2022

BOTANY — HONOURS

Paper : cc-Il
(Cell and Molecular Biology)

Full Marks : 50
The figures in the margin indicate full marks.

Candidates are required to give their answers in their own words as far as practicable.

1. Answer any five questions from the following

(a) Chromatin is composed of DNA (30-40%), RNA (1-10%) and proteins (50-60%). These constituents vary in different organisms and even in the different tissues of the same species[123].

(b) Blunt and staggered cuts refer to the types of cuts made in DNA by restriction enzymes. A blunt cut results in DNA ends where both strands are of equal length, leaving no unpaired bases on either strand. A staggered cut, on the other hand, generates two sticky ends or overhanging ends[45678].

© The end-replication problem refers to the inability of DNA polymerase to replicate the ends of linear chromosomes, leading to their gradual shortening over time[91011].

(d) Both chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) contain genes that participate in essential cellular processes. For instance, the genes in cpDNA participate in photosynthesis, while the genes in mtDNA take part in oxidative phosphorylation and protein synthesis[121314].

(e) The nuclear lamina is an essential component of metazoan cells. It is involved in most nuclear activities including DNA replication, RNA transcription, nuclear and chromatin organization, cell cycle regulation, cell development and differentiation, nuclear migration, and apoptosis[151617].

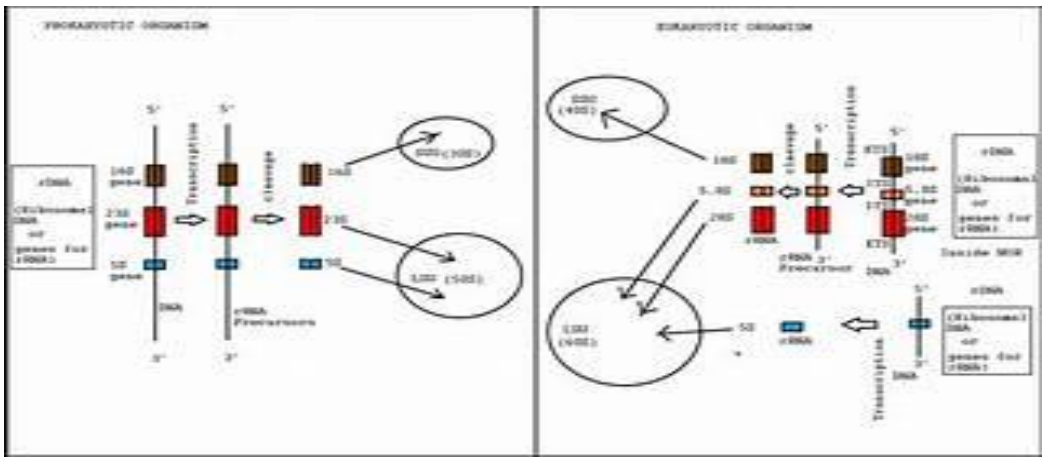
(f) Riboswitches are specific components of an mRNA molecule that regulate gene expression. They bind to small target molecules and directly regulate their own expression[1819].

(g) The concept of the RNA world came into existence to explain a hypothetical stage in the evolutionary history of life on Earth, in which self-replicating RNA molecules proliferated before the evolution of DNA and proteins[20212223]. The full form of PNA is Peptide Nucleic Acid[2425].

(h) The p53 gene plays a crucial role in controlling the cell cycle. When the p53 protein is mutated, it can lead to unrestricted cell cycle progression and the replication of damaged DNA, resulting in uncontrolled cell proliferation and potentially cancerous tumors[2627].

1. Answer any two questions from the following :

(a)



Explore

Ribosome biogenesis in eukaryotes is a complex and highly coordinated process that involves the production and correct assembly of four rRNAs and about 80 ribosomal proteins[12]. Here is a detailed explanation:

1. **Transcription**: In eukaryotic cells, three of the mature rRNA species are co-transcribed as a single transcript by RNA polymerase I[3]. The 5S rRNA is synthesized separately by RNA polymerase III[3].

