1. (a) Define (any four):
    - (i) Microsatellite
        - Microsatellites are short, tandemly repeated DNA sequences, typically 1-6 base pairs in length, found throughout the genome.
        - They are highly polymorphic due to variations in the number of repeat units and are used as genetic markers.
    - (ii) Inducer
        - An inducer is a molecule that initiates gene expression by binding to a regulatory protein, thereby changing its conformation and allowing transcription to occur.
        - In operons, inducers can bind to repressors, inactivating them and promoting transcription, or bind to activators, activating them.
    - (iii) RNAi
        - RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules.
        - It is a form of gene regulation that involves small interfering RNAs (siRNAs) and microRNAs (miRNAs).
    - (iv) Combinatorial Control
        - Combinatorial control refers to the regulation of gene expression by the combined action of multiple regulatory proteins or elements.
        - This allows for fine-tuned and highly specific control over gene transcription.
    - (v) Cooperativity

- Cooperativity is a phenomenon where the binding of one ligand to a macromolecule (like an enzyme or a regulatory protein) influences the binding affinity of subsequent ligands to other binding sites on the same macromolecule.
- In gene regulation, it can lead to a sharp, switch-like response to changes in ligand concentration.

2. (b) Justify as true or false (any five):
    - (i) Ara is an inducible operon.
        - True. The *ara* operon is an inducible operon because its expression is turned on in the presence of arabinose, which acts as an inducer.
    - (ii) Gene density is directly proportional to the complexity of an organism.
        - False. There is generally an inverse relationship between gene density and organismal complexity; more complex organisms tend to have lower gene density due to larger introns and more non-coding DNA.
    - (iii) Pre-miRNAs are present only in introns.
        - False. While some pre-miRNAs are derived from introns, many are transcribed from independent miRNA genes located in intergenic regions or exons.
    - (iv) Ribosomal Proteins are translational repressors of their own synthesis.
        - True. Ribosomal proteins often act as translational repressors, binding to their own mRNA and inhibiting translation when their concentration exceeds the amount needed for ribosome assembly.

- (v) Genes that are continually being expressed in most cells are referred to as inducible genes.
    - False. Genes that are continually being expressed in most cells are referred to as housekeeping genes or constitutive genes, not inducible genes. Inducible genes are expressed only when induced.
- (vi) DNA foot printing can be used to detect protein binding site.
    - True. DNA footprinting is a molecular biology technique used to identify the specific DNA sequence to which a DNA-binding protein is bound.

3. (c) Expand the following:
    - (i) RdRP
        - RNA-dependent RNA Polymerase
    - (ii) UAS
        - Upstream Activating Sequence
    - (iii) LINES
        - Long Interspersed Nuclear Elements
    - (iv) HLH
        - Helix-Loop-Helix
    - (v) LTR
        - Long Terminal Repeat
    - (vi) MAPK
        - Mitogen-Activated Protein Kinase
    - (vii) ESE
        - Exonic Splicing Enhancer

4. Distinguish Between (any three):
    - (i) STAT vs MAPK signal transduction pathway
        - STAT (Signal Transducers and Activators of Transcription) Pathway:
            - Directly activated by cytokines and growth factors binding to cell surface receptors (e.g., cytokine receptors).
            - STAT proteins are phosphorylated, dimerize, and directly translocate to the nucleus to act as transcription factors.
            - Provides a relatively direct and rapid route for gene activation in response to extracellular signals.
        - MAPK (Mitogen-Activated Protein Kinase) Pathway:
            - Activated by a wide variety of extracellular stimuli, including growth factors, stress, and cytokines, through receptor tyrosine kinases (RTKs) or G-protein coupled receptors.
            - Involves a cascade of phosphorylation events (MAPKKK → MAPKK → MAPK).
            - MAPKs phosphorylate various target proteins, including transcription factors, leading to a more diverse and often slower cellular response.
    - (ii) Heterochromatin and Euchromatin
        - Heterochromatin:
            - Highly condensed and transcriptionally inactive form of chromatin.

- Rich in repetitive DNA sequences and often found near centromeres and telomeres.
- Replicates late in the S phase.
- Characterized by histone modifications such as methylation (e.g., H3K9me3) and low levels of acetylation.

