine-deficient medium.
- The number of revertant colonies is directly proportional to the mutagenicity of the test chemical. A background level of spontaneous mutations always occurs, so an increase significantly above this background indicates mutagenicity.

vi. **Controls:** Positive controls (known mutagens) and negative controls (solvent only) are always included to ensure the validity of the test.

- **Significance:**

i. **Carcinogen Screening:** The primary significance is its ability to serve as a **cost-effective and rapid pre-screen for potential carcinogens**. While not all mutagens are carcinogens, and not all carcinogens are mutagens, there is a strong correlation between mutagenicity (especially genotoxicity) and carcinogenicity.

j. **Risk Assessment:** Helps in identifying hazardous chemicals in the environment, food, pharmaceuticals, and industrial products, contributing to public health and safety.

k. **Drug Development:** Used in the early stages of drug development to screen out compounds with potential mutagenic side effects.

l. **Understanding Mutagenesis:** Contributes to a fundamental understanding of how different chemicals interact with DNA and induce mutations.

m. **Reduced Animal Testing:** Provides an initial tier of testing that can reduce the need for more expensive, time-consuming, and ethically complex animal carcinogenicity studies. However, it's important to note that the Ames test is a *screening* tool and positive results often warrant further *in vivo* testing.

(b) Write a note on types of mutations.

Mutations are heritable changes in the genetic material (DNA or RNA). They can range from single base changes to large chromosomal rearrangements. Here are the main types of mutations:

- **1. Point Mutations:**
    - These involve a change in a single nucleotide base pair.
    - **a. Substitution Mutations:** One base pair is replaced by another.
        - **Transition:** A purine is replaced by another purine (A ↔ G) or a pyrimidine is replaced by another pyrimidine (C ↔ T).
        - **Transversion:** A purine is replaced by a pyrimidine, or a pyrimidine is replaced by a purine (A/G ↔ C/T).
    - **b. Insertion Mutations:** One or more extra nucleotide base pairs are added into a DNA sequence.

- **c. Deletion Mutations:** One or more nucleotide base pairs are removed from a DNA sequence.
- **Consequences of Point Mutations in Coding Regions (mRNA/Protein):**
  - **Silent (Synonymous) Mutation:** A base change that results in no change in the amino acid sequence of the protein due to the degeneracy of the genetic code.
  - **Missense Mutation:** A base change that results in a codon specifying a different amino acid, potentially altering protein function (e.g., sickle cell anemia).
  - **Nonsense Mutation:** A base change that converts an amino acid-coding codon into a premature stop codon, leading to a truncated and usually non-functional protein.
  - **Frameshift Mutation:** Insertions or deletions of nucleotides (not in multiples of three) that shift the reading frame of the mRNA, leading to a completely different amino acid sequence downstream and often a premature stop codon. Highly deleterious.

- **2. Chromosomal Mutations (Large-Scale Mutations):**
  - These involve changes in the structure or number of chromosomes.
  - **a. Deletion:** A segment of a chromosome is lost. Can lead to significant loss of genetic material.
  - **b. Duplication:** A segment of a chromosome is repeated. Can lead to extra copies of genes.
  - **c. Inversion:** A segment of a chromosome is reversed end-to-end. The order of genes is changed, but no genetic material is lost or gained.

- **d. Translocation:** A segment of one chromosome breaks off and attaches to a different, non-homologous chromosome.
    - **Reciprocal Translocation:** Exchange of segments between two non-homologous chromosomes.
    - **Robertsonian Translocation:** Fusion of two acrocentric chromosomes, with loss of short arms.
- **e. Aneuploidy:** Abnormal number of chromosomes (e.g., monosomy, trisomy like Down syndrome - trisomy 21).
- **f. Polyploidy:** More than two complete sets of chromosomes (e.g., triploid, tetraploid). Common in plants, lethal in humans.

- **3. Dynamic Mutations (Trinucleotide Repeat Expansions):**
    - Involve the amplification of specific three-nucleotide repeat sequences. The number of repeats can increase across generations (e.g., Huntington's disease, Fragile X syndrome).

