MB502 – MOLECULAR BIOLOGY

FINAL TERM MOST IMPORTANT QUESTIONS

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GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. Write a short note on CRISPR.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a revolutionary gene-editing technology derived from a natural defense mechanism in bacteria. It uses a protein called Cas9, guided by a piece of RNA, to locate and cut specific DNA sequences. Scientists can then add, delete, or modify genes with precision. CRISPR is widely used in medicine, agriculture, and genetic research due to its accuracy, efficiency, and low cost.

2. What is plasmid and where it is found?

A plasmid is a small, circular, double-stranded DNA molecule that replicates independently of the chromosomal DNA of the host cell. Unlike the main bacterial chromosome, plasmids are not essential for the survival of the organism under normal conditions, but they often carry beneficial genes, such as those that confer resistance to antibiotics, enable the metabolism of unusual substances, or provide virulence factors in pathogenic bacteria.

Plasmids are most commonly found in bacteria, where they can be present in multiple copies per cell. They can also occur in some archaea and, rarely, in eukaryotic cells (especially in yeast). Plasmids can be transferred between bacteria through processes like conjugation, making them important agents in horizontal gene transfer. In biotechnology and genetic engineering, plasmids are widely used as vectors to insert and express genes in host cells for research, medicine, and industrial applications.

3. Why plasmid are not regarded as cell's genome?

Plasmids are not regarded as part of a cell's genome because they are **extrachromosomal**—they exist separately from the main chromosomal DNA. While the genome includes all the genetic material necessary for the normal growth, development, and reproduction of the organism, plasmids usually carry non-essential genes that provide additional advantages (like antibiotic resistance) but are not required for basic cellular functions. Moreover, plasmids replicate independently and can be transferred between cells, further distinguishing them from the stable, inherited genome of the organism.

4. Who were the scientists that observed that free and bound ribosomes appear to be the same, leading to the proposal of Signal Hypothesis?

The scientists who observed that free and bound ribosomes appear to be the same were George Palade and his colleagues. Their observations in the 1950s and 1960s, particularly through electron microscopy, showed that ribosomes attached to the endoplasmic reticulum (ER) and those free in the cytoplasm were structurally identical.

This finding contributed to the development of the Signal Hypothesis, which was later proposed in 1971 by Günter Blobel and David Sabatini. The Signal Hypothesis explains how certain proteins are directed to the ER by a signal sequence, despite all ribosomes being structurally the same. Blobel was awarded the Nobel Prize in 1999 for this work.

5. What is the role of RHO protein in termination of transcription?

The Rho protein is involved in Rho-dependent termination of transcription in prokaryotes. It functions as an ATP-dependent helicase that binds to a specific RNA sequence called the rut site (Rho utilization site) on the newly synthesized RNA strand. Once attached, Rho moves along the RNA toward the RNA polymerase.

When the RNA polymerase reaches a termination region and pauses, Rho catches up and uses its helicase activity to disrupt the RNA-DNA hybrid within the transcription bubble. This leads to the release of the RNA transcript and the dissociation of RNA polymerase from the DNA, thereby ending transcription.

6. Write five points about the Operon model.

Here are five key points about the Operon model:

1. ***Definition****:* The operon model explains how a group of genes in prokaryotes is regulated and transcribed together under the control of a single promoter.
2. ***Structure****:* An operon typically includes a promoter, operator, and structural genes. The regulatory gene (located nearby or elsewhere) produces a repressor protein that can influence the operon's activity.
3. ***Regulation****:* Operons can be inducible (like the *lac operon*, activated in the presence of lactose) or repressible (like the *trp operon*, turned off when tryptophan is abundant).
4. ***Function****:* The operon system allows bacteria to efficiently regulate gene expression in response to environmental changes, conserving energy and resources.
5. ***Scientific Contribution****:* The model was first proposed by François Jacob and Jacques Monod in 1961 based on their work with the *lac operon* in *E. coli*, earning them a Nobel Prize.

7. Explain mechanism of RNA editing.

RNA editing is a molecular process through which the nucleotide sequence of an RNA molecule is altered after it is transcribed from DNA, resulting in an RNA sequence that differs from the original DNA template. This can change the information content and influence the final protein product.

