Question 1:

(a) Define the following (any nine): (2×9=18)

(i) Polycistronic transcription

(ii) Catenation

(iii) Ribozyme

(iv) Okazaki fragments

(v) Split genes

(vi) Replicators

(vii) Linker DNA

(viii) Replicon

(ix) Ori C

(x) Transcription bubble

(xi) Pribnow box

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(a) Definitions:

- **(i) Polycistronic transcription:**
    - Polycistronic transcription is a characteristic feature of prokaryotic gene expression where a single messenger RNA (mRNA) molecule carries the genetic information for the synthesis of more than one polypeptide chain.
    - This mRNA is transcribed from a single promoter and contains multiple open reading frames (cistrons), each coding for a different protein.
    - These genes often code for proteins involved in the same metabolic pathway or functional complex, allowing for coordinated expression.
    - The classic example is the lac operon in *E. coli*.

- **(ii) Catenation:**
    - Catenation, in the context of DNA, refers to the topological interlinking of two or more circular DNA molecules, forming a chain-like structure.
    - These interlinked circles cannot be separated without breaking one or both DNA strands in at least one of the molecules.
    - Catenation can occur during DNA replication, particularly in circular chromosomes or plasmids, where the newly synthesized daughter molecules become interlinked.
    - Topoisomerase enzymes (specifically Type II topoisomerases) are responsible for resolving these catenated structures by transiently breaking and rejoining DNA strands.
- **(iii) Ribozyme:**
    - A ribozyme is an RNA molecule that possesses catalytic activity, meaning it can catalyze specific biochemical reactions in a manner similar to protein enzymes.
    - Ribozymes are typically involved in RNA processing reactions, such as splicing (removing introns from RNA) or cleavage of RNA.
    - A prominent example is the ribosomal RNA (rRNA) within the ribosome, specifically the 23S rRNA in prokaryotes (and 28S rRNA in eukaryotes), which catalyzes the formation of peptide bonds during protein synthesis.
    - The discovery of ribozymes challenged the long-held belief that only proteins could act as enzymes.
- **(iv) Okazaki fragments:**
    - Okazaki fragments are short, newly synthesized DNA strands that are formed discontinuously on the lagging strand during DNA replication.

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- Due to the antiparallel nature of DNA strands and the 5' to 3' synthesis direction of DNA polymerase, the lagging strand template (3' to 5') is replicated in short segments.
- Each Okazaki fragment typically consists of an RNA primer followed by a stretch of DNA (around 1000-2000 nucleotides in prokaryotes, 100-200 in eukaryotes).
- These fragments are subsequently joined together by DNA ligase after the RNA primers are removed and replaced with DNA.

- **(v) Split genes:**
  - Split genes (also known as interrupted genes) are genes in eukaryotes (and some archaea and viruses) that are not continuous sequences but are composed of coding regions called **exons** interspersed with non-coding regions called **introns**.
  - Both exons and introns are transcribed into a primary RNA transcript.
  - During RNA processing, the introns are removed from this primary transcript, and the exons are accurately spliced together to form a mature messenger RNA (mRNA) molecule that codes for the protein.
  - This feature allows for alternative splicing, producing multiple protein isoforms from a single gene.
- **(vi) Replicators:**
  - Replicators are specific DNA sequences that contain all the necessary information to initiate and control DNA replication.
  - They typically include the origin of replication (ori), where DNA synthesis begins, and sequences that bind initiator proteins to recruit the replication machinery.
  - In eukaryotes, replicators are often referred to as autonomously replicating sequences (ARS) in yeast or origins of replication in higher eukaryotes.

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- The binding of specific initiator proteins to the replicator marks the site for the assembly of the pre-replication complex.

- **(vii) Linker DNA:**
  - Linker DNA refers to the stretch of DNA that connects adjacent nucleosomes in eukaryotic chromatin.
  - Each nucleosome consists of approximately 147 base pairs of DNA wrapped around a core of eight histone proteins.
  - The linker DNA, typically 20-60 base pairs long, provides the space between these nucleosome "beads" and is associated with histone H1, which helps to further compact the chromatin structure.
  - It is more accessible to nucleases than the DNA wrapped around the histone core.

- **(viii) Replicon:**
  - A replicon is a unit of DNA (or RNA, in some viruses) that contains an origin of replication and is replicated as a single entity.
  - In prokaryotes, the entire circular chromosome (and plasmids) often constitutes a single replicon, as replication initiates from a single origin and proceeds around the entire molecule.
  - In eukaryotes, given their large and linear chromosomes, there are multiple origins of replication, and each segment of DNA replicated from a single origin is considered a replicon. Eukaryotic chromosomes therefore consist of multiple replicons.

- **(ix) Ori C:**
  - Ori C (Origin of Chromosomal replication) is the single, specific DNA sequence that serves as the unique origin of replication for the circular chromosome in *Escherichia coli* and other bacteria.
  - It is a ~245 base pair region that is AT-rich and contains several conserved DNA sequences, including DnaA boxes (binding sites for

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the DnaA initiator protein) and AT-rich regions that facilitate DNA unwinding.

- The binding of DnaA protein to Ori C initiates the replication process by promoting the unwinding of the DNA helix and loading of the helicase.

- **(x) Transcription bubble:**
  - The transcription bubble is a localized region of unwound DNA that forms during transcription, allowing RNA polymerase to synthesize an RNA molecule complementary to one of the DNA strands.
  - Within this bubble, approximately 12-17 base pairs of DNA are transiently separated.
  - RNA polymerase moves along the DNA, unwinding the helix ahead of it and reannealing it behind, creating a moving transcription bubble.
  - This bubble contains the actively transcribing RNA polymerase, the DNA template strand, the non-template strand, and the nascent RNA molecule.

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- **(xi) Pribnow box:**
  - The Pribnow box is a conserved DNA sequence found in the promoter region of prokaryotic genes, typically located at approximately **-10 base pairs** upstream from the transcription start site (+1).
  - Its consensus sequence is TATAAT.
  - This sequence is recognized and bound by the sigma (σ) subunit of RNA polymerase, playing a crucial role in initiating transcription by facilitating the unwinding of the DNA double helix at the transcription start site.
  - It is analogous to the TATA box in eukaryotic promoters.

