dissociation constant of the repressor-operator complex? What is the significance of this result ? 2

M. Sc. Bio-Technology Part I Examination, 2019

Molecular Biology

Paper : MSBT-1/3

Time : Four hours Full Marks : 100

PART - I

Answer Question No. ***1*** and ***any four*** Questions taking two from each group.

1. Answer ***all*** questions :

   a) Define polar and non-polar molecules in terms of dipole moments. Do van der Waals interactions occur between polar or non-polar molecules ? 2

   b) Given the following sequence of RNA, propose the potential hairpin structure for this RNA. Indicate base pairing with a dotted line. 2

   5′ -AGGACCCUUCGGGGUUCU- 3′

   c) Although an E. coli mutant of PolA gene (encoding DNA polymerase I) is week but viable, a mutant of DnaE gene (encoding the alpha subunit of DNA polymerase III) is lethal. Why ? 2

   d) For the following amino acids, suggest whether they are more likely to be found buried or exposed in a stably

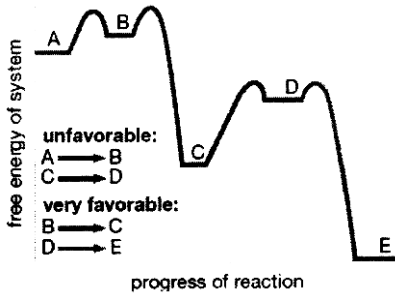
folded protein domain : isoleucine, valine, lysine and glutamic acid. 2

e) Explain why serine and tyrosine recombinases do not require an external source of energy such as ATP hydrolysis for catalysis. 2

GROUP - A

(Answer ***any Two*** questions)

2. a) Carefully examine the following figure :



Mention what fundamental principle of biochemistry is depicted in the above figure ? 3

b) The globular structure of myoglobin and each hemoglobin monomer involves eight α -helical segments. Is it the primary, secondary, tertiary, or quaternary structure that differs most between these two proteins ? Explain. 2

b) If you want to measure the amount of β -galctosidase and lac-mRNA produces by E.coli what assay principle you will follow. Graphically show the amount of β -galactosidase and lac-mRNA production with time for a E.coli (lac^+) grown in presence of lactose. 2+2

c) What are the different cis-acting and trns-acting elements are for eukaryotic regulation of gene expression ? Mention mechanism of gene regulation by any one of the cis-acting and trans-acting elements. 1+2

18. a) Write the names of different experimental tecnhiques that can be used to find out the operator DNA binding protein molecule in a prokaryotic organism ? Describe any one of the technique with a labelled diagram. 1+3

b) Write the names of different DNA-binding domain (DBD) structural motif present in the proteins that are involved in the regulation of gene expression. Describe any one of them with example and labelled diagram. 1+3

c) The dissociation constant for a particular repressor-operator complex is very low, about 10^{-13} M. An E. coli cell (volume 2 x 10^{-12} mL) contains 10 copies of the repressor. Calculate the cellular concentration of the repressor protein. How does this value compare with the

d) Which protein domain(s) recognize the acetylated as well as methylated histone amino-terminal tails ? 2

4. a) What is activation energy of a chemical reaction ? What is the origin of the activation energy ? 3

b) You isolate a supercoiled 10,000-bp plasmid from E.coli cells. Assume the plasmid is covalently closed circular DNA (cccDNA). You treat the 10,000-bp plasmid with DNase I under conditions such that the DNase I nicks on average once per DNA molecule.

i) Name the bond that DNase I breaks.

ii) How does this treatment change the topological state of the 10,000-bp plasmid ? 2

c) Most globular proteins are denatured and lose their activity when briefly heated to 65°C. However, globular proteins that contain multiple disulfide bonds often must be heated longer at higher temperatures than 65°C to denature them. Explain the reason for this difference. 2

d) What is the net charge of histidine at pH 1, 4, 8 and 12 ? For each pH, will histidine migrate toward the anode (+) or cathode (-) when placed in an electric field ? 3

5. a) Classically, when a chemical bond is made energy is released and when a bond is broken energy is absorbed. How then the breaking/hydrolysis of a high-energy phosphate bond such as that of an ATP releases so much energy ? 2

b) What are isoaccepting tRNAs ? What is meant by Glu-$tRNA^{Glu}$ and Glu-$tRNA^{Gln}$ and where are they found ? Can they be interconverted ? 4

c) How puromycin inhibits prokaryotic translation ? 2

d) The largest known polypeptide chain made by any cell is a protein called titin (made by mammalian muscle cells), and it has a molecular weight of 3,000,000 Daltons.