Mechanism of RNA editing:

1. ***Types of RNA editing:*** The most common types are **base modifications**, such as:
    - **A-to-I editing** (adenosine to inosine) by enzymes called ADARs (adenosine deaminases acting on RNA).

- **C-to-U editing** (cytidine to uridine) by enzymes like APOBEC.

2. ***Enzyme action:*** Specific enzymes recognize target RNA sequences and chemically modify particular bases without altering the RNA backbone.
3. ***Effect on coding:*** For example, inosine (I) is interpreted as guanosine (G) during translation, which can alter codons, affect splicing sites, or influence RNA stability.
4. ***Functional consequences:*** RNA editing can diversify protein products, regulate gene expression, or correct genetic mutations at the RNA level.
5. ***Examples:*** Editing is critical in some neurotransmitter receptor transcripts in the brain and in plant mitochondria and chloroplast RNAs.

8. Explain translocation. At which subunit it occurs?

Translocation is a step during protein synthesis (translation) where the ribosome moves one codon forward along the mRNA after a new amino acid has been added to the growing polypeptide chain. This shifts the tRNA carrying the polypeptide from the **A site** (aminoacyl site) to the **P site** (peptidyl site), freeing the A site for the next charged tRNA.

Translocation occurs at the large subunit of the ribosome. In prokaryotes, this is the 50S subunit, and in eukaryotes, the 60S subunit. The movement is facilitated by elongation factors (such as EF-G in bacteria) and requires energy from GTP hydrolysis.

9. Write importance, functions and availability of CRISPR.

CRISPR is a powerful gene-editing tool with wide-ranging importance, functions, and availability:

Importance:

- Allows precise editing of DNA sequences, revolutionizing genetics and molecular biology.
- Enables development of gene therapies for genetic disorders.
- Accelerates research by making gene modification faster and cheaper.
- Has potential applications in agriculture for creating disease-resistant or higher-yield crops.

Functions:

- Targets specific DNA sequences using a guide RNA.
- Cuts DNA at precise locations with the Cas9 enzyme.
- Enables deletion, insertion, or correction of genes.
- Used in basic research, medical treatments, and biotechnology

Availability:

- CRISPR technology is widely accessible in research labs worldwide.
- Available through commercial kits and open-source platforms.
- Ethical and regulatory frameworks vary by country, influencing clinical and agricultural applications.
- Ongoing improvements are making it more efficient and safer for broader use.

10. Write contribution of Franklin and Maurice Williams towards Molecular Biology.

Rosalind Franklin and Maurice Wilkins made crucial contributions to molecular biology, particularly in understanding the structure of DNA. Together, their work was fundamental in discovering the double helix structure of DNA, which is key to understanding genetic information storage and replication. Wilkins, Watson, and Crick received the Nobel Prize in 1962, while Franklin's contributions were recognized posthumously.

- **Rosalind Franklin** used X-ray crystallography to capture high-resolution images of DNA fibers. Her famous "Photo 51" provided critical evidence of the helical structure of DNA, revealing its dimensions and the arrangement of its strands.
- **Maurice Wilkins** also worked on X-ray diffraction studies of DNA and shared Franklin's crucial data with James Watson and Francis Crick, aiding their model building of the DNA double helix.

11. What are primers? Write their functions.

Primers are short sequences of nucleotides (usually RNA or DNA) that provide a starting point for DNA synthesis during replication or other molecular biology processes.

Functions of primers:

1. ***Initiate DNA synthesis:*** DNA polymerases cannot start new strands on their own; primers supply a free 3'-OH group for the enzyme to begin adding nucleotides.
2. ***Guide replication:*** They bind to specific complementary sequences on the DNA template to define where DNA synthesis starts.
3. ***Enable PCR:*** In the Polymerase Chain Reaction, primers flank the target DNA region to amplify it selectively.
4. ***Assist DNA repair and sequencing:*** Primers are used in various techniques to start DNA copying or reading specific sequences.

12. Define SR protein. How it occurs in splice site?

SR proteins (Serine/Arginine-rich proteins) are a family of RNA-binding proteins involved in the regulation of pre-mRNA splicing. They play a key role in both constitutive and alternative splicing by influencing splice site selection.