- Euchromatin:
  - Less condensed and transcriptionally active form of chromatin.
  - Contains most of the active genes.
  - Replicates early in the S phase.
  - Characterized by histone modifications such as acetylation (e.g., H3K9ac) and lower levels of methylation.

- (iii) Cis-acting regulatory sequences and Trans-acting factors
  - Cis-acting regulatory sequences:
    - DNA sequences that are located on the same molecule of DNA as the gene they regulate.
    - They are not diffusible and must be physically linked to the gene to exert their effect.
    - Examples include promoters, enhancers, silencers, and insulators.
  - Trans-acting factors:
    - Molecules (typically proteins or RNA molecules) that bind to cis-acting sequences to regulate gene expression.

- They are diffusible and can act on genes located on different DNA molecules (e.g., different chromosomes).
- Examples include transcription factors, RNA polymerase, and regulatory RNA molecules.

- (iv) Inducible and Repressible operon
  - Inducible Operon:
    - Normally "off" and gene expression is turned "on" in the presence of a specific molecule (inducer).
    - The inducer typically binds to a repressor protein, inactivating it and allowing transcription.
    - Example: Lac operon (induced by lactose).
  - Repressible Operon:
    - Normally "on" and gene expression is turned "off" in the presence of a specific molecule (corepressor).
    - The corepressor typically binds to an inactive repressor protein, activating it and causing it to bind to the operator, thereby inhibiting transcription.
    - Example: Trp operon (repressed by tryptophan).

5. (a) How is translation of iron binding protein Ferritin regulated?
   - The translation of the iron-binding protein Ferritin is regulated at the post-transcriptional level, primarily by the Iron Regulatory Proteins (IRPs) binding to Iron Response Elements (IREs) located in the 5' untranslated region (5' UTR) of ferritin mRNA.
   - In low iron conditions:
     - IRPs are active and bind to the IREs in the 5' UTR of ferritin mRNA.

        - This binding sterically blocks the ribosome's access to the start codon, thereby inhibiting the translation of ferritin mRNA.
        - As a result, less ferritin protein is produced, which is appropriate when iron levels are low and storage is not needed.
    - In high iron conditions:
        - Iron binds to IRPs, causing a conformational change that reduces their affinity for IREs.
        - IRPs dissociate from the ferritin mRNA.
        - This allows ribosomes to freely initiate translation, leading to the synthesis of more ferritin protein.
        - The increased ferritin then sequesters the excess iron, preventing its toxic effects.

6. (b) Describe how bacterial genes are regulated by riboswitch that responds to metabolites like S-adenosylmethionine.
    - Bacterial genes can be regulated by riboswitches, which are RNA elements usually located in the 5' untranslated region (5' UTR) of an mRNA molecule.
    - Riboswitches directly bind small molecule metabolites (like S-adenosylmethionine, or SAM) without the involvement of proteins, and this binding causes a conformational change in the mRNA, thereby affecting gene expression.
    - For SAM-responsive riboswitches:
        - The riboswitch has an aptamer domain that specifically binds SAM.
        - In the absence of SAM:

- The riboswitch adopts a conformation that allows transcription or translation to proceed. For example, in some cases, an anti-terminator structure might form, allowing RNA polymerase to continue transcription through the gene.

- In the presence of SAM:
  - SAM binds to the aptamer domain of the riboswitch.
  - This binding induces a conformational change in the riboswitch.
  - This conformational change often leads to the formation of a terminator structure in the nascent mRNA, causing premature termination of transcription by RNA polymerase.
  - Alternatively, in other cases, the conformational change might sequester the ribosome binding site (Shine-Dalgarno sequence) in the mRNA, thus inhibiting translation initiation.
- This mechanism allows bacteria to precisely regulate the synthesis of enzymes involved in SAM metabolism or other SAM-dependent processes based on the cellular concentration of SAM.

7. What is the functional significance of the following (any five):
   - (i) Spacer in CRISPR
     - Spacers in CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are segments of DNA derived from invading phages or plasmids.
     - They serve as a "memory" of past infections, allowing the bacterial or archaeal cell to recognize and neutralize future attacks by the same foreign genetic elements.