i) Calculate the time required to synthesize a titin mRNA from the corresponding gene of titin ?

ii) Calculate time required for muscle cell to translate an mRNA coding for titin ? (Assume standard rate of translation and sandard rate of transcription for eukaryotic cells). 2

GROUP - B

15. a) Draw a labelled diagram of the structure of lac operon with all of its elements. In the lac operon, explain the probable effect on gene expression due to the presence of the following :

i) Mutations in the lac operator.

ii) Mutations in the lacI gene.

iii) Mutations in the promoter. 1+3

7. a) Explain why the deamination of 5-methylcytosine leads to hot spots for spontaneous mutations more than the deamination of cytosine in DNA does. 2

   b) List the different ways by which thymine dimmers in the cells can be repaired. Briefly outline one of these processes. 3

   c) "As a result of DNA helicase activity, topoisomerases are also required during replication". Explain how topoisomerases help DNA helicases function more efficiently. Is toposiomerase involved in any other phase of DNA replication ? 3

   d) Which enzyme is required for resolution of Holliday Structure and how does it act ? 2

8. a) List the different enzymatic activities that RecBCD catalyzes and describe the significance of each activity in the steps of homologous recombination (via the DSB-repair pathway). 4

   b) Infection of E. coli with bacteriophage involves integrative recombination for the phage to enter the lysogenic state and excisive recombination for it to enter lytic growth. Which enzymes has a role in the integrative recombination and excisive recombination. Between which region of bacterial genome and phage genome this kind recombination take place ? 4

   g) Bacteria that become lysogenic for bacteriophage λ are immune to subsequent λ lytic infections. Why ? 1

   h) In the leader region of the trp mRNA, what would be the effect of removal of sequence 4 ? 1

   i) What is SAGA and what its role in transcription ? 1

   j) What is the role of Dicer and Argo in gene regulation ? 1

GROUP - A

11. a) Promoter clearance of RNA polymerase involves an inherent problem, in that the energetic forces that attract the polymerase to the promoter in the first place during initial binding will tend to hold it at the promoter, thereby preventing transcription elongation. In bacteria, how can the machinery overcome this problem and enable the polymerase to clear the promoter ? 4

    b) Predict the likely effects of a mutation in the sequence 5'-AAUAAA-3' in a eukaryotic mRNA transcript. 2

    c) Mention the full name and mechanism of action of the following factors eukaryotic pre-mRNA : CPSF, CstF, PAP, PAB2. 4

c) List the major differences between the 'Holiday Model' and 'Double Strand Break Repair Model' of Recombination. 2

9. a) With necessary diagrams, compare and contrast the cut-and-paste mechanism of transposition with the replicative mechanism of transposition. 4

b) What are the major difference of actions between the serine and tyrosine recombinases. 2

c) How would you isolate a DNA-replication deficient mutation in E. coli ? 4

PART - II

Answer Question No. ***10*** and ***any four*** Questions taking at least ***one*** from each group.

10. Answer ***all*** questions :

a) How does the enzyme polynucleotide phosphorylase differ from DNA and RNA polymerases ? 1

b) A short oligopeptide is encoded in this sequence of RNA.

5′ GACUAUGCUCAUAUUGGUCCUUUGACAAG

Where does it start and stop, and how many amino acids are encoded ? 1

c) What is the role of the sigma factor in transcription, and how does it accomplish this ? 1

d) A new RNA polymerase activity is discovered in crude extracts of cells derived from an exotic fungus. The RNA polymerase initiates transcription only from a single, highly specialized promoter. As the polymerase is purified, its activity is observed to decline. The purified enzyme is completely inactive unless crude extract is added to the reaction mixture. Suggest an explanation for these observations. 1

e) Methionine is one of the two amino acids having only one codon. yet the single codon for methionine can specify both the initiating residue and interior Met residues of polypeptides synthesized by *E. coli*. Explain exactly how this is possible. 1

f) A preparation of ribosomes in the process of synthesizing the polypeptide insulin was incubated in the presence of all 20 radiolabeled amino acids, tRNA's, aminoacyl-t-RNA synthetases and other components required for protein synthesis. All the amino acids have the same specific radioactivity (counts per minute per nanomole of amino acid). It takes ten minutes to synthesize a complete insulin chain (from initiation to termination) in this system. After incubation for 1 minute, the completed insulin chains were cleaved with trypsin and the radioactivity of the fragments determined. Which tryptic fragment has the highest specific activity ? 1