How SR proteins occur in splice sites:

- SR proteins recognize and bind **to** exonic splicing enhancers (ESEs)—specific sequences within exons near splice sites.
- By binding these sites, SR proteins recruit and stabilize components of the spliceosome at the correct splice junctions.
- They help define and promote the use of nearby splice sites, ensuring accurate removal of introns and joining of exons.
- SR proteins also mediate interactions between different spliceosomal components, facilitating spliceosome assembly and splicing catalysis.

13. Write numbers of uvrA, uvrB and uvrC in E. coli.

In *Escherichia coli*, the genes uvrA, uvrB, and uvrC each typically occur as a single copy in the bacterial genome. These genes encode proteins essential for the nucleotide excision repair (NER) system, which repairs DNA damage caused by ultraviolet (UV) light.

14. What is Torpedo model?

The Torpedo model explains how transcription termination occurs in eukaryotes for RNA polymerase II. After the pre-mRNA is cleaved at the polyadenylation site, a specialized 5'→3' exonuclease (called Xrn2 in humans) binds to the leftover RNA still being transcribed downstream of the cleavage site. This exonuclease rapidly degrades the RNA, chasing the RNA polymerase along the DNA like a "torpedo." When Xrn2 catches up, it disrupts the transcription complex, causing RNA polymerase II to detach and thus terminating transcription.

15. Explain Watson and Crick Model of DNA.

The Watson and Crick model of DNA describes the structure of DNA as a double helix composed of two antiparallel strands twisted around each other. Key features of the model are:

1. ***Double helix:*** DNA consists of two long strands forming a spiral staircase-like structure.
2. ***Antiparallel strands:*** The two strands run in opposite directions (5' to 3' and 3' to 5').
3. ***Sugar-phosphate backbone:*** Each strand's backbone is made of alternating sugar (deoxyribose) and phosphate groups.
4. ***Base pairing:*** Nitrogenous bases pair specifically — adenine (A) with thymine (T) via two hydrogen bonds, and guanine (G) with cytosine (C) via three hydrogen bonds.
5. ***Complementary strands:*** The base pairing ensures each strand can serve as a template for replication.

16. What is transcriptional promoter?

A transcriptional promoter is a specific DNA sequence located near the start site of a gene that serves as the binding site for RNA polymerase and other transcription factors. It signals where transcription should begin, controlling the initiation of gene expression. Promoters contain key elements like the -10 (TATA box) and -35 regions in prokaryotes, or the TATA box and other regulatory sequences in eukaryotes, which help recruit and position the transcription machinery.

17. Write role of RNA polymerase in elongation.

During elongation in transcription, RNA polymerase plays a central role by synthesizing the RNA strand:

- It moves along the DNA template strand in the 3' to 5' direction.
- RNA polymerase adds complementary ribonucleotides to the growing RNA chain in the 5' to 3' direction.
- It unwinds the DNA ahead of the transcription bubble and rewinds it behind as it progresses.
- The enzyme ensures accurate base pairing between the DNA template and incoming RNA nucleotides.
- RNA polymerase maintains the transcription bubble, allowing continuous RNA synthesis until termination signals are reached.

18. Define alpha helix arrangement.

The alpha helix is a common structural motif in proteins where the polypeptide chain coils into a right-handed spiral or helix. In this arrangement:

- The backbone forms a helical structure stabilized by hydrogen bonds between the carbonyl oxygen of one amino acid and the amide hydrogen of another amino acid four residues earlier.
- Each turn of the helix contains about 3.6 amino acids.
- The side chains of amino acids project outward from the helix.
- This structure provides stability and flexibility to proteins and is important in their function.

19. What changes occur in nucleosomes during transcription?

During transcription, nucleosomes undergo several changes to allow RNA polymerase access to DNA:

1. ***Disassembly or sliding:*** Nucleosomes may partially disassemble or slide along the DNA to expose the promoter and coding regions.
2. ***Histone modifications:*** Chemical changes like acetylation of histone tails reduce their positive charge, loosening DNA-histone interactions and making DNA more accessible.
3. ***Histone chaperones:*** Specialized proteins help remove or reposition histones ahead of the polymerase and reassemble them afterward.
4. ***Chromatin remodeling complexes:*** These use ATP to reposition or restructure nucleosomes, facilitating transcription machinery binding.