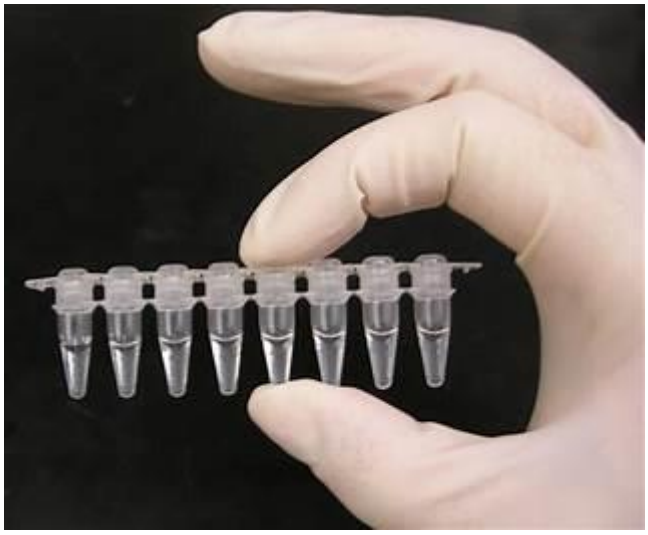
2. **Processing**: The rRNA transcript undergoes a series of nucleolytic processing steps to mature[34]. This maturation process involves the removal of external and internal transcribed spacer sequences to produce the mature 18S, 5.8S, and 28S rRNAs[34].

3. **Assembly**: Maturation of the rRNAs and recruitment of the ribosomal proteins occurs within a series of precursor ribosomal particles, or pre-ribosomes, within the nucleolus, nucleoplasm, and cytoplasm[3]. The systematic purification of pre-ribosomes has allowed the protein and rRNA composition of multiple intermediates to be elucidated and ordered into a ribosome assembly map[3].

4. **Modification and Folding**: A plethora of assembly factors, including those with predicted ATPase, GTPase, helicase, kinase, or nuclease activity, orchestrate the ordered modification, folding, and processing of rRNA, and the sequential recruitment of ribosomal proteins[3].

This process requires hundreds of factors not present in the mature particle[12]. In the absence of these proteins, ribosome biogenesis is stalled and cell growth is terminated even under optimal growth conditions[12]. Ribosome biogenesis is one of the most energetically demanding of cellular activities[4], and it is closely linked to other cellular activities like growth and division[4].

(b)



Explore

Polymerase Chain Reaction (PCR) is a technique used in molecular biology to amplify a specific DNA segment, making many copies of a particular region of DNA[1]. Here's a detailed explanation of the process and its applications:

Process of PCR:

1. **Denaturation**: The reaction mixture is heated to 94°C for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into single-stranded DNA[2].
2. **Annealing**: The reaction temperature is lowered to 54-60°C for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA[2].
3. **Extension**: The temperature is raised to 72°C. DNA polymerase synthesizes new strands of DNA, using the original strands as templates[2].

This cycle is repeated around 30 times to amplify the DNA[2].

Applications of PCR:

1. **Clinical Diagnosis**: PCR is highly useful for diagnosing various diseases in humans, including inherited disorders, viral diseases, bacterial diseases, etc[3].
2. **DNA Sequencing**: PCR is used for sequencing. For this purpose, single-strands of DNA are required[3].
3. **Gene Manipulation and Expression Studies**: PCR is used in gene manipulation and expression studies[3].
4. **Comparative Studies of Genomes**: PCR is used in comparative studies of genomes[3].
5. **Forensic Medicine**: PCR is used in forensic medicine for genetic fingerprinting[34].
6. **Comparison with Gene Cloning**: PCR is used in comparison with gene cloning[3].

PCR has revolutionized the field of molecular biology and has a wide range of applications in medical diagnostics, forensic science, and biological research[4].

