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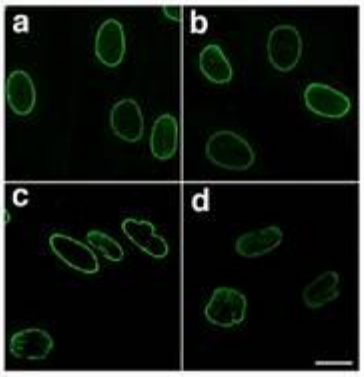


(a) The central dogma of living organisms illustrates the flow of genetic information from DNA to RNA to protein. It is defined as a process in which the information in DNA is converted into a functional product[123].

(b) The nuclear lamina is a dense fibrillar network inside the nucleus of eukaryote cells. It provides mechanical support, regulates important cellular events such as DNA replication and cell division, participates in chromatin organization, and anchors the nuclear pore complexes embedded in the nuclear envelope[4].

© DNA replication goes from 5' to 3' because DNA polymerase, which synthesizes the new DNA strand, can only add nucleotides to the 3' end of the strand. This directionality ensures that the new strand is formed in the 5' to 3' direction, complementary to the original 3' to 5' parent strand[567].

(d) Riboswitches are specific components of an mRNA molecule that regulate gene expression. They bind and target small target molecules, and an mRNA molecule containing a riboswitch directly regulates its own activity in response to the concentrations of its effector molecule[8910].

(e) When a cell reaches the G0 stage, it is not actively preparing to divide. The cell is in a quiescent (inactive) stage that occurs when cells exit the cell cycle. Some cells enter G0 temporarily until an external signal triggers the onset of G1. Other cells that never or rarely divide, such as mature cardiac muscle and nerve cells, remain in G0 permanently[11121314].

(f) Taq DNA polymerase is a thermostable enzyme used for DNA amplification. It is derived from Thermus aquaticus, an extremely thermophilic eubacterium which grows at temperatures above 70°C[15161718].

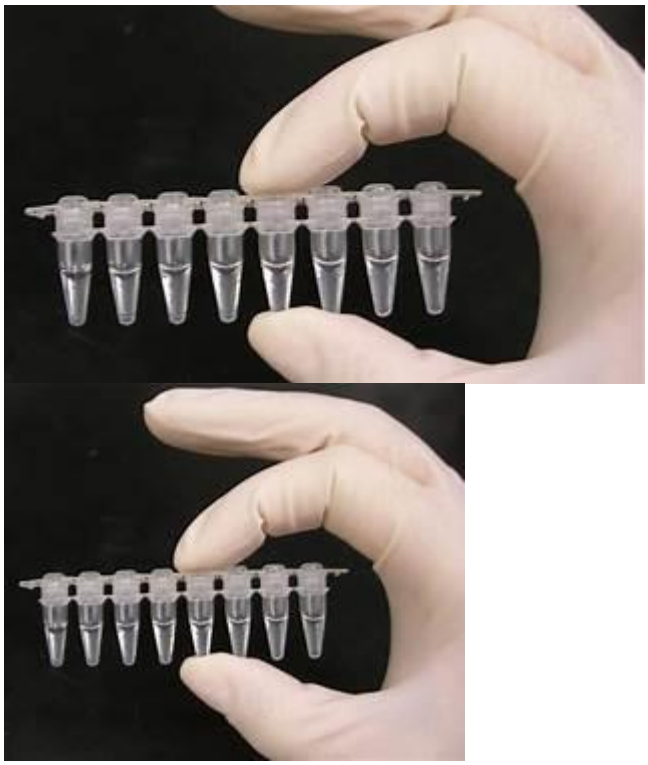
(g) Oncogenes and tumor suppressor genes play different roles in cell growth and division. Oncogenes, which are activated versions of proto-oncogenes, promote cell growth and division. When oncogenes are activated, cells can grow and divide

too quickly, potentially leading to cancer. On the other hand, tumor suppressor genes slow down cell division or tell cells to die at the right time. When tumor suppressor genes don't work properly, cells can grow out of control, which can also lead to cancer[19][20][21][22][23].

(h) Each nucleosome is composed of DNA wrapped around eight histone proteins, known as a histone octamer. Each histone octamer is composed of two copies each of the histone proteins H2A, H2B, H3, and H4[24][25].

(2)

(a) (a) Write a brief essay about application of PCR technology in biotechnology.



Explore

Polymerase Chain Reaction (PCR) is a revolutionary method developed by Kary Mullis in the 1980s. PCR is a technique used in molecular biology to amplify a single or a few copies of a specific DNA segment, enabling researchers to produce millions of copies of a specific DNA sequence[1].

PCR has a wide range of applications in biotechnology:

1. **Clinical Diagnosis**: PCR is highly useful for diagnosing various diseases, including inherited disorders, viral diseases, and bacterial diseases[2]. It provides direct information about DNA by amplifying the DNA of the relevant region, followed by the direct analysis of PCR products[2]. For

example, diseases like sickle-cell anemia, p-thalassemia, and phenylketonuria can be detected by PCR in prenatal diagnosis[2].