20. What are splicesomes?

Spliceosomes are large and complex molecular machines found in the nucleus of eukaryotic cells. They are responsible for removing introns (non-coding regions) from pre-mRNA during the process called RNA splicing.

Spliceosomes are made up of Small nuclear RNAs (snRNAs), which form complexes called snRNPs (small nuclear ribonucleoproteins) and numerous protein factors.

They recognize specific sequences at the intron-exon boundaries, bring these regions together, cut out the introns, and join the exons to produce mature, functional mRNA ready for translation.

21. Define terminator sequence.

A terminator sequence is a specific region of DNA that signals the end of transcription. When RNA polymerase reaches this sequence, it causes the enzyme to stop RNA synthesis and release the newly made RNA molecule, effectively ending transcription of that gene. Terminator sequences can function through different mechanisms, such as forming RNA hairpin loops (in prokaryotes) or involving specific proteins in eukaryotes.

22. Describe lac operon.

The lac operon in *E. coli* is an inducible system that controls the breakdown of lactose. It consists of structural genes (*lacZ*, *lacY*, *lacA*) responsible for lactose metabolism, a promoter where RNA polymerase binds, an operator where the lac repressor attaches, and a regulatory gene (*lacI*) that produces this repressor. When lactose is absent, the repressor binds to the operator, blocking transcription. However, when lactose is present, it binds to the repressor and changes its shape, causing it to release from the operator. This allows RNA polymerase to transcribe the structural genes, producing enzymes needed to digest lactose. Additionally, when glucose levels are low, cyclic AMP (cAMP) binds to the catabolite activator protein (CAP), which then enhances RNA

polymerase binding to the promoter, increasing transcription efficiency. This system ensures that the lac operon is only active when lactose is available and glucose is scarce.

23. How many CTD heptidase are there in yeast, worm, drosophila and humans?

The number of CTD (C-terminal domain) heptapeptide repeats in the largest subunit of RNA polymerase II varies among species:

- ***Yeast (Saccharomyces cerevisiae):*** Approximately 26 repeats
- ***Worm (Caenorhabditis elegans):*** Around 31 repeats
- ***Drosophila (fruit fly):*** About 44 repeats
- ***Humans:*** Around 52 repeats

24. Explain replication initiation process in prokaryotes.

In prokaryotes, replication initiation begins at a specific DNA region called the origin of replication (oriC)**.** The process starts when the initiator protein DnaA binds to repeated sequences within oriC, causing the DNA to unwind locally and form an open complex. This unwinding exposes single-stranded DNA regions, allowing the helicase (DnaB), loaded with the help of DnaC**,** to bind and further unwind the DNA helix. Single-strand binding proteins (SSBs) then attach to the separated strands to prevent re-annealing. Next, primase synthesizes short RNA primers on both strands, providing starting points for DNA polymerase III to begin DNA synthesis. This coordinated activity sets up the replication forks, allowing bidirectional replication of the bacterial chromosome.

25. Name three main processes of central dogma in molecular biology.

The three main processes of the central dogma in molecular biology are:

1. ***Replication*** – copying DNA to make identical DNA molecules.
2. ***Transcription*** – synthesizing RNA from a DNA template.
3. ***Translation*** – producing proteins by decoding the mRNA sequence.

26. How molecular biology has showed advancements in the field of agriculture?

Molecular biology has significantly advanced agriculture by enabling the development of genetically modified crops with improved traits such as pest resistance, herbicide tolerance, and enhanced nutritional value. Techniques like gene cloning and CRISPR gene editing allow precise manipulation of plant genomes, leading to higher yields and better stress tolerance against drought or salinity. Molecular markers and DNA fingerprinting improve crop breeding programs by speeding up selection for desirable traits. Additionally, molecular biology helps detect plant pathogens early and develop resistant varieties, reducing crop losses and improving food security.

27. How prokaryotic and eukaryotic transcription machinery different from each other?

Prokaryotic and eukaryotic transcription machinery differ in complexity and components. Prokaryotes have a single RNA polymerase that synthesizes all types of RNA, while eukaryotes have three main RNA polymerases (I, II, and III) specialized for different RNA types. Prokaryotic transcription requires fewer accessory proteins and uses simpler promoter sequences like the -10 and -35 regions. In contrast, eukaryotic transcription involves

many general transcription factors to help RNA polymerase II recognize complex promoters with elements like the TATA box. Additionally, eukaryotic transcription occurs in the nucleus with extensive RNA processing (capping, splicing, polyadenylation), whereas prokaryotic transcription and translation often happen simultaneously in the cytoplasm.