© The **Endosymbiotic Theory** is a key concept in cellular biology that explains the evolutionary origin of eukaryotic organelles, specifically mitochondria and chloroplasts[12]. Here's a more detailed explanation:

1. **Origins**: The theory posits that the first eukaryotic cell was probably an amoeba-like cell that obtained nutrients by phagocytosis and contained a nucleus that formed when a piece of the cytoplasmic membrane pinched off around the chromosomes[2].
2. **Engulfment**: Some of these amoeba-like organisms ingested prokaryotic cells that then survived within the organism and developed a symbiotic relationship[2]. Mitochondria formed when bacteria capable of aerobic respiration were ingested; chloroplasts formed when photosynthetic bacteria were ingested[2].
3. **Evolution**: Over time, these ingested prokaryotic cells lost their cell wall and much of their DNA because they were not of benefit within the host cell[2]. They eventually evolved into the organelles we now know as mitochondria and chloroplasts[12].
4. **Evidence**: The theory is supported by several pieces of evidence. For instance, mitochondria and chloroplasts have their own DNA that is circular, not linear[2]. They also have their own ribosomes that have 30S and 50S subunits, not 40S and 60S[2]. Additionally, these organelles are similar in size to prokaryotic cells and divide by binary fission[2].

The Endosymbiotic Theory has revolutionized our understanding of cell evolution and the complex relationships between different forms of life[12].

(d) The processing of mRNA in eukaryotes is a complex process that involves several steps[12]. Here's a detailed explanation:

1. **Transcription**: In eukaryotes, mRNA is transcribed on chromosomes in the nucleus[3]. Unlike prokaryotes, translation in eukaryotes takes place only after transcription has been completed[3].

2. **5' Capping**: While the pre-mRNA is still being synthesized, a 7-methylguanosine cap is added to the 5' end of the growing transcript by a 5'-to-5' phosphate linkage[1]. This moiety protects the nascent mRNA from degradation[1].

3. **Splicing**: Splicing involves a number of small ribonuclear proteins (snRNPs). snRNPs are particles composed of RNA and proteins. They bind to specific sites in an mRNA and then direct a sequential series of cuts and ligations (the splicing) necessary to process the mRNAs[2]. The role of snRNPs in splicing pre-mRNAs is illustrated below[2]. snRNP binding to a pair of splice sites flanking an intron in a pre-mRNA forms the spliceosome that completes the splicing, including removal of the lariat (the intermediate structure of the intron)[2]. The last step is to ligate exons into a continuous mRNA with all its codons intact and ready for translation[2].

4. **3' Polyadenylation**: A poly (A) tail is added to the 3' end of the pre-mRNA once elongation is complete[1]. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation[1].

5. **Export to the Cytoplasm**: After processing, the mRNA is shuttled through nuclear pores and into the cytoplasm[3].

This extensive processing of pre-mRNA in eukaryotes creates a molecule with a much longer half-life than a prokaryotic mRNA[1]. Eukaryotic mRNAs last for several hours, whereas the typical E. coli mRNA lasts no more than five seconds[1].

3. **Answer any three questions from the following** :

(a) An **operon** is a cluster of functionally-related genes that are controlled by a shared operator[12]. Operons consist of multiple genes grouped together with a promoter and an operator[12]. They are present in prokaryotes (bacteria and archaea), but are absent in eukaryotes[1]

Sure, here are the key differences between an inducible and a repressible operon:

1. **Regulation**: Inducible operons are usually turned off and require an inducer molecule to activate transcription[1]. In contrast, repressible operons are usually turned on and require a repressor molecule to stop transcription[1].
2. **Gene Expression**: Inducible operons control genes that are involved in catabolic pathways, which means the genes are expressed only when the substrate is present[1]. Repressible operons control genes that are involved in anabolic pathways, which means the genes are expressed until the product is synthesized[1].

3. **Feedback Inhibition**: Repressible operons are subject to feedback inhibition, which means that the end product of the pathway inhibits the synthesis of the enzymes involved in the pathway[1]. Inducible operons are not subject to feedback inhibition[1].

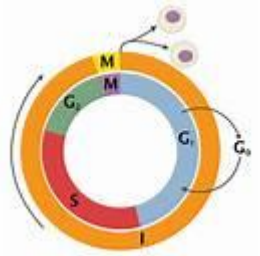
4. **Examples**: The lac operon is an example of an inducible operon, which is activated by the presence of lactose[1]. The trp operon is an example of a repressible operon, which is turned off when the end product of the pathway, tryptophan, is present[1].