2. **DNA Sequencing**: PCR is used for sequencing DNA. For this purpose, single-strands of DNA are required. In asymmetric PCR, preferential amplification of a single-strand is carried out[2].
3. **Gene Manipulation and Expression Studies**: The sequence of nucleotides in a piece of the gene (target DNA) can be manipulated and amplified by PCR[2].
4. **Comparative Studies of Genomes**: PCR is used in comparative studies of genomes[2].
5. **Forensic Medicine**: PCR is used in forensic medicine for DNA profiling[23].
6. **Comparison with Gene Cloning**: PCR is used in comparison with gene cloning[2].

PCR technology has revolutionized the field of biotechnology. It has not only made DNA sequencing more efficient but has also opened up new avenues in genetic engineering, disease diagnosis, forensic science, and many other areas of biotechnology[3].

OR/

The Application of PCR Technology in Biotechnology

Polymerase Chain Reaction (PCR) technology has revolutionized molecular biology and biotechnology since its development in the 1980s by Kary Mullis. PCR is a powerful technique that allows the amplification of specific DNA sequences, enabling researchers to analyze and manipulate genetic material with unprecedented precision. The applications of PCR technology in biotechnology are diverse and span various fields, including medicine, agriculture, forensic science, and environmental studies.

One of the primary applications of PCR is in **Molecular Diagnostics**. PCR enables the detection and diagnosis of genetic diseases, infectious agents, and other health-related conditions. It allows the amplification of specific DNA regions associated with pathogens or genetic mutations, facilitating early and accurate diagnosis. Techniques such as quantitative PCR (qPCR) further enhance the diagnostic capabilities by quantifying the amount of DNA present, providing insights into disease progression and treatment effectiveness.

In **Forensic Science**, PCR plays a crucial role in DNA profiling and analysis. Forensic scientists use PCR to amplify tiny DNA samples from crime scenes, allowing the generation of sufficient material for analysis. DNA fingerprinting, based on variable regions of the genome, has become a cornerstone in criminal investigations, paternity testing, and the identification of individuals in mass disasters.

PCR technology has also been instrumental in advancing **Genetic Engineering and Biopharmaceuticals**. In the field of genetic engineering, PCR is utilized to amplify specific genes for subsequent cloning and manipulation. It forms the basis for techniques like site-directed mutagenesis, facilitating the precise modification of DNA sequences. Additionally, PCR is employed in the production of recombinant proteins and therapeutic agents, allowing for the rapid and efficient amplification of gene constructs for expression in host organisms.

In **Agriculture and Food Industry**, PCR is employed for various applications. One notable application is the detection of genetically modified organisms (GMOs). PCR allows the identification and quantification of specific DNA sequences associated with genetically modified crops, ensuring compliance with regulatory standards and providing transparency to consumers. Additionally, PCR is used in pathogen detection in crops and livestock, aiding in disease management and control.

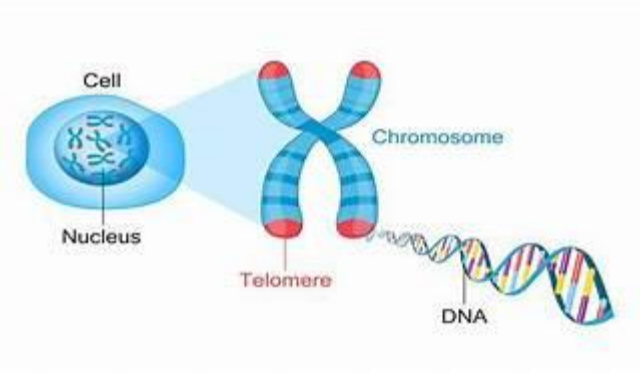
Environmental studies benefit from PCR technology in the analysis of microbial communities, biodiversity, and pollutant detection. Environmental scientists use PCR to amplify and study specific DNA markers, shedding light on microbial diversity, ecological interactions, and the impact of environmental changes.

The continuous evolution of PCR technology has led to the development of novel variants and applications, such as reverse transcription PCR (RT-PCR) for studying gene expression, digital PCR for absolute quantification, and metagenomic PCR for analyzing complex microbial communities.

In conclusion, the application of PCR technology in biotechnology has transformed the landscape of molecular biology and various scientific disciplines. Its versatility,

precision, and efficiency make PCR an indispensable tool for researchers, clinicians, forensic scientists, and professionals across diverse fields, contributing significantly to advancements in science, medicine, agriculture, and environmental studies. As technology continues to evolve, PCR remains at the forefront of innovation, opening new possibilities for understanding and manipulating the genetic blueprint of life.

(b) Discuss the role of telomerase in eukaryotic DNA replication and mention its importance.