28. What determines where the editing system should add or delete UMPs?

The site-specific addition or deletion of UMPs (uridine monophosphates) during RNA editing is determined by guide RNAs (gRNAs)**.** These small RNA molecules base-pair with the target pre-edited mRNA, directing the editing machinery to precise locations where U nucleotides need to be inserted or removed. The gRNAs act as templates that specify the exact pattern of editing, ensuring accurate modification of the RNA sequence.

29. Name the two 5'-3' RNA modification.

The two common 5' to 3' RNA modifications are:

1. ***5' Capping*** – Addition of a 7-methylguanosine cap to the 5' end of eukaryotic mRNA.
2. ***3' Polyadenylation*** – Addition of a poly (A) tail at the 3' end of the mRNA.

30. What is the function of beta galactosidase in lactose metabolism?

Beta-galactosidase is an enzyme that breaks down lactose into its two simpler sugars, glucose and galactose, which can then be used by the cell for energy. It cleaves the β-1,4-glycosidic bond in lactose, making lactose metabolism possible in organisms like *E. coli*.

31. How different types of UV radiations reach the earth differently and how they affect the life on earth?

UV radiations are classified mainly into three types based on their wavelength: UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm). They reach the Earth differently because of the atmosphere's filtering effect:

- ***UVC:*** Almost completely absorbed by the ozone layer and oxygen in the atmosphere, so very little or none reaches the Earth's surface.
- ***UVB:*** Mostly absorbed by the ozone layer, but a small portion penetrates to the surface. It is responsible for sunburn and can damage DNA, increasing the risk of skin cancer.
- ***UVA:*** Least absorbed by the atmosphere and reaches the Earth's surface in large amounts. It penetrates deeper into skin and contributes to aging and indirect DNA damage.

Effects on life:

- UVC, though filtered out, is the most harmful and can kill microorganisms, which is why it's used for sterilization.
- UVB causes direct DNA damage, leading to mutations and skin cancers but also helps produce vitamin D in humans.
- UVA causes oxidative stress and indirect DNA damage, contributing to aging and some cancers.

32. How group I intron splice the DNA?

Group I introns are self-splicing RNA sequences that remove themselves from RNA transcripts without needing proteins or spliceosomes. They do not splice DNA directly but act at the RNA level during RNA processing.

Mechanism of Group I intron splicing:

1. The intron RNA folds into a specific 3D structure forming an active site.
2. A free guanosine nucleotide (GTP, GDP, or free guanosine) acts as a cofactor and attacks the 5' splice site, cleaving the exon-intron junction.
3. The 3'-OH of the released exon then attacks the 3' splice site, joining the two exons together and releasing the intron as a linear RNA.

33. Write down the function of class II release factor.

Class II release factors in translation assist the termination process by helping class I release factors detach from the ribosome after polypeptide release. They are GTP-binding proteins that use energy from GTP hydrolysis to promote release factor recycling and ribosome disassembly, ensuring efficient termination and preparation for the next round of translation.

34. How gene expression is controlled by regulatory proteins?

Gene expression is controlled by regulatory proteins that influence transcription by interacting with specific DNA sequences near genes. These proteins include activators and repressors:

- Activators bind to enhancer or promoter regions, helping recruit or stabilize RNA polymerase and other transcription factors, increasing gene transcription.
- Repressors bind to operator or silencer sequences, blocking RNA polymerase binding or preventing transcription initiation, thereby reducing or stopping gene expression.

35. Differentiate between Ribose and Deoxyribose sugar.

Ribose and deoxyribose are both five-carbon sugars (pentoses) found in nucleic acids, but they differ slightly in structure and function. Ribose, found in RNA, has a hydroxyl group (-OH) attached to its 2' carbon, making it more reactive and less chemically stable. In contrast, deoxyribose, found in DNA, lacks the oxygen atom at the 2' carbon, having only a hydrogen atom instead. This absence of oxygen makes DNA more stable and better suited for long-term genetic storage. The chemical formulas also reflect this difference: ribose is $C_5H_{10}O_5$, while deoxyribose is $C_5H_{10}O_4$. This small structural variation plays a crucial role in the differing properties and functions of DNA and RNA.