Negative Control in Lac-Operon:
The lac operon is a well-known example of an inducible gene network that regulates the transport and metabolism of lactose in Escherichia coli[4]. It encodes the genes for the internalization of extracellular lactose and then its conversion to glucose[4].
In the absence of lactose, the transcription of the lac operon genes is blocked by a repressor protein[4]. This repressor protein is encoded by the lacI gene, which is located upstream of the lac operon and also consists of its own promoter[4]. The repressor protein binds tightly to the lac operator site, Olac, preventing transcription[5].
When lactose is available in the medium for the bacteria, the regulatory gene is activated[6]. The inducer (lactose) binds to the repressor protein and renders it inactive, which allows transcription of the operon[6]. Thus, the lac operon is negatively regulated in this case[6]

(b) In the yeast cell cycle, there are several checkpoints that ensure the proper progression of the cell through the cycle[123]. These include:

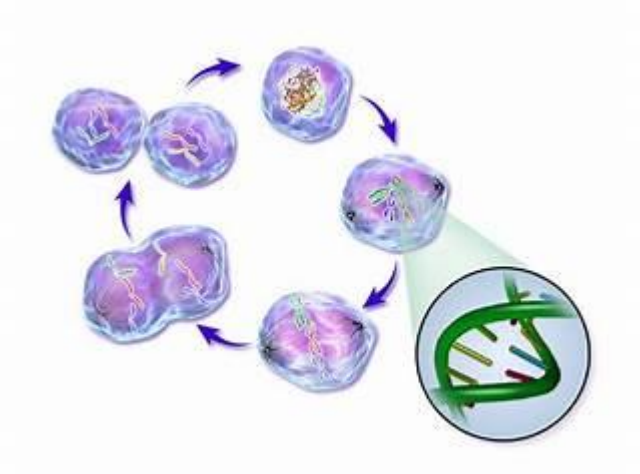
1. **G1 Checkpoint**: This checkpoint determines whether the cell enters the cell cycle or not[1]. It is present between the G1 phase and S phase and is responsible for the entry of the cell in the division phase[1].

2. **G2 Checkpoint**: This checkpoint ensures that DNA replication during the S phase did not produce any mistakes[2]. It is present at the transition from the G2 phase to the M phase[2].



3. **Spindle Checkpoint**: This checkpoint is at the transition from metaphase to anaphase[2]. It ensures that all chromosomes are properly attached to the spindle before the cell proceeds to anaphase[2]

MPF, or Maturation-Promoting Factor, is also known as mitosis-promoting factor or M-Phase-promoting factor[1]. It is a cyclin-Cdk complex that was first discovered in frog eggs[1]. MPF stimulates the mitotic and meiotic phases of the cell cycle[1]. This ensures that the cell cycle progresses in a regulated and orderly manner[1].



Explore

Maturation-Promoting Factor (MPF) is a cyclin-Cdk complex that plays a crucial role in cell cycle regulation[12]. Here's a detailed explanation:

1. **Formation of MPF**: MPF is formed when a cyclin molecule binds to a cyclin-dependent kinase (Cdk)[2]. The cyclin molecule activates the Cdk, which is otherwise inactive[2].
2. **Role in G2/M Transition**: MPF is particularly important for the transition from the G2 phase to the M phase of the cell cycle[3]. When mitotic cyclins bind to Cdks in G2, the resulting complex is known as MPF[4]. This complex acts as the signal for the G2 cell to enter mitosis[4].
3. **Phosphorylation of Proteins**: MPF promotes the entrance into mitosis (the M phase) from the G2 phase by phosphorylating multiple proteins needed during mitosis[1]. The MPF is also called the M phase kinase because of its ability to phosphorylate target proteins at a specific point in the cell cycle and thus control their ability to function[1].
4. **Degradation of Cyclin**: Once the mitotic cyclin degrades, MPF is inactivated and the cell exits mitosis by dividing and re-entering G1[4].