Question 2:

(a) Differentiate between the following (any four): (4×4=16)

(i) Topoisomerase type I and type II

(ii) DNA polymerase I and DNA polymerase III

(iii) Prokaryotic promoter and Eukaryotic promoter

(iv) GTFs and Transcription factors

(v) Z and A type of DNA

(b) Give any one role/significance of the following : (1×2=2)

(i) DNA ligase

(ii) Rho

(a) Differentiate between the following (any four):

- **(i) Topoisomerase Type I and Type II:**
    - **Topoisomerase Type I:**
        - **Mechanism:** Creates a **single-strand break** (nicks one strand) in the DNA backbone.
        - **Action:** Passes the intact DNA strand through the break and then religates the broken strand.
        - **Energy Requirement:** Does **not** require ATP hydrolysis for its activity. It uses the energy from the phosphodiester bond it breaks to reseal the DNA.
        - **Function:** Relieves **negative supercoils** (unwinds DNA) or **positive supercoils** (relaxes overwound DNA) one turn at a time.
        - **Effect on Linking Number (Lk):** Changes Lk by ±1.
        - **Examples:** Topoisomerase I in *E. coli*, eukaryotic Topoisomerase I.
    - **Topoisomerase Type II:**

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- **Mechanism:** Creates a **double-strand break** (breaks both strands) in the DNA backbone.
- **Action:** Passes an intact duplex DNA segment through the break and then religates the broken strands.
- **Energy Requirement:** Requires **ATP hydrolysis** for its activity, as it performs a more complex manipulation of DNA topology.
- **Function:** Can introduce or remove **negative supercoils** (unwinding) or **positive supercoils** (unwinding) by two turns at a time. Essential for decatenation (separation of interlinked circular DNA) after replication.
- **Effect on Linking Number (Lk):** Changes Lk by ±2.
- **Examples:** DNA gyrase (a bacterial Type II topoisomerase), eukaryotic Topoisomerase II.

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- **(ii) DNA Polymerase I and DNA Polymerase III (in *E. coli*):**
  - **DNA Polymerase I (Pol I):**
    - **Primary Role:** Primarily involved in **DNA repair** and **primer removal/gap filling** during replication.
    - **Processivity:** Low processivity (synthesizes only a short stretch of DNA before detaching).
    - **Exonuclease Activity:** Possesses three enzymatic activities:
      - 5' → 3' polymerase activity (adds nucleotides).
      - 3' → 5' exonuclease activity (proofreading).
      - **5' → 3' exonuclease activity (unique to Pol I), crucial for removing RNA primers and DNA repair.**
    - **Speed:** Relatively slow (around 10-20 nucleotides/second).
    - **Abundance:** Relatively abundant in the cell.

- **DNA Polymerase III (Pol III):**
  - **Primary Role:** The **main replicative enzyme** responsible for the bulk of DNA synthesis during replication (both leading and lagging strands).
  - **Processivity:** High processivity (can synthesize very long stretches of DNA without detaching, thanks to its β sliding clamp).
  - **Exonuclease Activity:** Possesses 5' → 3' polymerase activity and 3' → 5' exonuclease activity (proofreading). **Lacks 5' → 3' exonuclease activity.**
  - **Speed:** Very fast (around 250-1000 nucleotides/second).
  - **Abundance:** Less abundant than Pol I, but highly efficient.

- **(iii) Prokaryotic Promoter and Eukaryotic Promoter:**
  - **Prokaryotic Promoter:**
    - **Structure:** Relatively simple, typically consists of two main consensus sequences upstream of the transcription start site (+1).
    - **Key Elements:**
      - **Pribnow box (or -10 region):** Consensus sequence TATAAT, located around -10 bp. Involved in DNA unwinding.
      - **-35 region:** Consensus sequence TTGACA, located around -35 bp. Recognized by the sigma subunit of RNA polymerase.
    - **RNA Polymerase Binding:** Directly recognized and bound by the RNA polymerase holoenzyme (core enzyme + sigma factor).

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        - **Transcription Factors:** Generally fewer and simpler transcription factors (e.g., activators, repressors) are involved, often binding near or overlapping the promoter.
        - **Gene Arrangement:** Can initiate transcription of polycistronic mRNAs (operons).
    - **Eukaryotic Promoter:**
        - **Structure:** More complex and highly variable, often involving multiple *cis*-acting elements.
        - **Key Elements (Core Promoter):**
            - **TATA box:** Consensus sequence TATAAA, typically located around -25 to -30 bp (in many, but not all, genes). Recognized by TATA-binding protein (TBP), a subunit of TFIID.
            - **Inr (Initiator Element):** Surrounds the transcription start site.
            - **DPE (Downstream Promoter Element):** Located downstream of the start site (e.g., +28 to +34).
        - **RNA Polymerase Binding:** RNA polymerase (specifically Pol II for protein-coding genes) does not directly recognize the core promoter. It requires the binding of multiple **General Transcription Factors (GTFs)**.
        - **Transcription Factors:** Requires a large and complex set of GTFs to assemble at the core promoter to recruit RNA polymerase. Also influenced by distal *cis*-acting elements like enhancers and silencers, bound by specific transcription factors.
        - **Gene Arrangement:** Typically initiates transcription of monocistronic mRNAs (one gene per mRNA).

- **(iv) GTFs and Transcription Factors:**

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- **GTFs (General Transcription Factors):**
  - **Function:** Essential protein factors required for the accurate initiation of transcription by eukaryotic RNA polymerases (especially RNA Pol II) at all protein-coding gene promoters.
  - **Specificity:** Non-specific in terms of gene regulation; they are required for the basal level of transcription of *all* genes transcribed by a particular RNA polymerase.
  - **Role:** Form the pre-initiation complex (PIC) at the core promoter, which recruits RNA polymerase and positions it correctly for transcription initiation. They are involved in unwinding DNA and facilitating the transition to elongation.
  - **Examples:** TFIID, TFIIB, TFIIE, TFIIF, TFIIH.
- **Transcription Factors (Specific/Regulatory Transcription Factors):**
  - **Function:** Proteins that bind to specific DNA sequences (e.g., enhancers, silencers, UAS elements) located outside the core promoter region to regulate the rate of transcription (either activating or repressing it) of specific genes.
  - **Specificity:** Highly specific in terms of gene regulation; they activate or repress the transcription of particular sets of genes in response to developmental cues or environmental signals.
  - **Role:** Interact with GTFs, co-activators, or co-repressors to modulate the activity of RNA polymerase, influencing gene expression levels.
  - **Examples:** Steroid hormone receptors, activators like AP-1, repressors like LacI.

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- **(v) Z-DNA and A-DNA:**
  - **Z-DNA:**

- **Handedness:** Left-handed helix.
- **Helix Sense:** Left-handed.
- **Appearance:** Zig-zag backbone, narrower and more elongated than B-DNA.
- **Base Pairs per Turn:** 12 bp/turn.
- **Helix Diameter:** ~1.8 nm.
- **Grooves:** Narrow and deep major groove, shallow minor groove.
- **Hydration:** Forms under specific conditions, often in sequences with alternating purine-pyrimidine repeats (e.g., GCGCGC). Requires high salt concentrations or negative supercoiling for stability.
- **Biological Significance:** Transiently exists in cells; proposed roles in gene regulation, recombination, and protecting DNA from certain nucleases.