- These spacers are transcribed into guide RNAs that direct the Cas enzymes to target and cleave complementary foreign DNA, providing adaptive immunity.

- (ii) Xist
  - Xist (X-inactive specific transcript) is a long non-coding RNA (lncRNA) crucial for X-chromosome inactivation in female mammals.
  - It coats one of the two X chromosomes, triggering a cascade of events that lead to its transcriptional silencing and formation of a Barr body, ensuring dosage compensation of X-linked genes between males and females.
- (iii) Drosha
  - Drosha is a nuclear RNase III enzyme that plays a critical role in microRNA (miRNA) biogenesis.
  - It processes primary miRNA transcripts (pri-miRNAs) into precursor miRNAs (pre-miRNAs) by cleaving them into a hairpin structure, which is then exported to the cytoplasm for further processing by Dicer.
- (iv) OxyS
  - OxyS is a small regulatory RNA (sRNA) in *E. coli* that is induced under oxidative stress conditions.
  - It acts as a post-transcriptional regulator by binding to target mRNAs and altering their translation or stability, thereby helping the cell to respond and adapt to oxidative stress by regulating genes involved in defense mechanisms.
- (v) IRE

- IRE (Iron Response Element) is a conserved RNA stem-loop structure found in the untranslated regions (UTRs) of mRNAs encoding proteins involved in iron metabolism (e.g., ferritin, transferrin receptor).
- It acts as a binding site for Iron Regulatory Proteins (IRPs), thereby regulating the translation or stability of these mRNAs in response to cellular iron levels.

- (vi) CpG island
  - CpG islands are regions of DNA with a high frequency of CpG dinucleotides (a cytosine nucleotide followed by a guanine nucleotide, linked by a phosphate bond).
  - They are typically unmethylated and found in the promoter regions of approximately 60-70% of human genes.
  - Their methylation status plays a crucial role in regulating gene expression; unmethylated CpG islands are associated with active gene transcription, while methylation often leads to gene silencing.

8. (a) In detail with examples and diagrams (any two):
   - (i) Histone modifications, such as acetylation and deacetylation, regulate chromatin structure and gene expression. Illustrate the role of HATs and HDACs in transcriptional regulation, and explain how these modifications influence chromatin remodelling.
     - Histone modifications, particularly acetylation and deacetylation, are crucial epigenetic mechanisms that profoundly influence chromatin structure and gene expression.
     - Histone Acetylation:

- Performed by Histone Acetyltransferases (HATs).
- HATs add acetyl groups to specific lysine residues in the N-terminal tails of histones (e.g., H3K9, H4K16).
- The addition of an acetyl group neutralizes the positive charge of the lysine, reducing the electrostatic interaction between the histones and the negatively charged DNA.
- This leads to a more open, relaxed chromatin structure (euchromatin).
- This "open" conformation makes the DNA more accessible to transcription factors and RNA polymerase, thereby promoting gene transcription.
- Example: Gcn5, p300/CBP are common HATs that are often recruited to gene promoters by transcriptional activators.

- Histone Deacetylation:
  - Performed by Histone Deacetylases (HDACs).
  - HDACs remove acetyl groups from lysine residues on histone tails.
  - This restores the positive charge on the lysine, increasing the electrostatic interaction between histones and DNA.
  - This leads to a more compact, condensed chromatin structure (heterochromatin).
  - This "closed" conformation makes the DNA less accessible to transcription machinery, thereby repressing gene transcription.

        - Example: Rpd3, Hda1 are common HDACs often recruited by transcriptional repressors.

    - Influence on Chromatin Remodeling:

        - Histone acetylation and deacetylation directly influence chromatin remodeling, which involves ATP-dependent movement or restructuring of nucleosomes.

        - Acetylation creates a "loose" chromatin environment that facilitates the binding and activity of ATP-dependent chromatin remodelers (e.g., SWI/SNF complex). These remodelers can then slide, eject, or restructure nucleosomes to expose regulatory DNA sequences.

        - Deacetylation creates a "tight" chromatin environment that hinders chromatin remodelers and transcription factors from accessing the DNA.