Through these mechanisms, MPF ensures that the cell cycle progresses in a regulated and orderly manner[12].

Explore

© The **genetic code** is defined as the set of rules used by living cells to translate information encoded within genetic material (DNA or mRNA sequences) into proteins[1]. It is the sequence of nucleotides in DNA and RNA that determines the amino acid sequence of proteins[2].

Properties of Genetic Code:

1. **Triplet Code**: A codon or a code word is defined as a group of bases that specify an amino acid. There is strong evidence, which proves that a sequence of three nucleotides codes for an amino acid in the protein, i.e., the code is a triplet[3].
2. **Non-Ambiguous and Universal**: The genetic code is universal since similar codons are assigned to identical amino acids along with similar START and STOP signals in the majority of genes in microorganisms and plants[3].
3. **Degenerate Code**: There are 64 codons for 20 amino acids since every codon for one amino acid means that there exist more than one code for the same amino acid[3].
4. **Nonoverlapping 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[3].

5. **Commaless**: No room for punctuation in between which indicates that every codon is adjacent to the previous one without any nucleotides between them[3].
6. **Start and Stop Codons**: The genetic code includes specific codons that signal the start and end of protein synthesis[3].
7. **Polarity**: Each triplet is read from 5' → 3' direction and the beginning base is 5' followed by the base in the middle then the last base which is 3'. This implies that the codons have a fixed polarity[3].

Exceptions to Genetic Code:

The universality of the genetic code conveys that similar codons are assigned to identical amino acids, along with similar start and stop signals in the genes of most microorganisms and plants. Out of the few exceptions that were discovered, most of them include assigning one or two stop codons to an amino acid[3].

Decipherance of Codons:

Deciphering or cracking of the genetic code is the outcome of research of various scientists like Marshal Nirenberg, Steve Ochoa, Hargobind Khorana, Francis Crick, and their associates. They discovered that the order in which the nucleotides are arranged – mRNA would determine the sequence of amino acids in polypeptides[4]. Nirenberg and Mattaei gave the first experimental proof for the triplet codon. They used artificial mRNA made of only uracil nucleotides (Poly U) in a cell-free system. It resulted in the synthesis of a polypeptide chain made up of only one kind of amino acid, phenylalanine. It was concluded that the codon for phenylalanine was uridylic acid bases (uracil), UUU[4].

(d) Sure, here are the roles of protooncogenes and tumor suppressor genes in cancer development:

Protooncogenes:

1. **Normal Function**: Protooncogenes are genes that normally help cells grow and divide to make new cells, or to help cells stay alive[1].
2. **Mutation**: When a proto-oncogene mutates (changes) or there are too many copies of it, it can become turned on (activated) when it is not supposed to be[1].
3. **Oncogene Formation**: At this point, it's now called an oncogene[1].
4. **Uncontrolled Growth**: When this happens, the cell can start to grow out of control, which might lead to cancer[1].
5. **Gas Pedal Analogy**: A proto-oncogene normally functions in a way much like the gas pedal on a car. It helps the cell grow and divide. An oncogene is

like a gas pedal that is stuck down, which causes the cell to divide out of control[1].

Tumor Suppressor Genes:

1. **Normal Function**: Tumor suppressor genes are normal genes that slow down cell division or tell cells to die at the right time (a process known as apoptosis or programmed cell death)[2].
2. **Mutation**: When tumor suppressor genes don't work properly, cells can grow out of control, which can lead to cancer[2].
3. **Brake Pedal Analogy**: A tumor suppressor gene is like the brake pedal on a car. It slows down cell division. If the brake pedal is broken, the cell can divide out of control[2].
4. **Types**: Tumor suppressor genes come in three main types. Each type has a different function: telling cells to slow down and stop dividing, repairing damage to cellular DNA that results from dividing and could lead to cancer, and causing damaged cells to start a process called programmed cell death, or apoptosis[2].
5. **Oncogenes vs. Tumor Suppressor Genes**: Cancer can be related to problems with either the accelerator or the brakes, but often, damage to both oncogenes and tumor suppressor genes occurs before cancer develops[2].