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- **A-DNA:**
  - **Handedness:** Right-handed helix.
  - **Helix Sense:** Right-handed.
  - **Appearance:** Shorter and wider than B-DNA.
  - **Base Pairs per Turn:** 11 bp/turn.
  - **Helix Diameter:** ~2.3 nm.
  - **Grooves:** Deep and narrow major groove, wide and shallow minor groove.
  - **Hydration:** Forms in conditions of **low water content** (dehydrated state), or when DNA is associated with proteins in certain complexes. Found in DNA-RNA hybrids and double-stranded RNA.

- **Biological Significance:** Important for RNA structure (dsRNA) and DNA-RNA hybrids (e.g., during transcription or reverse transcription); not thought to be the predominant form of DNA in living cells.

(b) Give any one role/significance of the following:

- **(i) DNA ligase:**
  - **Role/Significance:** DNA ligase catalyzes the formation of a phosphodiester bond to join two DNA fragments, effectively sealing nicks in the DNA backbone.
  - **In Replication:** It is crucial for joining the Okazaki fragments on the lagging strand, converting them into a continuous DNA strand. It also repairs single-strand breaks that arise during DNA repair processes.
- **(ii) Rho (Rho factor):**
  - **Role/Significance:** Rho is a prokaryotic protein factor involved in **Rho-dependent termination of transcription**.
  - **Mechanism:** It acts as an ATP-dependent helicase that binds to a Rho utilization (rut) site on the nascent mRNA, moves along the RNA towards the RNA polymerase, and unwinds the RNA-DNA hybrid within the transcription bubble, causing the release of the mRNA and termination of transcription.
  - **Significance:** Provides a regulated mechanism for terminating transcription, often in operons or for specific genes.

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Question 3:

(a) Explain Meselson and Stahl's experiment demonstrating semiconservative nature of DNA replication. (6)

(b) What is linking number? Explain with suitable examples diagrammatically. Calculate the linking number of a covalently closed circular DNA of 16000 base pairs having 08 negative supercoils. (4+3=7)

(c) What is denaturation of DNA? Explain various factors which influence the denaturation and how it changes the properties of DNA. (5)

(a) Explain Meselson and Stahl's experiment demonstrating semiconservative nature of DNA replication.

- **Experiment Objective:** To determine the precise mechanism by which DNA replicates, specifically whether it is conservative, semiconservative, or dispersive. The prevailing hypothesis was semiconservative.
- **Key Principle:** The experiment relied on differentiating DNA molecules based on their density using isotopes of nitrogen and density gradient centrifugation.
    - **15N (heavy isotope):** DNA synthesized in a medium containing 15N would be denser.
    - **14N (light isotope):** DNA synthesized in a medium containing 14N would be lighter.
- **Steps of the Experiment (using *E. coli* as a model organism):**
    1. **Preparation of Heavy DNA (Parental Generation, G0):**
        - *E. coli* cells were grown for many generations in a culture medium containing **15NH4Cl** (ammonium chloride as the sole nitrogen source).
        - This ensured that all the nitrogenous bases in the DNA of these bacteria contained the heavy 15N isotope.
        - DNA extracted from these cells, when subjected to cesium chloride (CsCl) density gradient centrifugation, formed a single, dense band at the bottom of the tube.
    2. **First Generation (G1) of Replication:**
        - The 15N-labeled bacteria from G0 were then transferred to a fresh culture medium containing **14NH4Cl** (light isotope) and

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allowed to grow and divide for exactly one generation (one round of DNA replication).

- DNA was extracted from these cells.
- When this G1 DNA was subjected to CsCl density gradient centrifugation, it formed a **single band at an intermediate density**, precisely halfway between the positions of the heavy (15N/15N) and light (14N/14N) DNA.

3. **Second Generation (G2) of Replication:**
    - The bacteria were allowed to grow for a second generation (two rounds of replication in total) in the same 14N medium.
    - DNA was extracted from these G2 cells.
    - CsCl density gradient centrifugation of this DNA revealed **two bands**:
        - One band at the **intermediate density** (same as G1).
        - A second band at the **light density** (corresponding to 14N/14N DNA).
    - The intensity of the light band was approximately equal to that of the intermediate band.

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- **Interpretation of Results:**
    - **Intermediate Band in G1:** The single intermediate band in G1 strongly supported the **semiconservative model**. If replication were conservative, two distinct bands (heavy and light) would have been observed. If it were dispersive, a single, broad band between heavy and light would have been seen, but its precise intermediate position was more indicative of semiconservative. The intermediate band indicated that each DNA molecule in G1 consisted of one old (15N) strand and one newly synthesized (14N) strand.

- **Two Bands in G2:** The appearance of two bands (intermediate and light) in G2 further confirmed the semiconservative model. The intermediate band represented the hybrid molecules from G1 that had replicated again. The new light band represented molecules where the 14N strand from the hybrid served as a template, leading to the synthesis of entirely new 14N/14N DNA molecules. The equal intensity of the two bands indicated that half the DNA molecules were hybrid and half were entirely light.

- **Conclusion:** The results perfectly matched the predictions of the **semiconservative model of DNA replication**, where each new DNA molecule consists of one parental strand and one newly synthesized strand. This elegant experiment provided conclusive evidence for the fundamental mechanism of DNA inheritance.

(b) What is linking number? Explain with suitable examples diagrammatically. Calculate the linking number of a covalently closed circular DNA of 16000 base pairs having 08 negative supercoils.

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- **Linking Number (Lk):**
  - The linking number is a topological property of a covalently closed circular (CCC) DNA molecule. It is an integer that represents the total number of times one strand of the DNA double helix wraps around the other strand when the DNA is constrained to lie in a plane.
  - It is a topological invariant, meaning its value can only be changed by breaking at least one of the phosphodiester bonds in the DNA backbone.
  - **Lk = Tw + Wr**
    - **Tw (Twist):** The number of helical turns of one DNA strand around the other. For B-DNA, there is approximately 1 twist per 10.5 base pairs.
    - **Wr (Writhe):** The number of superhelical turns or supercoils (the coiling of the DNA helix upon itself). Negative supercoils

are right-handed (underwound), and positive supercoils are left-handed (overwound).

- Suitable Examples Diagrammatically:

I cannot generate diagrams. However, I can describe them:

- **Relaxed CCC DNA:** Imagine a perfectly flat, circular DNA molecule. If it's B-DNA, it will have a certain number of helical turns (Tw) based on its length. In a relaxed state, the Wr (writhe) is 0. So, Lk = Tw. For example, a 105 bp relaxed B-DNA circle would have 10 helical turns (Tw=10) and 0 supercoils (Wr=0), so Lk=10.
- **Negatively Supercoiled CCC DNA:** If you take the 105 bp DNA and underwind it by one turn (e.g., break a strand, uncoil by one turn, then reseal), it will acquire a negative supercoil. Its Tw might reduce (e.g., to 9), but to compensate, it will introduce a supercoil (Wr = -1). So, Lk = Tw + Wr = 9 + (-1) = 8. The molecule will look like it's twisted on itself, often forming a figure-eight or more complex intertwined structure.
- **Positively Supercoiled CCC DNA:** If you take the 105 bp DNA and overwind it by one turn, it will acquire a positive supercoil. Its Tw might increase (e.g., to 11), and it will introduce a supercoil (Wr = +1). So, Lk = Tw + Wr = 11 + (+1) = 12. The molecule will appear more tightly wound or braided.