Explore

Role of Telomerase in Eukaryotic DNA Replication[1234]:

Telomerase is an enzyme found in eukaryotic cells that plays a crucial role in DNA replication[1]. It helps solve the end-replication problem, a challenge posed by the linear nature of eukaryotic chromosomes[1].

During DNA replication, the DNA at the very end of the chromosome cannot be fully copied in each round of replication, resulting in a slow, gradual shortening of the chromosome[1]. This is because one of the two new strands of DNA at a replication fork, known as the lagging strand, is produced in many small pieces called Okazaki fragments, each of which begins with its own RNA primer[1]. When the replication fork reaches the end of the chromosome, there is a short stretch of DNA that does not get covered by an Okazaki fragment[1]. Also, the primer of the last Okazaki fragment that does get made can't be replaced with DNA like other primers[1].

This is where telomerase comes in. Telomerase, which has an inbuilt RNA template, extends the ends by copying the RNA template and extending one end of the chromosome[234]. DNA polymerase can then extend the DNA using the primer[234]. In this way, the ends of the chromosomes are protected[234].

Importance of Telomerase[1567]:

Telomerase has several important functions:

1. **Protection of Genetic Information**: By extending the telomeres, telomerase prevents the loss of important genetic information during DNA replication[1].
2. **Cellular Aging**: Telomerase activity is associated with cellular aging. The gradual shortening of telomeres as cells divide is believed to contribute to the aging process[5].
3. **Cancer**: Telomerase is often active in cancer cells, allowing them to divide indefinitely[6]. This has led to interest in telomerase as a potential target for cancer treatments[6].
4. **Stem Cells and Immune Cells**: Telomerase is also active in stem cells and immune cells, which need to divide frequently[7].

In summary, telomerase plays a crucial role in maintaining the integrity of our genetic information and has significant implications for aging and disease[1567].

(c) Enumerate the properties of genetic code mentioning the exceptions, if any.

Explore

The genetic code is a set of rules that dictate how the information encoded within genetic material (DNA or mRNA sequences) is translated into proteins by living cells[1]. Here are the key properties of the genetic code[12345]:

1. **Triplet Code**: Each genetic code is a triplet formed of three nucleotide bases that code for a particular amino acid[12345].
2. **Degenerate Code**: The code is degenerate, meaning there are more codons than there are amino acids. Therefore, most amino acids are encoded by more than one codon[12345].
3. **Non-overlapping Code**: The code is read sequentially in a group of three and a nucleotide which becomes a part of triplet never becomes part of the next triplet[12345].
4. **Commaless Code**: There is no room for punctuation in between which indicates that every codon is adjacent to the previous one without any nucleotides between them[12345].
5. **Non-ambiguous Code**: The code is non-ambiguous, meaning each codon specifies only one amino acid[12345].

6. **Universal Code**: The genetic code is universal, meaning it is the same in all organisms, from bacteria to humans[12345].
7. **Start and Stop Codons**: The code contains specific codons that signal the start and stop of protein synthesis[12345].
8. **Polarity**: Each triplet is read from 5' → 3' direction[12345].

Exceptions to the Genetic Code[6718]:

While the genetic code is nearly universal, there are a few exceptions. Most of these exceptions involve assigning one or two of the three STOP codons to an amino acid instead[6718]. Some exceptions have been reported for the mitochondrial genome and in unicellular eukaryotes for the synthesis of nonstandard proteins such as selenocysteine and pyrolysine[6718]. Despite these exceptions, the genetic code remains largely universal across all forms of life[6718].

(d) How does the initiation of translation occur in prokaryotes?

Translation is the process by which the information encoded in messenger RNA (mRNA) is used to synthesize a polypeptide chain. In prokaryotes, such as bacteria, the initiation of translation involves specific sequences on the mRNA, the small ribosomal subunit, initiator tRNA, and initiation factors. The process can be summarized in the following steps:

1. **mRNA Binding to the Small Ribosomal Subunit:**

- The small ribosomal subunit (30S in prokaryotes) binds to the mRNA at a specific region called the Shine-Dalgarno sequence.

- The Shine-Dalgarno sequence is a purine-rich sequence on the mRNA that base pairs with a complementary sequence near the 3' end of the 16S ribosomal RNA (rRNA) in the small ribosomal subunit.

2. **Initiation Factors:**

- Initiation factors (IFs) play a crucial role in the initiation of translation. They help in assembling the components needed for the initiation complex.

- One of the initiation factors, IF-3, prevents the large (50S) ribosomal subunit from binding prematurely to the small subunit.

3. **Formation of the Initiation Complex:**

- The small ribosomal subunit, along with the initiation factors, forms the initiation complex.

- The small subunit, mRNA, and initiator tRNA carrying the amino acid formyl-methionine (fMet) (in prokaryotes) come together to form the initiation complex.

4. **Binding of the Initiation Complex to the mRNA:**

- The initiation complex binds to the mRNA at the Shine-Dalgarno sequence, positioning the ribosome at the correct start codon (usually AUG) on the mRNA.