- **4. Somatic vs. Germline Mutations:**
    - **Somatic Mutations:** Occur in non-reproductive (somatic) cells. They are not inherited by offspring but can affect the individual (e.g., contribute to cancer).
    - **Germline Mutations:** Occur in germ cells (sperm or egg). They can be transmitted to offspring and passed down through generations.
- **5. Spontaneous vs. Induced Mutations:**
    - **Spontaneous Mutations:** Occur naturally due to errors in DNA replication, tautomeric shifts of bases, or spontaneous chemical changes (e.g., deamination of cytosine to uracil).
    - **Induced Mutations:** Caused by exposure to environmental mutagens (e.g., chemicals, radiation, UV light).

Understanding these different types of mutations is crucial for studying genetic diseases, evolution, and the mechanisms of DNA repair.

(c) Explain DNA damage caused by hydrolysis, alkylation, oxidation and radiation.

DNA is constantly exposed to various agents, both endogenous (from cellular metabolism) and exogenous (from the environment), that can cause damage.

- **1. DNA Damage by Hydrolysis:**
    - **Mechanism:** Hydrolytic reactions involve the addition of water to break chemical bonds. In DNA, two common hydrolytic damages are deamination and depurination/depyrimidination.
    - **Deamination:** The removal of an amino group from a base.
        - **Cytosine deamination:** Converts cytosine to uracil. Uracil pairs with adenine, so if not repaired, a C-G base pair becomes a T-A base pair after replication (a transition mutation).
        - **Adenine deamination:** Converts adenine to hypoxanthine. Hypoxanthine pairs with cytosine, leading to an A-T to G-C transition.
        - **Guanine deamination:** Converts guanine to xanthine. Xanthine blocks replication.
    - **Depurination/Depyrimidination:** The cleavage of the N-glycosidic bond that links a purine (adenine or guanine) or a pyrimidine (cytosine or thymine) base to the deoxyribose sugar in the DNA backbone.
        - **Depurination:** Much more common than depyrimidination. Leaves an **AP site (apurinic/apyrimidinic site)**. If unrepaired, DNA

polymerase may insert a random nucleotide opposite the AP site during replication, typically adenine, leading to a mutation.

- **2. DNA Damage by Alkylation:**
    - **Mechanism:** Alkylating agents are chemicals that add alkyl groups (e.g., methyl, ethyl) to the bases or phosphate backbone of DNA. This often occurs at nucleophilic sites on the bases (e.g., N7 of guanine, N3 of adenine).
    - **Consequences:**
        - **Misreplication:** Some alkylated bases (e.g., O6-methylguanine) can mispair during replication (O6-methylguanine pairs with thymine instead of cytosine), leading to G-C to A-T transition mutations.
        - **Block Replication:** Other alkylated bases can physically impede the movement of DNA polymerase, blocking DNA replication.
        - **DNA Strand Breaks:** Alkylation can weaken the N-glycosidic bond, leading to depurination and subsequent strand breaks.
    - **Example:** Nitrogen mustards, ethyl methanesulfonate (EMS).
- **3. DNA Damage by Oxidation:**
    - **Mechanism:** Reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2), are generated during normal cellular metabolism (e.g., mitochondrial respiration) or by exogenous factors (e.g., ionizing radiation). These ROS can attack DNA bases and the deoxyribose sugar.
    - **Consequences:**

- **Base Modifications:** The most common is the formation of **8-oxoguanine (8-oxoG)** from guanine. 8-oxoG can mispair with adenine during replication, leading to a G-C to T-A transversion mutation.
- **Sugar Damage:** Oxidation of the deoxyribose sugar can lead to DNA strand breaks (single-strand or double-strand breaks).
- **Cross-links:** Formation of DNA-protein cross-links or interstrand DNA cross-links.