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- Calculation:

Given:

- Length of DNA = 16000 base pairs (bp)
- Number of negative supercoils (Wr) = -08 (negative supercoils are usually denoted by a negative sign for writhe)

Assumptions:

- The DNA is in the B-DNA form, which has approximately 10.5 base pairs per helical turn.

Steps:

1. Calculate the number of helical turns (Twist, Tw) in a relaxed state:

Twrelaxed=Base pairs per turn of B-DNATotal base pairs

Twrelaxed=10.5 bp/turn16000 bp

Twrelaxed≈1523.81 turns

2. Calculate the Linking Number (Lk):

The formula is Lk=Tw+Wr.

Here, Tw represents the actual number of helical turns in the supercoiled molecule, and Wr is the number of supercoils.

For a supercoiled molecule, the actual twist (Tw) is what the DNA would have if it were relaxed (Twrelaxed) adjusted by the supercoils. More precisely, Lk is the topological invariant.

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Since we are given the number of supercoils, and assuming these supercoils *cause* a change from the relaxed state, the linking number is the inherent property defined by the number of helical turns if the molecule were relaxed, plus the writhe.

However, the question implies finding the Lk of the supercoiled molecule based on its relaxed state and existing supercoils.

So, if the DNA started relaxed, and then 8 negative supercoils were introduced, the Lk would be reduced from its relaxed state.

Lk=Twrelaxed+Wr

Lk=1523.81+(−08)

Lk=1523.81−8

Lk=1515.81

Therefore, the linking number of the covalently closed circular DNA of 16000 base pairs having 08 negative supercoils is approximately 1515.81.

Note: Linking numbers are always integers for covalently closed circles, but the calculation here results in a decimal due to the 10.5 bp/turn average for B-DNA. In reality, the Lk would be an integer, and the remaining fractional turn would be accommodated by a slight adjustment in twist or writhe. For practical purposes, it's often rounded to the nearest integer.

(c) What is denaturation of DNA? Explain various factors which influence the denaturation and how it changes the properties of DNA.

- **Denaturation of DNA:**
    - Denaturation of DNA (also known as DNA melting or unwinding) is the process by which the double-stranded DNA molecule separates into two single strands.
    - This occurs due to the breakage of the hydrogen bonds between complementary base pairs (A-T and G-C) and the stacking interactions between adjacent bases, while the phosphodiester covalent bonds within each strand remain intact.
    - The process is typically reversible; under appropriate conditions (e.g., slow cooling), the single strands can re-anneal or re-nature to form the double helix.
- **Factors Influencing Denaturation:**
    1. **Temperature:**
        - **Primary Factor:** Increased temperature is the most common way to denature DNA. As temperature rises, the thermal energy overcomes the hydrogen bonds and stacking forces holding the strands together.
        - **Melting Temperature (Tm):** The melting temperature (Tm) is the temperature at which 50% of the DNA in a sample has denatured. Tm is a characteristic property of a given DNA molecule.
    2. **GC Content:**

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- **Higher GC content leads to higher Tm (more stable DNA).**
- Reason: Guanine-cytosine (G-C) base pairs are held together by three hydrogen bonds, whereas adenine-thymine (A-T) base pairs are held by two hydrogen bonds. More hydrogen bonds mean more energy (higher temperature) is required to break them, thus a higher G-C content makes the DNA more stable against denaturation.

3. **pH:**
   - **Extreme pH values (both acidic and alkaline) promote denaturation.**
   - **Acidic pH:** At very low pH, protons (H+) bind to the nitrogenous bases, disrupting the hydrogen bonding and leading to strand separation.
   - **Alkaline pH:** At very high pH, the bases become deprotonated, also disrupting hydrogen bonds and leading to denaturation. DNA is generally stable between pH 5 and 9.

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4. **Ionic Strength (Salt Concentration):**
   - **Higher salt concentration (e.g., Na+ or K+) increases DNA stability and Tm.**
   - Reason: The phosphate backbone of DNA is negatively charged. Cations (like Na+) in the solution neutralize these negative charges, reducing the electrostatic repulsion between the two strands and allowing the hydrogen bonds and stacking interactions to be more stable.
   - **Lower salt concentration decreases Tm and promotes denaturation.**

5. **Presence of Denaturants:**

- Certain chemical agents, called denaturants, can disrupt hydrogen bonds and hydrophobic interactions, thereby lowering the Tm and promoting denaturation.
- **Examples:** Urea, formamide, dimethyl sulfoxide (DMSO). These agents interfere with the hydrogen bonding network and solvation shells around DNA.

- **How Denaturation Changes the Properties of DNA:**

1. **Increased UV Absorbance (Hyperchromic Effect):**
   - Double-stranded DNA absorbs UV light (at 260 nm) less efficiently due to the stacking of bases in the helix.
   - Upon denaturation, the base stacking is disrupted, and the bases become more exposed to the solvent. This leads to a significant increase (typically 30-40%) in the absorption of UV light, a phenomenon called the **hyperchromic effect**. This property is used to monitor DNA denaturation.
2. **Decreased Viscosity:**
   - Double-stranded DNA is a long, rigid rod-like molecule, giving its solutions high viscosity.
   - Upon denaturation, the DNA separates into two more flexible, random-coil single strands, which reduces the frictional resistance and thus the viscosity of the solution.
3. **Increased Buoyant Density:**
   - Denatured (single-stranded) DNA has a slightly higher buoyant density than double-stranded DNA due to changes in its hydration shell and conformation. This can be observed using density gradient centrifugation.
4. **Loss of Biological Activity:**

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- Denatured DNA can no longer serve as an effective template for replication or transcription because the base pairing is lost, and the necessary recognition sites for enzymes are disrupted. However, if conditions revert, renatured DNA can regain its activity.

5. **Separation of Strands:** The most direct change is the physical separation of the two complementary DNA strands.

Question 4:

(a) Give a detailed account of structure of DNA organization in prokaryotes. (3.5)

(b) Expand and give significance of the following (any five) : (2×5=10)

ARS

ORC

TBP

TFIID

TERT

Cot value

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(c) Describe the contribution of the following scientists : (1.5×3=4.5)

(i) Elizabeth H. Blackburn

(ii) Arthur Kornberg

(iii) Rosalind Franklin

(a) Give a detailed account of structure of DNA organization in prokaryotes.

Prokaryotic DNA organization, while much simpler than that in eukaryotes, is still highly organized and compacted within the bacterial cell, forming a structure called the nucleoid. Prokaryotes typically have a single, circular chromosome, although some may have multiple circular chromosomes or even linear ones.