12. a) Describe the process of stepwise assembly of eukaryotic transcription initiation complex with labelled diagram. 4

b) What is the significance of CTD of the largest submit of RNA pol II in the transition of the enzyme from the initiation to elongation phase ? 3

c) Mention the functions of the following enzymes in capping enzyme complex : RNA terminal phophatase, Guanylyl transferase. 3

13. a) "Eukaryotic genes are split in nature" – explain. Describe an experiment to prove the presence of introns. 2+3

b) What is mRNA editing ? Among the various kinds of RNA editing reaction, mention just one fundamental reaction. Which enzyme plays vital role in this process and briefly discuss it function. 3

c) Molecular weight of a prokaryotic double stranded DNA molecule is 6×10^9. If a polypeptide chain contains 1,000 amino acid residues, calculate the number of such polypeptide chains that can be synthesised from the above DNA. 2

14. a) Which position of the codon can be most easily changed without effect on the encoded amino acid ? Why this particular nucleotide so easily alterable ? 2

b) List the forces, which play critical role in stabilizing the DNA/RNA double helices. Do you know any force that tends to destabilize the double helix ? 2

c) What is the approximate molecular weight of a protein (assume that it consists of a single polypeptide chain), which is encoded by 2481 base pair long prokaryotic gene ? 3

d) Explain with necessary diagrams, which structural part of the core histone plays crucial role in structural transition from 10 nm to 30 nm fibre of the chromatin ? Can you prove this experimentally. 3

GROUP - B

(Answer ***Any Two*** questions)

6. a) Design an experiment by which it was demonstrated that DNA replication is actually 'semi-discontinuous'. 6

b) A loss of function mutation happens in *dam* gene (encoding the Dam methylase) in *E. coli.* Predict the phenotype you would observe with respect to initiation of replication in this mutant. Briefly explain your answer. 2

c) Why telomerase is not required in *E.coli* cells but is essential for humans ? Justify your answer. 2

b) Describe with a diagram how a RNA regulates gene expression after transcription initiation of a operon in *E.coli* ? 4

c) What phenotype you would anticipate of the following E.coli strain in terms of β-galactosidase production in absence and presence of IPTG, also explain why :

i) $I^+O^+Z^-A^+/F'I^-O^+Z^+A^+$

ii) $I^+O^+Z^-A^+/F'(I^-O^CZ^+A^+)$. 2

16. a) Write the name of a prokaryotic protein which acts as negative as well as positive regulator of gene expression in *E.coli*. Describe the mechanism of negative and positive regulation of gene expression by that protein. 4

b) How a protein hormone and a non-protein hormone regulate gene expression in eukaryote ? 4

c) What is the smallest number of molecules of ATP and GTP consumed in the synthesis of a protein having molecular mass 48 Kd, starting from amino acids ? 2

17. a) Compare the growth pattern of wild type *E.coli* in the normal M9 medium with the presence of following C-source, with labelled diagram : (i) only glucose, (ii) only lactose, (iii) both glucose and lactose. 3

c) Name the major types of bonds that occur between histone proteins and DNA and the specific region of DNA where these bonds form. Are these interactions sequence-specific ? Explain why or why not. 3

d) Are the helical structure of DNA and the helical structure of double-stranded RNA identical ? If not how do they differ ? 2

3. a) For any given set of base-pair, state which groove (major or minor) of DNA contains more useful chemical information for DNA-protein interaction. From your answer, state which groove is preferred by major classes of sequence-specific DNA binding proteins (such as a *lac* repressor) to interact with DNA. Justify your answer. 3

b) The pI of histones is very high, about 10.8. Predict, which amino acid residues must be present in relatively large numbers in histones ? How these residues contribute to the strong binding of histones to DNA ? 3

c) What is the primary type of bond responsible for each of the following interactions :

i) One DNA strand interacting with another strand of DNA in double-stranded DNA.

ii) A dipeptide of two amino acids. 2