        - These modifications also serve as docking sites for "reader" proteins (e.g., bromodomain-containing proteins bind acetylated histones) which recruit other complexes involved in gene activation or repression.

- (iii) Describe the process of nonsense-mediated mRNA decay (NMD) in eukaryotes and its role in quality control of mRNA. Use a diagram to show how premature stop codons trigger the decay process and prevent the production of faulty proteins.

    - Nonsense-mediated mRNA decay (NMD) is a crucial eukaryotic mRNA surveillance pathway that detects and degrades mRNAs containing premature termination codons (PTCs). Its primary role is to ensure the quality control of mRNA by preventing the translation of truncated, potentially harmful proteins.

- Process of NMD:
    - mRNA Splicing and Exon Junction Complexes (EJCs): During mRNA splicing, multiprotein complexes called exon junction complexes (EJCs) are deposited about 20-24 nucleotides upstream of exon-exon junctions. These EJCs remain bound to the mRNA as it is exported from the nucleus to the cytoplasm.
    - First Round of Translation (Pioneer Round): The mRNA undergoes a "pioneer" round of translation in the cytoplasm. During this initial translation, the ribosome scans the mRNA.
    - PTC Recognition: If the ribosome encounters a stop codon more than approximately 50-55 nucleotides upstream of the last EJC, it is recognized as a premature termination codon (PTC). This distance rule is critical: normal termination codons are typically located downstream of all EJCs (or in the last exon with no downstream EJCs).
    - Recruitment of NMD Factors: Upon encountering a PTC, the stalled ribosome recruits a complex of NMD factors, including Upf1, Upf2, and Upf3.
    - Activation of Degradation: This complex triggers a series of events leading to the degradation of the faulty mRNA. Upf1, when phosphorylated, interacts with decapping enzymes and deadenylases.
    - mRNA Degradation: The mRNA is then rapidly degraded, typically by removal of the 5' cap (decapping) and subsequent 5' to 3' exonucleolytic decay, and/or by shortening of the poly(A) tail

(deadenylation) followed by 3' to 5' exonucleolytic decay.

- Role in Quality Control:
  - Prevents Production of Truncated Proteins: NMD's main function is to eliminate mRNAs that would produce truncated proteins due to nonsense mutations, frameshift mutations, or errors in splicing.
  - Avoids Dominant-Negative Effects: Truncated proteins can be non-functional or even toxic, acting in a dominant-negative manner to interfere with the function of normal proteins. By degrading these mRNAs, NMD prevents such detrimental effects.
  - Maintains Cellular Homeostasis: It ensures that the cell maintains a healthy proteome, contributing to overall cellular homeostasis and preventing disease. NMD defects are implicated in various genetic disorders.

9. (b) How does the ubiquitin-proteasome system ensure selective protein degradation, and why is this important for cellular function?
   - The ubiquitin-proteasome system (UPS) is the primary pathway for selective degradation of intracellular proteins in eukaryotes. Its selectivity is achieved through a precise, multi-step tagging process involving ubiquitin, a small 76-amino acid protein.
   - Mechanism of Selective Degradation:
     - Ubiquitination Cascade:
       - Ubiquitin Activation (E1): Ubiquitin is first activated by an E1 ubiquitin-activating enzyme in an ATP-dependent reaction, forming a thioester bond with E1.

- Ubiquitin Conjugation (E2): The activated ubiquitin is then transferred from E1 to an E2 ubiquitin-conjugating enzyme.
- Ubiquitin Ligation (E3): The crucial step for selectivity lies with the E3 ubiquitin ligase. E3 enzymes recognize specific protein substrates destined for degradation. There are hundreds of different E3 ligases, each with unique substrate specificities. E3 facilitates the transfer of ubiquitin from E2 directly to the lysine residues of the target protein, or indirectly via its own active site.
- Polyubiquitination: Multiple ubiquitin molecules are typically attached to the target protein, forming a polyubiquitin chain (usually at least four ubiquitins linked via K48). This polyubiquitin chain acts as a degradation signal.