- **1. The Nucleoid:**
    - Instead of a membrane-bound nucleus, prokaryotic DNA is localized to a region within the cytoplasm called the **nucleoid**.
    - The nucleoid is not surrounded by a lipid bilayer membrane, but it is distinct from the rest of the cytoplasm and contains the bacterial chromosome, along with associated proteins and RNA.
- **2. Single Circular Chromosome:**
    - The vast majority of prokaryotes possess a single, double-stranded, circular DNA molecule as their main chromosome.
    - This chromosome is typically much smaller than eukaryotic chromosomes (e.g., *E. coli* chromosome is about 4.6 million base pairs).
- **3. Supercoiling:**
    - To fit the very long DNA molecule (e.g., *E. coli* DNA is ~1 mm long, while the cell is only a few micrometers) into the small cell, the DNA is extensively **supercoiled**.
    - Supercoiling involves the coiling of the DNA double helix upon itself, much like twisting a rubber band.
    - Most bacterial DNA is **negatively supercoiled**, meaning it is underwound relative to its relaxed state. This negative supercoiling helps in:
        - **Compaction:** Tightly compacting the DNA.
        - **Accessibility:** Making the DNA more accessible for processes like replication and transcription by facilitating strand unwinding.
    - Enzymes called **topoisomerases** are responsible for regulating the level of supercoiling:

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    - **DNA Gyrase (Type II topoisomerase):** Introduces negative supercoils (requires ATP).
    - **Topoisomerase I:** Relaxes negative supercoils.

- **4. DNA-Binding Proteins (Nucleoid-Associated Proteins, NAPs):**
    - Unlike eukaryotes, which use histones, prokaryotes use a variety of non-histone, basic (positively charged) proteins called **Nucleoid-Associated Proteins (NAPs)** to help compact and organize their DNA.
    - These NAPs bind to the DNA, causing it to bend, loop, and condense.
    - **Examples of NAPs:**
        - **HU protein:** Dimer that bends DNA, aiding in compaction.
        - **IHF (Integration Host Factor):** Another DNA-bending protein.
        - **H-NS (Histone-like Nucleoid Structuring protein):** Involved in gene silencing and condensation.
        - **Fis (Factor for Inversion Stimulation):** Involved in DNA inversion and transcription regulation.
    - These proteins organize the chromosome into an array of **supercoiled domains** or **looped domains** (typically 50-100 kb each in *E. coli*), which are anchored to a central scaffold or RNA-protein complex. Each loop is independently supercoiled.
- **5. Lack of Introns and Simpler Gene Organization:**
    - Prokaryotic genes typically lack introns and are continuous coding sequences.
    - They are often organized into **operons**, where multiple genes involved in a single metabolic pathway are transcribed together as a single polycistronic mRNA molecule from a common promoter. This allows for coordinated gene expression.

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- **6. Plasmids (Extrachromosomal DNA):**
    - In addition to the main chromosome, many prokaryotes carry smaller, circular, autonomously replicating DNA molecules called **plasmids**.
    - Plasmids are typically not essential for basic cell survival but can carry genes that provide advantageous traits (e.g., antibiotic resistance, virulence factors, metabolic capabilities).
    - They are also supercoiled and replicate independently of the main chromosome.

In summary, prokaryotic DNA is highly compacted into a nucleoid through supercoiling, facilitated by DNA gyrase and nucleoid-associated proteins, and organized into looped domains. This compact yet accessible structure allows efficient replication and transcription within the confines of the bacterial cell.

(b) Expand and give significance of the following (any five):

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- **ARS (Autonomously Replicating Sequence):**
    - **Expansion:** Autonomously Replicating Sequence.
    - **Significance:** ARS elements are specific DNA sequences in yeast chromosomes that function as origins of replication. They enable DNA segments to replicate autonomously (independently of chromosomal replication) when introduced into yeast cells on a plasmid. Their discovery was crucial for understanding eukaryotic DNA replication initiation and for developing yeast artificial chromosomes (YACs) for cloning large DNA fragments. They typically contain an AT-rich unwinding element and binding sites for the Origin Recognition Complex (ORC).
- **ORC (Origin Recognition Complex):**
    - **Expansion:** Origin Recognition Complex.
    - **Significance:** ORC is a multiprotein complex that binds to eukaryotic origins of replication (like ARS elements in yeast) in an ATP-dependent manner. It acts as the initiator protein for DNA replication

in eukaryotes. ORC's binding is the first step in the assembly of the pre-replication complex (pre-RC), which licenses the origin for replication during the G1 phase of the cell cycle, ensuring that DNA replication occurs only once per cell cycle.

- **TBP (TATA-binding Protein):**
    - **Expansion:** TATA-binding Protein.
    - **Significance:** TBP is a subunit of the General Transcription Factor TFIID. It is a key protein in eukaryotic transcription initiation. TBP binds specifically to the TATA box consensus sequence in the core promoter of many protein-coding genes. Its binding causes a significant bend in the DNA, serving as a landmark and platform for the assembly of other General Transcription Factors (GTFs) and RNA polymerase II, thereby initiating the formation of the pre-initiation complex.
- **TFIID (Transcription Factor II D):**
    - **Expansion:** Transcription Factor II D.
    - **Significance:** TFIID is a large, multi-subunit General Transcription Factor for RNA Polymerase II. It is the first GTF to bind to the core promoter, typically interacting with the TATA box via its TBP subunit. TFIID's binding serves as a critical nucleation step for the assembly of the entire pre-initiation complex (PIC), which then recruits RNA Polymerase II to the promoter. It also plays a role in regulating gene expression by interacting with various activator and repressor proteins.
- **TERT (Telomerase Reverse Transcriptase):**
    - **Expansion:** Telomerase Reverse Transcriptase.
    - **Significance:** TERT is the catalytic protein subunit of the enzyme telomerase. Telomerase is a ribonucleoprotein enzyme that synthesizes telomeric DNA repeats at the ends of linear eukaryotic chromosomes. TERT contains the reverse transcriptase activity, using

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an RNA template (a component of telomerase, TERC) to synthesize DNA. Its function is crucial for maintaining telomere length, which counters the "end-replication problem" and is essential for chromosome stability and the indefinite proliferation of cells (e.g., stem cells, cancer cells).

- **Cot value:**
    - **Expansion:** Concentration (of DNA) x Time.
    - **Significance:** The Cot value (C0t) is a measure used in molecular biology to characterize the complexity and repetitive nature of a DNA genome. It represents the product of the initial concentration of single-stranded DNA (C0) and the time (t) required for 50% of the DNA to re-anneal (hybridize) back into double-stranded DNA under specific conditions. DNA with highly repetitive sequences re-anneals faster (lower Cot value) because complementary strands are more likely to find each other. Non-repetitive (unique) sequences re-anneal much slower (higher Cot value). Cot analysis was historically used to determine the proportion of unique and repetitive DNA sequences in a genome and to estimate genome size.