- **4. DNA Damage by Radiation:**
  - **Mechanism:**
    - **UV Radiation (Ultraviolet Light):** Primarily causes damage to pyrimidine bases (thymine and cytosine). Its energy is absorbed by these bases, leading to the formation of abnormal covalent bonds between adjacent pyrimidines on the same DNA strand.
      - **Pyrimidine Dimers:** The most common are **cyclobutane pyrimidine dimers (CPDs)** and **(6-4) photoproducts**. These dimers distort the DNA helix, block DNA replication and transcription, and can cause mutations if unrepaired.
    - **Ionizing Radiation (X-rays, Gamma rays, Alpha/Beta particles):** Has much higher energy and can directly or indirectly cause severe DNA damage.
      - **Direct Effect:** Ionizing radiation can directly ionize DNA molecules, leading to radical formation.
      - **Indirect Effect:** More commonly, it ionizes water molecules in the cell, producing highly reactive hydroxyl radicals ($OH^{\cdot}$).

- **Consequences:** The primary and most dangerous lesions are **DNA single-strand breaks (SSBs)** and, more critically, **DNA double-strand breaks (DSBs)**. It can also cause base modifications and DNA-protein cross-links. DSBs are particularly dangerous because they can lead to chromosomal rearrangements, loss of genetic material, and cell death if not repaired accurately.

These different types of DNA damage highlight the continuous threat to genome integrity and underscore the importance of robust DNA repair mechanisms in all living organisms.

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5. (a) Explain the elongation phase of the replication in E. coli.

The elongation phase of DNA replication in *E. coli* is the stage where the DNA polymerase synthesizes new DNA strands using the existing parental strands as templates. It occurs at the replication fork, which is the Y-shaped structure formed when the DNA double helix is unwound.

- **1. Unwinding and Stabilization of the Replication Fork:**
    - **DnaB Helicase:** The DnaB helicase unwinds the double-stranded DNA ahead of the replication fork, separating the two parental strands. This process requires ATP hydrolysis.
    - **Single-Strand Binding Proteins (SSBs):** Once unwound, SSBs bind cooperatively to the single-stranded DNA (ssDNA) regions. This prevents the separated strands from re-annealing, protects them from degradation by nucleases, and keeps them in an extended conformation accessible for DNA polymerase.
    - **DNA Topoisomerases (Gyrase):** As the DNA unwinds, positive supercoiling builds up ahead of the replication fork. DNA gyrase (a type II topoisomerase in *E. coli*) introduces

negative supercoils to relieve this torsional stress, allowing replication to proceed smoothly.

- **2. Leading Strand Synthesis (Continuous Synthesis):**
    - The leading strand is synthesized continuously in the **5' to 3' direction**, following the direction of the replication fork movement.
    - Once a short RNA primer is synthesized by **DnaG primase** at the origin, **DNA Polymerase III (Pol III) holoenzyme** takes over.
    - Pol III remains tightly bound to the template by its β **sliding clamp (processivity factor)**, which forms a ring around the DNA, preventing the polymerase from dissociating. This high processivity allows for rapid and uninterrupted synthesis of the entire leading strand.
- **3. Lagging Strand Synthesis (Discontinuous Synthesis - Okazaki Fragments):**
    - The lagging strand is synthesized discontinuously in short fragments called **Okazaki fragments**. This is because DNA polymerase can only synthesize DNA in the 5' to 3' direction, but the lagging strand template runs 5' to 3' relative to the fork.
    - For each Okazaki fragment:
        i. **Primase Activity: DnaG primase** synthesizes a new short (approx. 10-12 nucleotides) RNA primer on the lagging strand template, periodically as the fork unwinds.
        ii. **Pol III Synthesis: DNA Polymerase III holoenzyme** binds to the primer and extends it in the 5' to 3' direction, synthesizing a new Okazaki fragment (approx. 1000-2000 nucleotides long in *E. coli*) until it encounters the 5' end of the preceding Okazaki fragment's primer.