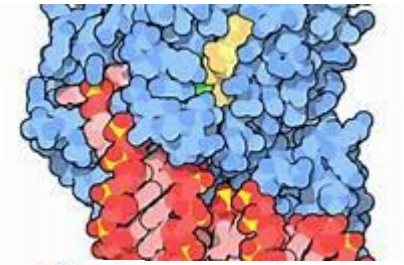
The **main types of cancer** include breast cancer, lung cancer, prostate cancer, colorectal cancer, melanoma, bladder cancer, non-Hodgkin lymphoma, kidney cancer, endometrial cancer, leukemia, pancreatic cancer, thyroid cancer, and liver cancer[78].
The **stages of cancer** refer to the size of the initial tumor and whether or not the cancer has spread to other areas of the body[3]. The stages are as follows[39]:

- **Stage 0**: The cancer is where it started (in situ) and hasn't spread.
- **Stage 1**: The cancer is small and hasn't spread anywhere else.
- **Stage 2**: The cancer has grown, but hasn't spread.
- **Stage 3**: The cancer is larger and may have spread to the surrounding tissues and/or the lymph nodes.
- **Stage 4**: The cancer has spread to other parts of the body.

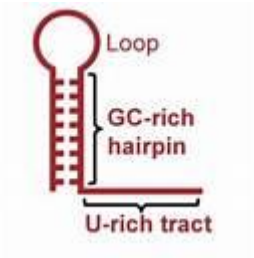
Carcinogenic agents are substances that can cause cancer. Some examples of carcinogens include tobacco, radon, asbestos, formaldehyde, ultraviolet rays, arsenic, chloroform, coal dust and emissions, cobalt, Epstein-Barr virus, estrogen-progestogen combined oral contraceptives[10]

(e) Sure, I can explain the process of transcription in prokaryotes in detail. Transcription is the process through which a DNA sequence is enzymatically copied by an RNA polymerase to produce a complementary RNA[1]. The process occurs in three stages: Initiation, Elongation, and Termination[12].

1. **Initiation:** In bacteria, transcription begins with the binding of RNA polymerase to the promoter in DNA[1]. The RNA polymerase is a core enzyme[1]. The DNA strand which is used in RNA synthesis is called the template strand[1].



2. **Elongation:** As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy[1]. The RNA thus synthesized is called messenger RNA (mRNA), because it carries a genetic message from the DNA to the protein-synthesizing machinery of the cell[1].



3. **Termination:** The process ends when a terminator sequence is reached[1]. The RNA polymerase, as well as the transcript of RNA, break away from DNA[2].

Please note that these are high-level descriptions. The actual processes are much more complex and involve many other proteins and enzymes. For more detailed information, you may want to refer to a molecular biology textbook or other specialized resources[12].

Sure, I can explain the roles of helicase, gyrase, and SSB protein in DNA replication in detail:

1. **Helicase:** This enzyme disrupts hydrogen bonds between two strands of DNA to separate the template DNA strands at the replication fork[1]. It unwinds and separates the double-stranded DNA by breaking the hydrogen bonds between base pairs[2]. This occurs at specific regions (origins of replication), creating a replication fork of two strands running in antiparallel directions[2].

2. **Gyrase (Topoisomerase II):** This enzyme relaxes the supercoiled chromosome, which is crucial for bacterial DNA replication to begin[3]. It also reduces the torsional strain created by the unwinding of DNA by helicase[2]. An example of a topoisomerase is DNA gyrase[1].

3. **Single-Strand Binding Proteins (SSBPs):** These proteins bind to unwound single-stranded regions of DNA to keep the template strands apart during replication[1]. They allow effective replication, protect from nucleases, promote DNA polymerase binding, and interact with other proteins[4]. SSB stands for Single-Stranded Binding Proteins. As the name suggests their function is to remain attached to the single-stranded DNA. It is significantly found during the process of replication[4].

Please note that these are high-level descriptions. The actual processes are much more complex and involve many other proteins and enzymes. For more detailed information, you may want to refer to a molecular biology textbook or other specialized resources[45123]…