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(c) Describe the contribution of the following scientists:

- **(i) Elizabeth H. Blackburn:**
    - **Contribution:** Elizabeth H. Blackburn is a molecular biologist who, along with Carol W. Greider and Jack W. Szostak, was awarded the Nobel Prize in Physiology or Medicine in 2009 for their discovery of **how chromosomes are protected by telomeres and the enzyme telomerase**.
    - Specifically, Blackburn co-discovered **telomerase**, the enzyme that synthesizes telomere DNA, in 1984. She conducted pioneering research on the structure and function of telomeres, the protective caps at the ends of chromosomes, demonstrating their role in maintaining genomic integrity and their link to aging and cancer.

- **(ii) Arthur Kornberg:**
    - **Contribution:** Arthur Kornberg was an American biochemist who was awarded the Nobel Prize in Physiology or Medicine in 1959 (shared with Severo Ochoa) for his discovery of the **mechanisms in the biological synthesis of deoxyribonucleic acid (DNA)**.
    - He is most famous for his groundbreaking work in the 1950s, particularly the **isolation and characterization of DNA polymerase I** (often called Kornberg enzyme) from *E. coli*. This was the first enzyme shown to synthesize DNA *in vitro*, providing strong evidence for how DNA replication might occur in living cells and laying the foundation for modern molecular biology techniques.
- **(iii) Rosalind Franklin:**
    - **Contribution:** Rosalind Franklin was a British biophysicist and X-ray crystallographer whose critical work in the early 1950s provided the essential experimental data that led to the discovery of the DNA double helix structure.
    - Her most significant contribution was the production of exceptionally high-quality **X-ray diffraction images of DNA**, particularly the famous "Photo 51." This image, along with her detailed analysis of it, provided crucial information about the helical nature, dimensions (pitch and diameter), and repeating patterns of the DNA molecule. Although her direct role in the final model building by Watson and Crick was controversial due to the manner in which her data was accessed, her experimental findings were indispensable for deducing the double helical structure of DNA.

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Question 5:

(a) Name any five types of proteins/enzymes involved in DNA replication fork in prokaryotes. (5)

(b) Draw the structure of tRNA. Name any two unusual bases present in tRNA. (3+2=5)

(c) How the fidelity is maintained at various levels during replication? (5)

(d) Define processivity of DNA polymerase enzyme. Explain its significance. (3)

(a) Name any five types of proteins/enzymes involved in DNA replication fork in prokaryotes.

1. **DNA Helicase (e.g., DnaB):** Unwinds the DNA double helix, separating the two strands at the replication fork.
2. **Single-Strand Binding Proteins (SSBs):** Bind to the separated single DNA strands to prevent them from reannealing, protect them from degradation, and prevent secondary structure formation.
3. **DNA Polymerase III (Pol III):** The primary enzyme responsible for synthesizing new DNA strands in the 5' to 3' direction on both the leading and lagging strands.
4. **Primase (DnaG):** An RNA polymerase that synthesizes short RNA primers, providing a free 3'-OH group for DNA polymerase to initiate DNA synthesis.
5. **DNA Ligase:** Joins the Okazaki fragments on the lagging strand by forming phosphodiester bonds, sealing the nicks.
6. **DNA Topoisomerase (e.g., DNA Gyrase, Topoisomerase I):** Relieves supercoiling (torsional stress) ahead of the replication fork caused by DNA unwinding.

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(b) Draw the structure of tRNA. Name any two unusual bases present in tRNA.

I cannot generate diagrams. However, I can describe the cloverleaf and L-shaped structure of tRNA.

- **Structure of tRNA (Transfer RNA):**
    - tRNA molecules are relatively small RNA molecules (typically 73-93 nucleotides long) that play a crucial role as adaptors in protein synthesis, bringing specific amino acids to the ribosome based on mRNA codons.

- **1. Cloverleaf Secondary Structure:** When drawn in 2D, tRNA forms a characteristic "cloverleaf" structure due to intramolecular base pairing, creating several stem-loop structures:
  - **Acceptor Stem (Amino Acid Arm):** Formed by the base pairing of the 5' and 3' ends of the molecule. The 3' end always has the sequence CCA-OH, and this is where the specific amino acid is covalently attached by aminoacyl-tRNA synthetase.
  - **D Loop:** Contains several dihydrouridine (D) residues. It is involved in recognition by aminoacyl-tRNA synthetases and overall tRNA folding.
  - **Anticodon Loop:** Contains the three-nucleotide **anticodon** sequence, which base-pairs (codon-anticodon interaction) with the complementary codon on the mRNA during translation.
  - **TΨC Loop (T loop):** Contains a modified base pseudouridine (Ψ) and a ribothymidine (T). It is important for ribosome binding and interaction with ribosomal proteins.
  - **Variable Loop (Extra Arm):** A loop of varying size between the anticodon loop and the TΨC loop. Its size varies significantly among different tRNAs.
- **2. L-shaped Tertiary Structure:** In its functional 3D conformation, the cloverleaf folds into a compact L-shaped structure. The acceptor stem and the TΨC stem form one arm of the 'L', and the D stem and anticodon stem form the other arm. This specific 3D structure is essential for its interaction with aminoacyl-tRNA synthetases and the ribosome.

- **Two Unusual (Modified) Bases Present in tRNA:**

1. **Pseudouridine (Ψ):** A common modified nucleoside where the glycosidic bond is formed with C5 of uracil instead of N1, changing the sugar-base linkage. Found in the TΨC loop.

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2. Dihydrouridine (D): A reduced form of uridine, lacking a double bond in the uracil ring, which makes the base non-planar. Found in the D loop.

(Other examples include Inosine (I), Ribothymidine (T), Methylguanosine, Methylcytosine, etc.) These modifications are introduced post-transcriptionally and are crucial for tRNA stability, accurate aminoacylation, and proper interaction with the ribosome.

(c) How the fidelity is maintained at various levels during replication?

- Maintaining the high fidelity (accuracy) of DNA replication is critical to prevent mutations and ensure genetic stability. This fidelity is achieved through multiple mechanisms operating at different levels:

1. **Base Pairing Specificity (Initial Selectivity):**
    - **Mechanism:** DNA polymerase has a strong preference for forming correct Watson-Crick base pairs (A with T, G with C) over incorrect ones. The active site of DNA polymerase is structured to preferentially accommodate correctly paired nucleotides based on their size and hydrogen bonding potential.
    - **Fidelity Contribution:** This inherent specificity in hydrogen bonding provides the first line of defense against misincorporation. While not 100% accurate on its own, it significantly reduces the error rate.
2. **Proofreading by DNA Polymerase (3' → 5' Exonuclease Activity):**
    - **Mechanism:** Most DNA polymerases (e.g., DNA Pol III in prokaryotes, Pol δ and Pol ε in eukaryotes) possess an intrinsic **3' → 5' exonuclease activity**. This activity allows the polymerase to "proofread" its newly synthesized strand. If an incorrect nucleotide is incorporated, it creates a distortion in the double helix and a mismatched base pair at the 3' end of the growing strand.