iii. **Loop Formation (Trombone Model):** The lagging strand template is thought to form a loop to allow Pol III to synthesize DNA in the 5' to 3' direction while still moving in the same overall direction as the replication fork. When Pol III reaches the previous fragment, the loop is released, and a new loop forms for the next fragment.

iv. **Primer Removal:** When Pol III reaches the RNA primer of the previous Okazaki fragment, it dissociates. **DNA Polymerase I (Pol I)** then binds. Pol I uses its **5' to 3' exonuclease activity** to remove the RNA primer nucleotide by nucleotide, simultaneously filling the gap with deoxyribonucleotides using its 5' to 3' polymerase activity.

v. **Ligation:** After Pol I removes the primer and fills the gap, a single-strand break (nick) remains between the newly synthesized DNA and the adjacent Okazaki fragment. **DNA ligase** seals this nick by forming a phosphodiester bond, completing the continuous lagging strand.

- **Overall Co-ordination (Replisome):**
    - The leading and lagging strand synthesis are coordinated by a large multi-enzyme complex called the **replisome**.
    - The replisome includes two molecules of Pol III (one for each strand), DnaB helicase, primase, SSBs, and the β sliding clamps. This ensures efficient and coordinated synthesis of both strands simultaneously at the replication fork.

This continuous unwinding, priming, synthesis, primer removal, and ligation ensures that both strands of the parental DNA are replicated accurately and efficiently in *E. coli*.

(b) Explain the experimentation to prove that DNA replication is semi-conservative in nature.

The semi-conservative nature of DNA replication was famously demonstrated by **Matthew Meselson and Franklin Stahl in 1958** using *E. coli* bacteria. Their elegant experiment provided strong evidence against conservative and dispersive models of replication.

- **Hypotheses about DNA Replication:**

    n. **Conservative:** The original DNA double helix remains intact, and an entirely new double helix is synthesized.

    o. **Semi-conservative:** Each new DNA molecule consists of one original (parental) strand and one newly synthesized strand.

    p. **Dispersive:** Each new DNA molecule contains a mixture of original and newly synthesized DNA on both strands, interspersed throughout the molecule.

- **Experimental Design (Meselson-Stahl Experiment):**

    1. Labeling Parental DNA with Heavy Nitrogen (N^{15}): * Meselson and Stahl grew *E. coli* bacteria for many generations in a growth medium containing **heavy isotope nitrogen,** N^{15} (instead of the common N^{14}). * Since nitrogen is a major component of DNA bases, the DNA of these bacteria became **uniformly labeled with** N^{15}, making it denser than normal N^{14}-containing DNA. * They isolated this N^{15}-labeled DNA and centrifuged it in a **cesium chloride (**CsCl**) density gradient**. The dense N^{15}-DNA formed a single band at a **high density position** in the gradient.

    2. Shifting to Light Nitrogen (N^{14}) Medium and Replication: * They then transferred the N^{15}-labeled *E. coli* cells to a fresh growth medium containing only **light isotope nitrogen,** N^{14}. * The bacteria were allowed to grow and divide for specific time periods (corresponding to rounds of DNA replication).

3. Analysis of DNA Density after Replication: * At various time points (after 1 generation, 2 generations, etc.), DNA was extracted from the bacterial cultures. * This extracted DNA was then subjected to CsCl **density gradient centrifugation**. * The position of the DNA band(s) in the gradient would reveal its density, thereby indicating the composition of the DNA strands.

- **Results and Interpretation:**
    - **After 0 Generations (Parental DNA):** A single band of DNA was observed at the **high-density position** (100% N^{15} DNA). This served as the initial control.
    - **After 1 Generation of Replication (in N^{14} medium):**
        - Meselson and Stahl observed a **single band of DNA at an intermediate density position**, precisely halfway between the high-density (N^{15}-DNA) and low-density (N^{14}-DNA) positions.
        - **Interpretation:** This result strongly supported the **semi-conservative model**.
            - It ruled out the **conservative model**, which would have predicted two distinct bands: one high-density (original parent) and one low-density (newly synthesized).
            - It was consistent with the **semi-conservative model**, where each new DNA molecule contained one N^{15} (parental) strand and one N^{14} (newly synthesized) strand, resulting in a hybrid density.
            - It was also initially consistent with the dispersive model, as a mixture of heavy and light DNA would also yield an intermediate density.
    - **After 2 Generations of Replication (in N^{14} medium):**