36. What are charged and uncharged amino acids?

Charged amino acids are those that carry a net positive or negative charge on their side chains at physiological pH (~7.4). They are divided into:

- ***Positively charged (basic):*** Lysine, Arginine, and Histidine.
- ***Negatively charged (acidic):*** Aspartic acid and Glutamic acid.

These amino acids are often involved in ionic interactions and are typically found on the surface of proteins.

Uncharged amino acids, on the other hand, have side chains that do not carry a net charge at physiological pH. They can be:

- ***Polar uncharged:*** Serine, Threonine, Asparagine, Glutamine, etc., which can form hydrogen bonds.
- ***Nonpolar (hydrophobic):*** Alanine, Valine, Leucine, Isoleucine, Phenylalanine, etc., which tend to cluster inside proteins away from water.

37. Name two proteins necessary for lactose metabolism.

Below are given two proteins necessary for lactose metabolism. Both are encoded by the *lacZ* and *lacY* genes of the lac operon in *E. coli*.

1. ***Beta-galactosidase*** – breaks down lactose into glucose and galactose.
2. ***Lactose permease*** – transports lactose into the bacterial cell.

38. What is translocation of Amino acids? And where it takes place?

Translocation of amino acids refers to the movement of the ribosome along the mRNA during translation, specifically after a new amino acid has been added to the growing polypeptide chain. During this process, the ribosome shifts one codon forward (in the 5' to 3' direction on the mRNA), moving the tRNA with the growing peptide from the A site to the P site, and the now-empty tRNA from the P site to the E site, where it exits the ribosome.

This translocation step occurs in the large subunit of the ribosome and is assisted by elongation factors (such as EF-G in prokaryotes or eEF2 in eukaryotes). It is essential for positioning the next codon in the A site for the next round of amino acid addition.

39. What is importance of EBR?

EBR (Enhanced Biological Remediation) is important because it improves the natural ability of microorganisms to break down pollutants and contaminants in soil, water, or air. It is used in environmental cleanup processes such as treating oil spills, heavy metals, and industrial waste. EBR enhances microbial activity through nutrient addition, genetic engineering, or environmental adjustments, making the cleanup faster, more efficient, and eco-friendly. This method is cost-effective and sustainable compared to physical or chemical methods of remediation.

40. What are functions of regulatory proteins?

Regulatory proteins control the expression of genes by interacting with DNA or other molecules involved in transcription and translation. Their main functions include:

1. Activating or repressing transcription by binding to promoter or operator regions near genes.
2. Responding to environmental signals to turn genes on or off as needed.
3. Assisting in chromatin remodeling in eukaryotes, making DNA more or less accessible.
4. Interacting with RNA polymerase or other transcription factors to regulate gene expression.
5. Maintaining cellular homeostasis by controlling when and how much of a protein is produced.

41. Explain elongation step of transcription.

The elongation step of transcription is the phase where RNA polymerase synthesizes the RNA strand by adding nucleotides complementary to the DNA template strand. After initiation, RNA polymerase moves along the DNA, unwinding the double helix and reading the template strand in the 3' to 5' direction, while synthesizing RNA in the 5' to 3' direction. As it progresses, it incorporates ribonucleotides (A, U, C, G) into the growing RNA chain. A temporary RNA-DNA hybrid forms inside the transcription bubble, and the RNA strand is elongated one nucleotide at a time. During this phase, RNA polymerase ensures high fidelity by selecting correct bases, and the DNA behind it re-anneals as the enzyme moves forward. No primer is needed, and elongation continues until a termination signal is reached.

42. Write function of DNA helicase.

DNA helicase is an essential enzyme that unwinds the double-stranded DNA during replication. Its main function is to break the hydrogen bonds between the two DNA strands, creating a replication fork and allowing each strand to serve as a template for new DNA synthesis. This unwinding is crucial for DNA polymerase to access and copy the DNA sequence.

43. What is alternative splicing?

Alternative splicing is a process in eukaryotic gene expression where a single pre-mRNA transcript is spliced in different ways to produce multiple mature mRNA variants from the same gene. During this process, different combinations of exons are joined together while introns are removed, allowing a single gene to encode multiple proteins with diverse functions. This increases protein diversity and allows cells to respond to different developmental stages, tissues, or environmental signals without needing more genes.