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- The polymerase detects this mismatch, pauses, and the 3' end of the DNA is transiently transferred to the exonuclease active site. The exonuclease removes the incorrectly paired nucleotide (hydrolyzing the phosphodiester bond) from the 3' end.
- **Fidelity Contribution:** This proofreading mechanism dramatically reduces the error rate of replication by correcting mistakes almost immediately after they occur.

3. **Mismatch Repair (Post-Replication Repair):**
   - **Mechanism:** Even with base pairing specificity and proofreading, a small number of errors (mismatches) can escape detection. Mismatch repair (MMR) systems identify and correct these remaining errors after replication is complete.
   - In *E. coli*, the MMR system distinguishes the newly synthesized strand from the parental strand (e.g., by transient lack of methylation on the new strand). It then excises a segment of the new strand containing the mismatch and resynthesizes the correct sequence using the methylated parental strand as a template.
   - **Fidelity Contribution:** MMR acts as a crucial "spell-checker" that further reduces the overall mutation rate, often by another 100- to 1000-fold.
4. **Balanced Deoxyribonucleotide Pool:**
   - **Mechanism:** Maintaining appropriate and balanced intracellular concentrations of dNTPs (dATP, dCTP, dGTP, dTTP) is important.
   - **Fidelity Contribution:** Imbalances in dNTP pools (e.g., an excess of one dNTP) can increase the frequency of misincorporation, as the polymerase might be more likely to incorporate an incorrect but abundant nucleotide. Cells regulate these pools to minimize such errors.

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These multi-layered mechanisms ensure that DNA replication is an extraordinarily accurate process, vital for maintaining genomic stability and preventing mutations that could lead to disease or dysfunction.

(d) Define processivity of DNA polymerase enzyme. Explain its significance.

- **Definition of Processivity of DNA Polymerase Enzyme:**
    - Processivity refers to the average number of nucleotides added by a DNA polymerase enzyme to a growing DNA strand before it dissociates from the template DNA.
    - A highly processive polymerase can synthesize very long stretches of DNA without detaching, while a low processive polymerase dissociates frequently after adding only a few nucleotides.
- **Significance:**
    - **Efficiency of Replication:** High processivity is absolutely crucial for efficient and rapid DNA replication, especially for long chromosomal DNA molecules. If a polymerase had low processivity, it would constantly detach and re-attach, wasting time and energy in finding the replication fork, leading to extremely slow replication. A highly processive polymerase ensures continuous and speedy synthesis.
    - **Completeness of Replication:** High processivity ensures that the entire chromosome can be replicated in a timely manner, preventing incomplete replication, which can lead to chromosomal instability and cell cycle arrest.
    - **Role of Sliding Clamps:** The high processivity of the main replicative polymerases (e.g., DNA Pol III in prokaryotes, Pol δ and ε in eukaryotes) is primarily conferred by accessory proteins called **sliding clamps** (e.g., the β clamp in *E. coli*). These clamps form a ring that encircles the DNA and tethers the polymerase to the template, preventing it from dissociating.
    - **Specialized Roles:** Polymerases with low processivity, like DNA Pol I in *E. coli*, are suited for their specialized roles in DNA repair and

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primer removal/gap filling, where they need to synthesize only short stretches of DNA before moving on. Their low processivity allows them to quickly replace a few nucleotides and then be replaced by DNA ligase.

Question 6:

(a) Write short note on (any four) : (4×4=16)

(i) Nucleosomes

(ii) RNA polymerases in Eukaryotes

(iii) D loop replication

(iv) Mechanism of origin activation in prokaryotes

(v) Termination of transcription in prokaryotes

(b) What are telomeric repeats? Mention their role.

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(a) Write short note on (any four):

- **(i) Nucleosomes:**
    - Nucleosomes are the fundamental repeating units of chromatin structure in eukaryotes. They represent the first level of DNA compaction within the nucleus.
    - **Structure:** Each nucleosome consists of a core of eight histone proteins (an octamer) around which approximately 147 base pairs of double-stranded DNA are wrapped in a left-handed superhelical turn. The histone octamer comprises two copies each of four core histones: H2A, H2B, H3, and H4.
    - **Linker DNA:** Adjacent nucleosomes are connected by a segment of "linker DNA," typically 20-60 base pairs long, which is bound by a fifth histone, H1, helping to further compact the nucleosomes into higher-order structures.
    - **Function:**

- **Compaction:** Compresses the enormous length of eukaryotic DNA (e.g., ~2 meters in a human cell) to fit into the microscopic nucleus.
- **Regulation of Gene Expression:** The packaging of DNA into nucleosomes influences gene expression. Tightly packed chromatin (heterochromatin) is generally transcriptionally inactive, while less condensed chromatin (euchromatin) is more accessible for transcription. Post-translational modifications of histones play a key role in regulating this accessibility.
- **Protection:** Helps protect DNA from damage.

- **(ii) RNA polymerases in Eukaryotes:**
  - Eukaryotic cells possess three distinct nuclear RNA polymerases, each responsible for transcribing different classes of genes, unlike prokaryotes which typically have only one RNA polymerase.
  - **RNA Polymerase I (Pol I):**
    - **Location:** Nucleolus.
    - **Function:** Synthesizes large ribosomal RNA (rRNA) precursors (e.g., 45S pre-rRNA in mammals), which are then processed into 18S, 5.8S, and 28S rRNAs. These rRNAs are structural and catalytic components of the ribosome.
    - **Sensitivity to α-amanitin:** Insensitive.
  - **RNA Polymerase II (Pol II):**
    - **Location:** Nucleoplasm.
    - **Function:** Responsible for synthesizing all messenger RNAs (mRNAs) that code for proteins, as well as some small nuclear RNAs (snRNAs) and microRNAs (miRNAs). This is the most studied RNA polymerase due to its role in gene expression.

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        - **Sensitivity to α-amanitin:** Highly sensitive (inhibited by very low concentrations).
    - **RNA Polymerase III (Pol III):**
        - **Location:** Nucleoplasm.
        - **Function:** Synthesizes transfer RNAs (tRNAs), 5S ribosomal RNA, and other small RNAs (e.g., some snRNAs).
        - **Sensitivity to α-amanitin:** Moderately sensitive (inhibited by high concentrations).
    - These specialized polymerases, along with their distinct sets of transcription factors, allow for complex and highly regulated gene expression in eukaryotes.