- Two distinct bands of DNA were observed:
    - One band at the **intermediate density position** (still representing N^{15}/N^{14} hybrid DNA).
    - One band at the **low-density position** (100% N^{14} DNA).
- **Interpretation:** This result was crucial and definitively ruled out the **dispersive model**.
    - The dispersive model would have predicted only a single band, with density gradually decreasing over generations as the N^{15} content became more diluted and interspersed.
    - The observation of two distinct bands (intermediate and light) perfectly fit the **semi-conservative model**: the original intermediate hybrid molecules had replicated again in N^{14} medium, producing more hybrid molecules and now also entirely light N^{14}/N^{14} molecules.

- **Conclusion:** The Meselson-Stahl experiment provided elegant and conclusive evidence that DNA replication is **semi-conservative**, meaning that each new DNA double helix consists of one strand from the original molecule and one newly synthesized strand. This fundamental mechanism ensures that genetic information is faithfully passed from one generation to the next.

(c) Explain an experiment to prove that DNA is a genetic material.

The definitive experiment proving DNA as the genetic material was performed by **Alfred Hershey and Martha Chase in 1952**, using bacteriophages (viruses that infect bacteria). This experiment built upon

earlier work by Griffith (transformation) and Avery, MacLeod, and McCarty (identifying the transforming principle as DNA).

- **Background:** By the early 1950s, it was known that genes resided on chromosomes, which were composed of both DNA and protein. However, it was unclear which of these two macromolecules carried the genetic information. Proteins were considered strong candidates due to their diverse functions and complex structures.
- **Experimental Design (Hershey-Chase Experiment):**
    - **Tool:** Bacteriophage T2, a virus that infects *E. coli*.
        - T2 phage consists of a protein coat surrounding a DNA core.
        - When T2 infects a bacterium, it injects its genetic material into the host cell, while most of its outer structure remains outside. The host cell then produces new phage particles.
    - **Strategy: Differential Labeling of DNA and Protein:**
        - Hershey and Chase used **radioactive isotopes** to specifically label either the DNA or the protein of the T2 phage.
        - **Labeling DNA:** They grew one batch of T2 phages in a medium containing **radioactive phosphorus (**P^{32}**)**. Since phosphorus is present in DNA (in the phosphate backbone) but not in protein, the DNA of these phages was labeled.
        - **Labeling Protein:** They grew another batch of T2 phages in a medium containing **radioactive sulfur (**S^{35}**)**. Since sulfur is present in some amino acids (methionine, cysteine) but not in DNA, the protein coat of these phages was labeled.
    - **Procedure:**

i. **Infection:** They mixed the labeled phages (either P^{32}-labeled DNA or S^{35}-labeled protein) with separate cultures of unlabeled *E. coli* bacteria. The phages adsorbed to the bacterial surface and injected their genetic material.

ii. **Blending:** After allowing sufficient time for infection, the phage-bacterium mixtures were subjected to vigorous agitation in a kitchen blender. This step was crucial to shear off the empty phage coats (capsids) that remained attached to the outside of the bacterial cells.

iii. **Centrifugation:** The mixtures were then centrifuged.

- The heavier bacterial cells (and anything inside them) pelleted at the bottom of the tube.
- The lighter phage particles (empty coats) remained in the supernatant.

iv. **Measurement of Radioactivity:** The radioactivity in both the pellet (bacterial cells) and the supernatant (phage coats) was measured for both experiments.

