Ex/MSBT/1/3/53/2017

M. Sc. (Biotechnology) Part I Examination, 2017

Molecular Biology

MSBT 1/3

Full Marks – 100
Time 4 Hours

Answer Question No. 1 and any *eight* Questions taking at least *one* from each group

1. Answer ***all*** questions 10×2=20

(a) What enzymatic activities are associated with DNA polymerase I? What is the principal physiological role of this enzyme? 2

(b) What is a 'Chi' site? How do 'chi' sites protect *E. coli* against foreign DNA? 2

(c) How does mismatch repair system ensures that the nucleotide that is replaced is the erroneous one and not the original nucleotide in the template DNA? 2

(d) Assume that 32 million histone octamers are required to package human genome, how many histone molecules must be transported per second per complex in cells whose nuclei contains 3000 nuclear pores and are dividing once per day. 2

(e) Nucleoside triphosphates labeled with [^{32}P] at the α, β, γ position are useful for monitoring various aspects. For the specific process listed below, give the position of the label that is appropriate for examining that step.

(i) Labeling the 5' end of linear DNA
(ii) Transcription Elongation by eukaryotic RNA polymerase II. 2

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

(g) You treat a protein with the denaturant urea. For each interaction or bond below, state if the interaction or bond is disrupted by the urea treatment.

(i) Ionic bonds.
(ii) Hydrogen bonds.
(iii) Disulfide bonds.
(iii) Peptide bonds. 2

(h) Explain from where the energy comes for the formation of peptide bond? 2

(i) What prevents sigma factor to bind to promoter sequence in absence of core RNA polymerase? 2

(j) Define "Exon Definition". What is its role in pre-mRNA splicing? 2

Group - I

2.(a) Consider the following reaction:

$$\text{Glutamate} + NH_3 \leftrightarrow \text{Glutamine} + H_2O \ (\Delta G = +3.4 \text{ kcal/mol.})$$

Would coupling this reaction to ATP hydrolysis allow glutamine formation to be favored? Explain why or why not. Write the overall reaction. 3

(b) Explain why nucleoside triphosphates rather than nucleoside di-phosphates are used in DNA synthesis. 2

(c) 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

(d) Justify the biological reason for the presence of uracil in RNA but not in DNA. 2

3.(a) How do enzymes enhance the rate of a reaction? 2

(b) The oxygen-binding proteins hemoglobin and myoglobin differ in that hemoglobin functions as a **tetramer in red blood cells**, whereas myoglobin functions as a monomer in muscle cells. 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. 3

(c) Explain why is RNA polynucleotide is chemically more prone to spontaneous hydrolysis at high alkaline pH whereas DNA. 2

(d) What is Ribozyme? Describe the properties shared between a protein-enzyme and a ribozyme. 3

4.(a) 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

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

5′-AGGACCCUUCGGGGUUCU-3′ 2

(d) 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 to denature them. Explain the reason for this difference. One such protein is bovine pancreatic trypsin inhibitor (BPTI), which has 58 amino acid residues in a single chain and contains three disulfide bonds. On cooling a solution of denatured BPTI, the activity of the protein is restored. What is the molecular basis for this property? 4

Group – II

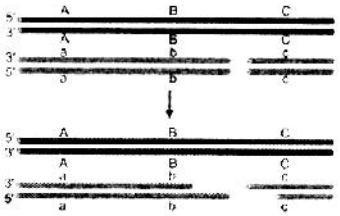
5.(a) Describe the various components of a nucleosome. Name the major types of bonds that occur between histone proteins and DNA and the specific region of DNA where these bonds form. Are these interactions sequence-specific? Explain why or why not. 4

(b) What is Okazaki fragment? Why these fragments generate during DNA replication? Using necessary diagrams and flowchart briefly describe the experiment (long description is not necessary), which Okazaki designed to detect the existence of these smaller fragments. Why was it important to use a short exposure to the radioactive label rather than a longer one in this experiment? 4

(a) 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

6.(a) Just mention with necessary diagram what happens if the replication machinery encounters a lesion in the template DNA that prevents its progression? What options exist for getting past the lesion? Which of these is the option of last resort? Why? 4

(b) Following the formation of a double strand break which enzyme process the double stranded DNA to form single stranded DNA as shown in following figure.

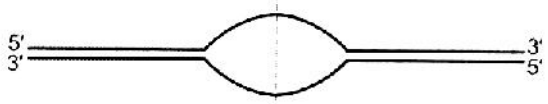


Does it matter if resection occur in the 5′ to 3′ direction or in the 3′ to 5′ direction? Justify your answer. 4

(c) Explain with the help of suitable diagram why the replication machinery is incapable of completely replicating the ends of the chromosomes. 2

7.(a) How does mismatch repair system ensures that the nucleotide that is replaced is the erroneous one and not the original nucleotide in the template DNA? 2

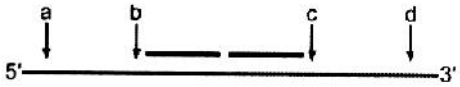
(b) Below is the diagram of a single origin of replication in Eukaryotic Cell. With respect to the dotted line, in which direction (right, or left) does the replication fork proceed. Present your answer with necessary diagram. Does the replication fork move the same way in prokaryotes?