- **(iii) D-loop replication:**
    - D-loop replication is a specific mechanism of DNA replication, most notably observed in **mitochondrial DNA (mtDNA)** in eukaryotes and in some chloroplast DNAs. It is a distinctive asynchronous mode of replication for circular DNA molecules.
    - **Mechanism:**
        1. Replication begins at a single origin of replication (the heavy-strand origin, OH) on one strand (the heavy strand).
        2. A new heavy strand is synthesized, displacing the original heavy strand. The displaced parental heavy strand forms a looped-out structure called the **D-loop (displacement loop)**, as it remains single-stranded and hydrogen-bonded to the new daughter strand.
        3. Replication continues for a significant portion of the genome (about two-thirds in mammals).
        4. Only after this initial synthesis has progressed substantially does replication initiate on the light strand (at the light-strand

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origin, OL), which is located roughly two-thirds of the way around the circle from OH.

5. This asynchronous replication continues from both origins until the two replication forks meet and the two daughter molecules are complete.

- **Significance:** This unique mechanism allows for the efficient replication of mitochondrial DNA, which has its own genes and replication machinery distinct from nuclear DNA. The D-loop itself is also a region involved in mtDNA transcription regulation and is prone to mutations.

- **(iv) Mechanism of origin activation in prokaryotes:**
  - The mechanism of origin activation in prokaryotes (e.g., *E. coli* at its *oriC*) is a tightly regulated process that ensures DNA replication initiates only once per cell cycle.
  - **Steps:**

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1. **DnaA Binding:** The process begins with the binding of multiple copies of the initiator protein **DnaA** to specific 9-bp repeats (DnaA boxes) within the *oriC* region. DnaA is an ATP-binding protein.

2. **DNA Unwinding (Initial Melting):** ATP-bound DnaA proteins oligomerize and interact with the AT-rich 13-bp repeats within *oriC*. This interaction causes localized unwinding or "melting" of the DNA helix at the AT-rich region, creating an open complex.

3. **Helicase Loading:** The unwound region provides a loading site for the **DnaB helicase** (the main replicative helicase). DnaC (helicase loader) helps to load DnaB onto the single-stranded DNA. DnaB hexamers are loaded onto each separated strand, forming a bidirectional replication bubble.

4. **Primer Synthesis and Replisome Assembly:** Once DnaB helicase is loaded and unwinding DNA, **primase (DnaG)** is recruited. Primase synthesizes short RNA primers on both leading strands. These primers provide free 3'-OH ends for **DNA polymerase III** to begin synthesizing new DNA strands.
5. **Replication Fork Establishment:** With the helicase unwinding DNA and polymerases synthesizing DNA, the complete replisome (replication machinery) is assembled at the replication forks, and bidirectional replication proceeds.

- **Regulation:** Origin activation is tightly regulated by factors like ATP/ADP ratio, DnaA concentration, and DNA methylation to ensure once-per-cell-cycle replication.

- **(v) Termination of transcription in prokaryotes:**
  - Termination of transcription in prokaryotes is the process by which RNA synthesis stops, and the newly synthesized RNA molecule is released from the DNA template, along with the RNA polymerase. There are two main mechanisms:
  - **1. Rho-Independent (Intrinsic) Termination:**
    - **Mechanism:** This type of termination relies solely on specific sequences in the DNA template and the resulting RNA transcript, without the involvement of any protein factors like Rho.
    - The DNA template contains a region that codes for an RNA sequence that can form a **stable hairpin loop** (or stem-loop structure) in the nascent mRNA, immediately followed by a stretch of **about 6-8 uridine residues** (poly-U sequence) in the RNA transcript.
    - The formation of the hairpin loop causes RNA polymerase to pause. The weak A-U base pairs in the poly-U sequence, which

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are unstable, cannot hold the RNA-DNA hybrid together during the pause.

- The instability leads to the dissociation of the RNA transcript from the DNA template and the release of RNA polymerase.
- **Example:** Termination signals often found at the end of operons.

- **2. Rho-Dependent Termination:**
  - **Mechanism:** This type of termination requires the participation of a protein called the **Rho factor**, which is an ATP-dependent helicase.
  - Rho binds to a specific C-rich, G-poor sequence called the **Rho utilization (rut) site** on the nascent mRNA transcript.
  - Rho then moves along the RNA transcript towards the RNA polymerase, unwinding the RNA.
  - When Rho catches up to the paused RNA polymerase (often at a GC-rich region), its helicase activity unwinds the RNA-DNA hybrid within the transcription bubble, leading to the dissociation of the RNA transcript and RNA polymerase from the DNA.
  - **Example:** Found in some phage genes and bacterial operons (e.g., *trp* operon in some contexts).

(b) What are telomeric repeats? Mention their role.

- **Telomeric Repeats:**
  - Telomeric repeats are specific, highly conserved, short, tandemly repeated DNA sequences found at the very ends of linear eukaryotic chromosomes.

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- In humans, the consensus sequence of the telomeric repeat is **5'-TTAGGG-3'**, which is repeated thousands of times, generating stretches of DNA tens of kilobases long.
- One strand of the telomere (the G-rich strand in humans) typically extends beyond the C-rich strand, forming a 3' overhang. This overhang can fold back on itself to form a protective loop structure called a T-loop, stabilized by telomere-binding proteins.

- **Role (Significance):**

1. **Protect Chromosome Ends from Degradation:** Telomeres act as protective caps, preventing the ends of chromosomes from being recognized as double-strand breaks by DNA repair enzymes. Without telomeres, chromosomes would be prone to degradation or fusion, leading to genomic instability.
2. **Solve the "End-Replication Problem":** Due to the unidirectional nature of DNA polymerase and the requirement for an RNA primer, conventional DNA replication cannot fully replicate the very 3' end of the lagging strand of linear chromosomes. This leads to progressive shortening of chromosomes with each round of cell division.
    - **Telomerase**, an enzyme containing both a protein subunit (TERT, with reverse transcriptase activity) and an RNA template (TERC), solves this problem. Telomerase uses its RNA template to extend the 3' G-rich overhang of the telomere, adding new telomeric repeats.
    - This extension allows DNA polymerase to synthesize the complementary lagging strand, thus compensating for the loss of sequence at the ends and preventing net shortening of the chromosome.
3. **Cellular Senescence and Aging:** Telomere length acts as a "mitotic clock." In most somatic cells, telomerase activity is very low or absent, leading to gradual telomere shortening with each cell division. Once telomeres reach a critically short length, the cell enters a state of

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replicative senescence (stops dividing) or undergoes apoptosis (programmed cell death). This mechanism contributes to the normal aging process of an organism.

4. **Cancer:** In contrast to normal somatic cells, cancer cells often reactivate or upregulate telomerase activity. This allows them to maintain telomere length, overcome the cell's natural senescence mechanisms, and achieve indefinite proliferation, which is a hallmark of cancer.
5. **Chromosome Segregation:** Telomeres also play a role in proper chromosome segregation during cell division, possibly by influencing the interaction of chromosomes with the nuclear matrix.

In essence, telomeric repeats and telomerase are crucial for maintaining the integrity and stability of eukaryotic chromosomes, with profound implications for cellular lifespan, aging, and disease.

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