- **Results:**
  - **Experiment with S^{35}-labeled phages (protein labeled):**
    - Most of the S^{35} radioactivity was found in the **supernatant** (associated with the empty phage coats).
    - Very little S^{35} was found in the bacterial **pellet**.
    - Furthermore, the progeny phages produced by these bacteria were **not radioactive**.
  - **Experiment with P^{32}-labeled phages (DNA labeled):**

- A significant amount of the P^{32} radioactivity was found in the bacterial **pellet** (inside the bacterial cells).
- Very little P^{32} was found in the **supernatant** (associated with the empty phage coats).
- Crucially, the progeny phages produced by these bacteria **were radioactive with** P^{32}.

- **Conclusion:**
  - The results clearly showed that **DNA (labeled with** P^{32}**) entered the bacterial cells**, while most of the protein (S^{35}) remained outside.
  - Since the infected bacteria subsequently produced new, infectious phage particles containing P^{32}-labeled DNA, it logically followed that the injected **DNA must carry the genetic information** necessary for phage replication and the synthesis of new phage components.
  - This experiment provided compelling and widely accepted evidence that **DNA, not protein, is the genetic material**.

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6. (a) Write the difference between simple transposon and complex transposon.

Transposons (also known as transposable elements or "jumping genes") are DNA sequences that can move from one location in the genome to another. They are broadly classified into simple and complex transposons.

- **Simple Transposons (Insertion Sequences - IS elements):**
  - **Structure:**
    - **Minimalist:** These are the simplest type of transposons.

        - **Composition:** Typically consist only of the gene(s) encoding the necessary enzyme(s) for transposition (usually **transposase**) flanked by short, inverted repeat (IR) sequences at their ends.
        - **Size:** Generally small, ranging from about 700 to 2000 base pairs.
    - **Genetic Information:** Carry **no genes other than those involved in their own transposition**. They do not carry genes that confer a phenotypic advantage to the host (e.g., antibiotic resistance).
    - **Target Site Duplication:** Like other transposons, when they insert into a new target DNA site, they create a direct repeat of the target site DNA immediately flanking the inserted IS element.
    - **Mechanism of Transposition:** Can move via cut-and-paste (non-replicative) or copy-and-paste (replicative) mechanisms, depending on the specific IS element.
    - **Examples:** IS1, IS2, IS10, IS50 (part of Tn5).
- **Complex Transposons (Composite Transposons or Tn elements):**
    - **Structure:**
        - **Modular:** More elaborate structures than simple transposons.
        - **Composition:** Consist of two copies of an **Insertion Sequence (IS) element** (often identical or nearly identical) flanking a central region.
        - **Inverted Repeats:** The entire complex transposon is delimited by the inverted repeats of the outer IS elements.

        - **Central Region:** This region contains **additional genes** that are unrelated to transposition, often conferring a selective advantage to the host bacterium.
    - **Genetic Information:** Carry **genes for their own transposition (transposase from the flanking IS elements) *and* other accessory genes**, such as genes for antibiotic resistance (e.g., ampicillin resistance, kanamycin resistance), heavy metal resistance, or metabolic enzymes.
    - **Transposition:** Transposition is mediated by the transposase encoded within the flanking IS elements. The entire unit (flanking IS elements plus the central region) moves as a single transposable element.
    - **Size:** Larger than simple transposons, often several kilobases (kb) in length, depending on the content of the central region.
    - **Examples:**
        - **Tn10:** Contains the gene for tetracycline resistance, flanked by two copies of IS10.
        - **Tn5:** Contains genes for kanamycin, bleomycin, and streptomycin resistance, flanked by two copies of IS50.
        - **Tn3:** Contains the gene for ampicillin resistance. (Note: Tn3 is sometimes called a "resolvase-mediated transposon" or "complex non-composite transposon" because its transposition involves a resolvase, but it still fits the general idea of carrying accessory genes).

In essence, simple transposons are "selfish" DNA elements focused solely on their own movement, while complex transposons are like "mobile genetic islands" that carry and transfer useful genes along with their transposition machinery.

(b) Write a note on Direct transposition and Replicative transposition.

Transposons can move to new locations in the genome through different mechanisms. The two main types are direct (non-replicative or "cut-and-paste") transposition and replicative (copy-and-paste) transposition.