5. **IF-3 Release and Binding of the Large Ribosomal Subunit:**

- After the initiation complex is formed, IF-3 is released.

- The large ribosomal subunit (50S) can then join the complex, forming the complete 70S ribosome.

6. **Start Codon Recognition:**

- The initiator tRNA carrying fMet pairs with the start codon on the mRNA through complementary base pairing.

7. **GTP Hydrolysis and IF-2 Release:**

- The initiation factor IF-2, which helps in the binding of initiator tRNA, hydrolyzes GTP to GDP and inorganic phosphate.

- This GTP hydrolysis step is important for the release of initiation factors and the stabilization of the fully assembled ribosome on the mRNA.

8. **Formation of the Peptidyl Transferase Center (PTC):**

- The PTC, located in the large ribosomal subunit, is ready for the formation of peptide bonds between amino acids.

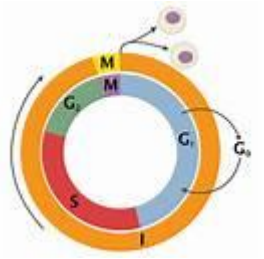
Once these steps are completed, the elongation phase of translation begins, where amino acids are added to the growing polypeptide chain. The initiation of translation in prokaryotes is a highly regulated and coordinated process, ensuring the accurate start of protein synthesis at the correct start codon on the mRNA.

(3)

(a) State the events occur during the G1 – S and G2 – M checkpoints in yeast cell cycle. Describe the role of MPF during cell-cycle progression.

(a) **Events during the G1-S and G2-M Checkpoints in Yeast Cell Cycle**[123]:

1. **G1-S Checkpoint**: This is the main decision point for a cell – that is, the primary point at which it must choose whether or not to divide[2]. Once the cell passes the G1 checkpoint and enters S phase, it becomes irreversibly committed to division[2]. At the G1 checkpoint, a cell checks whether internal and external conditions are right for division[2]. Here are some of the factors a cell might assess: Size, Nutrients, Molecular signals, and DNA integrity[2].



2. **G2-M Checkpoint**: To make sure that cell division goes smoothly (produces healthy daughter cells with complete, undamaged DNA), the cell has an additional checkpoint before M phase, called the G2 checkpoint[2]. At this stage, the cell will check: DNA integrity[2].

(b) **Role of MPF during Cell-Cycle Progression**[45678]:

Maturation promoting factor (MPF) is a cell cycle checkpoint that regulates the passage of a cell from the G2 growth phase to the M phase[6]. It is also known as the G2 checkpoint, and ensures that DNA replication during the S phase did not produce any mistakes[6].

MPF is a protein complex made of a cyclin (M cyclin) and a cyclin dependent kinase[4]. The cyclin dependent kinase is activated upon binding to the M cyclin and

can phosphorylate targets that promote the cell to move through G2 phase into mitosis, or M phase[4].

During mitosis, active MPF activates another protein complex, APC[4]. APC causes the M cyclins to be destroyed, thus deactivating MPF and allowing the new daughter cells to exit mitosis into G1[4].

OR/

The cell cycle is a highly regulated process that governs the growth and division of eukaryotic cells. Two crucial checkpoints in the cell cycle are the G1–S checkpoint and the G2–M checkpoint. Here's a brief description of the events that occur during these checkpoints in the yeast cell cycle, along with the role of the Mitosis-Promoting Factor (MPF).

G1–S Checkpoint:

Events:

1. **Cell Growth and Normal Cellular Functions:**

   - In the G1 (Gap 1) phase, the cell undergoes normal cellular functions and grows in size.

2. **Check for Cell Size and Nutrient Availability:**

   - The cell monitors its size and checks for the availability of nutrients. If the conditions are favorable, the cell proceeds to the S (Synthesis) phase.

3. **DNA Damage Check:**

   - The cell checks for DNA damage. If the DNA is damaged, the cell cycle may be halted to allow for repair before DNA replication.

4. **Activation of Cyclin-Dependent Kinases (CDKs):**

- Cyclin-dependent kinases (CDKs) are activated, and their association with specific cyclin proteins triggers the transition from G1 to S phase.

5. **Initiation of DNA Synthesis:**

- Once conditions are suitable, and the cell has passed the checks, DNA synthesis is initiated in the S phase.

G2–M Checkpoint:

Events:

1. **Completion of DNA Replication:**

- DNA replication is completed during the S phase.

2. **Check for DNA Replication Errors:**

- The cell checks for errors in DNA replication and ensures that the DNA has been faithfully duplicated.

3. **Activation of CDK-Cyclin Complexes:**

- CDKs associate with specific cyclins, forming complexes known as MPF (Mitosis-Promoting Factor). These complexes drive the cell into mitosis.

4. **Check for Cell Size:**

- The cell checks its size to ensure that it has grown adequately before entering mitosis.