4

(c) 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

8.(a) Shown below is a long template strand of DNA, where lagging strand DNA synthesis is occurring. The short horizontal lines represent two Okazaki fragments that have already been made. In the context of the replication fork, select the letter (a–d) that indicates where primase will synthesize the next RNA primer. Why did you choose that location? Justify your answer.



4

(b) What do you mean by heteroduplex DNA? Explain with necessary diagram how heteroduplex DNA forms following a double strand break in the chromosome. 4

(c) What is the role of base flipping in DNA repair? What does the repair machinery accomplish by relying on this phenomenon? 2

Group – III

9.(a) Consider a bacterial promoter with –35 and –10 elements. What assay is the best method to show that RNA polymerase binds at regions centered on the –35 and –10 positions upstream of the start site of transcription?

3

(b) Are the 5′-splice site and 3′-splice site labeled 5′ and 3′ with respect to the ends of the intron or the ends of the exons? In addition to the 5′ and 3′ splice sites, what other sequence is required in splicing? Where is this sequence located? 3

(c) How does the number and size of introns in the average gene make splice site selection such a challenge for cells? Describe two different mechanisms that cells use to increase the accuracy of splice site selection. 4

10. (a) Explain why the mediator and nucleosome modifiers are required for high levels of transcription in eukaryotic cells but not in vitro. 2

(b) What is the role of Pol II CTD phosphorylation in the regulation of eukaryotic transcription and mRNA biogenesis events? 4

(c) Consider the Rho-independent terminator sequence

5′-CCCAG**CCC**GCCUAAUGAGC**GGG**CUUUUUUUU-3′

Why does a point mutation at any one of the boldfaced nucleotides disrupt termination of transcription? How would you test your conclusion? 4

11(a) Draw the diagram of a pre-mRNA as it exists in a spliceosome just before the second step of splicing. Show the interactions with U2, U5 and U6 SnRNPs. Just draw the diagram only. No description is necessary. If you prefer you can color code each component
3

(b) Compare the self-splicing reactions carried out by group I and group II introns. Which is most similar to the reaction carried out by the spliceosome on pre-mRNAs?
4

(b) Which region(s) of sigma factor were shown to interact with the -10 and -35 region of the *E. coli* promoter? Suggest an experiment by which you can demonstrate your answer experimentally.
4

12(a) Explain the basis of interaction between small nuclear ribonuclear proteins (snRNPs) and the pre-mRNA splicing substrate. 2

(c) Are there similarities between the DNA replication bubbles and the transcription bubbles? Explain. 2

(d) A human gene has three exons and two introns. The exons are 456 and 524 bp, while the introns are 2.3 kb and 4.6 kb.

(i) Draw this gene, showing the promoter, introns, exons, and transcription start and stop sites.

(ii) Surprisingly, it is found that this gene encodes not one but two mRNAs that are completely different in sequence. The first mRNA is 980 nucleotides, while the second is 3.2kb. Use your drawing to show how it is possible for this one region of DNA to encode these two transcripts.
6

Group -IV

13(a) Which tRNA synthetase would you expect to have an editing pocket to hydrolyze an aminoacyl-tRNA mischarged with glycine? Explain your choice. 2

(c) Describe what are the A, P, E sites and decoding and peptidyl-transferase center of the ribosome. Present schematic diagram to show where are they located? What occupies each of the sites after peptidyl-transfer, but before translocation is completed by EF-G? 6

(d) You are given the following DNA sequence located in the middle of a gene 5′-ACCGTTTCGGCTAGG-3′ from E. coli. This strand represents the coding strand. How many possible polypeptides that this sequence can encode. 2

14. (a) Describe the different mechanisms used to ensure translation accuracy, beginning with the charging of the tRNA and continuing up to the transfer of the amino acid to the growing polypeptide chain. 4

(b) What phenotype you would anticipate of the following *E. coli* strain in terms of β-galctosidase production (i) in absence of lactose and (ii) in its presence: $i^+o^+Z^-Y^-A^+$ and $i^+o^cZ^+Y^+A^+$. 2

(c) In a genetic screen, researchers isolated mutants of E. coli that constitutively expressed the genes from the *lacZYA* operon.
(i) Describe what constitutive expression means in terms of the *lacZYA* operon.
(ii) Give an example of a mutation that could lead to constitutive expression of the *lacZYA* genes. 4

15. (a) What direction does the ribosome move along the mRNA during translation? What drives this movement---why doesn't the ribosome just bind the mRNA and stay in one place? 2

(b) "*araC* can act as both repressor and activator"- Justify the statement. 4

(c) "trp attenuator in *E. coli* is an example of riboswitch" – Justify the statement 4

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

(b) The most common type of regulation of gene expression occurs at the level of transcription. Name other types of regulation for gene expression in eukaryotic cells. 2

(c) List different types of DNA binding motifs are found in eukaryotic transcription activators. What is the most common structural features shared by these motifs which are used in DNA binding? 2

(d) What do you mean by the terms "signal integration" and "combinatorial control"? Cite one example of each one. 2

(e) What is "RISC"? How this component contributes to regulation of gene expression in eukaryotes? 2