- **1. Direct Transposition (Non-Replicative or "Cut-and-Paste" Transposition):**
    - **Mechanism:** In this mode, the transposable element (transposon) is **excised (cut out)** from its original donor site and then **inserted (pasted)** into a new target site in the genome. There is no replication of the transposon itself during the process.
    - **Steps:**
        v. **Excision:** The transposase enzyme (encoded by the transposon) recognizes the inverted repeat (IR) sequences at the ends of the transposon. It makes staggered double-strand breaks at the ends of the transposon in the donor DNA and also at the target site DNA.

        vi. **Transposon Cleavage:** The transposon is completely excised from the donor DNA, leaving a gap.

        vii. **Target Site Insertion:** The excised transposon then inserts into the target DNA site where the staggered cuts were made.

        viii. **Gap Filling:** The gaps created at the integration site are filled in by host DNA polymerase, using the overhanging single-stranded regions as templates. This results in the characteristic **direct repeats** of the target site DNA flanking the inserted transposon.

        ix. **Donor Site Repair:** The empty donor site (where the transposon was excised) is typically repaired by host DNA

repair mechanisms. This can involve homologous recombination if a sister chromatid is available, or non-homologous end joining (NHEJ) which might lead to small deletions or mutations at the donor site.

- **Outcome:** The number of transposon copies in the genome **does not increase** through this mechanism (unless the donor site repair process involves copying from another chromosome). The original copy is simply relocated.
- **Examples:** Many bacterial IS elements and Tn10 (a complex transposon) transpose primarily by a cut-and-paste mechanism. Some eukaryotic transposons (e.g., P elements in Drosophila) also use this method.

- **2. Replicative Transposition (Copy-and-Paste Transposition):**
  - **Mechanism:** In this mode, the transposable element is **replicated (copied)**, and the copy is then inserted into a new target site, while the **original copy remains at the donor site**. This effectively increases the number of transposon copies in the genome.
  - **Steps (e.g., Tn3 family):**
    x. **Staggered Cuts & Strand Transfer:** Transposase makes single-strand cuts at the ends of the transposon (donor DNA) and also staggered single-strand cuts at the target DNA site. The free 3'-OH ends of the transposon are then ligated to the 5'-phosphate ends of the target DNA, forming a **coblestone structure** or **Shapiro intermediate** (also known as a "cointegrate").

    xi. **Replication:** Host DNA replication machinery is recruited to this cointegrate intermediate. DNA synthesis proceeds from the 3'-OH ends (provided by the transposon) through the entire transposon and into the donor and target DNA, effectively **replicating the transposon** and forming a

fused molecule containing two copies of the transposon. This intermediate is called a **cointegrate**.

xii. **Resolution (by Resolvase):** A separate enzyme called **resolvase** (encoded by the transposon) then mediates site-specific recombination between the two identical copies of the transposon within the cointegrate. This resolves the cointegrate into two separate molecules: the original donor molecule and the new target molecule, each now containing one copy of the transposon.

- **Outcome:** The number of transposon copies in the genome **increases** with each successful transposition event.
- **Examples:** Many bacterial transposons, particularly those belonging to the Tn3 family (e.g., Tn3, TnA), utilize replicative transposition.

In summary, direct transposition moves the element without increasing its copy number, while replicative transposition makes a new copy and inserts it elsewhere, increasing the overall copy number of the transposon in the genome.

(c) Write the role of any three inhibitors : Ciprofloxacin, 5-FU, Mitomycin C, Quinolones.

I will discuss Ciprofloxacin, 5-FU, and Mitomycin C. (Note: Quinolones are a class of antibiotics that includes Ciprofloxacin, so discussing Ciprofloxacin covers the main mechanism of quinolones).

- **1. Ciprofloxacin (A type of Quinolone):**
  - **Role/Mechanism:** Ciprofloxacin is a synthetic broad-spectrum antibiotic belonging to the **fluoroquinolone class**. Its primary mechanism of action is to **inhibit bacterial DNA gyrase (DNA topoisomerase II) and topoisomerase IV**.