5. **Check for DNA Integrity:**

- Integrity of the replicated DNA is confirmed.

6. **Mitotic Spindle Formation:**

- MPF stimulates the formation of the mitotic spindle, a structure that separates chromosomes during cell division.

7. **Entry into Mitosis (M Phase):**

- Once all conditions are met and the cell has passed the checks, it enters the M phase, where chromosomes are segregated into daughter cells.

Role of MPF (Mitosis-Promoting Factor):

Composition:

- MPF is composed of a cyclin protein (specifically cyclin B) and a cyclin-dependent kinase (CDK), which are tightly bound together.

Function:

- MPF is a key regulator that promotes the transition from the G2 phase to the M phase of the cell cycle.

- The activation of MPF is a critical step for initiating mitosis.

- The CDK component of MPF phosphorylates various target proteins, triggering events such as condensation of chromosomes, breakdown of the nuclear envelope, and formation of the mitotic spindle.

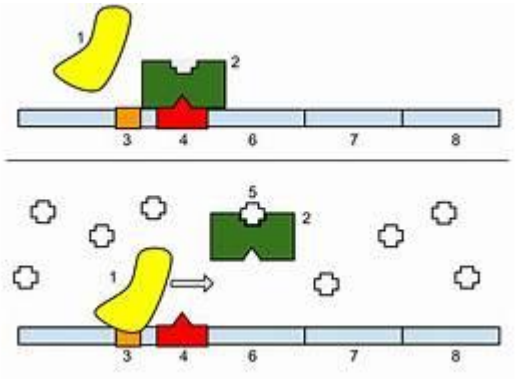
Regulation:

- The activity of MPF is regulated by the concentration of cyclin B. Cyclin B accumulates during the G2 phase and associates with CDK to form active MPF complexes.

- The activation and subsequent inactivation of MPF are tightly regulated by the phosphorylation and dephosphorylation of specific amino acid residues on CDK.

In summary, the G1–S and G2–M checkpoints ensure the orderly progression of the cell cycle by monitoring factors such as cell size, nutrient availability, and DNA integrity. MPF, composed of cyclin B and CDK, plays a crucial role in driving the cell through the G2–M checkpoint and initiating mitosis.

(b) What is an operon? Describe the structure of lac-operon. Discuss with diagrams the negative control of lac-operon.



An **operon** is a functioning unit of DNA containing a cluster of genes under the control of a single promoter[123]. The genes are transcribed together into an mRNA strand and either translated together in the cytoplasm, or undergo splicing to create monocistronic mRNAs that are translated separately[123]. This results in the genes contained in the operon being either expressed together or not at all[123]. Operons are present in prokaryotes (bacteria and archaea), but are absent in eukaryotes[123].

The **lac operon** is a classic example of an operon. It is a cluster of three structural genes encoding proteins involved in lactose metabolism and the sites on the DNA involved in the regulation of the operon[4]. The lac operon consists of a promoter (P) and operator (O) region followed by three structural genes lacZ, lacY, and lacA[45]. A regulatory gene lacI (I) preceding the lac operon is responsible for producing a repressor ® protein[45].

The **structure of the lac operon** is as follows[45]:

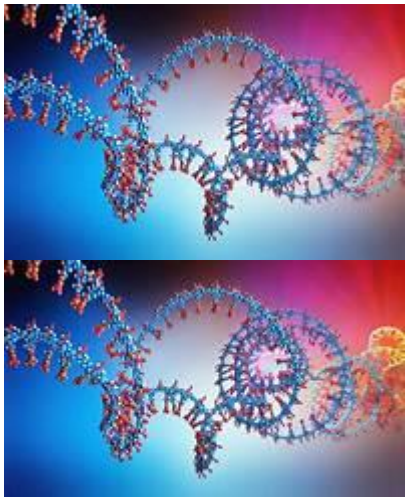
- **lacZ**: Encodes β-galactosidase, an enzyme that cleaves the disaccharide lactose into glucose and galactose[45].

- **lacY**: Encodes lactose permease, a protein that transports lactose into the cell across the cell membrane[45].
- **lacA**: Encodes thiogalactoside transacetylase[45].

The **negative control of the lac operon** involves the lac repressor[678]. In the absence of lactose, the lac repressor binds to the operator site, blocking RNA polymerase from transcribing the lac operon[678]. This prevents the production of the enzymes when lactose is not available[678]. When lactose is present, it is converted into allolactose, which binds to the lac repressor[678]. This changes the shape of the repressor so it can no longer bind to the operator[678]. RNA polymerase can then move along the DNA and transcribe the lac operon, leading to the production of the enzymes needed to metabolize lactose[678].

(c) Describe briefly about the events that led to transition from RNA to DNA world. What is endosymbiosis? How endosymbiotic theory helps to explain the origin of eukaryotic cell?