- Proteasomal Degradation:
  - 26S Proteasome Recognition: The polyubiquitinated protein is recognized by the 19S regulatory particle of the 26S proteasome, a large multi-subunit protease complex.
  - Unfolding and Translocation: The protein is unfolded by ATPases within the 19S particle and threaded into the 20S catalytic core of the proteasome. Ubiquitin molecules are typically recycled.
  - Proteolysis: Inside the 20S core, the protein is cleaved into small peptides (typically 3-25 amino acids long) by proteases. These peptides can then be further degraded into amino acids or used for antigen presentation.

- Importance for Cellular Function:
  - Removal of Damaged/Misfolded Proteins: The UPS identifies and degrades proteins that are misfolded, damaged, or otherwise aberrant, preventing their accumulation which can be toxic and lead to diseases (e.g., neurodegenerative disorders like Alzheimer's and Parkinson's).
  - Regulation of Cell Cycle Progression: Key cell cycle regulators (e.g., cyclins, cyclin-dependent kinase inhibitors) are degraded by the UPS at specific points in the cell cycle, ensuring unidirectional progression through different phases.
  - Control of Transcriptional Regulation: Many transcription factors and co-regulators are short-lived proteins whose activity is modulated by targeted degradation, allowing for rapid and precise changes in gene expression.
  - Immune Response: The UPS generates antigenic peptides for presentation by MHC class I molecules, which is crucial for the adaptive immune response against intracellular pathogens and cancer cells.
  - Signal Transduction: It plays a vital role in terminating or modulating signaling pathways by degrading signaling components (e.g., receptors, kinases).
  - Development and Differentiation: Controlled protein degradation is essential for numerous developmental processes, cell differentiation, and tissue remodeling.

10. Comment on the following (any three):

- (i) Nonsense mediated decay
  - Nonsense-mediated mRNA decay (NMD) is a crucial mRNA surveillance pathway in eukaryotes responsible for

detecting and degrading mRNAs that contain premature termination codons (PTCs). Its primary function is to prevent the synthesis of truncated, potentially harmful proteins that could have dominant-negative effects or lead to cellular dysfunction. NMD achieves this by coupling translation to mRNA quality control; ribosomes encountering a PTC upstream of a specific landmark (often an exon junction complex) trigger the recruitment of NMD factors, leading to rapid degradation of the aberrant mRNA. This system is vital for maintaining proteome integrity and plays a role in various genetic disorders.

- (ii) CRISPR-CAS as gene editing system
    - CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins) has revolutionized gene editing by providing a precise, efficient, and relatively easy-to-use tool for modifying genomes. It leverages a bacterial adaptive immune system where guide RNAs (gRNAs), derived from viral DNA spacers, direct the Cas9 nuclease to specific complementary DNA sequences. Cas9 then creates a double-strand break (DSB) at the target site. The cell's natural DNA repair mechanisms (non-homologous end joining for gene knockout or homology-directed repair for precise gene insertion/correction) are then exploited to achieve the desired genetic modification. This system has vast potential in research, gene therapy for genetic diseases, and agriculture.
- (iii) tmRNA
    - tmRNA (transfer-messenger RNA) is a unique bacterial RNA molecule that functions as both a tRNA and an mRNA, primarily involved in rescuing stalled ribosomes on mRNAs lacking a stop codon. When a ribosome stalls

at the end of an mRNA without a stop codon, tmRNA enters the A site, acting like a tRNA carrying alanine. It then functions as an mRNA, providing a short coding sequence followed by a stop codon. This process, known as trans-translation, adds a short "tag" peptide to the C-terminus of the nascent polypeptide, targeting it for degradation by cellular proteases, and also releases the stalled ribosome, preventing cellular resources from being tied up.

- (iv) X-inactivation
    - X-inactivation, also known as lyonization, is a dosage compensation mechanism in female mammals where one of the two X chromosomes in each somatic cell is largely silenced to balance the gene dosage between males (XY) and females (XX). This process occurs early in embryonic development and is largely random for each cell, resulting in a mosaic expression pattern in heterozygous females. The inactivation is mediated by the Xist long non-coding RNA, which coats the future inactive X chromosome, leading to extensive epigenetic modifications (e.g., DNA methylation, histone deacetylation, histone methylation) that compact the chromosome into a transcriptionally inactive Barr body.