        - **DNA Gyrase:** In *E. coli* and other bacteria, DNA gyrase is essential for relieving positive supercoiling that builds up ahead of the replication fork and for introducing negative supercoils, which are crucial for DNA replication, transcription, and recombination.
        - **Topoisomerase IV:** This enzyme is primarily involved in decatenation (uncoupling) of daughter chromosomes after replication.
    - **Inhibition:** Ciprofloxacin binds to the DNA-enzyme complex, stabilizing the "cleavable complex" intermediate. This prevents the re-ligation step of the topoisomerase reaction, leading to accumulation of **DNA double-strand breaks**.
    - **Consequences:** These breaks trigger bacterial cell death. By preventing essential DNA topological changes, Ciprofloxacin effectively halts bacterial DNA replication and transcription, thus inhibiting bacterial growth and proliferation.
    - **Clinical Use:** Used to treat a wide range of bacterial infections, including urinary tract infections, respiratory tract infections, and gastrointestinal infections.

- **2. 5-FU (5-Fluorouracil):**
    - **Role/Mechanism:** 5-Fluorouracil (5-FU) is an **antimetabolite chemotherapeutic agent** widely used in cancer treatment. Its mechanism involves interfering with nucleic acid synthesis, particularly DNA replication and repair.
    - **Activation:** 5-FU is a prodrug that needs to be anabolized intracellularly into active metabolites, primarily 5-fluorodeoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP).
    - **Inhibition of Thymidylate Synthase: FdUMP** is a potent and irreversible inhibitor of **thymidylate synthase (TS)**. TS is a

critical enzyme in the *de novo* synthesis pathway of deoxythymidine monophosphate (dTMP), which is a precursor for dTTP (one of the four DNA building blocks). By inhibiting TS, 5-FU depletes the cellular pools of dTTP.

- **Consequences of dTTP Depletion:** This leads to an imbalance in nucleotide pools, severely impacting DNA synthesis and repair. Cells attempt to incorporate uracil (which is now in excess relative to thymine) into DNA, leading to DNA damage and repair errors.

- **Incorporation into RNA and DNA:**
  - **FUTP** can be incorporated into RNA, interfering with RNA processing and protein synthesis.
  - **F dUTP** (derived from 5-FU) can be incorporated into DNA instead of dTTP, leading to DNA strand breaks and replication fork arrest.
- **Clinical Use:** Primarily used in the treatment of various cancers, especially colorectal, breast, gastric, and pancreatic cancers, often in combination with other chemotherapeutic agents.

- **3. Mitomycin C (MMC):**
  - **Role/Mechanism:** Mitomycin C is an **antibiotic and antineoplastic (anti-cancer) agent** that functions as a DNA damaging agent. It is a prodrug that requires bioreduction (activation) within the cell.
  - **DNA Cross-linking:** Once activated, Mitomycin C forms highly reactive intermediates that act as potent **DNA alkylating agents**. Its most significant effect is the formation of **DNA interstrand cross-links (ICLs)**, which are covalent bonds between complementary DNA strands. It can also form mono-adducts and DNA-protein cross-links.

- **Consequences:**
  - **Blocks DNA Replication:** ICLs physically block the separation of DNA strands, making it impossible for DNA polymerase to synthesize new DNA during replication. This leads to replication fork arrest.
  - **Blocks Transcription:** ICLs also impede the progression of RNA polymerase, thereby inhibiting transcription.
  - **Induces DNA Damage Response and Apoptosis:** The severe DNA damage caused by ICLs activates DNA damage checkpoints and repair pathways. If the damage is extensive or unrepaired, it triggers programmed cell death (apoptosis).
- **Clinical Use:** Used in the treatment of various solid tumors, including gastric, pancreatic, bladder, and breast cancers. It is often administered in combination with other chemotherapy drugs. Due to its toxicity, its use is carefully managed.