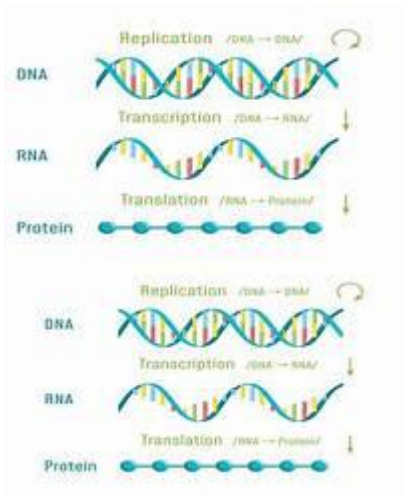
© The transition from an RNA to a DNA world is a significant event in the history of life. The RNA world hypothesis posits that before DNA, life was based on RNA molecules[1]. Here are some key events that led to the transition from RNA to DNA[1]:



1. **RNA Replication**: In the RNA world, RNA molecules were capable of both storing genetic information and catalyzing chemical reactions[1]. An RNA enzyme or 'ribozyme' capable of copying RNA molecules existed early in evolution[1].

2. **Emergence of DNA**: The theory suggests that DNA genomes evolved from RNA genomes. In the RNA world, RNA polymerase ribozymes could have produced single-stranded complementary DNA and then converted it into stable double-stranded DNA genomes[1].

3. **Protein Enzymes**: Protein enzymes could have evolved before DNA genomes. These protein-based enzymes, called reverse transcriptases, can copy RNA to produce molecules of complementary DNA[1].



4. **DNA Replication**: Once DNA emerged, it took over the role of information storage, due to its superior stability compared to RNA. This allowed RNA to specialize in other functions, such as coding for proteins[1].

The term **endosymbiosis** refers to a symbiotic relationship where one organism (the endosymbiont) lives inside the body or cells of another organism (the host)[2]. Both organisms benefit from this relationship[2].

The **endosymbiotic theory** is a widely accepted theory that explains the origin of eukaryotic cells from prokaryotic organisms[345]. According to this theory, certain organelles in eukaryotic cells, specifically mitochondria and chloroplasts, originated from free-living prokaryotic cells that were engulfed by ancestral eukaryotic cells[345]. This led to a mutually beneficial symbiotic relationship[345]. Over time, these endosymbionts lost some of their independence and became integrated parts of the eukaryotic cell[345]. This theory is supported by several lines

of evidence, including the fact that mitochondria and chloroplasts have their own DNA and ribosomes, which are more similar to those of prokaryotes than eukaryotes[345].

OR,

Transition from RNA to DNA World:

The transition from an RNA world to a DNA world is a significant milestone in the evolution of life. The RNA world hypothesis proposes that early life forms relied on RNA for both information storage and catalytic functions. However, as life evolved, DNA gradually replaced RNA as the primary genetic material, likely due to the greater stability and information storage capacity of DNA.

1. **RNA World:**

- In the RNA world, RNA molecules served as both genetic material and catalysts (ribozymes).

- RNA can store genetic information and catalyze biochemical reactions, making it a plausible precursor to DNA.

2. **Emergence of DNA:**

- Over time, the emergence of DNA offered several advantages. DNA is more stable than RNA and less prone to spontaneous hydrolysis.

- DNA also provided a more robust and efficient way to store genetic information, as its double-stranded structure offers redundancy and error-checking mechanisms during replication.

3. **RNA as Intermediary:**

- It is theorized that RNA played an intermediary role during this transition, with RNA molecules facilitating the synthesis of DNA.

4. **Selective Pressure:**

- The selective pressure for improved genetic stability and information storage likely favored the transition from an RNA-based system to a DNA-based one.

5. **Evolutionary Advantage:**

- DNA's enhanced stability and information storage capacity would have provided organisms with an evolutionary advantage, leading to the predominance of DNA as the genetic material in modern life.

Endosymbiosis:

Endosymbiosis is a theory that proposes the origin of eukaryotic cells through the symbiotic relationship between different species of prokaryotic cells. According to the endosymbiotic theory, ancestral eukaryotic cells formed through the engulfment of free-living prokaryotic cells by a host cell. Over time, these engulfed cells established a symbiotic relationship within the host cell, giving rise to the organelles found in eukaryotic cells today.

Key Points of Endosymbiotic Theory:

1. **Mitochondria and Chloroplasts Originated from Prokaryotes:**

- Mitochondria and chloroplasts, the energy-producing organelles in eukaryotic cells, share similarities with free-living bacteria. It is believed that mitochondria originated from an ancestral aerobic bacterium, while chloroplasts originated from an ancestral photosynthetic bacterium.

2. **Evidence of Endosymbiosis:**

- The endosymbiotic theory is supported by various lines of evidence, including the resemblance of mitochondria and chloroplasts to bacteria, the presence of their own circular DNA (similar to bacterial DNA), and the ability of these organelles to replicate independently of the host cell.