44. Why mRNA has short life span?

mRNA has a short life span to allow cells to tightly regulate gene expression and respond quickly to changes in their environment. A short-lived mRNA ensures that proteins are produced only when needed, preventing unnecessary or excessive protein synthesis, which could waste resources or disrupt cellular functions. Additionally, rapid mRNA degradation allows for quick shutdown of gene expression, enabling precise control over protein levels during development, stress responses, or cell signaling.

45. Define allotropy.

Allotropy is the existence of two or more different physical forms or structural modifications of the same chemical element in the same physical state. These different forms, called allotropes, have distinct molecular or crystal structures and different physical or chemical properties. For example, carbon exists as allotropes like diamond, graphite, and graphene.

46. What is bacteriophage? Write its chemical composition.

A bacteriophage (or phage) is a virus that specifically infects bacteria. It attaches to a bacterial cell, injects its genetic material, and uses the bacterial machinery to reproduce new phage particles, often destroying the host cell in the process.

Chemical composition of bacteriophage:

- ***Nucleic acid:*** Either DNA or RNA (usually double-stranded DNA in many phages)
- ***Protein coat (capsid):*** Made of protein subunits that protect the nucleic acid and help attach to the host bacterium
- ***Tail structure:*** Composed of proteins, used for attachment and injecting genetic material into the host
- Some bacteriophages also have enzymes like lysozyme to help penetrate the bacterial cell wall.

47. Write steps of RNA replication.

Here are the basic steps of RNA replication (as seen in RNA viruses):

1. ***Initiation:*** The RNA-dependent RNA polymerase (RdRp) binds to the RNA template strand.
2. ***Unwinding:*** The RNA template is prepared for copying by separating any secondary structures.
3. ***Elongation:*** RdRp synthesizes a complementary RNA strand by adding ribonucleotides in the 5' to 3' direction, using the original RNA as a template.
4. ***Termination:*** When the entire RNA template is copied, synthesis stops, and the new RNA strand is released.
5. ***Processing:*** The replicated RNA may undergo modifications or folding necessary for its function.

48. What is significance of RFLP?

The significance of RFLP (Restriction Fragment Length Polymorphism) lies in its ability to detect variations in DNA sequences among individuals. RFLP is a powerful tool for analyzing genetic variation because it detects differences in restriction enzyme cutting sites in DNA. It is widely used for:

1. ***Genetic mapping*** – helping locate genes on chromosomes.
2. ***DNA fingerprinting*** – identifying individuals in forensic science.
3. ***Disease diagnosis*** – detecting mutations linked to genetic disorders.
4. ***Paternity testing*** – establishing biological relationships.
5. ***Studying genetic diversity*** – in populations and breeding programs.

49. What are binding sites of ribosomes?

The main binding sites of ribosomes involved in translation are:

1. ***A site (Aminoacyl site):*** Where the incoming aminoacyl-tRNA carrying the next amino acid binds.
2. ***P site (Peptidyl site):*** Holds the tRNA attached to the growing polypeptide chain.
3. ***E site (Exit site):*** Where the now empty tRNA exits the ribosome after transferring its amino acid.

50. What is minor splicing and its function?

Minor splicing is a specialized RNA splicing process that removes a rare class of introns called U12-type introns from pre-mRNA. Unlike the common major splicing (which removes U2-type introns), minor splicing is carried out by a distinct, smaller spliceosome composed of unique small nuclear RNAs (snRNAs) and proteins.

Minor splicing ensures accurate removal of these rare introns, which are found in some essential genes involved in important cellular functions. Proper minor splicing is crucial for producing correct mRNA transcripts and functional proteins, affecting gene expression and cell viability.

51. What is transcription initiation complex?

The transcription initiation complex is a large assembly of proteins and enzymes that forms at the promoter region of a gene to start transcription. In eukaryotes, it includes RNA polymerase II and several general transcription factors (GTFs) such as TFIID, TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH. These factors help RNA polymerase bind to the promoter, unwind the DNA, and begin RNA synthesis. The complex precisely positions RNA polymerase at the transcription start site, ensuring accurate initiation of mRNA production.