3. **Genetic Similarities:**

- The genetic material (DNA) found in mitochondria and chloroplasts is more similar to bacterial DNA than nuclear DNA in eukaryotic cells.

4. **Double Membrane Structure:**

- Mitochondria and chloroplasts have a double membrane structure, which is consistent with the idea that they were engulfed by an ancestral host cell.

5. **Independent Replication:**

- Both mitochondria and chloroplasts have the ability to replicate independently of the host cell, similar to free-living bacteria.

6. **Phylogenetic Evidence:**

- Phylogenetic analyses comparing the DNA sequences of mitochondria, chloroplasts, and bacteria provide additional support for the endosymbiotic theory.

The endosymbiotic theory provides a compelling explanation for the origin of eukaryotic cells, suggesting that the symbiotic relationship between different prokaryotic cells laid the foundation for the complexity and diversity of eukaryotic life.

(d) What are the structural differences between hnRNA and mRNA? What is spliceosome? Describe the prerequisite condition for DNA replication in prokaryotes.

(d) **Structural Differences between hnRNA and mRNA**[1234]:

Heterogeneous nuclear RNA (hnRNA) and messenger RNA (mRNA) are both types of RNA, but they have different structures and roles in the cell[1234].

- **hnRNA**: It is the primary transcript that is synthesized from DNA during transcription[13]. hnRNA is much larger than mRNA and contains both exons (coding regions) and introns (non-coding regions)[13]. It undergoes various processing steps, including capping, tailing, and splicing, to become mRNA[13].
- **mRNA**: It is the processed form of hnRNA[124]. Introns are removed, and exons are joined together during the splicing process[124]. The mRNA

molecule is smaller and more stable than hnRNA, and it carries the genetic code from DNA to the ribosomes, where proteins are synthesized[124].

Spliceosome[567]:

A spliceosome is a large ribonucleoprotein (RNP) complex found primarily within the nucleus of eukaryotic cells[567]. The spliceosome is assembled from small nuclear RNAs (snRNA) and numerous proteins[567]. The spliceosome removes introns from a transcribed pre-mRNA, a type of primary transcript[567]. This process is generally referred to as splicing[567].

Prerequisite Condition for DNA Replication in Prokaryotes[89101112]:

For DNA replication to occur in prokaryotes, several conditions must be met[89101112]:

1. **Origin of Replication (OriC)**: DNA replication begins at a specific site on the chromosome known as the origin of replication (OriC)[89101112].
2. **Unwinding of DNA**: An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the base pairs[89101112].
3. **Primer**: A primer is needed to provide a free 3'-hydroxyl group to which nucleotides can be added[11].
4. **dNTPs**: Deoxyribonucleotide triphosphates (dNTPs) are required as the building blocks of the new DNA strand[11].
5. **DNA Polymerase**: DNA polymerase is the enzyme that adds nucleotides to the growing DNA strand[89101112].
6. **Single-Strand Binding Proteins**: These proteins stabilize the newly formed replication bubble[89101112].
7. **Topoisomerases**: These enzymes relieve the tension in the DNA molecule that is created by the unwinding of the double helix[89101112].

OR/

Structural Differences between hnRNA (Heterogeneous Nuclear RNA) and mRNA (Messenger RNA):

1. **Location and Processing:

- **hnRNA:** Synthesized in the cell nucleus. It undergoes various modifications, including capping, splicing, and polyadenylation, before becoming mature mRNA.

- **mRNA:** Transcribed from the DNA template and processed in the nucleus. Mature mRNA is transported to the cytoplasm for translation.

2. **Introns and Exons:

- **hnRNA:** Contains both introns (non-coding regions) and exons (coding regions).

- **mRNA:** After splicing, contains only exons, as introns are removed.

3. **Length:

- **hnRNA:** Generally longer and includes non-coding regions.

- **mRNA:** Shorter, as it lacks introns after splicing and contains only the coding regions.

4. **5' Cap and Poly-A Tail:

- **hnRNA:** Gains a 5' cap and a poly-A tail during post-transcriptional modifications.

- **mRNA:** Retains the 5' cap and poly-A tail, which are essential for mRNA stability and translation in the cytoplasm.

5. **Transport to Cytoplasm:

- **hnRNA:** Stays in the nucleus and undergoes processing.

- **mRNA:** Transported to the cytoplasm for translation after processing.

Spliceosome:

The spliceosome is a complex cellular machinery responsible for the removal of introns from pre-mRNA during the process of RNA splicing. RNA splicing is crucial for the maturation of mRNA by removing non-coding introns and ligating together the coding exons. The spliceosome consists of small nuclear

ribonucleoproteins (snRNPs) and other protein components. The splicing process involves the following steps:

1. **Recognition of Intron-Exon Boundaries:**

   - The spliceosome recognizes the junctions between introns and exons in the pre-mRNA.

2. **Formation of the Spliceosome:**

   - snRNPs, small nuclear RNA-protein complexes, assemble with other proteins to form the spliceosome.

3. **Intron Removal (Splicing):**

   - The spliceosome catalyzes the removal of introns by forming a lariat structure, connecting the 5' end of the intron to a branch point within the intron.

4. **Ligation of Exons:**

   - The exons are then ligated together to form the mature mRNA molecule.

5. **Release of Intron Lariat:**

   - The excised intron forms a lariat structure and is released from the mRNA.

6. **Formation of Mature mRNA:**

   - The ligated exons constitute the mature mRNA ready for translation.

The spliceosome is a dynamic and highly regulated machinery that ensures the accurate removal of introns and the precise joining of exons, contributing to the diversity of mRNA transcripts and the proteome.

Prerequisite Conditions for DNA Replication in Prokaryotes:

DNA replication is a highly regulated process that ensures the faithful transmission of genetic information during cell division. In prokaryotes, such as bacteria, several prerequisite conditions are essential for DNA replication:

1. **Initiation Proteins:**

   - Initiation of DNA replication requires specific initiator proteins that bind to the origin of replication (oriC) in the bacterial chromosome.

2. **Origin of Replication (oriC):**

   - Presence of the origin of replication, a specific DNA sequence where replication begins.

3. **Single-Stranded DNA Binding Proteins:**

   - Single-stranded DNA binding proteins stabilize the separated DNA strands by preventing them from reannealing.

4. **DNA Gyrase:**

   - DNA gyrase (topoisomerase) relieves the tension generated ahead of the replication fork by introducing negative supercoils.

5. **RNA Primers:**

- RNA primers are required to initiate DNA synthesis by providing a 3'-OH group for DNA polymerase to start adding nucleotides.

6. **DNA Polymerase:**

- DNA polymerase synthesizes the complementary DNA strand using the template strand as a guide.

7. **dNTPs (Deoxynucleotide Triphosphates):**

- Availability of dNTPs (deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate) as building blocks for DNA synthesis.

8. **Helicase:**

- Helicase unwinds the DNA double helix at the replication fork, creating single-stranded templates for DNA synthesis.

9. **DNA Ligase:**

- DNA ligase seals the nicks between Okazaki fragments on the lagging strand.

10. **Proper Cellular Environment:**

- The cell must be in a suitable environment for DNA replication, with appropriate pH, temperature, and availability of cofactors.

The coordination of these factors ensures the accurate and efficient replication of the bacterial genome in prokaryotic cells.

(E) What is cancer? Mention the different stages of cancer. What are the probable causes of cancer? Mention the role of 'Ras' gene in cancer.

What is Cancer?

Cancer is a disease characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissue[1][2][3]. It can start almost anywhere in the human body, which is made up of trillions of cells[1]. When cells grow old or become damaged, they die, and new cells take their place. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn't[1]. These cells may form tumors, which are lumps of tissue[1]. Tumors can be cancerous or not cancerous (benign)[1].

Different Stages of Cancer:

Cancer stages are usually described by the TNM system[4][5][6][7]:

- **T** refers to the size and extent of the main tumor.
- **N** refers to the number of nearby lymph nodes that have cancer.
- **M** refers to whether the cancer has metastasized, meaning it has spread from the primary tumor to other parts of the body.

The stages are often broken down into the following:

- **Stage 0**: This is used to describe cancer in situ, which means "in place." Stage 0 cancers are still located in the place they started and have not spread to nearby tissues[6].
- **Stage I**: This stage is usually a small cancer or tumor that has not grown deeply into nearby tissues. It also has not spread to the lymph nodes or other parts of the body. It is often called early-stage cancer[6].
- **Stage II and III**: These stages indicate larger cancers or tumors that have grown more deeply into nearby tissue. They may have also spread to lymph nodes but not to other parts of the body[6].
- **Stage IV**: This stage means that the cancer has spread to other organs or parts of the body. It may also be called advanced or metastatic cancer[6].

Probable Causes of Cancer:

Cancer is caused by changes (mutations) to the DNA within cells[2][8][9][10]. The DNA inside a cell is packaged into a large number of individual genes, each of which contains a set of instructions telling the cell what functions to perform, as well as how to grow and divide[2]. Errors in the instructions can cause the cell to stop its normal function and may allow a cell to become cancerous[2]. Some of the probable causes of cancer include:

- Smoking and tobacco use
- Alcohol
- Lack of physical activity
- Being overweight or obese
- Poor diet

- Sun exposure
- Radiation exposure
- Virus infections and other infections[9]

Role of 'Ras' Gene in Cancer:

The main members of the RAS gene family— KRAS, HRAS, and NRAS — encode proteins that have a pivotal role in cell signaling[11121314]. When RAS genes are mutated, cells grow uncontrollably and evade death signals[11121314]. RAS mutations also make cells resistant to most available cancer therapies[11]. It has been known for more than three decades that about a third of all human cancers, including a high percentage of pancreatic, lung, and colorectal cancers, are driven by mutations in RAS genes[11].